State of Hawaii
DEPARTMENT OF LAND AND NATURAL RESOURCES
Division of Aquatic Resources
Honolulu, Hawaii 96813

April 24, 2009

Board of Land
and Natural Resources
Honolulu, Hawaii

Request for Authorization and Approval to Issue a Papahānaumokuākea Marine National Monument Research Permit to Dr. Robert Toonen, University of Hawaii, Hawaii Institute of Marine Biology, for Access to State Waters to Conduct Reef Invertebrates Genetic Survey Activities.

The Division of Aquatic Resources (DAR) hereby submits a request for your authorization and approval for issuance of a Papahānaumokuākea Marine National Monument research permit to Dr. Robert Toonen, assistant researcher, University of Hawaii, Hawaii Institute of Marine Biology, pursuant to § 187A-6, Hawaii Revised Statutes (HRS), chapter13-60.5, Hawaii Administrative Rules (HAR), and all other applicable laws and regulations.

The research permit, as described below, would allow entry and research activities to occur in the Papahānaumokuākea Marine National Monument (Monument), including the NWHI State Marine Refuge and the waters (0-3 nautical miles) surrounding the following sites:

- Nihoa Island,
- Necker Island (Mokumanamana),
- French Frigate Shoals,
- Gardner Pinnacles,
- Maro Reef
- Laysan Island,
- Lisianski Island, Neva Shoal,
- Pearl and Hermes Atoll,
- Kure Atoll State Seabird Sanctuary

The activities covered under this permit would occur from May 15, 2009 through November 15, 2009.

The proposed activities are largely a renewal of work previously permitted and conducted in the Monument.

INTENDED ACTIVITIES

The purpose of these activities is to conduct a population genetic survey of common reef invertebrates. This survey is an on-going effort to determine patterns of connectivity or isolation

among each reef ecosystem throughout the Hawaiian Archipelago with a focus on locations within the Monument, as well as between the Monument and the main Hawaiian Islands.

To carry out this survey, the applicant is requesting to collect target invertebrates by hand and take tiny tissue biopsy samples prior to release of the live animals back to the environment. Tissue biopsy samples are typically obtained as small fragments for corals (1 cm 2), arm clips for sea stars, brittle stars and sea cucumbers, or dactyl (toe) cuttings from crustaceans. The targeted species are locally abundant and widespread in the area, easy to identify, and easy to collect. Despite the fact that only extremely abundant species are targeted, for which removal of 50 individuals has no detectable population effect, the applicant would use non-lethal tissue samples to further minimize impact whenever possible.

The applicant is requesting 50 specimens/species/location and notes that this number is cumulative for his past and future cruises, meaning once 50 samples of a species are gathered at one location, no additional specimens will be taken on subsequent cruises. The activities described above are part of a multiyear project, and to date the applicant has completed collections for 4 of the 26 target species. A new aspect of this years work is the request for an additional set of samples (50/species/location) from the deep reefs. New species that have not previously been included in the applicant's collection list are several species of black coral. Small (3-5cm) samples would be taken and analyzed against samples that have already been collected from the MHI.

See F-5c for a table of species and current collection status.

Tissue samples would be preserved in >70% ethanol and transported back to HIMB for genetic analyses.

The applicant notes that researchers at HIMB have coordinated to share samples where appropriate.

This research would help identify the specific location of genetic breaks throughout the archipelago and determine which portions of the Monument are isolated ecosystems and which are components of a larger meta-population that can draw on individual and resources at other locations in times of stress. It would also address the issue about whether the NWHI serves as a source of larvae to replenish depleted stocks in the main Hawaiian Islands.

The activities proposed by the applicant directly support the Monument Management Plan's priority management needs 3.1 – Understanding and Interpreting the NWHI (through action plan 3.1.1 – Marine Conservation Science).

The activities described above may require the following regulated activities to occur in State waters:

- ☒ Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving Monument resource
- ☒ Touching coral, living or dead

☒ Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

REVIEW PROCESS:

The permit application was sent out for review and comment to the following scientific and cultural entities: Hawaii Division of Aquatic Resources, Hawaii Division of Forestry and Wildlife, Papahānaumokuākea Marine National Monument (NOAA/NOS), NOAA Pacific Islands Regional Office (NOAA-PIRO), United States Fish and Wildlife Service Hawaiian and Pacific Islands National Wildlife Refuge Complex Office, and the Office of Hawaiian Affairs (OHA). In addition, the permit application has been posted on the Monument Web site since March 11th, giving the public an opportunity to comment. The application was posted within 40 days of its receipt, in accordance with the Monument's Public Notification Policy.

Comments received from the scientific community are summarized as follows:

Scientific reviews support the acceptance of this application.

Concerns raised were:

1. What the anticipated long-term timeline for collections in the Monument looks like

Comments received from the Native Hawaiian community are summarized as follows:

Cultural reviews support the acceptance of this application. No concerns were raised.

Additional reviews and permit history:

Are there other relevant/necessary permits or environmental reviews that have or will be issued with regard to this project? (e.g. MMPA, ESA, EA) Yes ☒ No ☐
If so, please list or explain:

- The proposed activities are in compliance with the National Environmental Policy Act.

Has Applicant been granted a permit from the State in the past? Yes ☒ No ☐
If so, please summarize past permits:

- The applicant was granted permits DLNR/NWHI/06R005, PMNM-2007-033, and PMNM-2008-047 to conduct similar work in 2006 through 2008.

