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: 5/1/11 - 10/31/11
Proposed Method of Entry (Vessel/Plane): RV Hi'ialakai
Proposed Locations: Shallow reefs and mesophotic reef habitats (1 - 400 feet depth), focused on Kure, Midway, Pearl & Hermes, Lisianski, Laysan, Maro Reef, Gardner Pinnacles, French Frigate Shoals, Mokumanmana, 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:
7
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 11 shallow reef fishes, plus 8 mesophotic reef fishes, 9 mesophotic invertebrates, and one mesophotic plant species, designed to address the level of isolation between shallow and deep reef ecosystems across the Hawaiian Archipelago, and especially throughout the Papahanaumokuakea Marine National Monument.

Specimens will be collected on deep reefs to evaluate the hypothesis that these mesophotic 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 periods in 2009 - 2010 were 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 19 fishes, 9 invertebrate, and one plant species at locations across the entire archipelago, using polespears and narcotics to collect fish, nonlethal tissue biopsies for invertebrates (except the oysters which are collected whole) and small biopsies from a plant, and using DNA sequencing technology to resolve novel evolutioanry lineages, genetic diversity and connectivity among reef habitats.

c.) This activity would help the Monumen**t** 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.

For example, the most recent findings from this research indicate that the Yellow Tang (Zebrasoma flavescens) is divided into 4-7 isolated populations within the Hawaiian Archipelago, including three populations in the PMNM (Eble et al. Accepted). This fish is heavily harvested for the ornamental fish trade, and so findings will realign management units for this species. Findings also indicate some connectivity between the Main Hawaiian Islands and the lower NWHI.

Other information or background: To preserve biodiversity, it is important to know how it is arises (Bowen & 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 & 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; Weersing & Toonen 2009). 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: Research Professor

1a. Intended field Principal Investigator (See instructions for more information):
Randall Kosaki for cruise August - September 2011

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

Phone: [redacted]

Fax: [redacted]

Email: [redacted]

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

3. Affiliation (institution/agency/organization directly related to the proposed project):
Hawaii Institute of Marine Biology, School of Ocean and Earth Science and Technology, University of Hawai

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):
Randall Kosaki (Ph.D., Research Diver, PMNM), Dan Wagner (Graduate student, Research diver, HIMB), Ray Boland (Research diver and divemaster, NMFS), Richard Pyle (Research diver and collector, Bishop Museum), Greg McFall (Research diver and collector, ONMS), Anthony Montgomery (Research diver and collector, USFWS), Iria Fernandez (Post-doctoral

scientist, research diver and collector, HIMB), Richard Coleman (Graduate student, Research diver and collector, HIMB), Joseph DiBattista (Post-doctoral scientist, research diver and collector, HIMB), Carl Meyer (Post-doctoral scientist, research diver and collector, HIMB), Christie Wilcox (Graduate student, Research diver and collector, HIMB), Derek Skillings (Graduate student, Research diver and collector, HIMB), Robert Toonen (Associate Professor, Research diver and collector, HIMB), Keo Lopes (Research diver and collector, UH Dive safety Program), William Love (Research diver and collector, UH Dive safety Program)

The core HIMB team covered under this permit application for fish, invertebrate, and plant collections include Bowen, Wagner, Fernandez, Coleman, DiBattista, Wilcox, Skillings, and Toonen (although only two to four will actually participate on any single cruise). Kosaki has agreed to be field P.I. in the event that Bowen cannot participate on the September cruise. Boland, Pyle, McFall, and Montgomery are members of the mesophotic team, covered under a separate permit application, who have agreed and are qualified to collect specimens. Carl Meyer has a separate permit application to tag and monitor large predators, and has agreed to collect fish where possible. Lopes and Love are UH dive safety officers who will participate in multiple activities including trimix dives (under Kosaki), predator monitoring (under Meyer) and tissue collections for genetic analysis (under Bowen and field P.I. Kosaki).

Section B: Project Information

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

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

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

Location Description:

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, invertebrates, and one common plant, primarily designed to address the issue of population connectivity across the PMNM. We will survey approximately 29 species across the entire archipelago, using DNA 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.

Objectives: The objective of this permit request is a genetic (mtDNA) survey of fish and invertebrates across the NWHI to assess the level of connectivity among isolated reef habitats. We can accomplish this with samples of 30 - 50 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 nets, polespears, and fish narcotic (clove oil or quinaldine). 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.

