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: Robert J. Toonen
Affiliation: Hawaii Institute of Marine Biology, University of Hawaii at Manoa

Permit Category: Research
Proposed Activity Dates: 05/15/10 through 11/15/10
Proposed Method of Entry (Vessel/Plane): R/V Hi'ialakai
Proposed Locations: Shallow water habitats (< 100 feet depth), focused on: 1) completing connectivity sampling in locations that were excluded due to weather conditions, cruise constraints or time in previous years, and 2) taxonomic studies of scleractinian corals of the genera Montipora and Pocillopora in collaboration with Maragos (USFWS). In addition to completing the last of the connectivity collections, we also propose to begin exploration of black corals at mesophotic depths (30 to 250m) which remain largely unexplored outside the MHI.

Estimated number of individuals (including Applicant) to be covered under this permit: One berthing position for my research team on each of 2 different research cruises, plus available members of researchers from other permitted activities who can collect opportunistically on our behalf.
Estimated number of days in the Monument: Up to approximately 60 days spread across 2 research cruises

Description of proposed activities: (complete these sentences):

a.) The proposed activity would…

collect non-lethal tissue biopsy samples of common reef invertebrates to conduct a population genetic survey. This survey is an attempt to complete the on-going effort to determine patterns of connectivity or isolation among each reef ecosystem throughout the Hawaiian Archipelago with a focus on specific species and locations of special interest that have been missed due to circumstances beyond our control in previous years. The primary goal of this work will be to: 1) complete collections of spiny lobsters, and 2) to determine the specific location of limited exchange detected between Papahānaumokuākea Marine National Monument and the Main Eight Hawaiian Islands. The sites of the NWHI between Kauai and French Frigate Shoals have been missed in collection efforts in previous years due to cruise constraints or weather, and I am unsure that they will be collected this year either, but we will continue to submit permit

applications until we are able to collect at these sites to determine the location of the barrier between the Main and NWHI.

Additionally we propose to begin taxonomic work on several groups of corals whose identity is a subject of debate. In particular, we propose to collaborate with Jim Maragos (USFWS) to collect voucher specimens and characterize the Hawaiian Montipora and Pocillopora species.

Finally, we also propose to study black corals at mesophotic depths in the Monument. This research will involve collection of vouchers for comparison with established type specimens to confirm or refute the morphological identification of the Hawaiian antipatharian (black coral) fauna. The black corals are already under revision because the species previously identified as Antipathes dichotoma from Hawaii do not match specimens from the type locality of A. dichotoma in the Mediterranean Sea; as a result, the Hawaiian "A. dichotoma" has now been assigned the new name of Antipathes griggi (Opresko 2009). Likewise, our study over the past year has resulted in the redescription of Antipathes grandis (Wagner et al., in review) from the Main Hawaiian Islands. Last year, during the first technical diving to the appropriate depths in the Monument, we discovered 4 species of black corals never before reported from the NWHI and now seek to obtain voucher collections of these animals to determine whether the species identity is correct (or a misidentification due as described for A. dichotoma above). In addition to technical diving to collect samples, we request permission to 1) deploy an ROV for exploration and video surveys of appropriate habitats to estimate the distribution and abundance of each species within the Monument, and 2) deploy temperature loggers at technical dive sites in order to check whether differences in faunal composition across sites correspond to latitudinal differences in temperature. The temperature loggers will be recovered on a subsequent cruise in the 2011.

b.) To accomplish this activity we would ….
collect target invertebrates by hand. Connectivity studies require only a tiny tissue biopsy sample be collected prior to release of the live animals back to the environment. These samples are identified in a sample database and tissues are preserved for future DNA analyses to determine patterns of genetic structure among locations and infer the level and magnitude of exchange among those populations. Coral samples for taxonomic study require high resolution photographs of the colony prior to sampling, and then collection of small portions of colonies for subsequent DNA and morphological study as well as museum archiving. Likewise, black coral samples from mesophotic depths will be preserved for DNA, morphological, histological and electron microscopic work to identify the species and sex of the sampled colonies in addition to subsequent archiving in appropriate museums (we propose the Bishop and Smithsonian museums as the obvious choices). Finally, we hope to use an ROV at depth to perform video transects of appropriate habitat to estimate the abundance and distribution of the antipatharian species within the Monument.

c.) This activity would help the Monument by …
identifying the location of the reduced connectivity between the Main and Northwestern Hawaiian Islands to determine whether the location corresponds to the border of the Monument or not. Results from this study to date indicate that there are regions of both high exchange and

of strong isolation within the Archipelago that need to be considered in management strategies. In particular, there is a barrier to dispersal between the Main and Northwestern Hawaiian Islands (particularly between Kauai and French Frigate Shoals) that is poorly understood because of mechanical failure, limited field opportunities, and poor weather in previous years. We seek to complete the sampling of a few remaining species and identify the specific location of the Main to NWHI barrier.

Knowing which species occur and where is fundamental to the management of the resources, and the first dives to mesophotic depths in the NWHI have already extended the ranges of black coral in Hawaii throughout large areas of the Monument. Studies in the MHI have called the taxonomy of the antipatharians into question, and we propose to survey the habitats by ROV to estimate the extent of these valuable resources within Monument boundaries, and collect voucher specimens to confirm which species are present in which areas. Likewise, there is debate among coral taxonomists regarding the species designations of Montipora and Pocillopora under Monument jurisdiction, and we propose to begin taxonomic collections for detailed study of the morphospecies of contention.

