

**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

or <http://nps.gov/papaha>

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:** 05/15/08 through 09/15/08

**Proposed Method of Entry (Vessel/Plane):** R/V Hi'ialakai

**Proposed Locations:** Shallow water habitats (< 200 feet depth), focused on Kure, Midway, Pearl & Hermes, and Nihoa. However, we request latitude to sample other regions as weather and opportunity dictate.

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

19

**Estimated number of days in the Monument:** 54

**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 on-going effort to determine patterns of connectivity or isolation among each reef ecosystem throughout the Hawaiian Archipelago with a focus on locations with 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. This study of connectivity will help determine whether the Monument is a series of isolated ecosystems that must each stand or fall on their own (i.e., a series of relatively fragile units) or 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: Assistant Researcher, Hawaii Institute of Marine Biology

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

L. Scott Godwin

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

Hawaii Institute of Marine Biology  
[REDACTED]

Phone: [REDACTED]

Fax: [REDACTED]

Email: [REDACTED]

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

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 will include fewer than five individuals on the cruise, we list all likely participants here to provide maximum flexibility in assembling scientific teams for this research.

Randy Kosaki (Ph.D., Research Diver, MNM), Elizabeth Keenan (Ph.D., Research Diver, MNM), Carl Meyer (Ph.D., Research Diver, HIMB), Robert Toonen (Ph.D., Research Diver, HIMB), Scott Godwin (M.S., Research Diver, HIMB), Luiz Rocha (Ph.D., Research Diver, HIMB), Michael Stat (Ph.D., Research Diver, HIMB), Stephen Karl (Ph.D., Research Diver, HIMB), Matthew Craig (Ph.D., Research Diver, HIMB), Erik Franklin (Research Diver, HIMB), Toby Daly-Engel (Graduate student, Research Diver, HIMB), Greg Concepcion (Graduate student, Research Diver, HIMB), Michelle Gaither (Graduate student, Research Diver, HIMB), Jenny Schultz (Graduate student, Research Diver, HIMB), Matt Iacchei (Graduate student, Research Diver, HIMB), Frederique Kandel (Graduate student, Research Diver, HIMB), Daniel Wagner (Graduate student, Research Diver, HIMB), Derek Skillings (Graduate student, Research Diver, HIMB), Derek Smith (Water Safety Officer & Research Diver, HIMB).

**Section B: Project Information**

**5a. Project location(s):**

<input checked="" type="checkbox"/> Nihoa Island	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Necker Island (Mokumanamana)	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> French Frigate Shoals	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Gardner Pinnacles	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Maro Reef			
<input checked="" type="checkbox"/> Laysan Island	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Lisianski Island, Neva Shoal	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Pearl and Hermes Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Midway Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Kure Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input type="checkbox"/> Other			

**Ocean Based**

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

Location Description:

As outlined above, we anticipate visiting Kure, Midway, Pearl & Hermes, and Nihoa, but list all possible sites for maximum flexibility due to weather or unforeseen delays in cruise departure dates. 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 project will continue three years of efforts to complete a comprehensive genetic survey of common coral reef invertebrates to identify stock structure and estimate population connectivity among atolls and banks of the NWHI. Both researchers and resource managers around the globe generally 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. A survey of connectivity among common coral reef species is therefore needed to define the relevant units of management for the Monument and to assess the degree of interconnection among geographically isolated populations of the same species found throughout the Monument jurisdiction. Although the Monument prohibits extraction of living resources from protected waters, no law can prevent natural and human-made disasters such as hurricanes, warm/cold-water events, coral bleaching, marine debris, oil spills, disease outbreaks, and so forth. Thus, it is of critical importance to resource managers to know how populations will respond to these impacts and how such impacts can be mitigated. Connectivity is of direct relevance to this question: 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 such 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 effects at any given location should have no long-term ramifications at any other location. In stark contrast to predictions of resilience, the effects of an alien species hitch-hiking on marine debris would have the opposite effect: slow spread and 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 will provide an answer as to where along this continuum various locations within the Monument lie.

