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: Matthew Iacchei
Affiliation:
University of Hawaii, Manoa
Department of Zoology
Hawaii Institute of Marine Biology (HIMB)

Permit Category: Research
Proposed Activity Dates:
On board Hi'ialakai: tentatively, 06/04/08 - 06/28/08 and 07/31/08 - 08/28/08
On board Oscar Elton Sette: tentatively, 08/14/08 - 09/02/08
On board NMFS/PIFSC lobster tagging cruise: tentatively, 08/02/08 - 09/05/08

Proposed Method of Entry (Vessel/Plane):
HIMB-NWHI Cruise and Maritime Heritage Cruise: Hi'ialakai
PSD Monk Seal Camps Cruise: Oscar Elton Sette
NMFS/PIFSC lobster tagging cruise: Vessel to be determined by NMFS/PIFSC lab

Proposed Locations:
All activity will take place between 5 and 65 meters depth.

All possible locations: Nihoa Island, Necker Island, French Figate Shoals, Gardener Pinnacles, Maro Reef, Laysan Island, Lisianski Island, Pearl and Hermes Atoll, Midway Atoll, Kure Atoll.

Maritime Heritage cruise will visit: French Frigate Shoals, Pearl and Hermes Atoll, Midway Atoll, Kure Atoll

PSD Monk Seal Camps Cruise will visit: TBD

HIMB-NWHI Cruise will visit: TBD

NMFS/PIFSC lobster tagging cruise will visit: Necker Island, Maro Reef, Laysan Island, Gardener Pinnacles

Estimated number of individuals (including Applicant) to be covered under this permit: 25

Estimated number of days in the Monument: 109 total ship days in the monument (this represents all vessel days, for instance counting 2 days when two vessels are in the Monument on the same day)

Hi'ialakai HIMB NWHI Cruise: 25 days
Hi'ialakai Maritime Heritage Cruise: 29 days
Oscar Elton Sette PSD Monk Seal Camps Cruise: 20 days
NMFS/PIFSC lobster tagging cruise: 35 days

Description of proposed activities: (complete these sentences):

a.) The proposed activity would…

The proposed activity would utilize mitochondrial and microsatellite DNA markers to identify stock structure and estimate population connectivity among atolls and banks for six lobster species throughout the Hawaiian archipelago: Panulirus marginatus, Panulirus penicillatus, Scyllarides squammosus, Scyllarides haanii, Parribacus antarcticus, and Arctides regalis. In addition this work will assess temporal and spatial variability in genetic diversity for P. marginatus and S. squammosus at four banks in the NWHI: Maro Reef, Necker Island, Laysan Island, and Gardener Pinnacles.

b.) To accomplish this activity we would ….

To accomplish this activity I would use both trapping and SCUBA diving methods to non-lethally collect 50 tissue samples (lobster legs) per species per bank for all banks in the NWHI. DNA would be extracted from each tissue sample, and each lobster would be genotyped using 1 mitochondrial and a minimum of 10 nuclear microsatellite DNA markers. These genotypes would then undergo statistical analyses to determine for each species whether or not stock structure exists in the Hawaiian archipelago or if the species has one panmictic population in Hawaii. For those species with stock structure, I would determine the relative magnitude and direction of connectivity among the banks and atolls in the archipelago.

c.) This activity would help the Monument by …

This activity would help the Monument by directly addressing one of the principal management needs of the Monument: to understand archipelago-wide connectivity of coral reef species. P. marginatus is an endemic lobster species in Hawaii that historically supported a valuable marine fishery in the state (along with S. squammosus and S. haanii). Ongoing lobster tagging studies targeting P. marginatus and S. squammosus at four banks in the NWHI (Laysan, Gardener, Maro, Necker) have yet to find any evidence of adult individuals of either species moving between banks over multiple years (O'Malley et al unpublished data). This suggests that any exchange of individuals between island populations in the Monument occurs during the larval

