

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: Bowen, Brian W.

Affiliation: Hawaii Institute of Marine Biology

Permit Category: Research

Proposed Activity Dates: 6/1/09 - 10/31/09

Proposed Method of Entry (Vessel/Plane): RV Hi'ialakai

Proposed Locations: Shallow water habitats (< 200 feet depth), focused on Kure, Midway, Pearl & Hermes, Laysan, Maro, Gardner, 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:

16

Estimated number of days in the Monument: 55

Description of proposed activities: (complete these sentences):

a.) The proposed activity would...

be a genetic survey of reef fishes, designed to address the level of isolation between reef ecosystems across the Hawaiian Archipelago, and especially throughout the Papahānaumokuākea Marine National Monument.

One aspect of the project is that we will use DNA technology to examine a parasite that may have been accidentally introduced in the 1950's. This parasitic nematode, *Spirocamallanus istiblenni*, apparently hitchhiked to Hawaii with the blueline snapper *Lutjanus kasmira* and has subsequently spread to native fishes. While the introduction occurred on Oahu, the parasite has been detected in the Monument but has an uncertain distribution.

A new aspect of the project is to measure genetic diversity in five parrotfish species, to compare healthy stocks in the NW Hawaiian Islands with depleted stocks in the main Hawaiian Islands, and previously healthy stocks in the main Hawaiian Islands. This will be accomplished by sequencing mtDNA from parrotfish remains in middens (trash pits) dating back 100-1000 years. To accomplish this we need a maximum of 50 parrotfish specimens from each species, at collection sites spread across the Monument. No more than 10 specimens/species will be taken

at any single island or atoll, and every effort will be made to sample nonlethally. The benefit of this research is to determine whether there has been a historical decrease in genetic diversity in these parrotfishes due to human predation.

Another new aspect to the project is the expansion of diving efforts to greater than 130 feet depth. Specimens will be collected on deep reefs to evaluate the hypothesis that deep reefs can serve as refugia to replenish shallow reefs. Genetic studies can validate or refute this hypothesis, which has clear implications for management and conservation of biological resources. Deep dives during the permit period in 2009 will be used to select species for genetic analysis (ones that are abundant and feasible to collect). In addition, we wish to collect specimens of any new species encountered at depths greater than 130 feet, for genetic characterization, description, and vouchering in the Bishop Museum (see Appendix 1, opportunistic collections). This is an essential activity to characterize the biodiversity of the Monument, and will only be invoked in cases where species are sufficiently abundant (encounter rate of 5+ per hour) to sustain collections without adverse impact.

b.) To accomplish this activity we would
survey approximately 30 fish species at locations across the entire archipelago, using polespears and traps to collect fish, and using mtDNA sequencing technology to resolve novel evolutionary lineages, genetic diversity and connectivity among reef habitats.

c.) This activity would help the Monument by ...
determining whether the Monument is a series of relatively fragile (isolated) ecosystems, or whether individual reef habitats are connected in a larger and more robust ecosystem. There is also a concern about whether the NWHI serves as a source of larvae to replenish depleted fisheries in the main Hawaiian Islands. The assays of population connectivity outlined here will address these issues in a format that has statistical power and scientific credibility.

Other information or background: To preserve biodiversity, it is important to know how it arises (Bowen and Roman 2005). While the main objective is to assess genetic connectivity among shallow reef habitats, a “value added” component is that we can assess the age and origin of Hawaiian fauna as well as the age and origins of populations on each island. A genealogical approach to relationships among mtDNA haplotypes will indicate whether the closest relatives to the Hawaiian fauna lie predominantly to the West (Ogasawara Arch, Wake Island, or Marshall Islands) or to the South (Johnston Atoll, Line Islands; Gosline 1955; Maragos and Jokiel 1986; Maragos et al. 2004). In these cases, populations of the widespread Indo-Pacific species will be compared to the Hawaiian endemic. The geographic source of the Hawaiian form (especially Hawaiian endemics) will be resolved with parsimony networks and phylogenetic tools (see Methods), and the age of colonization events will be estimated with the mtDNA molecular clock.

Reef fauna typically have a pelagic phase (eggs and larvae), which lasts 20-60 days, followed by settlement onto a reef where they remain through juvenile and adults stages. Long distance dispersal is accomplished almost exclusively during the pelagic larval phase. However, the

geographic limits of such dispersal are uncertain (Bowen et al. 2006a; 2006b). Recent research shows that effective dispersal of marine larvae can fall short of their potential (Swearer et al. 2002). This may be particularly true of the damselfishes, as recent evidence indicates (Ramon et al. 2008), and so we have added four damselfish species to this investigation.