Have there been any a) violations: Yes ☐ No ☒
b) Late/incomplete post-activity reports: Yes ☐ No ☒

Are there any other relevant concerns from previous permits? Yes ☐ No ☒

RESPONSE:

1. The Applicant points out that they are nearly done with the sampling, and are now simply trying to just finish up collections from the sites at which they have been unable to collect due to poor weather or research limitations in the past. The results from previous years suggest areas of specific interest to resolve the exact site of genetic barriers within the Hawaiian Archipelago, and some of those are among the least thoroughly sampled locations, so they believe that this is important to complete. He also states that his research is driven by the needs of the managers at the Monument and he will respond to and try to meet those needs as they arise. The logical next step to this work would be understand and predict how global climate change will alter the patterns that they are currently uncovering.

STAFF OPINION:

DAR staff is of the opinion that Applicant has properly demonstrated valid justifications for his application and, conditional on development of a peer-reviewed Monument transport protocol, should be allowed to enter the NWHI State waters and to conduct the activities therein as specified in the application with the following special instructions and conditions, which are in addition to the Papahānaumokuākea Marine National Monument Conservation and Management Permit General Conditions. The following special conditions have been vetted through the legal counsel of the Co-Trustee agencies.

1. This permit is not to be used for nor does it authorize the sale of collected organisms. Under this permit, the authorized activities must be for noncommercial purposes not involving the use or sale of any organism, by-products, or materials collected within the Monument for obtaining patent or intellectual property rights.

2. The permittee may not convey, transfer, or distribute, in any fashion (including, but not limited to, selling, trading, giving, or loaning) any coral, live rock, or organism collected under this permit without the express written permission of the Co-Trustees.

3. To prevent introduction of disease or the unintended transport of live organisms, the permittee must comply with the disease and transport protocols attached to this permit.

4. Tenders and small vessels must be equipped with engines that meet EPA emissions requirements.

5. Refueling of tenders and all small vessels must be done at the support ships and outside the confines of lagoons or near-shore waters in the State Marine Refuge

6. No fishing is allowed in State Waters except as authorized under State law for subsistence, traditional and customary practices by Native Hawaiians.

MONUMENT MANAGEMENT BOARD OPINION:

The MMB is of the opinion that the Applicant has met the findings of Presidential Proclamation 8031 and this activity, conditional on development of a peer-reviewed Monument transport protocol, may be conducted subject to completion of all compliance requirements. The MMB concurs with the special conditions recommended by DAR staff.

RECOMMENDATION:

"That the Board authorize and approve, with stated conditions, a Research Permit to Dr. Robert Toonen, University of Hawaii, Hawaii Institute of Marine Biology."

Respectfully submitted,

for DAN POLHEMUS
Administrator

APPROVED FOR SUBMITTAL

LAURA H. THIELEN
Chairperson

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: 05/15/09 through 11/15/09
Proposed Method of Entry (Vessel/Plane): R/V Hi'ialakai
Proposed Locations: Shallow water habitats (< 100 feet depth), focused on completing connectivity sampling in locations that were excluded dur to weather conditions, cruise constraints or time in previous years. Our Particular focus will be: 1) the center of the NWHI chain between French Frigate Shoals and Pearl and Hermes Atolls and 2) the near NWHI between Kauai and French Frigate Shoals. However, as with previous years, we request latitude to sample other regions as weather and opportunity dictate. There may also be the potential for some deep water sampling (>100ft) via technical diving operations but all shallower than 500ft.

Estimated number of individuals (including Applicant) to be covered under this permit: 5 berthing positions for my research team spread across up to 3 different research cruises, plus available members of crew from other permitted activities who can collect opportunistically on our behalf.
Estimated number of days in the Monument: Up to approximately 90 days spread across 3 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 locations with little previous sampling effort. The primary goal of this work is to determine patterns of connectivity and identify regions of limited exchange throughout Papahānaumokuākea Marine National Monument and between the Monument and the Main Eight Hawaiian Islands.

b.) To accomplish this activity we would ....
collect target invertebrates by hand and take tiny tissue biopsy samples 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.

c.) This activity would help the Monument by ...
determining the degree of connectivity or isolation among each atoll, reef or bank across the Monument. Preliminary results from this study indicate that there are regions of both high exchange and of strong isolation within the Archipelago that need to be considered in management strategies. We seek to complete the sampling of areas of interest to determine the specific locations of genetic breaks throughout the archipelago to determine which portions of the Monument are isolated ecosystems that must each stand or fall on their own (i.e., a series of relatively fragile units) and which portions are individual components of a larger meta-population that can draw on individuals and resources at other locations in times of stress (i.e., a more robust ecosystem). Additionally, this study will address the long-standing question of whether or not the populations in the Monument will serve as a source of recruits to replenish exploited stocks in the Main Hawaiian Islands.

Other information or background: Virtually every management agency on the planet is seeking to better understand patterns of connectivity because assays such as those outlined here increase decision-making capacity, and provide critical management information with solid statistical basis and great scientific credibility.

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]

Phone: [redacted]

Fax: [redacted]

Email: [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 five participating individuals spread across three cruises, we request permission for all permitted participants of each research cruise to be included as potential collection assistants here to provide maximum flexibility in the field depending on weather conditions and

site. Because the permit request covers 3 separate cruises through to October, we request flexibility in assembling the scientific teams for this research, but we expect that cruise participants will be drawn from the following list.

Rob Toonen (Ph.D., Research Diver, HIMB), Greg Concepcion (Graduate student, Research Diver, HIMB), Michelle Gaither (Graduate student, Research Diver, HIMB), Matt Iacchei (Graduate student, Research Diver, HIMB), Jon Puritz (Graduate student, Research Diver, HIMB), Daniel Wagner (Graduate student, Research Diver, HIMB), Derek Skillings (Graduate student, Research Diver, HIMB), Zoltan Szabo (PhD, Research Diver, HIMB), Derek Smith (Water Safety Officer & Research Diver, HIMB), Jack Kittinger (Graduate student, Research Diver, UH Manoa).