phase. My research will determine whether each of these banks is self-sustaining or exchanging larval individuals with other banks in the Northwestern or Main Hawaiian Islands. New analysis techniques will enable me to determine both a direction and a relative magnitude of this exchange wherever it occurs. This information will be beneficial for all lobster species in the study. Managers will gain a better understanding of whether the protection of the Monument will allow the rejuvenation of lobster stocks in the NWHI, and if the Monument may also enhance lobster populations in the main islands. While extraction of lobsters is no longer permitted in the NWHI, there is no way to prevent natural or broad scale anthropogenic effects that may damage lobster stocks (i.e. oil spills or disease outbreaks) from occurring. Knowledge of whether and how lobster populations on various banks are connected will enable managers to rapidly implement the most beneficial contingency plan in case one of these events occurs.

In addition to the direct benefits to the management of the Monument discussed above, my research will utilize the uniqueness of this large and isolated marine reserve and the lobster species contained within to address some of the most critical gaps in the science of designing effective marine reserves.

Other information or background:

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Iacchei, Matthew, J

Title: Mr.; Graduate Student, University of Hawaii at Manoa, Department of Zoology

1a. Intended field Principal Investigator (See instructions for more information):
Matthew Iacchei, CV attached;
Joseph O'Malley, CV attached
NOAA, NMFS, PIFSC, JIMAR
[redacted]
Please see Joe's permit application for further information

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:
Dr. Robert Toonen
[redacted]

3. Affiliation (institution/agency/organization directly related to the proposed project):
University of Hawaii at Manoa
Department of Zoology
Hawaii Institute of Marine Biology (HIMB)

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):

Please see attached excel file. Additional assistant(s) to be named later if berths permit – names of individuals and all contact information will be forwarded as soon as they are identified.

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:
Sampling will take place in typical spiny and slipper lobster habitat between 5 and 65 meters depth at all banks checked off above. Typical lobster habitat for these species in this depth range generally consists of rock rubble, sand, and bedrock with minimal topographical relief but containing small cave openings. Due to the nature of the research, the precise locations where samples will be collected at each bank will remain unknown until sampling takes place.

Specific GPS coordinates for trapping transects conducted during the NMFS/PIFSC lobster tagging cruise will be provided before the tagging vessel enters the monument.

GPS coordinates outlining boundaries for all planned activities for the Hi'ialakai and Sette cruise are included below. Specific collection locations will be reported during/after the collection of specimens:

Location	Longitude	Latitude
Kure Atoll	-178.19706	28.55825
	-178.19624	28.29958
	-178.45988	28.29958

	-178.46071	28.55742
Midway Atoll	-177.19638	28.37420
	-177.19721	28.13377
	-177.52801	28.13460
	-177.52801	28.37420
Pearl and Hermes Atoll	-176.08851	28.04643
	-175.63289	28.04540
	-175.63289	27.70729
	-176.08954	27.70626
Lisianski Island	-173.67293	26.25151
	-173.67293	25.83943
	-174.23095	25.83943
	-174.23095	26.25151
Laysan Island	-171.47900	25.96027
	-171.47725	25.65597
	-171.97918	25.65772
	-171.97918	25.96202
Maro Reef	-170.86374	25.60396
	-170.07997	25.60395
	-170.06693	25.14212
	-170.86172	25.10014
Gardner Pinnacles	-167.74832	25.26071
	-167.75087	24.34878
	-168.36222	24.35133
	-168.36477	25.26071
French Frigate Shoals	-166.55721	24.00867
	-166.51198	23.51256
	-165.87083	23.56711
	-165.81116	24.05429
Necker Island	-165.05630	23.77200
	-165.07200	23.16591

	-164.15132	23.19059
	-164.20934	23.74897
Nihoa Island	-161.66032	23.23817
	-161.66287	22.94013
	-162.05005	22.94268
	-162.05260	23.23562

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:***
Purpose:

The primary purpose of my research is to identify stock structure and estimate population connectivity among atolls and banks for six lobster species throughout the Hawaiian archipelago: Panulirus marginatus, Panulirus penicillatus, Scyllarides squammosus, Scyllarides haanii, Parribacus antarcticus, and Arctides regalis. I will accomplish this goal using both mitochondrial and at least 10 nuclear microsatellite DNA markers. In addition this work will assess temporal and spatial variability in genetic diversity for P. marginatus and S. squammosus at four banks in the NWHI: Maro Reef, Necker Island, Laysan Island, and Gardener Pinnacles.