Other information or background: There appear to be 4 primary areas of restricted larval exchange across the Hawaiian Archipelago, resulting in 5 areas of moderate connectivity. The findings of our connectivity research have been presented at numerous public meetings and to all the co-trustee agency partners. One of the primary remaining questions from the work to date is the specific location of the Main to NWHI barrier, which we have not been able to address due to the difficulty of collecting samples at the near NWHI sites. We hope to solve that last remaining question this year. We have also been asked to complete work on the spiny lobster connectivity, and propose to collect the samples necessary to address that question as well.

In addition to hopefully completing the connectivity collections, we propose to survey the species diversity and reproductive status of black corals found at mesophotic depths. This area was explored for the first time in the Monument last year, and we discovered four species of black coral never before reported from the NWHI. These include Antipathes griggi (formerly A. dichotoma) off the islands of Necker and Laysan in 59-70 m, Myriopathes ulex off Necker Island and Pearl & Hermes Atoll in 58-69 m, Cirrhipathes cf. anguina off the island of Necker and Laysan in 59-69 m, and Cirrhipathes sp. off Necker Island in 58 m. We request permission to collect vouchers of each so that we can examine these samples in comparison to the museum collections and in consultation with the world taxonomic experts to confirm whether the species identifications are correct or whether these are potential new species. Whenever possible, use of an ROV for video transects will help to estimate the distribution and abundance of these species among sites throughout the Monument waters.

We also propose to begin taxonomic work on scleractinian corals whose identity is a subject of debate. In particular, we propose to collaborate with Jim Maragos (USFWS) to collect voucher specimens and characterize the Hawaiian Montipora and Pocillopora species. Our current work in the MHI to examine the Species of Concern, Montipora dilatata, shows 3 distinct genetic groups: I) M. patula/M. verilli, II) M. dilatata/M. turgescens/M. flabellata and III) M. capitata. Alternate explanations for these results

could include either: 1) these genetic groups represent closely related but distinct species with occasional hybridization, and 2) there are only three morphologically plastic species which have been split into 6 different named taxa. Validation of these initial findings and determination of which alternate is true requires collection of a few voucher specimens of all known species of Montipora across the Hawaiian Archipelago to establish the taxonomy of these species (Forsman et al. in prep). Likewise, initial testing of the coral Pocillopora meandrina suggests that the Hawaiian form is genetically distinct from P. meandrina from other locations throughout the Pacific, and may be an unrecognized species restricted to Hawaii. If this were true, that would greatly alter its priority for management. We propose to collaborate on voucher specimen collections with Maragos to identify and sample a few colonies of all known morphospecies of Montipora and Pocillopora corals (some of which are restricted to the NWHI) across the Hawaiian Archipelago for taxonomic studies.

Finally, we are developing a new DNA-based technology for the detection of alien invasive species (the ReefChip), and we request permission to collect samples of any alien invasive species located within the Monument while performing these other activities to ensure that those species are included on our detection chip.

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Robert J. Toonen

Title: Associate Researcher, Hawaii Institute of Marine Biology

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

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

For students, major professor's name, telephone and email address:
Rob Toonen, HIMB
[redacted]

3. Affiliation (institution/agency/organization directly related to the proposed project):
Hawaii Institute of Marine Biology,
School of Ocean & Earth Science & Technology,
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):
Although our actual research team is expected to include only one participant on each cruise, we request permission for permitted participants of each research cruise to be included as potential collection assistants on this project to provide maximum flexibility in the field depending on weather conditions and site. We expect that cruise

participants for this project will be Daniel Wagner (Graduate student, Research Diver, UH Manoa) and Roxanne Haverkort (Graduate student, Research Diver, UH Manoa). We ask that researchers on other projects on the cruise be able to assist with the collection when not otherwise occupied with their own work. Although I cannot predict the exact composition of the other teams, I expect that those participants will most likely be drawn from the following list.

Jim Maragos (USFWS Coral expert & Research Diver), Derek Smith (Water Safety Officer & Research Diver, HIMB), Megan Donahue (Assistant Research Faculty, Research Diver, HIMB), Michael Stat (Post-doc, Research Diver, HIMB), Randall Kosaki (PMNM, Research Diver), Scott Godwin (PMNM, Research Diver), Kelly Gleason (PMNM, Research Diver), Ray Boland (NOAA, Research Diver), Greg McFall (NOAA, Research Diver), Carl Meyer (Ph.D., Research Diver, HIMB), Jon Dale (Graduate student, Research Diver, HIMB), Yannis Papastamatiou (Graduate student, Research Diver, HIMB), Steve Karl (Ph.D., Research Diver, HIMB), Jonathan Whitney (Graduate student, Research Diver, HIMB), Nyssa Silbiger (Graduate student, Research Diver, HIMB), and/or John Fitzpatrick (Graduate student, Research Diver, HIMB).