Management benefits: These data will provide information on connectivity required for management, and can also detect cryptic endemic species (DiBattista et al. 2011) 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 taxa, we can determine if there are general patterns that can guide management decisions for understudied species (Toonen et al. 2011). The genetic surveys of connectivity among reef habitats substantially augment the scientific foundation for conservation measures. Specifically, this research will establish the boundaries of isolated reef ecosystems of the NW Hawaiian Islands. Each ecosystem is an independent management unit.

This is a multiyear project for which the first round of data on shallow reef connectivity has been published (see references 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 (DiBattista et al. 2011). 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 practioners 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. Team members have already received some guidance in the first six 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 knowledgable 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.

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 there is no 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 and invertebrates, 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 (Toonen et al. 2011). 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 several more years of collecting activities.

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 and Robert Toonen will lead the project, with 20 years experience in this field, including four prior expeditions to the NWHI and over 100 scientific publications pertaining to reef biodiversity, endangered species, and conservation. They are known to the PMNM staff, DAR staff, and USFWS staff, and are clearly qualified to perform this research.

Field P.I. Randall Kosaki (Ph.D.) is the veteran of at least six previous PMNM expeditions and is thoroughly qualified to identify and capture permitted species with minimal collatoral damage.

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 yet to be determined ship time on the NOAA research vessel Hiialakai, a line item in the budget of the Monument. Subsequent lab-based research is supported by National Science Foundation (grants OCE-0903129 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 100 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, and 50/location for nonlethal invertebrate and plant sampling. All species in our list are common reef organisms 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 five years and through the entire history of the Monument. During these previous efforts, there have been no problems with permit violations by this 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 and invertebrate species listed in Appendix 1 inhabit shallow and mesophotic reefs and are accessible via snorkeling and scuba dives. The primary methodology for collecting fishes is with the use of Hawaiian polespears, but we wish to supplement this with a fish narcotic administered with small squirt bottles to collect fishes in crevasses (such as cardinalfish) and in mesophotic habitats where time is short and diver safety (due to sharks during long decompression stops) is a consideration. Abundant evidence from over a dozen field studies have shown that the fish narcotics clove oil and quinaldine do not harm corals or other invertebrates (Japp & Wheaton 1975; Munday & Wilson 1997; Robertson & Smith-Vaniz 2010), and these narcotics can be precisely directed at small schools of fish (cardinalfish and mesophotic damselfish in our case) to avoid bycatch. We wish to use both clove oil and quinaldine this year only; the latter is more effective at shallow depths, but we need to guage the performance of both at mesophotic depths under actual field conditions. Notably, fish that are dosed but not collected regain alertness within a couple of minutes and return to habitat; these narcotics are not lethal at the doses proposed here (approximately 30-50 ppm quinaldine, 100 ppm clove oil). Alternative methods such as hook and line fishing are not as selective, whereas narcotics and spearing allows us to collect only the species we need while avoiding unnecessary bycatch. For invertebrates we sample non-lethally and remove a rice-grain sized piece of tissue (biopsy of less than one square cm) and release the animal in the location from which it was collected. The single exception is the winged oyster, which we collect whole. We have made significant progress in nonlethal sampling, however most fish specimens are collected with polespears.

Algae specimens will be approximately one square cm harvested nonlethally from unrelated (spacially distinct) individuals, and stored in our standard salt-DMSO buffer, which has been shown to preserve the integrity of plant biomolecules (Shoaf 1976).

All coral collections, particularly black corals, will be made in compliance with state regulations.

Statistical rigor requires a minimum sample size of 30 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, and 50/location for nonlethal invertebrate and plant sampling.

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 collections. No samples will be provided to researchers outside HIMB until a material transfer agreement is available from the Monument.

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. Bowen will retain specimens until P.I. Franklin has a permit to possess and study the specimens. 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. Kimberly Tenggardjaja and Giacomo Bernardi at University of California Santa Cruz are conducting parallel studies of reef fish connectivity with damselfishes. Ms. Tenggardjaja participated in 2009 and 2010 cruises, and has spent two months in my lab processing samples, pending the development of a material transfer agreement by the Monument.

An electronic database of all samples is available, and will be updated 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, tissue biopsy tools, 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. Fish narcotics are clove oil (MSDS attached) and quinaldine (MSDS attached), neither are toxic to humans at the doses used here (30 - 100 ppm), although both can irritate eyes at high concentration.