Need:
This information will directly address one of the principal management needs of the Monument: to understand archipelago-wide connectivity of coral reef species. P. marginatus is an endemic lobster species in Hawaii that historically supported a valuable marine fishery in the state (along with S. squammosus and S. haanii). Ongoing lobster tagging studies targeting P. marginatus and S. squammosus at four banks in the NWHI (Laysan, Gardener, Maro, Necker) have yet to find any evidence of adult individuals of either species moving between banks over multiple years (O'Malley et al unpublished data). This suggests that any exchange of individuals between island populations in the Monument occurs during the larval phase. My research will determine whether each of these banks is self-sustaining or exchanging larval individuals with other banks in the Northwestern or Main Hawaiian Islands. New analysis techniques will enable me to determine both a direction and a relative magnitude of this exchange wherever it occurs. Managers will gain a better understanding of whether the protection of the Monument will allow the rejuvenation of lobster stocks in the NWHI, and if the Monument may also enhance lobster populations in the main islands. While extraction of lobsters is no longer permitted in the NWHI, there is no way to prevent natural or broad scale anthropogenic effects that may damage lobster stocks (i.e. oil spills or disease outbreaks) from occurring. Knowledge of whether and how lobster populations on various banks are connected will enable managers to rapidly implement the most beneficial contingency plan in case one of these events occurs.

Scope/Rationale:

Benthic marine invertebrates, such as both spiny and slipper lobsters, do not tend to move large distances as adults; consequently, most adult lobsters in Hawaii are confined to the island or bank they settle on as juveniles. Any relationship between distant island populations is through larval dispersal, as lobster larvae stay in the water column anywhere from 6 months to one year before settling in a new location. This long larval duration, coupled with the small size of larvae and the ability to swim against prevailing oceanographic currents make larvae extremely difficult to track. Much of the difficulty in successfully managing marine species arises from the lack of knowledge of population connectivity in organisms with this pelagic larval stage (Carr et al 2003). For example, here in Hawaii, the NWHI lobster fishery was closed in 2000 because of increasing uncertainty in population and stock assessment models, particularly with the disregard of spatial heterogeneity and assumption of synchronous dynamics among bank specific populations (Botsford et al. 2002). Estimates of population connectivity are necessary to develop models that more effectively represent bank specific population dynamics, and the use of genetic sampling is widely regarded as the cheapest and most robust way to answer questions of connectivity on the scale of the Hawaiian archipelago.

There is a paucity of research on the majority the species I propose to study in Hawaii (but, for Panulirus penicillatus see MacDonald and Thompson 1987; Johnson, M.W. 1968; for S. squammosus see DeMartini et al 2002, DeMartini and Williams 2001, and DeMartini et al 2005, for P. marginatus see below). None of these studies have examined population relationships or status of populations, which is not well known for any of the species in the Main Hawaiian Islands. Some aspects of P. marginatus have been fairly well examined in the NWHI due to its importance as a commercial fishery. Most work to date, however, has focused solely on populations at two banks: Maro Reef and Necker Island. Studies on how populations are related through larval dispersal have so far produced controversial results. Pollock hypothesizes phyllosoma larvae are mixed together in the Pacific subtropical gyre and remain there for up to four years before returning to settle on Hawaiian reefs (1992), while MacDonald contends that larvae are retained around the archipelago, and for shorter time periods before recruiting (1986). Polovina et al conclude that phyllosoma are driven by currents in a southeasterly direction until reaching Necker, at which time they travel

southwest (1999). Previous genetic studies have been unable to resolve these issues. A P. marginatus allozyme study with samples from a substantial range within the archipelago suggested homogeneity across the entire island chain (Shaklee and Samollow 1980). A later study examined just Maro and Necker and found significant differences between those two banks at one of seven allozyme loci (Seeb et al 1990). Neither of these studies, however, sampled the entire archipelago, and both focused solely on one species. Furthermore, new genetic techniques and analyses can provide more detailed insight into population relationships than allozymes.