In this survey, we are seeking to cover a broad range of species and locations to see whether the patterns and consequences of connectivity can be generalized 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 reasonable 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 an unforeseen management requirement, the absence of data must result in an uninformed

response from managers. There are currently precious little data to address this important question, and 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). Further, 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). These genetic data surveys have obvious and immediate management implications, and our survey may highlight the locations and species most likely to be at risk of extinction within the Monument jurisdiction.

These studies also 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.

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 of other locations beyond the Hawaiian Archipelago as propagule donors to Hawaii in general, and Papahānaumokuākea 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?

We are performing connectivity studies that allow resource managers to define the relevant units of management, and determine the scale of connectivity among geographically isolated portions of the Monument. The information from our work greatly increases the decision-making capacity of the managers, and similar work forms the basis of science-based resource management in virtually all marine reserves for which it is available. Throughout the world, understanding of connectivity is recognized as being essential for an adaptive management strategy to be implemented, and the same is true in the Monument. Further, we 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 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 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 land within the Monument, and we report but do not touch any submerged artifacts discovered during our diving activities.

In terms of cultural resources, each person on the team undergoes a cultural briefing to understand the significance of Papahānaumokuākea to the Hawaiian People and appropriate behavior within the Monument. We are requesting that the ship be blessed and we ask proper permission to enter the Monument waters and take from the Sea prior to the departure of the Hi'ialakai on our requested research trip. We have also begun discussions with members of the Native Community to establish a Cultural Practitioner Advisory Council that would provide input to researchers in this effort to ensure minimal impacts to cultural resources as well as understanding and sensitivity to cultural issues. Finally, we have begun to speak with the Monument staff about including a Native Hawaiian Cultural Practitioner as a direct collaborator on our research and possible team member for our future work to maximize the exchange of information and ensure proper safeguards are upheld to protect cultural resources within the Monument.

b. How will the activity be conducted in a manner compatible with the management direction of this proclamation, considering the extent to which the conduct of the activity may diminish or enhance Monument cultural, natural and historic resources, qualities, and ecological integrity, any indirect, secondary, or cumulative effects of the activity, and the duration of such effects?

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, we do not detect that our activities have any effect to diminish Monument resources, nor have indirect, secondary or cumulative effects on the ecosystem or resources therein.

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 that it should be self-evident that there is no practical alternative to sampling within the Monument when the goal of the research is to understand connectivity among disjunct 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, 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.

Scott Godwin will be the lead on this project, and is one of the most experienced invertebrate biologists to have visited the NWHI. He has been on numerous research cruises to the region, has performed the primary baseline alien species surveys, is well-known to the Monument co-trustees, and is clearly 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 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 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 these activities speaks to the outstanding quality of the research coming from our lab. Our rate of publication and the quality of journals in which those publications appear speaks to the general acceptance in the scientific community of the importance and quality of the work being performed. 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 natural resources which are the focus of the study for purposes of conservation.

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:**

The objective of this research is to survey 26 key reef species across the central and eastern Pacific to assess the level of connectivity among isolated reef habitats. These species are described in Appendix A. We will accomplish this with samples up to 50 invertebrate specimens per species per atoll, from common species found on Hawaiian reefs (Appendix 1). The "key" species in this case are locally abundant and widespread in the area, easy to identify, and easy to collect. We have refined our species list based on research findings and personal observation on previous cruises, and we only collecting species for which 50 individuals is a tiny fraction of the population at any site. Although some species may be listed as rare in the broad-scale NOWRAMP surveys, these species can still be very abundant in some locations, and we are only interested in sampling those locations where 50 individuals can be collected easily. Further, 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 – considering that the reef habitat surrounding most locations is well over one hundred thousand acres, if 50 individuals was less than 1% of the stock 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 A below) inhabit shallow reefs and are accessible via snorkeling, or scuba dives. Tissue biopsy samples are typically obtained as small fragments for corals (1cm<sup>2</sup>), arm clips for sea stars, brittle stars and sea cucumbers, or whole animals for small cryptic crabs. Despite the fact that we target only extremely abundant species, for which removal of 50 individuals has no detectable population effects, we still use non-lethal tissue samples to further minimize our impact whenever possible. Photographic monitoring of coral tissue sampling from the colonies from previous cruises was revisited each subsequent year to document whether the small damage inflicted during our collections has any detectable effect on the coral colony by the next research cruise. In each case, we had considerable difficulty determining that the coral had been sampled at all, and there were no signs of reduced growth, increased infection or bleaching, or mortality among any of the colonies that were revisited. Coral reef invertebrates are collected by hand using pliers, forceps or scissors depending on the species. We store tissue samples in >70% ethanol 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 a non-lethal 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 (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 such a reason. However, the data available from lobster tagging efforts conducted by NMFS suggests uniformly high survival from the variety of tag types employed. Given these examples, we have no reason to expect this sampling effort would have any detectable negative impact on the populations sampled. Exceptions to non-lethal sampling include only hermit crabs of the genus *Calcinus* in which we: 1) have preliminary data to document that there may be some cryptic species within the Monument, and we require the original specimens to sort out the taxonomy of the sampled animals after genetic analyses, 2) which are common by any measure of abundance across the NWHI archipelago, and 3) are requested for inclusion in the