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:
Major sampling for the shallow reef connectivity study was completed in 2010, and analysis of specimens is ongoing (e.g. Eble et al. 2009; Craig et al. 2010; Gaither et al. 2010; Wagner et al. 2010; Eble et al. 2011; Stat et al. 2011; Dibattista et al. 2011; Bird

et al. 2011, Forsmen et al. 2011, Toonen et al. 2011). Only a few shallow fishes species are requested for this permit, as we refocus our efforts on mesophotic reef connectivity. Data analysis and write-up usually take no more than an additional year, although the turn-around time for some journals can exceed 200 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 Hawaii DLNR 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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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 Collections for B.W. Bowen

The 2010 field season concluded major sampling efforts for the shallow reef connectivity studies. As the proposed activities below indicate, we request only a few fish collections, and no invertebrate collections, from the shallow reefs. The emphasis this year, for the few remaining shallow species, is on the reef habitats between French Frigate Shoals northwest to Pearl and Hermes Atoll (Maro, Laysan, Lisianski, Gardner). We know that some species show genetic breaks in this region, but existing samples are inadequate to define this more precisely. Notable aspects of this request include the following points:

1) In 2009 we completed sampling of 12 species, and removed them from the list. During the 2010 expeditions, we completed field efforts for an additional 13 fish species, including all remaining wrasses, parrotfishes, and blennies. Results from these species are published (e.g. endemic surgeonfishes, Eble et al. 2009; Montipora corals, Forsman et al. 2010, black corals, Wagner et al. 2010; blueline snapper, Gaither et al. 2010; endemic butterflyfishes, Craig et al. 2010; moray eels, Reece et al. 2010; Blueline Surgeonfish; DiBattista et al. 2011; Brown Surgeonfish, Eble et al. 2011, opihi, Bird et al. In press), in press (Yellow Tang, Eble et al.; Hawaiian seahorse, Szabo et al.), in an advanced state of preparation (Montipora corals, Concepcion et al.; vermetid gastropods, Faucci et al.; blennies, Szabo et al.; Oval Butterflyfish, Bowen et al.), or the subjects of ongoing DNA analyses.

   The shallow organisms remaining on the list below are all fishes, including five damselfishes, four goatfishes, and one butterflyfish. These remain on the list to allow one more effort at filling gaps in coverage. In addition, we have added one fish to the list, the common and abundant iridescent cardinalfish (*Pristiapogon kallopterus*). This fish may have limited dispersal due to the reproductive behavior of mouthbrooding, and so may help resolve the boundaries of isolated ecosystems in the Monument.

2) The second (possible) cruise to the Papahanaumokuakea Marine National Monument (September) will include a deep (>130 feet) diving component to assess the health and biodiversity of this reef fauna. We request permission to collect nine common invertebrates, eight common fishes, and one ubiquitous seaweed to further connectivity studies, both between mesophotic habitats and between mesophotic and shallow habitats. Invertebrate collections are made in situ with nonlethal sampling (except for the winged oyster) with a tissue sample about the size of a rice grain. Fish will be collected with polespears and clove oil and quinaldine, fish narcotics that do not harm corals and other invertebrates (Jaap & Wheaton 1975, Munday & Wilson 1997, Robertson & Smith-Vaniz 2010). We also request limited latitude to collect voucher specimens of new species that may be encountered at these depths (see below).

3) We propose to study black corals at mesophotic depths in the Monument. This research will involve collection of vouchers for comparison with established type specimens to confirm or refute the morphological identification of the Hawaiian antipatharian (black coral) fauna. The black corals are already under revision because the species previously identified as *Antipathes dichotoma* from Hawaii do not match specimens from the type locality of *A. dichotoma* in the Mediterranean Sea; as a result, the Hawaiian "*A. dichotoma*" has now been assigned the new name of *Antipathes griggi* (Opresko 2009). Likewise, our study over the past year has resulted in the redescription of *Antipathes grandis* (Wagner et al., in press) from the Main Hawaiian Islands, and on-going collaborations with State and Federal groups interested in the taxonomy and management of these precious corals. The first technical diving to the appropriate depths in the Monument revealed 4 species of black corals never before reported from the NWHI and we now seek to obtain opportunistic voucher collections of unusual colonies to determine how many species are present, and whether the initial species identifications were correct (or a misidentification as described for *A. dichotoma* above). All collections of black corals will comply with Hawaii state regulations.