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:
As outlined above, we anticipate our collection efforts will be concentrated in the shallow water reef habitats between Kauai and French Frigate Shoals to determine the location of the Main-NWHI barrier (see Appendix 1). Additional sampling effort and ROV surveys at mesophotic depths will be performed as cruise conditions permit at the sites targetted for technical diving operations - FFS, P&H and possibly Midway. Other priorities dictate that cruises will visit a variety of atolls, and we still need some samples of lobsters or voucher specimens of corals that could be collected at many of these sites. I therefore list all possible sites here for maximum flexibility due to weather or unforeseen changes to cruise schedules. 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
Necker Island	-164.13627752700	23.71705429230
Necker Island	-164.13373024500	23.20505064020
Necker Island	-164.92084033700	23.20505064020
Necker Island	-164.92338761900	23.71960157420
Nihoa Island	-161.66031956700	23.23816530420
Nihoa Island	-161.66286684900	22.94013332760
Nihoa Island	-162.05005369100	22.94268060940
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:***
This year we seek to complete previous efforts to develop a comprehensive genetic survey of common coral reef invertebrates across the Archipelago to estimate patterns and magnitude of exchange among locations. We seek to identify the specific point of limited dispersal detected between the Monument and the Main Hawaiian Islands. Although we have made considerable progress in understanding patterns of connectivity across the Hawaiian Archipelago over the past several years, the exact location of the Main-NWHI barrier remains unknown, and it is important to determine whether or not this barrier happens to coincide with the boundaries of the Monument or not.

Mesophotic coral reef ecosystems (MCE's) are warm water, light-dependent coral reef ecosystem occuring from the lower limits of traditional SCUBA diving depths (i.e., >30m) to the bottom of the photic zone which varies by location, but in some areas can be up to 265m deep. This area is one of the least explored on the planet, and the first technical dives conducted in the Monument discovered 4 previously unreported species of black corals (Wagner et al. in review). Although these species have been tentatively identified based on photographs, our study of black corals in the MHI has revealed that such identifications are tenuous, and we propose to collect voucher samples for positive ID and additional study from the waters of the Monument. This work will allow us to determine whether the colonies in the NWHI are in fact the species they have been tentatively identified as, or are some previously unknown species that are being misidentified. Our discussion of this issue with coral taxonomists at the Smithsonian lead us to believe the latter is the case for at least some of the antipatharians discovered on the cruise last summer.

Likewise, our genetic surveys allow for the possible identification of cryptic Hawaiian endemics that may not be currently recognized due to similarity in morphology to their broadly-occurring relatives. Our ongoing study with the Montipora species of concern, Montipora dilatata, requires vouchers of all known morphospecies of Montipora in Hawaii to evaluate alternative hypotheses that would either place it as: 1) a distinct species under critical threat and worthy of consideration for endangered species status, or 2) an unusual growth form of widely occuring species that should not be considered as distinct for the purposes of management. There is some support for both interpretations of the data, and we seek to address this critical issue to inform managers who are faced with making decisions about the impact of activities on these groups throughout Hawaii as well as within Papahānaumokoākea Marine National Monument. Along the same lines, our initial studies of Pocillopora suggest that P. meandrina in the NWHI is genetically distinct from other locations sampled in the Pacific. This raises the question of how the Main and NWHI populations of P. meandrina are related, whether the corals in Hawaii are in fact a distinct population or perhaps a previously unrecognized endemic species. The importance of this primary habitat coral to juvenile fishes has already been reported by Friedlander & DeMartini, but the management priority assigned to impacts facing this coral would differ if it were a common and

broadly distributed Pacific coral or a Hawaiian endemic. Thus we are requesting permission to collect voucher samples of Pocillopora species for the same type of study as outlined for the Montipora in collaboration with Maragos to evaluate the taxonomy of the Hawaiian Pocilloporids also.

Along these same lines we request permission to collect a voucher specimen of an individual organism that cannot be identified as a known species and/or may represent new geographic records or new species from the taxonomic groups under study in this permit. The voucher specimen would be used for taxonomic study to determine the species identity and would be included with the Bishop and/or Smithsonian museum permanent collections under the conditions set forth in the material transfer agreement.

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?
I and my team are conservation biologists who are both teaching and studying the science of how best to manage and conserve biological diversity in the sea. As such, minimizing our impact to the ecosystem we are trying to conserve is naturally and inherently a top priority for any research we conduct within the Monument. In particular, we focus on nonlethal sampling to minimize any potential unforeseen negative impacts to the system as outlined in the PROCEDURES 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 to the ecosystem, nor have we been able to detect any effect to date. As outlined in detail below, our sample size, choice of species, 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. In comparison to the estimated 30,000 tons of prey consumed per year by ulua at a single atoll (Sudekum et al. 1991; Friedlander & DeMartini 2002), it is not surprising that our non-lethal tissue sampling of common reef species shows no effect on the ecosystem.

We do not impact historic resources because 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 I explained in my permit application last year, it has taken time for me and my team to grow to understand the importance of Papahānaumokuākea to the Hawaiian People and what exactly the cultural resources of the place include. It is only after I have gone through the cultural briefings each year, and have come to know and collaborate closely with some Cultural Practitioners that I believe I am finally beginning to understand the significance of the place and what this question really means from the Hawaiian perspective. With that caveat, I have tried and will continue to ensure that I and my team do our best to have minimal impact on the

cultural resources of Papahānaumokuākea. We have on-going collaborations with several Native Hawaiian Cultural Practitioners, and last year our team completed the first joint scientific/cultural survey of opihi habitat in the Monument. We hope to continue that work in the future and include cultural practitioners as collaborators in our efforts to better understand the resources of the Monument. It is through our interactions with those cultural practitioners who have generously given their time and knowledge to work with us that our understanding of what constitute cultural resources has grown, and from which our efforts to minimize our impacts derive.

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 ensures that they also recognize the cultural significance of the place.