Other participants on the same cruise (but included on other permits) are requested to assist with our effots when possible. Because there are multiple cruises and these are months away, it is impossible to provide an exhaustive list at this time, but we anticipate those personnel to probably include: Randall Kosaki (PMNM, Research Diver), Scott Godwin (PMNM, Research Diver), Ann Mooney (PMNM, Research Diver), Kelly Gleason (PMNM, Research Diver), Ray Boland (NOAA, Research Diver), Hans van Tilberg (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), Jill Zamzow (Ph.D., Research Diver, HIMB), Kelvin Gorospe (Graduate student, Research Diver, HIMB), Kim Tice (Graduate student, Research Diver, HIMB), Michael Stat (Ph.D., 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 Main eight - NWHI divide and in the middle of the chain between French Frigate Shoals and Pearl & Hermes. Other priorities dictate that cruises will visit a variety of atolls, and we still need some samples to reach our final sample size of 50 individuals per site for many species (see Appendix 1). 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. We seek to identify stock structure and estimate population connectivity among atolls and banks of the NWHI as well as between the Monument and the Main Hawaiian Islands. Both researchers and resource managers around the globe agree that efforts to establish effective marine protected areas require detailed information regarding connectivity among disjunct populations of species (e.g., Botsford et al. 2001, Halpern & Warner 2003, Palumbi 2003, Roberts et al. 2003, Cowen et al. 2006). Although this information is needed by the co-trustees to effectively manage the resources of the Monument, there is very little information on the relative direction and magnitude of connectivity for any species within the Hawaiian Archipelago. Thus, this survey of connectivity among common coral reef species is the first of its kind in the Archipelago, and is needed to define the relevant units of management within the Monument. Additionally, this work seeks to assess the degree of interconnection among geographically isolated populations of the same species found throughout the Monument jurisdiction and between the Monument and the Main Eight Hawaiian Islands.

Monument protection status prohibits extraction of living resources from these waters, but no law can absolutely prevent natural and human-made disasters such as hurricanes, warm/cold-water events, coral bleaching, marine debris, alien species introductions, oil spills, disease outbreaks, global climate change, ocean acidification and so forth. Thus, it is of critical importance to resource managers to know what the boundaries are to interconnected populations, how those populations will respond to these impacts and how such impacts can be mitigated. In addition to delineating what constitutes a population, connectivity is of direct relevance to this question because on one end of the connectivity extreme, individual locations within the Monument are isolated, self-seeding (closed) populations with reduced genetic diversity, increased individual risk of extinction, few to no immigrants from other locations, and little hope of recovery from local disasters within the lifetime of a manager. On the other end of the extreme, the reefs are fully integrated (open) populations which exchange immigrants continuously which therefore stand or fall as an entire chain, and the local reductions at any given island or atoll should have no long-term ramifications at any other location. In stark contrast to predictions of resilience from local reductions, the effects of an alien species hitch-hiking on marine debris would have the exact opposite effect: little or slow spread and excellent potential for a management response in a highly isolated series of environments as opposed to rapid spread and little hope for containment in an open system. Our genetic survey is now beginning to provide an answer as to where along this continuum various locations within the Monument lie.

In this years application, we are seeking to fill gaps in our sampling of a broad range of species across sites throughout the Monument to determine what are the patterns and consequences of connectivity across the Archipelago. Further we are seeking to determine whether general patterns can be predicted by life history, taxonomy or

habitat, or whether each species appears to be a unique case that requires individual study to draw any management implications. This question can only be addressed by doing a broad survey of common species across each of these factors for statistical comparison. The management implications of such efforts are clear: if generalizations can be drawn among any such groups, then even in the absence of specific data, an informed management response can be launched quickly as the need arises. In contrast, if no such generalizations can be made, and the results must be determined on a case-by-case basis for each species, then in the event of a any management need, the absence of data must result in an uninformed response from managers. There are currently precious little data to address this important question, and beyond the preliminary data from this project, none of it comes from the Hawaiian Archipelago.

Further use for these data involves gaining an understanding of the standing genetic diversity by location throughout the Monument. Several recent studies have shown a direct link between demographic population structure and genetic population structure (Selkoe et al. 2006, Toonen & Grosberg In review). Frankham and colleagues have recently shown that across 170 of the most threatened species on the planet, 77% of them had significantly lower genetic diversity than that found in the closest-related nonthreatened species (Spielman et al. 2004, Frankham 2005b). Both subsequent empirical and theoretical work suggests that reductions in genetic diversity and the resultant inbreeding can speed the mean time to extinction by up to 78% over species without reduced genetic diversity (Frankham 2005a). Further Eble and colleagues (Eble et al. 2009) have recently shown that population structure is greater in Hawaiian endemics across the same spatial scale than in broadly distributed congeners. These data imply that endmic species have reduced dispersal potential and are more vulnerable than are closely-related species that are broadly distributed. Taken together, these genetic data surveys have obvious and immediate management implications, and our survey will highlight some of the locations and species most likely to be at risk within the Monument jurisdiction.

These studies are also beginning to provide direct inference to a long-standing question for managers in the State of Hawaii regarding the level of connection between the Main and NWHI. Our studies also include the MHI and these parallel efforts provide the comparison necessary to determine whether there is spill-over of individuals (either adults or larvae) from the Monument into the Main Eight Hawaiian Islands. For opihi we can say that there is little to no subsidy to the Main Hawaiian Islands from the NWHI (Bird et al. 2007), and completion of this study will determine if other species show the same trend, or if every species is unique.