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Bowen, Brian W.

Title: Associate Researcher

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

Bowen, Brian W. for cruise August 6 - September 4

Robert J. Toonen for cruise June 9 - July 3

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:

3. Affiliation (institution/agency/organization directly related to the proposed project):

Hawaii Institute of Marine Biology, School of Ocean and Earth Science and Technology,
University of Hawaii

4. Additional persons to be covered by permit. List all personnel roles and names (if known at time of application) here (e.g. John Doe, Research Diver; Jane Doe, Field Technician):

Randy Kosaki (Ph.D., Research Diver, PMNM), Elizabeth Keenan (Ph.D., Research Diver, PMNM), Ann Mooney (Ph.D., Research Diver, PMNM), Carl Meyer (Ph.D., Research Diver, HIMB), Robert Toonen (Ph.D., Research Diver, HIMB), Scott Godwin (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), Yannis Papastamatiou (Ph.D., Research Diver, HIMB), Jeff Eble (Graduate student, Research

Diver, HIMB), Kelvin Gorospe (Graduate student, Research Diver, HIMB), Tonatiuh Trejo (Graduate student, Research Diver, HIMB). Kimberly Tenggardjaja (Graduate student, Research Diver, University of California at Santa Cruz). Note: Our actual research team will include perhaps three individuals on the cruise 6/9/09 - 7/3/09, four individuals on the cruise 8/6/09 - 9/4/09, and two individuals on the RAMP cruise in September-October. This list is meant to provide flexibility in assembling scientific teams.

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:

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:*

The proposed research is a genetic survey of reef fishes, primarily designed to address the issue of population connectivity across the NWHI. We will survey approximately 30 species across the entire archipelago, using mtDNA sequencing technology.

Management need: An ongoing issue for management of the NWHI is whether this is a series of relatively fragile (isolated) ecosystems, or whether reef habitats are connected in a larger and more robust ecosystem. There is also a concern about whether the NWHI serves as a source of larvae to replenish depleted fisheries in the main Hawaiian Islands. The assays of population connectivity outlined here will address these issues in a format that has statistical power and scientific credibility.

The primary purpose of the proposed research is to define the level of isolation among reef communities in the NW Hawaiian archipelago. How fragile are the geographically isolated reef habitats of the NWHI? If these habitats are highly connected by larval dispersal, then any one of them can recover quickly from human or natural perturbation. If they are isolated, they have to recover without significant input from other islands and atolls.

A second goal of the research is to track the spread of a nematode believed to be introduced into Hawaii with the ta'ape (*Lutjanus kasmira*) about 50 years ago. This nematode is widespread in the main Hawaiian islands, but so far has not been observed west of French Frigate Shoals.

Objectives: The objective of this permit request is a genetic (mtDNA) survey fish species across the NWHI to assess the level of connectivity among isolated reef habitats. We can accomplish this with samples of up to 30 fish specimens/species/location. Each location is defined as an atoll or reef, and collections will be made at a low density of approximately 10 individuals per hectare with traps and polespears. The target species are chosen to be abundant and widespread in the archipelago, easy to identify, and easy to collect. Every effort is made to minimize the impact of these collections on the natural communities.

We are also developing a genetic assay to detect the putative introduced parasite, the nematode *Spirocamallanus istiblenni*, using the same mtDNA technology we apply to fish studies. We already know that this parasite occurs as far north as French Frigate Shoals (G. Aeby, pers. comm.), has not spread as far as Midway, but has an uncertain distribution in the Monument. Another unknown is how much the nematode has spread to other native fish species. For this reason our request includes 30 specimens/location from five goatfish, one surgeonfish (*Ctenochaetus strigosus*), and the original host (ta'ape, *Lutjanus kasmira*)

Management benefits: These data will provide information on connectivity required for management, and can also detect cryptic endemic species and document the patterns and history of species entering the Hawaiian Archipelago. Furthermore, by documenting the pattern and magnitude of connectivity in a diverse set of fish taxa, we can determine if there are general patterns that can guide management decisions for understudied species. The genetic surveys of connectivity among reef habitats will substantially augment the scientific foundation for

conservation measures. Specifically, this research will establish whether reef ecosystems of the NW Hawaiian Islands are isolated management units (as preliminary data indicate) or components of an interactive metapopulation. In the former case, each reef ecosystem will have to recover from environmental insults (whether human or natural) without significant input from other reef ecosystems. A corresponding conservation mandate would be that each ecosystem is an independent management unit.