Not being of Hawaiian descent, and not knowing the language, it is difficult for us to offer a chant upon entering the Monument. Likewise, because prayer is such a personal thing, I do not feel comfortable mandating that my team offer a prayer prior to entering the Monument. My solution is to request that my team meet on the bow of the ship to feel the environment and reflect in silence on why they are on this trip, what is the purpose of the trip, and how lucky they are to be able to go to this special place. It is respectful to provide an offering and to not go forth to take from the place with empty hands. Given concerns regarding transport of materials into the Monument, it is also difficult to present a proper offering in the form of a gift. In our discussion with William J. Aila, Jr., he suggested that water would be an appropriate offering, but simply buying a bottle of water and taking it along obviously misses the entire point of the offering. Thus, I ask that my team each collect their own rainwater and autoclave sterilize a bottle of fresh-caught rainwater (to ensure no biological transport) prior to bringing that along as our offering in return for the privilege of collecting samples in the Monument. After my team has had a chance to reflect on their intentions at entering the Monument and the privilege of going there, we will pour the water from the bow of the Hi'ialakai as our collective offering.

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?

This type of research is directly mandated by the Proclamation, and is necessary to both maintain ecosystem integrity and provide for adaptive ecosystem management in the face of natural or anthropogenic disasters and 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 we are concerned about exactly these sort of impacts, we have also conducted a threat assessment of the activities in the Monument (Selkoe et al. 2008) and compiled a cumulative impact threat map of the Monument (Selkoe et al. 2009) which has been provided to the co-trustees for use in future management decisions.

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.

We expect it is self-evident that there is no practical alternative to sampling within the Monument when the goal of the research is to understand connectivity among disjunt populations within the Monument. Likewise, surveys of biodiversity of the Monument which seek to determine the species present, their abundance and distribution are only possible within the bounds of the region of interest.

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

Given that we can detect no adverse effects of our activities on the resources of the Monument, we believe that the end value of this research clearly outweighs that imperceptible impact. Further, an understanding of connectivity across this region will identify potentially vulnerable locations and species, and (as outlined above) greatly increase the decision-making capacity of the co-trustees in dealing with unforeseen events within the Monument.

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

The cruise length is shorter than ideal, and is certainly no longer than 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 a PhD in Population Biology, and have published more than 50 research papers in peer-reviewed journals dealing specifically with the subject of conservation and management of Hawaiian natural resources over the past five years. I will be responsible for the conduct of my team and the field PIs on this project will be Daniel Wagner, who was on the technical diving cruise last year as a support diver, and has been involved in a number of research cruises ranging from Hawaii to the Antarctic. Roxanne Haverkort is a graduate student who seeks to train with Jim Maragos, and will be working with him directly in the field for the purpose of voucher collections of Montipora and Pocillopora species.

Our research to address these fundamental management questions began prior to the establishment of the Monument, and has been of considerable interest to both the science and management community of Hawaii throughout the establishment and subsequent management of Papahānaumokuākea Marine National Monument. This on-going project is well-known to the Monument co-trustees, and our research accomplishments are presented in regular meetings with the management community and semi-annual meetings. My accomplishments and qualifications to perform this research are further documented in my included CV.

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.

There are adequate finances in the Toonen-Bowen lab and the PMNM-HIMB partnership to conduct and complete all the research outlined herein. We have an established track record of completing and publishing the research conducted in the Papahānaumokuākea Marine National Monument on a reasonable time frame, and have every intention to continue that tradition. In addition to NOAA funds, there are NSF and private foundations who are supporting this on-going research as documented in the attached CV.

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 are 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 genetic methods outlined herein are employed routinely in the Toonen-Bowen lab, and are appropriate to the proposed activities. The fact that both Toonen and Bowen have been awarded highly-competitive NSF grants to expand the activities described here speaks to the outstanding quality of the research and the wide-spread scientific acceptance of work coming from our lab. Our rate of publication, the quality of journals in which those publications appear, and the rate of citation of our studies shows endorsement of the work being performed by the broader scientific community. The use of genetic sampling is widely regarded as the cheapest and most robust way in which to answer questions of connectivity on these scales, with the minimum of impact on the resources of the Monument. Minimizing this impact is critical to us because they are the focus of the study for purposes of conservation, and we do not want to detract from that we are seeking to conserve. Thus we employ non-lethal sampling whenever possible and take only the samples necessary to address the questions we are seeking to answer for the resource managers who guide this work.

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 using the NOAA vessel Hi'ialakai

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

There are no other factors that would make the issuance of the permit inappropriate

8. Procedures/Methods:

To date, we have completed sufficient collections for analysis on most of the target species in the northwestern chain beyond French Frigate Shoals. The exception is the spiny lobsters and boxer shrimp which remain to be sampled. In each year, we propose

to sample the islands between Kauai and French Frigate Shoals, and each year mechanical failure, cruise priorities or poor weather prevent us from being able to complete our proposed sampling. Thus, we still need to collect up to 50 tissue samples per species from these near NWHI sites (see Appendix 1). The objective of this research is to pinpoint the location of the barrier to dispersal we have detected between the Main and NWHI and determine whether or not it corresponds to the border of the Monument. To accomplish this goal, we continue to collect up to 50 tissue biopsy samples from each of the target invertebrate species per atoll. The species we target are common and easily collected species found on most Hawaiian reefs (see Appendix 1). The "key" or target species in this case are both locally and regionally abundant, widespread in most areas, easy to identify, easy to collect, and already sampled at most other locations throughout the Monument. As always, our criterion is that we do not sample any individuals if our collection would require us to handle more than 1% of the estimated standing population at any atoll. This consideration is both prudent and practical – we are conservation biologists studying how best to manage and conserve these populations and certainly do not want to have a negative impact on the system in the process. Also, considering that the reef habitat surrounding most locations is well over one hundred thousand acres, if 50 individuals was ever less than 1% of the population scattered across that area, we would simply not be able to locate the animals we were trying to sample.