Finally, additional research in our lab, funded by the National Science Foundation, leverages these studies to assess population connectivity of broadly occurring Hawaiian species across the central and eastern Pacific. This research is complementary to ongoing efforts within the Monument and has several direct management implications as well. First, our genetic surveys allow for the identification of cryptic Hawaiian endemics that may not be currently recognized due to similarity in morphology to their

broadly-occurring relatives. Similarly, these efforts allow us to refute putative Hawaiian endemics that have been identified on the basis of differing morphology or coloration, but lack any genetic isolation from other populations outside the Hawaiian Archipelago. Second, our research seeks to understand the importance that other locations beyond the Hawaiian Archipelago, such as Johnston Atoll, serve as propagule donors to Hawaii in general, and Papahānaumokoākea Marine National Monument in particular.

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 only common species for which we make every effort 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 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.

In terms of cultural resources, it is only now, after years of working with the Monument and Native Hawaiian Cultural Practitioners in the Main Hawaiian Islands, that I am finally beginning to comprehend what this question really means. Being a Canadian who was recruited to the University of Hawaii in 2002, I lacked much understanding of the Hawaiian culture and was frankly ignorant of the real meaning of this question in the past. Looking back at some of my responses from previous years, I expect that my inadequate answers were probably received as trite and insincere. I can now say that it is not insincerity but ignorance that was the issue. It has taken time 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 will submit a separate permit application for our proposed collaboration on that project. It is through our interactions with them 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 propose to collect pure 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 conducted 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.

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 roughly 40 research papers in peer-reviewed journals dealing with the subject of conservation and management of Hawaiian natural resources over the past five years. I will be the field PI on this project, and across the length of my career have spent more than a year at sea on research cruises such as this. My research to address these questions began prior to the establishment of the Monument, and has been of considerable interest to both the science and management community of Hawaii. This on-going project is well-known to the Monument co-trustees, and my accomplishments documented in my included CV prove that I am qualified to perform this research.

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.

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 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.

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 collections for only 4 of the 26 target species we selected for this work. Among the remaining 22 species, we still need to collect up to 50 tissue samples from many sites throughout the Monument (see Appendix 1). The objective of this research remains to survey all 26 common reef species we selected across the central and eastern Pacific to assess the level of connectivity among isolated reef habitats. 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, and easy to collect. We have refined our species list based on guidance from the research and Hawaiian community as well as our previous findings and personal observation. Furthermore, we only collecting species for which 50 individuals is a tiny fraction of the population at any site - most of these species occur in abundances of thousands of individuals per acre of reef. 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) and do not kill the animals that are sampled. Whenever possible, individuals are sampled in the least disruptive manner (i.e., we take a tiny tissue sample from the animal and release it alive in the same location). For example, lobster and some crab samples involve only the excision of a single dactyl (toe) from the animal before it is released. By comparison, the normal anti-predatory defense response of these species is autotomy, which involves the ready self-amputation of an entire walking leg 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. For example, in studies with porcelain crabs, and spiny lobsters in California, we showed a 100% survival rate among individuals kept in the lab for 2 months after being sampled in this way; we also found no increased mortality of American Lobsters sampled this way from the Maine Lobster fishery (Toonen, unpubl. data). Similar studies have not been repeated with animals from the NWHI because we have no reason to expect a different outcome, and cannot justify the transport of live animals from the Monument back to tanks on Oahu without good reason. Likewise, the data available from lobster tagging efforts conducted by NMFS suggests uniformly high survival from the variety of tag types employed. Given all of these examples, we have absolutely no reason to expect this sampling effort would have any detectable negative impact on the populations sampled. Samples are not killed intentionally for any of our sampling, and even accidental cases of lethal sampling are still justified because they are of direct influence on the management decisions of the Monument; for example, we may discover

previously unknown endemic species that may be at risk, but are only identified after genetic typing.

Any sample size greater than N = 1 can be informative in genetic analysis, but statistical rigor for mtDNA sequencing studies requires N > 25 and our target sample size is 30-50 per location (depending on the genetic marker being used – see Analysis below) for the target species. Target species are described in the table presented in Appendix 1. We seek to reach our ideal sample size with a collection of ~50 specimens per target species around each of the islands and atolls in the Monument. We have updated our total number of samples collected to date as well as the number of samples required to obtain a final sample size of 50 individuals per species per atoll in Appendix 1. Because we are likely to participate in at least two and possibly three cruises during this season, we have an extended date request, but the same total number of specimens required to complete our sampling since our initial permit request. Thus, although the number of samples seems large, it is important to note that this effort is spread across many years, species and sites and no additional samples will be collected once the maximum of 50 samples are reached on any cruise. As a result of previous sampling efforts, we have completed collections of 4 species to date, and they are therefore removed from our collection permit here. We also require flexbililty in our collections because our goal is to focus on the species that can be collected rapidly, safely and in sufficient number for statistical rigor, and those priorities will be dictated in part by field conditions. Thus, we do not know yet which cruise will allow us to sample the species or sites to allow us to complete our sampling. Again, I reiterate that these sample number requests are OVERALL totals and are NOT ADDITIVE per cruise.

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: 1) precious corals (Antipathes dichotoma and A. grandis, Myriopathes ulex, Cirrhipathes anguina and Stichiopathes echinulata), 2) sea cucumbers (Holothuria atra and H. whitmaei) and/or 3) brittle stars (Ophiocoma pica, O. erinaceus and O. dentata). The sea cucumbers and brittle stars are already included on our shallow water connectivity studies, but the precious corals are new to our permit request, so we justify that new request in more detail below.