4) To facilitate the evaluation of this request, we have divided the list of fish into a shallow reef component (near completion) and a deep reef component (still experimental).

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

SHALLOW REEF COLLECTIONS (< 130 feet depth)

Family Pomacentridae

Hawaiian Sargeant	*Abudefduf abdominalis*	27 all sizes Nihoa
		9 all sizes Mokumanamana
		0 all sizes French Frigate Shoals
		30 all sizes Gardner Pinnacles
		7 all sizes Maro Reef
		1 all sizes Laysan
		30 all sizes Lisianski
		0 all sizes Pearl and Hermes
		0 all sizes Midway
		0 all sizes Kure
Indo-Pacific Sargeant	*Abudefduf vaigiensis*	26 all sizes Nihoa
		23 all sizes Mokumanamana
		0 all sizes French Frigate Shoals
		30 all sizes Gardner Pinnacles
		30 all sizes Maro Reef
		16 all sizes Laysan
		30 all sizes Lisianski
		0 all sizes Pearl and Hermes
		0 all sizes Midway
		29 all sizes Kure
Vanderbilt's Chromis	*Chromis vanderbilti*	0 all sizes Nihoa
		16 all sizes Mokumanamana
		0 all sizes French Frigate Shoals
		30 all sizes Gardner Pinnacles
		30 all sizes Maro Reef
		10 all sizes Laysan
		30 all sizes Lisianski
		0 all sizes Pearl and Hermes
		8 all sizes Midway
		0 all sizes Kure
Three-spot Chromis	*Chromis verater*	0 all sizes Nihoa
		30 all sizes Mokumanamana
		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
	30 all sizes Midway
	0 all sizes Kure
Hawaiian Chromis *Chromis ovalis*	0 all sizes Nihoa
	0 all sizes Mokumanamana
	0 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	0 all sizes Maro Reef
	0 all sizes Laysan
	30 all sizes Lisianski
	0 all sizes Pearl and Hermes
	0 all sizes Midway
	27 all sizes Kure
Family Mullidae	
Yellowstripe goatfish *Mulloidichthys flavolineatus*	30 all sizes Nihoa
	15 all sizes Mokumanamana
	3 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	21 all sizes Maro Reef
	21 all sizes Laysan
	30 all sizes Lisianski
	0 all sizes Pearl and Hermes
	0 all sizes Midway
	0 all sizes Kure
Yellowfin goatfish *Mulloidichthys vanicolensis*	22 all sizes Nihoa
	22 all sizes Mokumanamana
	0 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	28 all sizes Maro Reef
	24 all sizes Laysan
	30 all sizes Lisianski
	0 all sizes Pearl and Hermes
	0 all sizes Midway
	0 all sizes Kure
Manybar goatfish *Parupeneus mulitfasciatus*	0 all sizes Nihoa
	8 all sizes Mokumanamana
	0 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	30 all sizes Maro Reef
	29 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*	29 all sizes Nihoa
	21 all sizes Mokumanamana
	0 all sizes French Frigate Shoals
	30 all sizes Gardner Pinnacles
	27 all sizes Maro Reef
	0 all sizes Laysan

	29 all sizes Lisianski
	4 all sizes Pearl and Hermes
	0 all sizes Midway
	0 all sizes Kure
Family Chaetodontidae	
Threadfin butterflyfish *Chaetodon auriga*	10 all sizes Nihoa
MAXIMUM OF 50 FISH TOTAL	10 all sizes Mokumanamana
	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 Apogonidae	
Iridescent cardinalfish *Pristiapogon kallopterus*	30 all sizes Nihoa
	30 all sizes Mokumanamana
	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

DEEP REEF COLLECTIONS (> 130 feet)