This is a multiyear project and progress to date includes completed collections for seven species, and the first round of data (see publications below). One outcome is that the endemic fish species seem to have more population structure than the widespread Pacific species (Eble et al. 2009). This somewhat counterintuitive finding indicates that the endemic species are poor dispersers. Once they colonize Hawaii, they are unable to maintain genetic connectivity with the source population outside the Hawaiian Archipelago (Eble et al. 2009). This finding, combined with the restricted range of endemic species, indicates a management concern for endemic species.

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?

Our first step is to consult Hawaiian cultural practitioners to identify the special locations and activities that could infringe on kanaka maoli spiritual beliefs. In pursuit of this goal, I have requested guidance from the Office of Hawaiian Affairs, and have reminded my research team that this training is essential to a successful project. My team members have already received some guidance in the first four years of this project, including a review of the kapu principles that have promoted ecosystem health and sustainability. We also recognize the tradition of the mano aumakuas, and for this reason we refrain from lethal sampling of sharks.

To protect natural resources, we abide by the principles of taking only the absolute minimum necessary to provide the information required by the Proclamation for protection of the Monument. This research team is very experienced and knowledgeable about what organisms are sensitive to touch or contact, and we minimize contact with live coral stands.

To provide adequate protection of historical locations and objects, we do not set foot on uninhabited islands, and we do not touch or disturb submerged artifacts. We maintain a strict policy of no contact.

To maintain cultural integrity, we seek advice from the Office of Hawaiian Affairs, and Hawaiian cultural practitioners. We restrict lethal sampling to common, widespread, and abundant species that number in the millions. We collect at low density in any one area and spread the collections across multiple locations. Our collections total a few kilograms per island

or atoll, and are miniscule when contrasted with the estimated 30,000 tons of fish taken by ulua and other large predators every year at a single atoll (Sudekum et al. 1991; Freidlander and DeMartini 2002).

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? We are fully compliant with conditions described in the Findings of Presidential Proclamation 8031, particularly Section 3.a.i.A-D concerning the compatibility with management direction of the proclamation. These concerns also include ecological integrity and minimal impact.

This research is mandated by the Proclamation directive to maintain ecosystem integrity.

One ongoing goal is develop (and in some cases pioneer) technology for nonlethal sampling of fishes. We have spent two years testing traps to catch fishes for nonlethal sampling. Our first set of nylon twine/steel frame traps were destroyed by sharks within minutes after we set them. However, our second set employed the F- plus plastic trap with much greater success. These traps have been tested rigorously around HIMB.

We also tested nets for nonlethal sampling during the 2006 NWHI collecting trip, with less success. Based on the expertise of Dr. Jill Zamzow, we found that net trapping was much less efficient than pole spearing, although we will continue to use this methodology where possible for some of the small fish such as blennies (Family Blenniidae).

In sum, we make every effort to maximize management benefits, and minimize negative impacts to the system, including decontamination between locations as outlined in the Procedures below. We believe that we have implemented every reasonable safeguard for the resources and ecological integrity of the Monument in our research, and we do not expect any detectable impact from our research sampling.

c. Is there a practicable alternative to conducting the activity within the Monument? If not, explain why your activities must be conducted in the Monument.

There is no practical alternative to conducting this research in the Monument because it is a description of the Monument from the perspective of connectivity and isolation among reef habitats. Clearly we have to sample habitats within the NWHI to resolve connectivity in this region.

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

The end value of the research clearly outweighs the imperceptible impacts from our sampling. Our collections of a few kilograms of fish, spread across thousands of hectares, are miniscule compared to the tens of thousands of tons harvested naturally by apex predators (Sudekum et al. 1991; Freidlander and DeMartini 2002). In contrast, reef connectivity data will have a direct

positive impact in the identification of vulnerable locations and species, and will inform the assessment of hazards for atolls and islands of the the Monument. The connectivity issue is identified as an essential foundation for reef management in the journal Science (Dawson et al. 2006).

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

This is an ongoing multi-year project that will require field efforts in 2009 and possibly 2010 in order to complete. The duration of the proposed activity is much shorter than the time needed to complete the project.