Black coral (mainly Antipathes dichotoma, A. grandis and M. ulex) is harvested commercially in the Main Hawaiian Islands for the jewelry industry. Traditionally, black corals have never been commercially fished within the NWHI, and are protected within the Monument from anthropogenic effects that impact fauna in the Main Hawaiian Island, such as fishing. Understanding the impacts of the fishery on natural black coral

populations is key to developing effective management strategies to ensure sustainability of the resource. However, 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 3-5cm piece inside Monument (colonies have already been sampled in the Main Hawaiian Islands). This amount of sampling has been shown to not have negative effects on reproductive output and growth of colonies in previous studies. Samples 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. Additionally, the Monument is the only stable population in the Islands 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 (3-5cm). Because colonies can reach well over 2m in height, 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. Black coral is important to Hawaii: (1) socially and culturally - it is the official gemstone of the State of Hawaii, (2) ecologically as black corals provide essential habitat for a myriad of fish and invertebrates on deep reefs, and (3) economically because the black coral fishery supports a $30M state-wide industry.

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).

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.

Genotyping of nuclear (microsatellite) markers will be used for reef corals. Development of these markers has followed standard techniques, and quality control testing is currently underway (Selkoe & Toonen 2006). 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).

Although there was some initial resistence to the large number of total samples we request in this application, 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 spread our sampling across many species and many locations such that there is no detectable impact at any given site. 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. Even for corals, which have been deemed by some to be the most sensitive members of the ecosystem, we sample 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 colonies shows clear growth and in each case it has not been possible to see remaining damage or locate the sampled portion visually in the subsequent year.

To date, we sampled a total of 4178 individuals across the 22 species listed in Appendix 1 - these samples are collected across several years and throughout multiple dive sites from each of the10 islands and atolls of the NWHI (with no more than 50 individuals per species from any atoll, as explained previously). With the exception of the hermit crabs (for which the entire animal must be collected to identify the species after collection), the remaining samples are virtually all collected as non-lethal tissue biopsy samples, and

even including the lethal sampling of hermit crabs (which have been completed and are removed from our current permit request) roughly 90% of our samples are non-lethal - i.e., the individuals left alive in the location from which they were initially collected.

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

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

Common name:

See Appendix 1

Scientific name:

See Appendix 1

& size of specimens:

See Appendix 1

Collection location:

See Appendix 1

☒ Whole Organism ☒ Partial Organism

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

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.

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.

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. Whenever possible, all researchers at HIMB share the same single collected sample. For example, research done by the Rappé, Karl, Gates and Toonen labs all rely on a single coral biopsy sample that is subsequently split among the labs for different genetic analyses. Further, we are developing a tissue sample bank that will be available for future research and will not require re-sampling of the individuals in our collection. An electronic database of all samples will be provided to the Monument upon completion of the studies outlined here. This database will be searchable against future permit requests and can reduce the need for return trips to collect tissue samples in the NWHI, and prevent duplicative sampling efforts. However, no samples are provided to anyone without the prior transfer of the responsibility for those samples from our permit to theirs and express written consent of the co-trustees.

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

We will collect samples by hand using forceps, scissors or hammer and chisel 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 collections and any individuals that can be located from collections made in previous years.

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. Once sampling is complete (if all goes as expected, this should be possible during the 2009 cruise season), then analysis of samples is usually completed within roughly a year. Data analysis and write-up usually take no more than 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. Regular monthly brown-bag luncheons at HIMB allow researchers to highlight important or interesting new results and discuss them with the agency members responsible for the Monument. In addition, we publish a semi-annual report that highlights the most recent findings of each of the researchers involved in the NWHI research. We hold a 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. 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 roughly 6 months of the data being collected.

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

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

Faucci, A., R.J. Toonen & M.G. Hadfield. In review. Colonization history of Hawaii and Pacific phylogeography of vermetid gastropods. Journal of Biogeography.

Faucci, A., R.J. Toonen & M.G. Hadfield. In review. Rare dispersal events, currents and volcanology shape patterns of population structure in marine gastropods in Hawaii. Evolution.

Andrews K., L. Karczmarski, W.W.L. Au, S.H. Rickards, C.A. Vanderlip, B.W. Bowen & R.J. Toonen. In review. Intraspecific variability in genetic structure corresponds with social structure and environment for the Hawaiian spinner dolphin (Stenella longirostris). Molecular Ecology.

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

Barshis, D.J., J.H. Stillman, R.D. Gates, R.J. Toonen, L.W. Smith, C. Birkeland. In review. Coral resistance to environmental extremes: a case for host adaptation. Molecular Ecology.

Bird, C.E., S.A. Karl & R.J. Toonen. In review. Comparison of genetic differentiation metrics for empirical studies. Evolution.

Weersing, K.A. & R.J. Toonen. In post-review revision. Population genetics, larval dispersal, and demographic connectivity in marine systems. Marine Ecology Progress Series.

Gaither, M.R., R.J. Toonen, D.R. Robertson, S. Planes & B.W. Bowen. In post-review revision. Endemism among Marquesan shore fishes: genetic perspectives from two widespread Indo-Pacific snappers (Lutjanus spp.). Journal of Biogeography.

Bird, C.E., E. Franklin, R.J. Toonen & C. Smith. In post-review revision. Between tide and wave marks: A new and unifying model of physical zonation on littoral shores. Marine Ecology Progress Series.