Techniques:

The target species (see Appendix 1 below) inhabit shallow reefs and are accessible via snorkeling, or scuba dives. Tissue biopsy samples are typically obtained as small fragments for corals (1cm^2), arm clips for sea stars, brittle stars and sea cucumbers, or dactyl (toe) cuttings from crustaceans. Despite the fact that we target only abundant species, for which removal of 50 individuals is not expected to have any detectable population effects, we still use non-lethal tissue samples to further minimize our impact whenever possible. Returning to the corals from which tissue samples were collected in the previous year, it was impossible to see where the sample was removed. There were no signs of reduced growth, increased infection or bleaching, or mortality among any of the colonies that were revistied. Coral reef invertebrates are collected by hand using pliers, forceps or scissors depending on the species. We store tissue samples in >70% ethanol or saturated salt buffer at room temperature in 2 to 50ml vials during fieldwork. Samples are archived in a database and prepared for long-term storage and future DNA use upon return from the field. These 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.

The tissue biopsy collections are typically 0.1 – 0.5 grams (roughly the size of a grain of rice to a pea) and do not kill the animals that are sampled. Whenever possible, individuals are sampled in the least disruptive manner (i.e., in some cases we take a tiny tissue sample from the animal without picking it up, and in others we capture and sample the animal prior to releasing it alive in the same location). For example, lobster

samples are obtained by grabbing an antennae and snapping off the tip without capturing the animal. By comparison, the normal anti-predatory defense response of these species is autotomy, which involves the ready self-amputation of an entire antennae or walking leg(s) to attract attention before the animal flees to safety. This ready self-amputation has resulted in removal of a walking leg becoming a standard sampling technique for crustaceans in most genetic surveys because survival is uniformly high from such treatment. Similar nonlethal sampling methods have been used for the other species on the list at sites throughout the Monument, and we now only need to collect those species at a few remaining sites to isolate the location the Main-NWHI barrier.

This year, we also request an additional set of samples to be collected from the deep reefs (below traditional SCUBA limits) to evaluate the idea that mesophotic reefs (the so-called "twilight" reefs between 100 and 600ft) may provide a refuge from overharvest, global climate change and ocean acidification impacts on shallow reefs. If there is opportunity to employ deep diving technology on any of the cruises, we also request an additional sample of up to 50 non-lethal tissue biopsy samples at each of those sites for comparison to the samples that we have collected from shallow waters to date. We expect that the most common and easily collected samples will include: sea cucumbers (Holothuria atra and H. whitmaei) and/or brittle stars (Ophiocoma pica, O. erinaceus and O. dentata). The sea cucumbers and brittle stars are already included on our shallow water connectivity studies, and have been collected from sites throughout the Monument for comparison to the deep locations.

Mesophotic coral reef ecosystems (depths of roughly 30m to 265m) remain one of the least explored areas on the planet, and the first technical dives conducted in the Monument discovered 4 previously unreported species of black corals at multiple sites (Wagner et al. in review). Black coral (mainly under the species names Antipathes dichotoma, A. grandis and M. ulex) are harvested commercially in the Main Hawaiian Islands for the jewelry industry. Black coral is important to Hawaii: (1) culturally – ʻĒkaha kū moana was traditionally used for medicinal purposes to remedy mouth sores and lung diseases (2) ecologically as black corals provide essential habitat for a myriad of fish and invertebrates on deep reefs, (3) socially and economically - it is the official gemstone of the State of Hawaii, and supports a $30M state-wide fishery. Black corals have never been commercially fished within the NWHI, and are protected within the Monument from the suite of anthropogenic effects that impact fauna in the Main Hawaiian Island, but our research in the MHI has exposed the need for taxonomic revision. Although the first reports of black corals from Monument waters have been tentatively identified based on photographs, our study of black corals in the MHI has revealed that such identifications are tenuous (Wagner et al. in review). Thus, we propose to collect up to 5 voucher samples per location for morphological, genetic, and reproductive study from the waters of the Monument in order to confirm the species identification of the specimens. This work will allow us to determine whether the colonies in the NWHI are in fact the species they have been tentatively identified as, or are some previously unknown species that are being misidentified. Our discussion of

this issue with coral taxonomists at the Smithsonian suggest the latter for at least some of the antipatharians first seen on the cruise last summer. Also, since this habitat has recently become dominated with invasive snowflake coral in the main Hawaiian Islands (Kahng & Grigg 2005) it will also be important to check and see if this invasive coral, Carijoa sp., is present in the NWHI at similar depths.