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

P.I. Bowen will lead the project, with 20 years experience in this field, including three prior expeditions to the NWHI and over 70 scientific publications pertaining to reef fishes, endangered species, and conservation. He is known to the MNM staff, DAR staff, and USFWS staff, 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.

The field-based component of this project is supported by a 25 day allocation of ship time (6/9/09 - 7/3/09), a 30 day allocation of ship time (8/6/09 - 9/4/09) on the NOAA research vessel Hiiialakai, a line item in the budget of the Monument. We may also have a small presence (1-3 individuals) on the RAMP cruise to the NWHI in September-October, also on the NOAA research vessel Hiiialakai. Subsequent lab-based research is supported by National Science Foundation (grants OCE-0453167 to B. Bowen), and the HIMB-NWHI Coral Reef Research Partnership (NMSP MOA 2005-008/66882).

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.

The genetic methods outlined herein have been employed by Bowen and Toonen in over 70 peer-reviewed publications, and are widely recognized as appropriate for the proposed activity. The fact that both Toonen and Bowen have been awarded highly-competitive NSF grants to expand these activities speaks to the quality of the research. The use of genetic sampling is widely regarded as the most efficient and robust way in which to answer questions of connectivity on these scales. To promote historical and cultural integrity, we completely avoid sacred sites and historical sites, we don't set foot on uninhabited islands, and we don't sample species that are designated as kapu (such as moi and mano).

Statistical rigor requires an optimum sample size of 30-50 should be obtained. Therefore, in the interest of maintaining statistical rigor while minimizing the number of samples collected, our target sample size is 30/location for most fish species. All species in our list are common reef fish that can easily sustain such collection pressure.

All scuba equipment is soaked in a bleach solution between sampling locations, in compliance with decontamination protocols.

i. Has your vessel has been outfitted with a mobile transceiver unit approved by OLE and complies with the requirements of Presidential Proclamation 8031?

Yes

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

This is a continuation of research efforts that have been conducted for three years and through the entire history of the Monument. During these previous efforts, there have been no problems with permit violations by my research team, no safety issues, and no complaints of offensive behavior. In these circumstances there are no other factors that would make the issuance of the permit inappropriate.

8. Procedures/Methods:

FIELD METHODS

The fish species listed in Appendix 1 inhabit shallow reefs and are accessible via snorkeling and scuba dives. The primary methodology for collecting fishes is with the use of Hawaiian polespears. Alternative methods such as hook and line fishing are not as selective and spearing allows us to collect only the species we need while avoiding unnecessary bycatch. Whenever possible, we sample non-lethally and remove a rice-grain sized piece of fin (biopsy of less than one square cm) and release the animal in the location from which it was collected. We have made significant progress in nonlethal sampling (see Section 7-B above), however most specimens are collected with polespears.

Statistical rigor requires a minimum sample size of 25 individuals per location. In studies examining the statistical power for inferring connectivity based on molecular tools, Ruzzante (1998) showed that sample sizes of less than 30-50 had significant bias and could be misleading. Therefore, in the interest of maintaining statistical rigor while minimizing the number of samples collected, our target sample size is 30/location for most fish species.

All scuba equipment is soaked in a bleach solution between sampling locations, in compliance with decontamination protocols.

LAB METHODS

The primary lab methodology in this study will be sequencing of mtDNA cytochrome genes. In most species, a segment of approximately 800 base pairs of the mtDNA cytochrome b or cytochrome oxidase 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 our lab. Genomic DNA aliquots will be maintained in long-term storage at HIMB so that the genetic material collected will be available for future studies.

Advances in population genetics, especially coalescence theory, will greatly enhance our analysis, elucidating the history of reef organisms, including the effective population size, founder events, and patterns of population collapse and recovery (Harpending et al. 1998, Beerli and Felsenstein 2001, Emerson et al. 2001).

DNA sequence variation will be summarized with standard diversity indices and with an analysis of molecular variance (AMOVA) using ARLEQUIN vers. 2 (Schneider et al. 2000). 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 and Felsenstein 2001).

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?

To the greatest extent possible, specimens will be frozen and vouchered so that future research efforts can use archived material instead of collecting new specimens. Preserved tissue samples suitable for DNA work will be archived at HIMB for future permitted uses. PI Bowen will be responsible for the database which will track each sample and will be the lead contact for persons wishing to access the tissue sample collection. No samples will be provided to researchers outside HIMB without prior consent of Permit Coordinators.

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?

Frozen fish and fin clips for genetic analysis will be transported in the RV Hiialakai.