Forsman, Z.H., D.J. Barshis, C.L. Hunter & R.J. Toonen. In press. Shape-shifting corals: Molecular markers show morphology is evolutionarily plastic in Porites. BMC Evolutionary Biology.

Selkoe, K.A., B.H. Halpern, C. Ebert, E. Franklin, E. Selig, K. Casey, J. Bruno, R.J. Toonen. In press. A map of cumulative impacts to a 'pristine' coral reef ecosystem, the Papahānaumokuākea Marine National Monument. Coral Reefs.

Wagner, D., S.E. Kahng & R.J. Toonen. In press. Observations on the life history and feeding ecology of a specialized nudibranch predator (Phyllodesmium poindimiei), with implications for biocontrol of an invasive octocoral (Carijoa riisei) in Hawaii. Journal of Experimental Marine Biology and Ecology.

Eble, J., R.J. Toonen & B.W. Bowen 2009. Endemism and dispersal: comparative phylogeography of three surgeonfishes across the Hawaiian Archipelago. Marine Biology. Online early.

Schulz, J.K., J.D. Baker, R.J. Toonen & B.W. Bowen. 2009. Extremely low genetic diversity in the endangered Hawaiian monk seal (Monachus schauinslandi). Journal of Heredity. 100(1):25-33.

Selkoe, K.A., B.S. Halpern & R.J. Toonen. 2008. Evaluating and ranking the vulnerability of regions within the Papahānaumokuākea Marine National Monument to anthropogenic threats. Aquatic Conservation: Marine and Freshwater Ecosystems. 18(7):1149-1165.

Concepcion, G., M. Crepeau, S.E. Kahng & R.J. Toonen. 2008. An alternative to ITS - a hypervariable, single-copy nuclear intron in corals, and its use in detecting cryptic species within the octocoral genus Carijoa. Coral Reefs. 27(2):323-336.

Toonen, R.J. & A.J. Tyre. 2007. If larvae were smart: A simple model for optimal settlement choices of competent larvae. Marine Ecology Progress Series.
Bird, C.E., B.S. Holland, B.W. Bowen & R.J. Toonen. 2007. Contrasting population structure in three endemic Hawaiian limpets (Cellana spp.) with similar life histories. Molecular Ecology. 16(15):3173-3187.

Wagner, D., S. Kahng & R.J. Toonen. 2007. New report of nudibranch predators of the invasive octocoral Carijoa riisei in the Hawaiian Islands. Coral Reefs. 26:411

Daly-Engel, T.S., R.D. Grubbs, B.W. Bowen & R.J. Toonen. 2007. Frequency of multiple paternity in an unfished tropical population of sandbar sharks (Carcharhinus plumbeus) and implications for management. Canadian Journal of Fisheries and Aquatic Sciences. 64:198-204.

Faucci, A., R.J. Toonen & M.G. Hadfield. 2007. Host shift and speciation in a coral-feeding nudibranch. Proceedings of the Royal Society, B – Biological Sciences. 274:111-119.

Wares, J., P.H. Barber, J. Ross-Ibarra, E. Sotka & R.J. Toonen. 2006. Mitochondrial DNA and Population Size. Science 314:1388-1389.

Concepcion, G., M. Medina & R.J. Toonen. 2006. Novel mtDNA intron primers from scleractinian corals. Molecular Ecology Notes. 6:1208–1211

Andrews, K.R., L. Karczmarski, W.W.L. Au, S.H. Rickards, C.A. Vanderlip, & R.J. Toonen. 2006. Patterns of genetic diversity of the Hawaiian spinner dolphin (Stenella longirostris). Atoll Research Bulletin. No. 543: 65-73.

Daly-Engel, T.S., R.D. Grubbs, K. Holland, R.J. Toonen & B.W. Bowen. 2006. Multiple paternity assessment in three congeneric species of carcharhinid sharks in Hawaii. Environmental Biology of Fishes. 76: 419-424.

Other Literature Cited:

Baums IB, Miller MW, Hellberg ME (2005) Regionally isolated populations of an imperiled Caribbean coral, Acropora palmata. Molecular Ecology 14:1377-1390

Baums IB, Paris CB, Chérubin LM (2006) A bio-oceanographic filter to larval dispersal in a reef-building coral. Limnology and Oceanography 51:1969-1981

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Science 98:4563-4568

Botsford LW, Hastings A, Gaines SD (2001) Dependence of sustainability on the configuration of marine reserves and larval dispersal distance. Ecology Letters 4:144-150

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science 311:522-527

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online

Frankham R (2005a) Conservation biology - Ecosystem recovery enhanced by genotypic diversity. Heredity 95:183-183

Frankham R (2005b) Genetics and extinction. Biological Conservation 126:131-140
Halpern BS, Warner RR (2003) Matching marine reserve design to reserve objectives. Proc R Soc Lond Ser B-Biol Sci 270:1871-1878

Jayewardene D, Birkeland C (2006) Fish predation on Hawaiian corals. Coral Reefs 25:328
Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecological Applications 13:S146-S158

Rivera MAJ, Kelley CD, Roderick GK (2004) Subtle population genetic structure in the Hawaiian grouper, Epinephelus quernus (Serranidae) as revealed by mitochondrial DNA analyses. Biological Journal of the Linnean Society 81:449-468

Roberts CM, Andelman S, Branch G, Bustamante RH, Castilla JC, Dugan J, Halpern BS, Lafferty KD, Leslie H, Lubchenco J, McArdle D, Possingham HP, Ruckelshaus M, Warner RR (2003) Ecological criteria for evaluating candidate sites for marine reserves. Ecological Applications 13:S199-S214

Selkoe KA, Gaines SD, Caselle JE, Warner RR (2006) Current shifts and kin aggregation explain genetic patchiness in fish recruits. Ecology 87:3082-3094

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences of the United States of America 101:15261-15264

Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, Massachusetts.