My project will utilize both a mitochondrial DNA marker, and at least 10 nuclear microsatellite DNA markers to investigate how intraspecific populations of 6 lobster species are related through larval dispersal in the Hawaiian archipelago: P. marginatus, P. penicillatus, S. squammosus, S. haanii, A. regalis, and P. antarcticus. My research will address more species, encompass more banks, and use more detailed genetic information than any of the previous lobster genetics studies in Hawaii, with the aim of resolving the issue of lobster connectivity in the archipelago.

Both researchers and resource managers around the globe generally agree that efforts to establish effective marine protected areas require detailed information regarding connectivity among disjunct populations of species (e.g., Botsford et al. 2001, Halpern & Warner 2003, Palumbi 2003, Cowen et al. 2006). The unique location and species chosen for this project provide an opportunity to address some of the most critical gaps in the knowledge required to design beneficial marine reserve networks: the most effective size and spacing of marine reserves, whether and how larval duration correlates with dispersal distance, and if connectivity patterns can be generalized across taxa or if each species of interest will have to be investigated individually.

One of the major questions in the design of marine reserves is the impact of size and spacing of reserves on their effectiveness. The Monument is one of the largest, and the most isolated marine protected area in the world. My research in the Monument will provide valuable information on what, if any additional benefits a large protected area may provide over one or several smaller areas. Depending on stock structure patterns, additional analyses may be able to decipher whether the small marine reserves in the Main Hawaiian islands are as effective in maintaining populations as is the large protected area of the Monument. If I find that areas of the Monument seed

populations in the main Hawaiian Islands, I may be able to get an idea of how long this now protected area will take to rejuvenate main Hawaiian Island populations that have undergone heavy fishing pressure in the past. Alternatively, I may find the opposite direction of connectivity: that main Hawaiian Islands seed atolls in the Monument. This conclusion would suggest that more or larger marine reserves may be necessary in the main Hawaiian Islands if management wants to rebuild lobster stocks both here and in the Monument. The number of species I propose to investigate, the varying fishing pressure they have experienced, and the differences in behavior some of the species exhibit allow for many such comparisons to be made and tested further depending on the connectivity patterns for each species.

Another important issue is whether each species exhibits different connectivity patterns or if generalizations can be made across taxonomic groups. By examining the connectivity of the majority of the spiny and slipper lobster species in the Hawaiian archipelago, my data will provide insights into whether taxonomy correlates with connectivity patterns, or if taxonomic group has no relation to connectivity, as has been shown in Hawaiian opihi (Bird et al 2007). The lobster species I will investigate are all in the same superfamily (Palinuroidea), but separate into two different families: Palinuridae (the spiny lobsters) and Scyllaridae (the slipper lobsters). Furthermore, the slipper lobsters fall into two subfamilies: Ibacinae (P. antarcticus) and Arctidinae (S. squammosus, S. haanii, A. regalis). Within the Arctidinae, there are two lobster species in the genus Scyllarides (S. squammosus and S. haanii) and one in the genus Arctides (A. regalis). Although there are not large replicates in any of the groupings, a comparison between their connectivity patterns may reveal certain taxonomic patterns of population relationships. The management implications of such efforts are clear: if generalizations can be drawn among any such groups, then even in the absence of specific data, an informed management response can be launched quickly as the need arises. In contrast, if no such generalizations can be made, and the results must be determined on a case-by-case basis for each species, then in the event of an unforeseen management requirement, the absence of data must result in an uninformed response from managers.