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

It is incumbent on us to make maximum use of specimens, especially when they are derived from lethal collections. Towards this end, we have coordinated fish species lists with a parallel project by Erik Franklin and Matt Craig. They can use the specimens collected initially for genetic analysis to resolve age, growth, diet, and other aspects of natural history. Further, the nonlethal sampling of apex predators (listed in Appendix 1) will make maximum use of the captures conducted by Carl Meyer and colleagues for tagging studies. We previously provided specimens for the Bishop Museum, for ciguatera research at University of Hawaii at Hilo, and for life history studies. We will continue this effort to make the most of precious specimens collected in the NWHI.

An electronic database of all samples will be available 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.

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

Materials include snorkel and scuba gear (mask, fins, snorkel, wetsuit, tank, BCD), collection bag, polespear, hand nets, fish traps, and a high resolution digital camera in an underwater housing to photo-document the collections.

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:

We expect sampling to be mostly complete in 2009-2010, and analysis of specimens is ongoing (Eble et al. 2009; Craig et al. in prep). Data analysis and write-up usually take no more than an additional year, although the turn-around time for some journals can exceed 300 days, so time to publication can still be considerable post-submission of the study.

Results from these studies are made available to Monument, FWS, and stat managers as quickly as possible. Brown-bag luncheons at HIMB allow researchers to highlight important or interesting new results and discuss them with the management personnel. In addition, we hold biannual symposia during which researchers present the most current findings from their ongoing research in the Monument. These efforts ensure that research results are provided to the Monument co-trustees as quickly as they become available.

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

Craig, M.T., J.A. Eble, D.R. Robertson, B.W. Bowen. 2007. High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). *Marine Ecology Progress Series* 334: 245–254.

Schultz, J.K., R.L. Pyle, E. DeMartini, and B.W. Bowen. 2007. Genetic homogeneity among color morphs of the flame angelfish, *Centropyge loriculus*. *Marine Biology* 151: 167-175.

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Schultz, 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* 100:25-33.

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Slattery, M., A. Baker, P. Bongaerts, B.W. Bowen, J. Leichter, M. Lesser, R. Nemeth, P. Yoshioka. Biological and physical connectivity of mesophotic coral reefs. Submitted

Andrews, K.R., L. Karczmarski, W.W.L. Au, B.W. Bowen, S. Rickards, R.J. Toonen. Intraspecific variability in genetic structure corresponds with social structure and environment for the Hawaiian spinner dolphin (*Stenella longirostris*). Submitted

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Ramon, M.L., P.A. Nelson, E. DeMartini, W.J. Walsh, G. Bernardi. 2008. Phylogeography, historical demography, and the role of post-settlement ecology in two Hawaiian damselfish species. *Mar Biol* 153:1207-1217.

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Toonen, R.J. 2001. *Molecular Genetic Analysis of Recruitment and Dispersal in the Intertidal Porcelain Crab, *Petrolisthes cinctipes**. Ph.D. Dissertation, Center for Population Biology, Section of Evolution and Ecology, University of California, Davis, CA. 325 pp.

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. Requested Fish Collections for B.W. Bowen

As the requested permit activities below indicate, we have completed collections for most shallow-reef fishes at most locations. The emphasis this year is on the reef habitats between French Frigate Shoals and Pearl and Hermes Atoll (Gardner, Maro, Laysan) to complete the sampling of many of the remaining species (see Oval butterflyfish *Chaetodon lunulatus* and Ornate wrasse *Halichoeres ornatus* below for example). We know that some species show a genetic break in this region, but existing samples are inadequate to define this more precisely. This list has changed from last year in the following points:

- 1) Previously we completed sampling of seven species, and have removed them from the list: Orangecheek surgeonfish *Acanthurus olivaceus*, Yellow tang *Zebrasoma flavescens*, Brown surgeonfish *Acanthurus nigrofuscus*, Blue-lined surgeonfish *Acanthurus nigroris*, Milletseed butterflyfish *Chaetodon miliaris*, Blueline butterflyfish *Chaetodon fremblii*, Pebbled butterflyfish *Chaetodon multicinctus*. Results from these species are either published (Eble et al. 2009) or in an advanced state of preparation (Craig et al. in prep). We removed the Orange goatfish *Mulloidichthys pflugeri* after determining that most individuals were too deep to sustain a population genetic study. This year we are requesting no further collections of moray eels (Reece and Bowen permit), as we have sufficient material to characterize the genetic diversity in Hawaiian species.
- 2) We request additional specimens of Goldring bristletooth *Ctenochaetus strigosus* and the introduced snappers (*Lutjanus kasmira* and *Lutjanus fulvus*) to test for the spread of the parasitic nematode that was introduced with ta'ape.
- 3) We request specimens from four species of damselfishes. Recent studies have demonstrated that damselfish have limited dispersal and may be particularly appropriate for resolving genetic partitions among ecosystems (Ramon et al. 2008). Notably, damselfish are among the most abundant fishes in coral ecosystems of the Monument, numbering in the millions. We therefore anticipate no impact of these collections on ecosystem function.
- 4) We request small samples (a maximum of ten per location, a maximum of 50 total) from five species of parrotfish, to determine whether overfishing has depressed genetic diversity in the main Hawaiian Islands. This will be accomplished by comparing parrotfish genetic diversity in the Monument, Main Hawaiian Islands, and ancient middens (trash pits) on Hawaii dating to 100-1000 years before present. The accomplishment of sequencing ancient DNA, by Yvonne Chan in our research group, allows a unique historical perspective on fishing, but can only be accomplished with complete surveys of contemporary genetic diversity.
- 5) We request specimens of the Blue Spine unicornfish *Naso unicornis* (a maximum of ten per location, a maximum of 50 total), to determine whether overfishing has altered the timing of first reproduction and population demography (fewer older fish) in the main Hawaiian Islands. This will be accomplished by comparing average age of sexual maturity and population age structure between the Monument, Oahu, and Hawai'i Island.
- 6) The second cruise to the Papahānaumokuākea Marine National Monument will include a deep (>130 feet) diving component to assess the health and biodiversity of this reef fauna. Therefore we request limited latitude to collect voucher specimens of new species that may be encountered at these depths (see below).

This permit request is based on the remaining sampling needs after three previous collecting trips, to bring sample size up to 30 per location. Most of the sampling for this project is complete, and we (happily) anticipate taking fewer fish this year.

As before, to make maximum use of specimens, samples obtained in this project will have multiple uses in other permitted studies, especially the life-history work by Matthew Craig and Eric Franklin, and the disease work by Greta Aeby (separate permit applications pending).

Common name	Scientific name	No., Size, Locations
Family Acanthuridae		
Goldring bristletooth	<i>Ctenochaetus strigosus</i>	30 all sizes Nihoa 30 all sizes French Frigate Shoals 30 all sizes Gardner Pinnacles 30 all sizes Maro Reef 30 all sizes Laysan 30 all sizes Lisianski 30 all sizes Pearl and Hermes 30 all sizes Midway 30 all sizes Kure
Blue Spine surgeonfish	<i>Naso unicornis</i>	10 all sizes Nihoa
MAXIMUM OF 50 FISH TOTAL		10 all sizes French Frigate Shoals 10 all sizes Gardner Pinnacles 10 all sizes Maro Reef 10 all sizes Laysan 10 all sizes Lisianski 10 all sizes Pearl and Hermes 10 all sizes Midway 10 all sizes Kure
Family Chaetodontidae		
Oval butterflyfish	<i>Chaetodon lunulatus</i>	30 all sizes Nihoa 1 all sizes French Frigate Shoals 30 all sizes Gardner Pinnacles 30 all sizes Maro Reef 27 all sizes Laysan 22 all sizes Lisianski 0 all sizes Pearl and Hermes 0 all sizes Midway 0 all sizes Kure
Family Labridae		
Ornate wrasse	<i>Halichoeres ornatissimus</i>	5 all sizes Nihoa 0 all sizes French Frigate Shoals 25 all sizes Gardner Pinnacles 30 all sizes Maro Reef 28 all sizes Laysan 30 all sizes Lisianski 0 all sizes Pearl and Hermes 0 all sizes Midway 0 all sizes Kure
Yellowtail coris	<i>Coris gaimard</i>	29 all sizes Nihoa 0 all sizes French Frigate Shoals 30 all sizes Gardner Pinnacles 30 all sizes Maro Reef 30 all sizes Laysan 30 all sizes Lisianski 29 all sizes Pearl and Hermes 30 all sizes Midway 30 all sizes Kure
Slow wrasse	<i>Coris venusta</i>	26 all sizes Nihoa