Toonen RJ, Grosberg RK (In review) Effects of coastal upwelling on genetic structure in an intertidal crab with planktonic larvae. Evolution

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

Appendix 1

All Samples Collected to date

	Acropora cytherea	*Fungia scutaria*	*Montipora capitata*	*Porites lobata*	*Pocillopora damicornis*	*Pocillopora meandrina*	*Pavona varians*	*Panulirus marginatus*	*Panulirus penicillatus*	*Scyllarides squammosus*	*Stenopus hispidus*	*Acanthaster planci*	*Colobocentrotus atratus*	*Eucidaris metularia*	*Heterocentrotus mamillatus*	*Holothuria atra*	*Holothuria whitmaei*	*Linckia multiflora*	*Ophiocoma erinaceus*	*Ophiocoma pica*	*Octopus cyanea*	*Cellana sandwicensis*		Total by Atoll
Nihoa	0	0	25	34	0	41	0	0	0	0	3	3	0	33	0	3	10	50	16	13	1	0		232
Necker	0	0	0	48	0	16	0	50	1	50	0	0	0	0	0	0	1	10	0	0	0	18		194
French Frigate Shoals	50	45	50	50	50	50	43	6	4	2	6	12	2	50	17	50	50	50	50	48	2	8		695
Gardner	0	0	1	48	0	47	1	50	0	50	1	4	10	3	0	3	1	17	1	24	0	0		261
Maro	0	0	47	50	8	32	0	50	1	50	0	0	0	1	2	1	9	0	2	4	0	0		257
Laysan	0	0	21	5	0	50	1	50	0	50	0	0	0	1	2	15	26	0	11	29	0	0		261
Lisianski	0	50	50	50	50	1	29	0	0	0	1	0	0	3	4	1	2	0	4	50	0	0		295
Pearl & Hermes	2	50	50	50	50	50	29	50	8	8	2	50	0	11	46	49	50	50	50	50	3	0		708
Midway	0	10	50	50	50	50	0	17	1	3	5	10	0	35	50	50	50	50	50	50	1	0		582
Kure	0	50	50	50	50	50	50	50	0	0	2	11	0	4	50	50	50	50	50	44	10	0		671
Total by species	52	205	344	435	258	387	153	323	15	213	20	90	12	141	171	222	249	277	234	312	17	26		4156

Samples needed to reach maximum sample size

Nihoa	50	50	25	16	50	9	50	50	50	50	47	47	50	17	50	47	40	0	34	37	49	50		868
Necker	50	50	50	2	50	34	50	0	49	0	50	50	50	50	50	50	49	40	50	50	50	32		906
French Frigate Shoals	0	5	0	0	0	0	7	44	46	48	44	38	48	0	33	0	0	0	0	2	48	42		405
Gardner	50	50	49	2	50	3	49	0	50	0	49	46	40	47	50	47	49	33	49	26	50	50		839
Maro	50	50	3	0	42	18	50	0	49	0	50	50	50	49	48	49	41	50	48	46	50	50		843
Laysan	50	50	29	45	50	0	49	0	50	0	50	50	50	49	48	35	24	50	39	21	50	50		839
Lisianski	50	0	0	0	0	49	21	50	50	50	49	50	50	47	46	49	48	50	46	0	50	50		805
Pearl & Hermes	48	0	0	0	0	0	21	0	42	42	48	0	50	39	4	1	0	0	0	0	47	50		392
Midway	50	40	0	0	0	0	50	33	49	47	45	40	50	15	0	0	0	0	0	0	49	50		518
Kure	50	0	0	0	0	0	0	0	50	50	48	39	50	46	0	0	0	0	0	6	40	50		429
Total by Species	448	295	156	65	242	113	347	177	485	287	480	410	488	359	329	278	251	223	266	188	483	474		6844

NOTE: These are maximum sample sizes requested, and cannot be collected in any single year. All samples collected to date (above) are still less than requested here.

Also note that some cells with 50 samples requested are outside the range of the target species (e.g., Cellana at P&H) so this total sample is the absolute maximum possible.

Papahānaumokuākea Marine National Monument
Compliance Information Sheet

1. Updated list of personnel to be covered by permit. List all personnel names and their roles here (e.g. John Doe, Diver; Jane Doe, Field Technician, Jerry Doe, Medical Assistant):

Rob Toonen – Principal Investigator and Diver, HIMB
Matthew Iacchei – Diver, HIMB
Zoltan Szabo – Diver, HIMB

Alternates in case of emergency:
Jon Puritz – Diver, HIMB
Derek Skillings – Diver, HIMB
Daniel Wagner – Diver, HIMB

Other participants on the same cruise (but included on other permits and listed in the respective compliance sheets) are also requested to assist with our efforts when possible. We likewise request latitude for our team to assist other cruise participants with their permitted work when not otherwise engaged.

2. Specific Site Location(s): (Attach copies of specific collection locations):

We are focused on completing our sampling in locations that were excluded in previous years due to weather conditions, cruise constraints or time; we are attempting to finalize the broad-scale collections of reef invertebrates in areas highlighted to be of greater management interest by results from our ongoing connectivity studies to date. Our particular focus will be: 1) the center of the NWHI chain between French Frigate Shoals and Pearl and Hermes Atolls and 2) the near NWHI between Kauai and French Frigate Shoals. Exact sites will be determined by cruise and weather conditions, and we request latitude to sample other regions as weather and opportunity dictate within the limits outlined in our permit request.