Understanding the life history, growth, and demographic characteristics of natural black coral populations is key to developing effective management strategies to ensure sustainability of the resource in areas under harvest. Studies to date have been based on incorrect taxonomy of antipatharian corals in the MHI, and this warrants a comparison of populations in both a pristine, unfished state to populations in an impacted, fished state. For this purpose colonies will be sampled non-lethally by clipping a roughly 5cm piece at locations throughout the Monument where found (colonies have already been sampled in the Main Hawaiian Islands). This amount of sampling has been shown to not significantly impact reproductive output and growth of colonies in previous studies in the MHI (Wagner et al. in prep). Subsamples from these voucher collections will be used for (1) the study of reproduction using standard histological techniques, and (2) genetic studies; both of these will allow for an assessment of the impacts of the fishery on natural populations (e.g. reproductive output, genetic diversity, etc). Additionally, genetic studies will be used to assess the level of connectivity of the NWHI populations to the populations with the Main Hawaiian Islands, to understand whether genetic exchange exist between these spatially isolated populations. Such information, would provide managers information on the minimum spacing needed when designing no-take zones if spatial management of precious coral resources was pursued. Additionally, the Monument is likely to be the only stable population in the Archipelago that has not been fished historically for black corals. Hence this comparison will address the question about how harvest pressure in the Main Hawaiian Islands may affect the life history of these populations. This research requires only a tiny tissue sample (~5cm). Because colonies can reach well over 3m in height and roughly the same in diameter, we expect the effect of sampling will be minimal to colonies; such sampling has been shown not to have any negative impacts on reproductive output and growth of colonies in previous studies in the MHI and there is no reason to expect any in the NWHI either. The information gained from this study will help managers to develop effective strategies to achieve sustainability of black coral in Hawaii. In particular, it will provide information on what impacts the fishery is having on natural populations and whether areas that are protected from the fishery are having any spill-over effects on fished populations.

Samples for coral taxonomy involve collecting portions of colonies that are not killed for the study. In general a sample of 5 to 10cm is necessary for museum vouchering, and the samples necessary for genetic, reproductive and microscopical study are tiny subsamples of the museum voucher sample. It is usually only necessary to collect about 3 to 5 individuals at each of a few sites for taxonomic study, and even if there were accidental cases of lethal sampling are still justified in this case because they are of direct influence on the management decisions of the Monument; for example, we

may discover previously unknown endemic species that are at risk, but are only identified after detailed taxonomic study.

Analysis:

Two lab methodologies will be employed in this study. One will be direct sequencing of mitochondrial (mtDNA) or nuclear (nDNA) genes using PCR methodology. In most species, a segment of approximately 600-800 base pairs of the mtDNA cytochrome c oxidase subunit I gene will be amplified and sequenced following protocols used daily in our laboratory. DNA sequences will be generated with an ABI 3100 automated DNA sequencer in the core facility at HIMB. Genomic DNA aliquots will be maintained in long-term storage at HIMB for future permitted studies without the need to recollect these samples. DNA sequence variation will be summarized with standard diversity indices and with an analysis of molecular variance (AMOVA) using ARLEQUIN vers. 3 (Excoffier et al. 2005) as well as new methods being developed and pioneered in collaboration with our lab (Jost 2008, Bird et al, in review).

The other is genotyping of nuclear (microsatellite) markers used for some species, such as the coral Montipora capitata. Development of these markers has followed standard techniques, and quality control testing is currently underway (Selkoe & Toonen 2006) for submission of the study in the near future. The results of this work have already been presented in multiple presentations to resource managers. Samples will be genotyped on an ABI 3100 automated sequencer. Data will be analyzed for standard genetic diversity indices using ARLEQUIN. Assignment tests implemented in the program STRUCTURE will be employed to detect the number of populations and patterns of gene flow, and MIGRATE and IM will also use coalescent approaches to estimate gene flow, including its directionality. This approach was successful in describing genetic diversity and gene flow, including some surprising and unexpected breaks in population structure which had major management implications, in an endangered Caribbean coral (Baums et al. 2005, Baums et al. 2006).

Coalescence approaches will be used to infer population histories, including growth rates, effective population size and age of founding populations. Phylogenetic methods will include neighbor joining and maximum likelihood algorithms in PAUP version 4.0 (Swofford 2002). Population separations will be defined with using Fst values and the maximum likelihood approach of MIGRATE vers. 1.7.3 (Beerli & Felsenstein 2001). The key innovation in MIGRATE (relative to conventional Nm estimates) is that it estimates asymmetric migration: cases where one region is a source and another is a recipient. The utility of this information for resolving dispersal pathways is readily apparent.

Although there has been some resistance to the large number of total samples in the study, the request is reasonable and carefully considered to avoid undue impacts to the natural, historical and cultural resources of the Monument. I emphasize that we focus on only abundant species, and sample no more than 50 individuals per species from

each atoll across the Archipelago to infer patterns of connectivity within these species. Even at the level of an atoll, we try to minimize any local impacts by sampling individuals across an average of 5-10 dive sites to minimize any undetected potential impact of biopsy sampling 50 individuals per species per atoll. We take minimal samples to have the least detectable effect on the animals and emphasize non-lethal sampling in all our efforts. For example, we sample far less than an average bite from the puffer Arothron meleagris, and the damage from such bites is virtually undetectable on Hawaiian corals within 30-40 days (Jayewardene & Birkeland 2006). Furthermore, revisiting previously sampled coral colonies shows clear growth and in each case it has not been possible to see remaining damage or even locate the sampled portion visually in the subsequent year.