	0 all sizes French Frigate Shoals
	29 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	0 all sizes Laysan
	27 all sizes Lisianski
	0 all sizes Pearl and Hermes
	0 all sizes Midway
	0 all sizes Kure
Blacktail wrasse <i>Thalassoma ballieui</i>	23 all sizes Nihoa
	0 all sizes French Frigate Shoals
	0 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	0 all sizes Laysan
	11 all sizes Lisianski
	0 all sizes Pearl and Hermes
	0 all sizes Midway
	0 all sizes Kure
Family Scaridae	
Spectacled parrotfish <i>Chlorurus perspicillatus</i>	10 all sizes Nihoa
MAXIMUM OF 50 FISH TOTAL	10 all sizes French Frigate Shoals
	10 all sizes Gardner Pinnacles
	10 all sizes Maro Reef
	10 all sizes Laysan
	10 all sizes Lisianski
	10 all sizes Pearl and Hermes
	10 all sizes Midway
	10 all sizes Kure
Bullethead parrotfish <i>Chlorurus spilurus</i>	10 all sizes Nihoa
MAXIMUM OF 50 FISH TOTAL	10 all sizes French Frigate Shoals
(formerly <i>C. sordidus</i>)	10 all sizes Gardner Pinnacles
	10 all sizes Maro Reef
	10 all sizes Laysan
	10 all sizes Lisianski
	10 all sizes Pearl and Hermes
	10 all sizes Midway
	10 all sizes Kure
Regal parrotfish <i>Scarus dubius</i>	10 all sizes Nihoa
MAXIMUM OF 50 FISH TOTAL	10 all sizes French Frigate Shoals
	10 all sizes Gardner Pinnacles
	10 all sizes Maro Reef
	10 all sizes Laysan
	10 all sizes Lisianski
	10 all sizes Pearl and Hermes
	10 all sizes Midway
	10 all sizes Kure
Ember parrotfish <i>Scarus rubroviolaceus</i>	10 all sizes Nihoa
MAXIMUM OF 50 FISH TOTAL	10 all sizes French Frigate Shoals
	10 all sizes Gardner Pinnacles
	10 all sizes Maro Reef
	10 all sizes Laysan
	10 all sizes Lisianski
	10 all sizes Pearl and Hermes

	10 all sizes Midway
	10 all sizes Kure
Palenose parrotfish <i>Scarus psittacus</i>	10 all sizes Nihoa
MAXIMUM OF 50 FISH TOTAL	10 all sizes French Frigate Shoals
	10 all sizes Gardner Pinnacles
	10 all sizes Maro Reef
	10 all sizes Laysan
	10 all sizes Lisianski
	10 all sizes Pearl and Hermes
	10 all sizes Midway
	10 all sizes Kure
Family Pomacentridae	
Hawaiian Sargeant <i>Abudefduf abdominalis</i>	30 all sizes Nihoa
	30 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	30 all sizes Laysan
	30 all sizes Lisianski
	30 all sizes Pearl and Hermes
	30 all sizes Midway
	30 all sizes Kure
Indo-Pacific Sargeant <i>Abudefduf vaigiensis</i>	30 all sizes Nihoa
	30 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	30 all sizes Laysan
	30 all sizes Lisianski
	30 all sizes Pearl and Hermes
	30 all sizes Midway
	30 all sizes Kure
Hawaiian Chromis <i>Chromis ovalis</i>	30 all sizes Nihoa
	30 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	30 all sizes Laysan
	30 all sizes Lisianski
	30 all sizes Pearl and Hermes
	30 all sizes Midway
	30 all sizes Kure
Vanderbilt's Chromis <i>Chromis vanderbilti</i>	30 all sizes Nihoa
	30 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	30 all sizes Laysan
	30 all sizes Lisianski
	30 all sizes Pearl and Hermes
	30 all sizes Midway
	30 all sizes Kure
Family Blenniidae	
Scarface blenny <i>Cirripectes vanderbilti</i>	15 all sizes Nihoa
	15 all sizes French Frigate Shoals