3. Other permits (list and attach documentation of all other related Federal or State permits):

None

3a. For each of the permits listed, identify any permit violations or any permit that was suspended, amended, modified or revoked for cause. Explain the circumstances surrounding the violation or permit suspension, amendment, modification or revocation.

None

4. Funding sources (Attach copies of your budget, specific to proposed activities under this permit and include funding sources. See instructions for more information):

This research is funded by NSF, NOAA and the PMNM-HIMB partnership as outlined in the permit application.

5. Time frame:
Activity start: 2005
Activity completion: 2011

Dates actively inside the Monument:
From: 9 June 2009
To: 3 July 2009

Describe any limiting factors in declaring specific dates of the proposed activity at the time of application:

All dates are tentative and dependent upon ship and weather conditions. Ocean conditions strongly influence the dates that vessels can enter Monument waters, as well as when research can be conducted while in the Monument waters. Dates are also dependent on vessel and personnel schedules. Co-trustees will be notified of any changes to the dates currently provided

Personnel schedule in the Monument:

All personnel will remain on the NOAA vessel Hi'ialakai (or on small boats that are transported to the Monument by the main vessel) throughout the cruise duration. No individual will be on land to conduct this research.

DATE	PORT	Departure time	Distance
6/09/09	Pearl Harbor	0900 hrs	500nm to FFS @9.5 kts ~ 53hrs
6/10/09	Transit	Transit	
6/11/09	Transit	Arrive FFS- half day of ops	
6/12/09	FFS	Full Day	
6/13/09	FFS	Full Day	
6/14/09	FFS	Full Day Depart 1830	257nm to Maro @9.5 kts ~ 27hrs
6/15/09	Transit	Arrive Maro pm	
6/16/09	Maro	Full Day	
6/17/09	Maro	Full Day depart 1830	317nm to P&H @ 9.5 kts ~33hrs
6/18/09	Transit	Arrive P&H pm	
6/19/09	P&H	Full Day	
6/20/09	P&H	Full Day	
6/21/09	P&H	Full Day depart 1830	85nm to Midway @ 9.5 kts ~9hrs

6/22/09	Midway	Arrive Midway-full day ops
6/23/09	Midway	Full Day
6/24/09	Midway	Full Day
6/25/09	Midway	Full Day
6/26/09	Midway	Half Day depart 1200 to Laysan 336nm to Laysan @9kts~37 hrs
6/27/09	Transit	Transit
6/28/09	Laysan	Arrive Laysan full day day
6/29/09	Laysan	Full day depart 1830 804nm to HNL @9 kts ~ 89hrs
6/30/09	Transit	Transit
7/01/09	Transit	Transit
7/02/09	Transit	Transit
7/03/09	Arrive	Pearl Harbor ~1000

6. Indicate (with attached documentation) what insurance policies, bonding coverage, and/or financial resources are in place to pay for or reimburse the Monument trustees for the necessary search and rescue, evacuation, and/or removal of any or all persons covered by the permit from the Monument:

All divers are requested to carry DAN insurance in addition to UH workers compensation that will cover any diving related injury or an accident that occurs while on a diving research cruise.

7. Check the appropriate box to indicate how personnel will enter the Monument:

☒ Vessel
☐ Aircraft

Provide Vessel and Aircraft information:

NOAA vessel Hi'ialakai

8. The certifications/inspections (below) must be completed prior to departure for vessels (and associated tenders) entering the Monument. Fill in scheduled date (attach documentation):

☐ Rodent free, Date:
☐ Tender vessel, Date:
☐ Ballast water, Date:
☐ Gear/equipment, Date:
☐ Hull inspection, Date:

9. Vessel information (NOTE: if you are traveling aboard a National Oceanic and Atmospheric Administration vessel, skip this question):
Vessel name:
Vessel owner:
Captain's name:
IMO#:
Vessel ID#:
Flag:
Vessel type:
Call sign:
Embarkation port:
Last port vessel will have been at prior to this embarkation:
Length:
Gross tonnage:
Total ballast water capacity volume (m3):
Total number of ballast water tanks on ship:
Total fuel capacity:
Total number of fuel tanks on ship:
Marine Sanitation Device:
Type:

Explain in detail how you will comply with the regulations regarding discharge in the Monument. Describe in detail. If applicable, attach schematics of the vessel's discharge and treatment systems:

Other fuel/hazardous materials to be carried on board and amounts:

Provide proof of a National Oceanic and Atmospheric Administration (NOAA) Office of Law Enforcement-approved Vessel Monitoring System (VMS). Provide the name and contact information of the contractor responsible for installing the VMS system. Also describe VMS unit name and type:

VMS Email:
Inmarsat ID#:

10. Tender information:

On what workboats (tenders) will personnel, gear and materials be transported within the Monument? List the number of tenders/skiffs aboard and specific types of motors:

TBD by NOAA crew aboard vessel Hi'ialakai. Generaly, two inbord jet boats and a zodiac with an outboard.

Additional Information for Land Based Operations

11. Proposed movement of personnel, gear, materials, and, if applicable, samples:

12. Room and board requirements on island:

13. Work space needs:

DID YOU INCLUDE THESE?

- ☐ Map(s) or GPS point(s) of Project Location(s), if applicable
- ☐ Funding Proposal(s)
- ☐ Funding and Award Documentation, if already received
- ☐ Documentation of Insurance, if already received
- ☐ Documentation of Inspections
- ☐ Documentation of all required Federal and State Permits or applications for permits