FISHES	
Goldring bristletooth *Ctenochaetus strigosus*	30 all sizes Nihoa
	30 all sizes Mokumanamana
	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 *Chromis ovalis*	30 all sizes Nihoa
	30 all sizes Mokumanamana
	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
Whitetail Chromis *Chromis leucura*	30 all sizes Nihoa
	30 all sizes Mokumanamana
	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
Three-spot Chromis *Chromis verater*	30 all sizes Nihoa
	30 all sizes Mokumanamana
	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
	28 all sizes Kure
Hawaiian Dascyllus *Dascyllus albisella*	30 all sizes Nihoa
	22 all sizes Mokumanamana
	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
Yellowfish soldierfish *Myripristis chryseres*	30 all sizes Nihoa
	30 all sizes Mokumanamana
	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 Bigeye *Priacanthus meeki*	30 all sizes Nihoa
	30 all sizes Mokumanamana
	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
Turkeyfish/Lionfish *Pterois sphex*	30 all sizes Nihoa
	30 all sizes Mokumanamana
	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
INVERTEBRATES		
Hawaiian Black Coral	*Antipathes griggi*	50 Nihoa 50 Mokumanamana 50 French Frigate Shoals 50 Gardner Pinnacles 50 Maro Reef 50 Laysan 50 Lisianski 50 Pearl and Hermes 50 Midway 50 Kure
Wire Black Coral	*Cirrhipathes anguina*	50 Nihoa 50 Mokumanamana 50 French Frigate Shoals 50 Gardner Pinnacles 50 Maro Reef 50 Laysan 50 Lisianski 50 Pearl and Hermes 50 Midway 50 Kure
Feather Black Coral	*Myriopathes ulex*	50 Nihoa 50 Mokumanamana 50 French Frigate Shoals 50 Gardner Pinnacles 50 Maro Reef 50 Laysan 50 Lisianski 50 Pearl and Hermes 50 Midway 50 Kure
Bicolor Gorgonian	*Acabaria bicolor*	50 Nihoa 50 Mokumanamana 50 French Frigate Shoals 50 Gardner Pinnacles 50 Maro Reef 50 Laysan 50 Lisianski 50 Pearl and Hermes 50 Midway 50 Kure
Rubber coral	*Palythoa caesia*	50 Nihoa 50 Mokumanamana 50 French Frigate Shoals 50 Gardner Pinnacles 50 Maro Reef

		50 Laysan
		50 Lisianski
		50 Pearl and Hermes
		50 Midway
		50 Kure
Rice Coral	*Montipora capitata*	50 Nihoa
		50 Mokumanamana
		50 French Frigate Shoals
		50 Gardner Pinnacles
		50 Maro Reef
		50 Laysan
		50 Lisianski
		50 Pearl and Hermes
		50 Midway
		50 Kure
Winged Pearl Oyster	*Pteria brunnea*	50 Nihoa
		50 Mokumanamana
		50 French Frigate Shoals
		50 Gardner Pinnacles
		50 Maro Reef
		50 Laysan
		50 Lisianski
		50 Pearl and Hermes
		50 Midway
		50 Kure
Black Sea Cucumber	*Holothuria atra*	50 Nihoa
		50 Mokumanamana
		50 French Frigate Shoals
		50 Gardner Pinnacles
		50 Maro Reef
		50 Laysan
		50 Lisianski
		50 Pearl and Hermes
		50 Midway
		50 Kure
Teatfish	*Holothuria whitmaei*	50 Nihoa
		50 Mokumanamana
		50 French Frigate Shoals
		50 Gardner Pinnacles
		50 Maro Reef
		50 Laysan
		50 Lisianski
		50 Pearl and Hermes
		50 Midway
		50 Kure
PLANTS		
Green seaweed	*Halimeda kanaloana*	50 Nihoa
		50 Mokumanamana
		50 French Frigate Shoals
		50 Gardner Pinnacles
		50 Maro Reef
		50 Laysan

50 Lisianski
50 Pearl and Hermes
50 Midway
50 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	*Galeocerdo cuvier*
Galapagos shark	*Carcharhinus galapagensis*
Grey reef shark	*Carcharhinus amblyrhynchos*
Blacktip shark	*Carcharhinus melanopterus*
Whitetip reef shark	*Triaenodon obesus*
Jack (Ulua)	*Caranx spp.*
Green jobfish	*Aprion virescens*
Barracuda	*Sphyraena barracuda*
Tuna	*Thunnus spp.*

Opportunistic sampling of new species

In 2011 the Hiialakai cruise in September (pending funding) will include deep diving (> 130 feet) with trimix scuba technology. In these circumstances we wish to collect specimens of new fish and invertebrate species, for genetic characterization, taxonomic description, and vouchering in the Bishop Museum. These collections will allow us to characterize the 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 species	A maximum of five specimens/species at each island or atoll