permanent collection of the Bishop museum once new species are identified. Such 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 A. We seek to complete our intended collection of ~50 specimens per target species around each of the atolls in the Monument. We have updated our maximum sample size for each species in Appendix A to reflect the number of samples required to obtain a final sample size of 50 individuals. Because we are likely to participate in two cruises during this season, we have an extended date request, but the same total number of specimens required to complete our sampling. Thus, it is important to note that no additional samples will be collected on the second cruise if 50 samples are reached on the first cruise. 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. Therefore, we have made the same request on each permit application, but these sample number requests are OVERALL totals and are NOT ADDITIVE.

#### 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).

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  $F_{st}$  values and the maximum likelihood approach of MIGRATE vers. 1.7.3 (Beerli & Felsenstein 2001). The key innovation in MIGRATE (relative to conventional  $N_m$  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 some have balked at the large number of total samples we request, I want to reiterate 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 sites to minimize any unforeseen and unrecorded 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.

Last year we sampled 2790 individuals across 7 atolls and 26 species (no more than 50 individuals per species from any atoll, as explained previously), of which 86% were taken as non-lethal biopsy samples – i.e., the individuals left alive in the location from which they were initially collected. As described above, the exceptions were hermit crabs (*Calcinus* spp.) for which the whole animal is needed to confirm field identification and from which we expect to discover new species that will subsequently become part of the Bishop Museum permanent collection.

**NOTE:** If land or marine archaeological 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 A

Scientific name:

See Appendix A

# & size of specimens:

See Appendix A

Collection location:

See Appendix A

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 such time as the Monument co-trustees request that they be returned.

**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 in >70% ethanol for genetic analyses 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 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 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 a 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 (expected in 2008), 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 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. Finally, we hold annual mini-symposia during which researchers present the most current findings from their ongoing research in the Monument Office to all the agency partners who wish to attend. In sum, these efforts ensure that research results are provided to the Monument co-trustees almost as quickly as they become available.

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

Concepcion, G., S.E. Kahng, M. Crepeau, E.C. Franklin, S. Coles & R.J. Toonen. In review. Molecular data refute a Caribbean introduction and suggest multiple origins for the invasive snowflake coral in Hawaii. *Molecular Ecology*.

Selkoe, K.A., B.S. Halpern & R.J. Toonen. Accepted. Evaluating and ranking the vulnerability of regions within the Northwest Hawaiian Islands Marine National Monument to anthropogenic threats. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Concepcion, G., M. Crepeau, D. Wagner, S.E. Kahng & R.J. Toonen. Online first. 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*. DOI: 10.1007/s00338-007-0323-x

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.

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

Selkoe, K.A. & R.J. Toonen. 2006. Microsatellites for Ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters* 9:615–629.

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

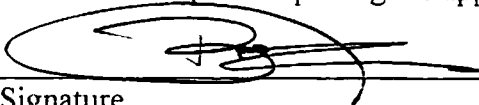
Selkoe KA, Gaines SD, Caselle JE, Warner RR (2006) Current shifts and kin aggregation explain genetic patchiness in fish recruits. *Ecology* 87:3082-3094

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

01 Feb 2008  
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