It is also worth noting that lobsters have some of the longest larval durations of any species. Conducting my work on this taxonomic group will provide invaluable insight into the difficult question of whether, and if so how larval duration affects connectivity of isolated areas. Prevailing theory suggests

that species with larval durations of this length should be panmictic across broad ranges (i.e. Shanks et al 2003, Siegel et al 2003). However, previous Panulirus sp. genetic studies have found some indications of local recruitment despite an 8-12 month larval duration (Johnson and Wernham 1999; Silberman and Walsh 1994). Additionally, P. marginatus is endemic to the Hawaiian Islands, while the other lobster species here are found throughout the West Pacific. A couple species (P. penicillatus, A. regalis) cross the East Pacific Barrier, and one is even found as far away as the Western Atlantic (P. antarcticus). By eventually surveying these species on a broader scale, I hope to understand the importance of other locations beyond the Hawaiian Archipelago as propagule donors and contributors to the unique biodiversity of Hawaii in general, and the North Western Hawaiian Islands Marine National Monument in particular. I will also be able to determine if the Monument serves as a stepping-stone for species to cross the East Pacific Barrier by comparing genetic signatures for lobsters in the Monument with those on either side of the Barrier.

Additionally, I will assess temporal and spatial variability in genetic diversity for P. marginatus and S. squammosus at four banks in the NWHI: Maro Reef, Necker Island, Laysan Island, and Gardener Pinnacles. I already have samples from these banks from the last two years from the NMFS/PFSC lobster tagging cruise. Several recent studies have shown a direct link between demographic population structure and genetic population structure (Selkoe et al. 2006, Toonen & Grosberg in review). Further, Frankham and colleagues have recently shown that across 170 of the most threatened species on the planet, 77% of them had significantly lower genetic diversity than that found in the closest-related non-threatened species (Spielman et al. 2004, Frankham 2005b). Both subsequent empirical and theoretical work suggests that reductions in genetic diversity and the resultant inbreeding can speed the mean time to extinction by up to 78% over species without reduced genetic diversity (Frankham 2005a). This work is especially important for P. marginatus and S. squammosus at Maro Reef and Necker island given that they experienced heavy commercial fishing pressure in a relatively short period of time (20 years). Laysan Island and Gardener Pinnacles, which were not as heavily fished, provide valuable control groups for this work. Continued sampling of these banks will help managers evaluate whether these stocks are recovering after the closure of the NWHI lobster fishery, or if the fishery caused rapid losses of genetic diversity that may factor in to a slowed recovery of populations or a failure to recover at all.

Finally, although extraction of lobsters from the Monument will likely never occur again, a recent threat analysis for the NWHIMNM indicated that the top six threats to the biological integrity of the Monument ecosystem have a global or Pacific-wide, rather than a local, origin (Selkoe et al. in press). Thus, it is of critical importance to resource managers to know how populations will respond to these impacts and how such impacts can be mitigated. Connectivity is of direct relevance to this question: on one end of the connectivity extreme, individual locations within the Monument are isolated, self-seeding (closed) populations with reduced genetic diversity, increased individual risk of extinction, few to no immigrants from other locations, and little hope of recovery from such local disasters within the lifetime of a manager. On the other end of the extreme, the reefs are fully integrated (open) populations that exchange immigrants continuously which therefore stand or fall as an entire chain, and the local effects at any given location should have no long-term ramifications at any other location. My genetic survey will provide an answer as to where along this continuum various locations within the Monument lie. The numerous valuable contributions of my work are reinforced by the fact that the data I require to attain these results involves virtually non-extractive and non-lethal methods that will entail only an insignificant impact on the Monument's cultural, historical and natural resources.

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?
Cultural:

The Northwestern Hawaiian Islands have always been considered a sacred place in Hawaiian tradition. Much of the knowledge we have of this place comes from oral and written histories, genealogies, and archaeological sites. For example, Nihoa and Mokumanamana Islands have some of the most numerous and important archaeological sites in the archipelago, with 88 and 52 recorded cultural sites respectively. These sites include ceremonial,

residential, and agricultural areas (NMFS lobster EIS 2007). My research can be conducted so as to avoid all sites of cultural significance since they are all terrestrial sites, and we can conduct all sampling efforts without going on land.