To date, we have completed most of the collections for most sites, but the location of the main-NWHI barrier remains unknown. Thus, we submit the same request as last year to complete the collections at Nihoa and Mokumanamana in the hopes of pinpointing the specific location of the barrier. Again, we are at the mercy of the weather and other cruise priorities in completing this collection, but we hope that this year we will be able to complete the final collections to wrap up the invertebrate connectivity study.

This year we also seek to sample each morphospecies of Montipora and Pocillopora coral found across the Hawaiian Archipelago as well as the antipatharian corals within the Monument for taxonomic study. In each case, there is good reason to expect that some of the species designations may change, and we are therefore proposing to collect up to 5 voucher specimens per species at each of 2 to 3 sites for further study. These samples will be subsampled for genetic, morphological and histological study as well as permanent inclusion in the Bishop and Smithsonian museum collections.

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 for connectivity samples.
1) black corals
2) rice corals
3) cauliflower coral

Scientific name:

Following above, see Appendix 1for connectivity samples
1) antipatharians tentatively identified as Antipathes griggi (formerly A. dichotoma), Myriopathes ulex, Cirrhipathes cf. anguina, Cirrhipathes sp.
2) Montipora species: M. capitata (=M. verrucosa), M. incrassata, M. patula, M. flabellata, M. turgescens, M. dilatata.
3) Pocillopora species: P. eydouxi, P. meandrina, P. molokensis, P. ligulata, P. damicornis.

& size of specimens:

Following above, see Appendix 1 for connectivity samples
1) up to 5 nonlethal voucher samples (no larger than 10cm) per species from each of 2-3 sites
2) up to 5 nonlethal voucher samples (no larger than 10cm) per species from each of 2-3 sites
3) up to 5 nonlethal voucher samples (no larger than 10cm) per species from each of 2-3 sites

Collection location:

See Appendix 1 for connectivity samples
for vouncher coral specimens (1-3 above) samples will be collected by the deep diving team at sites for technical diving operations (French Frigate Shoals, and Pearl & Hermes, plus possibly Midway).

☐ 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 Toonen is maintaining the database and providing for the storage of all preserved biospy tissue samples collected to date 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 and 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?

Tissue biopsy samples preserved for genetic analyses (in ethanol or saturated salt buffer, see details below) will be transported back to HIMB aboard the R/V Hi'ialakai. Voucher specimens will be subsampled for genetic analysis and stored frozen or in preservative prior to study.

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.

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

We will collect samples by hand using forceps, scissors, bone shears or hammer during SCUBA diving. Each collector will have standard SCUBA gear (mask, fins, snorkel, wetsuit, tank, BCD) and a collection bag in which to store gear and samples as they are collected. One diver will also have a high resolution digital camera in an underwater housing to photo-document the colonies prior to collection for colony form and taxonomic purposes.

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), MSDS attached, and saturated salt buffer with dimethylsulfoxide (DMSO), MSDS attached. Both EtOH and DMSO are commonly sold for human consumption, and should not pose any significant health or environmental risk.

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

None

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

Time to study completion depends on having a complete sampling of individuals and sites. Each year I think that we will get the samples to complete the survey, but something beyond our control prevents us from collecting at the near NWHI. However, once sampling is complete, then analysis of samples is usually completed within roughly a year or two. Data analysis and write-up usually take roughly an additional year, although the turn-around time for some journals now exceeds 800 days, so time to publication can still be considerable post-submission of the study.

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:

Selkoe, K.A., J. Watson, C. White, T. Ben-Horin, M. Iacchei, S. Miterai, D. Siegel, S.D. Gaines & R.J. Toonen. Complex ocean circulation predicts marine population genetic structure at small spatial scales. Molecular Ecology, Invited Contribution to Special Issue in Landscape Genetics – publication target August 2010. Accepted pending minor revision.

Barshis, D.J., J.H. Stillman, R.D. Gates, R.J. Toonen, L.W. Smith, C. Birkeland. 2010. Protein expression and genetic structure of the coral Porites lobata in an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity?. Molecular Ecology. In press

Kahng, S.E., H. Spalding, R. Garcia, E. Brokovich, D. Wagner, E. Weil, L. Hinderstein & R.J. Toonen. 2010. MCE: A review of community ecology of mesophotic coral reef ecosystems. Coral Reefs In press

White, C., J. Watson, D.A. Siegel, K.A. Selkoe, D.C. Zacherl & R.J. Toonen. 2010. Ocean currents help explain population genetic structure. Proceedings of the Royal Society B: Biological Sciences. In press

Gaither, M., B.W. Bowen, R.J. Toonen, S. Planes, V. Messmer, J. Earle & D.R. Robertson. 2010. Genetic consequences of introducing two divergent, allopatric lineages of Bluestripe Snapper (Lutjanus kasmira) to Hawaii. Molecular Ecology. Online Early.

Daly-Engel, T.S., R.D. Grubbs, K. Feldheim, B.W. Bowen, and R.J. Toonen. 2010. Is multiple mating beneficial or unavoidable? Low multiple paternity and genetic diversity in the shortspine spurdog (Squalus mitsukurii). Marine Ecology Progress Series. Online early

Gaither, M.R., R.J. Toonen, L. Sorenson, B.W. Bowen. 2010. Isolation and characterization of microsatellite markers for the Crimson Jobfish, Pristipomoides filamentosus (Lutjanidae). Conservation Genetic Resources Online early (doi: 10.1007/s12686-009-9119-3).