	15 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	11 all sizes Laysan
	12 all sizes Lisianski
	13 all sizes Pearl and Hermes
	0 all sizes Midway
	0 all sizes Kure
Fangblenny <i>Plagiotremus goslinei</i>	14 all sizes Nihoa
	7 all sizes French Frigate Shoals
	15 all sizes Gardner Pinnacles
	15 all sizes Maro Reef
	15 all sizes Laysan
	15 all sizes Lisianski
	15 all sizes Pearl and Hermes
	3 all sizes Midway
	7 all sizes Kure
Fangblenny <i>Plagiotremus ewaensis</i>	15 all sizes Nihoa
	14 all sizes Gardner Pinnacles
	14 all sizes Laysan
	15 all sizes Maro Reef
	15 all sizes Lisianski
	15 all sizes Pearl and Hermes
	11 all sizes Midway
	11 all sizes Kure
Family Mullidae	
Yellowstripe goatfish <i>Mulloidichthys flavolineatus</i>	30 all sizes Nihoa
	8 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	30 all sizes Laysan
	30 all sizes Lisianski
	30 all sizes Pearl and Hermes
	0 all sizes Midway
	7 all sizes Kure
Yellowfin goatfish <i>Mulloidichthys vanicolensis</i>	30 all sizes Nihoa
	6 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	30 all sizes Laysan
	30 all sizes Lisianski
	3 all sizes Pearl and Hermes
	0 all sizes Midway
	0 all sizes Kure
Manybar goatfish <i>Parupeneus multifasciatus</i>	30 all sizes Nihoa
	0 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	30 all sizes Laysan
	30 all sizes Lisianski
	0 all sizes Pearl and Hermes
	0 all sizes Midway
	0 all sizes Kure

Sidespot goatfish *Parupeneus pleurostigma* 30 all sizes Nihoa
 22 all sizes French Frigate Shoals
 30 all sizes Gardner Pinnacles
 30 all sizes Maro Reef
 30 all sizes Laysan
 30 all sizes Lisianski
 29 all sizes Pearl and Hermes
 0 all sizes Midway
 0 all sizes Kure

Family Lutjanidae

Blueline snapper (ta'ape) *Lutjanus kasmira* 30 all sizes Nihoa
 INTRODUCED SPECIES 30 all sizes French Frigate Shoals
 30 all sizes Gardner Pinnacles
 30 all sizes Maro Reef
 30 all sizes Laysan
 30 all sizes Lisianski
 30 all sizes Pearl and Hermes
 30 all sizes Midway
 30 all sizes Kure

Blacktail snapper *Lutjanus fulvus* 30 all sizes Nihoa
 INTRODUCED SPECIES 30 all sizes French Frigate Shoals
 30 all sizes Gardner Pinnacles
 30 all sizes Maro Reef
 30 all sizes Laysan
 30 all sizes Lisianski
 30 all sizes Pearl and Hermes
 30 all sizes Midway
 30 all sizes Kure

Family Serranidae

Peacock hind (roi) *Cephalopholis argus* 14 all sizes Nihoa
 INTRODUCED SPECIES 30 all sizes French Frigate Shoals
 30 all sizes Gardner Pinnacles
 30 all sizes Maro Reef
 30 all sizes Laysan
 30 all sizes Lisianski
 30 all sizes Pearl and Hermes
 30 all sizes Midway
 30 all sizes Kure

Opportunistic Sampling of Apex Predators

We request authority to collect and archive tissue specimens of less than one gram weight from the apex predators captured during tagging studies by Carl Meyer and colleagues. In the course of placing acoustic and satellite tags, a small piece of tissue is dislodged from the fish, and we seek to archive these under the mandate to make maximum use of sampling opportunities. **Under no circumstances will these species be subject to directed fishing effort.** Possible capture species include, but are not limited to:

Tiger shark	<i>Galeocerdo cuvier</i>
Galapagos shark	<i>Carcharhinus galapagensis</i>
Grey reef shark	<i>Carcharhinus amblyrhynchos</i>
Blacktip shark	<i>Carcharhinus melanopterus</i>
Whitetip reef shark	<i>Triaenodon obesus</i>
Jack (Ulua)	<i>Caranx spp.</i>
Green jobfish	<i>Aprion virescens</i>
Barracuda	<i>Sphyraena barracuda</i>
Tuna	<i>Thunnus spp.</i>

Opportunistic sampling of new species

In 2009 the Hiiialakai cruise August 6 – September 4 will include deep diving (> 130 feet) with trimix scuba technology. In these circumstances we wish to collect specimens of new fish species, for genetic characterization, taxonomic description, and vouchering in the Bishop Museum. These collections will allow us to characterize the fish biodiversity of the Monument, and will only be made in cases where species are sufficiently abundant (encounter rate of 5+ per hour) to sustain collections without adverse impact. Encounters with rarer species will be documented with photo-vouchers.

New fish species A maximum of five specimens/species at each island or atoll