Historic:
Throughout the NWHI, there are over 60 known ship losses and 67 identified aircraft crashes, with the earliest dating back to 1818. Many of these maritime heritage sites are both nationally and historically important, and a variety of mandates have been established to ensure their protection and preservation (NWHI lobster EIS 2007). I can avoid disturbing these historical sites during my research by ensuring that none of the diving or trapping will be conducted near any of these locations. If by chance anyone in my science team comes across an aircraft or vessel underwater that has not been documented, I can make sure they avoid the site, but make note of its GPS coordinates for historians to explore the cite in the future.

Natural Resources and Ecological Integrity:

I think history provides the best evidence that this research can be conducted with adequate safeguards for the natural resource and ecological integrity of the Monument, as well as the historical and cultural resources. Both trapping and diving have proven to be a non-destructive and virtually non-lethal and non-extractive way of sampling. Over 20 lobster community survey cruises have been conducted on board the Oscar Elton Sette, and 10 NMFS/PIFSC lobster tagging cruises have been conducted on various commercial vessels. Diving cruises have been utilized since 2004 to collect tissue samples from a variety of organisms including two lobster species, and in all cases, research has been conducted with the most adequate safeguards for the integrity and resources of the Monument. Impact on Monument resources has been negligible in the past. Most of the researchers who will be aiding in my sample collection are veterans of working in the Monument and fully aware of the best practices needed to protect this valuable resource, and they have consistently demonstrated that they can and will implement these best practices. Please see question 7h for further details on how my specific methods will minimize impacts to the ecological integrity of the Monument.

b. How will the activity be conducted in a manner compatible with the management direction of this proclamation, considering the extent to which the conduct of the activity may diminish or enhance Monument cultural, natural and historic resources, qualities, and ecological integrity, any indirect, secondary, or cumulative effects of the activity, and the duration of such effects?
My research will not diminish the Monument's cultural, natural and historic resources, qualities, and ecological integrity in any detectable manner. There are no foreseeable indirect, secondary, or cumulative effects of my research, other than to provide Monument staff with quantitative information regarding the connectivity of lobster populations in the Hawaiian archipelago, and all of the additional benefits that this knowledge will provide.

Cultural:

None of the research I propose will be conducted on land, and no participants will embark on the terrestrial area of any island, so we will not disturb any native Hawaiian cultural resource. However, I am aware that in ancient Hawaiian culture, lobsters were often utilized in place of pigs as a sacrifice to the gods, and they may still have cultural significance for native Hawaiians. I welcome and look forward to a consultation with the Office of Hawaiian Affairs (OHA) to more fully understand the role of lobsters in Hawaiian culture, and to learn how to conduct my research with the utmost respect for cultural sensitivities. I also hope that the knowledge I obtain from my research may benefit the OHA and enhance Hawaiian cultural identity. In addition, all scientists participating in my research will receive a Native Hawaiian cultural briefing before entering the monument.

Historic: In order to preserve the historical artifacts contained in the Monument, I will not conduct any trapping or diving activities near any potentially historical sites while carrying out my research activities. Any historical site happened upon accidentally will be recorded, but not disturbed.

Natural Resources and Ecological Integrity: I will continue to conduct my research in a virtually non-lethal and non-extractive manner. As a conservation biologist whose research goal is to determine how to best manage and conserve the biological diversity in the ocean, minimizing the impact of my research is always a top priority. I will follow the example of those who have conducted trapping and diving research in the Monument in the past and adhere to the widely regarded best practices for ensuring the ecological integrity of the Monument resources. The methods I intend to use

to conduct my research and how they specifically fit this objective are detailed in question 7h.

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.
This study purports to examine how populations of spiny and slipper lobster species are connected throughout the whole Hawaiian Island chain; therefore, it would be impossible to conduct the experiment solely outside of the Monument. This research would be uninformative without tissue samples from lobster populations within the Monument considering the extent of the Hawaiian archipelago that is located in Monument waters.

d. How does the end value of the activity outweigh its adverse impacts on Monument cultural, natural and historic resources, qualities, and ecological integrity?
As discussed in questions 7a, 7b, and 7h, there are no foreseeable adverse impacts of my research on the Monument's cultural, natural, or historic resources, qualities, and ecololological integrity. All efforts will be made to minimize the number of animals that are sacrificed for this research, and any organisms that are removed will cause a minimum impact on the ecosystem. The large majority of lobsters will only have a dactyl removed to fulfill my collection needs, and lobsters commonly release their appendages as a predatory defense. All legs will grow back within one year. Trapping and diving locations will be selected to avoid culturally and historically significant habitats. Trapping will occur in areas of low relief and minimal coral cover in order to avoid damaging any habitat, as well as to catch more lobsters in a shorter time frame. Diving will be conducted by professionals who follow best practices to prevent damaging or disturbing any of the ecological resources other than targeted collection species.