Concepcion, G., S.E. Kahng, M. Crepeau, E.C. Franklin, S. Coles & R.J. Toonen. 2010. Resolving natural ranges and marine invasions in a globally distributed octocoral (genus Carijoa). Marine Ecology Progress Series. In Press

Andrews, K.R., L. Karczmarski, W.W.L. Au, S. Rickards, C. Vanderlip, B.W. Bowen, E.G. Grau, and R.J. Toonen. 2010. Rolling stones and stable homes: social structure, habitat diversity and population genetics of the Hawaiian spinner dolphin (Stenella longirostris). Molecular Ecology Online early (doi: 10.1111/j.1365-294X.2010.04521.x)

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Gaither, M.R., R.J. Toonen, D.R. Robertson, S. Planes, and B.W. Bowen. 2010. Genetic evaluation of marine biogeographic barriers: perspectives from two widespread Indo-Pacific snappers (Lutjanus spp.). Journal of Biogeography Online early (doi: 10.1111/j.1365-2699.2009.02188.x).

Keever, C., J. Sunday, J.B. Puritz, J.A. Addison, R.J. Toonen, R.K. Grosberg & M.W. Hart. 2009. Discordant distribution of populations and genetic variation in a sea star with high dispersal potential. Evolution. Online early (doi:10.1111/j.1558-5646.2009.00801.x).

Kimokeo BK, Szabo Z, Saarman NP, Simison WB, Martin-Smith K, Barrows A, Baine M, Lourie S, Toonen RJ, Hamilton H. 2009. Taxonomy and phylogeography of Hippocampus kuda in Hawai‘i. Integrative and Comparative Biology. 49:E254.

Weersing, K.A. & R.J. Toonen. 2009. Population genetics, larval dispersal, and demographic connectivity in marine systems. Marine Ecology Progress Series, Feature Article 393:1-12

Chan, Y.L., X. Pochon, M. Fisher, D. Wagner, G.T. Concepcion, S. Kahng, R.J. Toonen and R.D. Gates. 2009. Host genotypes and endosymbiotic dinoflagellate diversity in the coral Leptoseris sampled between 60-100 meter depths. BMC Ecology, Featured Article 9:21.

Ben-Horin, T., M. Iacchei, K. Selkoe, T. Mai & R.J. Toonen. 2009. Characterization of eight polymorphic microsatellite loci for the California spiny lobster, Panulirus interruptus and cross-

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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

All Samples Collected to date																
	Fungia scutaria	*Pocillopora damicormis*	*Pocillopora meandrina*	*Pavona varians*	*Panulirus marginatus*	*Panulirus penicillatus*	*Scyllarides squammosus*	*Stenopus hispidus*	*Eucidaris metularia*	*Heterocentrotus mamillatu*	*Holothuria atra*	*Holothuria whitmaei*	*Linckia multiflora*	*Ophiocoma erinaceus*	*Ophiocoma pica*	Total by Atoll
Nihoa	0	0	41	0	0	0	0	3	35	0	3	14	50	50	31	227
Necker	0	0	16	0	50	5	50	0	0	1	50	13	10	0	0	195
Total by species	0	0	57	0	50	5	50	3	35	1	53	27	60	50	31	422
Samples needed to reach maximum sample siz																
Nihoa	50	50	9	50	50	50	50	47	15	50	47	36	0	0	19	523
Necker	50	50	34	50	0	45	0	50	50	49	0	37	40	50	50	555
Total request by Spec	100	100	43	100	50	95	50	97	65	99	47	73	40	50	69	1078
NOTE: These are maximum sample sizes requested, and history indicates the collection will be far smaller than this request.																

All Samples Collected to date									
	Panulirus marginatus	*Panulirus penicillatus*	*Scyllarides squammosus*	*Stenopus hispidus*	*Antipathes griggi*	*Cirrhipathes cf. anguina*	*Myriopathes ulex*	*Stichopathes cf. echinulata*	Total by Atoll
Nihoa	0	0	0	3	0	0	0	0	3
Necker	50	5	50	0	8	8	1	7	129
French Frigate Shoals	12	36	2	22	0	0	0	0	72
Gardner	50	0	50	1	0	0	0	0	101
Maro	50	5	50	0	0	0	0	0	105
Laysan	50	1	50	0	8	4	0	0	113
Lisianski	50	21	0	50	0	0	0	0	121
Pearl & Hermes	50	30	8	4	0	0	16	0	108
Midway	50	4	3	7	0	0	0	0	64
Kure	50	1	0	6	0	0	0	0	57
Total by species	412	103	213	93	16	12	17	7	821
Samples needed to reach maximum sample siz									
Nihoa	50	50	50	47	50	50	50	50	247
Necker	0	45	0	50	42	42	49	43	95
French Frigate Shoals	38	14	48	28	50	50	50	50	128
Gardner	0	50	0	49	50	50	50	50	99
Maro	0	45	0	50	50	50	50	50	95
Laysan	0	49	0	50	42	46	50	50	99
Lisianski	0	29	50	0	50	50	50	50	79
Pearl & Hermes	0	20	42	46	50	50	34	50	108
Midway	0	46	47	43	50	50	50	50	136
Kure	0	49	50	44	50	50	50	50	143
Total by Species	88	397	287	407					1179
NOTE: These are maximum sample sizes requested, and cannot be collected in any single year.									
	We will likely only collect at 3 or 4 of the listed sites								
Because we do not know where we will have the opportunity to sample, we are requesting all									
	sites, but will collect at only a subset of the locations.								