Given the virtually non-existent impacts of my research on the Monument's resources, the end value of my study certainly outweighs its impacts. My results will identify stock structure and estimate population connectivity of lobster species throughout the Hawaiian archpelago. Researchers and managers widely agree that knowledge of population connectivity is essential to establish effective marine protected areas, but many of the factors affecting connectivity among populations are not well understood. My study will help in deciphering some of the most critical gaps in the knowledge required to design beneficial reserve networks: the most effective size and spacing of marine reserves, whether and how larval duration correlates with

dispersal distance, and if connectivity patterns can be generalized across taxa or if each species of interest will have to be investigated individually. For more specific details on how my project will accomplish these goals, please refer to section 6: purpose/need/scope.

My research also directly addresses management needs of the Monument. P. marginatus is an endemic species in Hawaii that historically supported a valuable marine fishery in the state (along with S. squammosus and S. haanii). Ongoing lobster tagging studies targetting P. marginatus and S. squammosus at four banks in the NWHI (Laysan, Gardener, Maro, Necker) have yet to find any evidence of adult individuals of either species moving between banks over multiple years (O'Malley et al unpublished data). This suggests that any exchange of individuals between island populations in the Monument occurs during the larval phase. My research will determine whether each of these banks is self-sustaining or exchanging larval individuals with other banks in the Northwest or Main Hawaiian Islands. New analysis techniques will enable me to determine both a relative magnitude and direction of this exchange wherever it occurs. This information will be beneficial for all lobster species in the study. Managers will understand whether the establishment of the Monument will allow the rejuvination of lobster stocks in the NWHI, and if it may also enhance lobster populations in the main islands. While extraction of lobsters is no longer permitted in the NWHI, there is no way to prevent natural or broad scale anthropogenic effects that may damage lobster stocks (i.e. oil spills or disease outbreaks) from occurring. Knowledge of whether and how lobster populations on various banks are connected will enable managers to rapidly implement the most beneficial contingency plan in case one of these events occurs. Please see my pupose/need/scope section to more fully understand the end products of my work. The benefits greatly outweigh the imperceptible impact my work will have on the Monument.

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

Panulirus marginatus and Scyllarides squammosus have been on Dr. Rob Toonen's collection permit for the past two years, during which two collection cruises each year have taken place. In this time, the required number of lobsters of either species has not been collected by the HIMB team at any bank in the NWHI. Research suggests that if there was still a commercial fishery, the allowable bank-specific sustainable catch levels would be in excess

of 10,000 lobsters (slipper and spiny lobsters combined), which indicates that the abundances of these species is certainly high enough to obtain the required samples at each bank (<3% of the predicted allowable catch) (NMFS unpublished data 2007). Though these species have not been the specific focus of any HIMB collection cruise, the low sample number collected over two years demonstrates that the requested duration of the activity is certainly not excessive. It also speaks to the need of employing different methods than previously used to help obtain the required number of samples in the least amount of time necessary. The NMFS/PIFSC lobster tagging cruise has successfully provided the requested number of samples from four banks (Necker Island, Maro Reef, Gardener Pinnacles, Laysan Island) in the last two years. Prior to that, the Oscar Elton Sette lobster monitoring cruise also provided the requested number of samples from Maro Reef and Necker Island. These results suggest that the requested number of samples can be obtained within one summer using the trapping method. Once all requested sample numbers have been obtained, all collection of these species will be ceased to prevent the excessive duration of the proposed activity.

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

Prior to my graduate studies at UH Manoa, I conducted field research on the California spiny lobster (Panulirus interruptus) for the Wrigley Institute of Environmental Studies at the University of Southern California. During the two years I worked on this project, I spent >1500 hours working on small boats, trapping, handling, measuring, and taking tissue samples from spiny lobsters using commercial fishing traps. This experience taught me how to most effectively collect information on spiny lobsters while minimizing the impact to both the lobsters and their environment. In addition, this past summer, I worked on the annual NWHI lobster cruise (OES #07-05) to gain a fuller appreciation of how this type of research is conducted on larger vessels in this particularly sensitive environmental area. My proposed research activity has been funded by the Environmental Protection Agency's (EPA) Science to Achieve Results (STAR) grant, one of the top graduate fellowships in the biology field. Only a small percentage of proposals for this grant are funded annually, demonstrating both the value of the project and my ability as a researcher to achieve the stated results. Finally, the established reputation my advisor Dr. Rob Toonen as one of the top researchers in the field of invertebrate population genetics affirms the likelihood of the project's success.

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.
As a graduate student in the Toonen-Bowen lab, I have access to all of the lab equipment to successfully complete the analysis for this project. There are adequate finances in the lab from the NWHIMNM-HIMB grant to complete the laboratory portion of my work. My salary and any additional research needs are currently funded through my EPA STAR grant (see above). Additionally, I currently have funding from both the Watson T. Yoshimoto Foundation and the Charles H. and Margaret B. Edmondson Research Foundation to complete my collections in the Main Hawaiian Islands.

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.
I intend to use both mitochondrial and microsatellite DNA markers to understand the connectivity of lobster populations throughout the Hawaiian Archipelago. The use of genetic sampling is widely regarded as the cheapest and most robust way in which to answer questions of connectivity on these scales. My data will allow resource managers to define the relevant units of management, and determine the scale of connectivity among geographically isolated portions of the Monument. This information forms the basis of science-based resource management in virtually all marine reserves for which it is available, and is essential for an adaptive management strategy to be implemented in the Monument.

I have optimized my sample sizes to minimize impact while maximizing the information that will be gained from my research. Both empirical and theoretical studies examining reliability of genetic estimators of population structure suggest that sample sizes of 50 to 100 individuals are required to minimize bias and error variance from the data (e.g., Ruzzante 1998). I have weighed this bias against power analyses for the markers I will use (Ryman & Jorde 2001, Ryman et al. 2006) to ensure that I have the statistical power to detect population differentiation among locations if it occurs, and determined that for microsatellite data a sample size of 50 individuals per species per atoll is the statistically appropriate sample size to address connectivity issues within the Monument. Because of the diverse behaviors of the lobsters I intend to study, I am proposing two methods for collecting these individuals for my study: trapping and SCUBA diving.

Using either methodology, I will sample individuals in a non-lethal manner whenever possible. Except in the rare instance when a lobster is caught with a three-prong spear, only an appendage will be removed from each lobster to fulfill my sampling needs. Removal of a walking leg is standard sampling technique for crustacean genetic surveys because survival is uniformally high after such treatment. Studies of porcelain crabs and spiny lobsters in California showed a 100% survival rate among individuals kept in the lab for up to a year after being sampled this way (Toonen unpublished data; Iacchei unpublished data). In natural populations, lobsters commonly self-amputate an entire walking leg as an anti-predatory defense. These appendages typically grow back during the next molt, which generally occurs at least once yearly (Mykles 2001). This virtually non-lethal and non-extractive sampling methodology provides me with a sufficient amount of tissue to answer the questions I pose.

Below I discuss the appropriateness of each of the two methods I propose to use to obtain these samples, while addressing their impact (or lack thereof) on the Monument's cultural, historical, and ecological resources.

SCUBA diving:

Diving cruises have been utilized since 2004 to collect tissue samples from a variety of organisms including two lobster species, and in all cases, research has been conducted with the most adequate safeguards for the integrity and resources of the Monument.

In areas of high lobster abundance, diving can be a rapid and efficient method for obtaining samples. This is especially true for P. penicillatus, and A. regalis, which have not been shown to be easily attracted to or captured with commercial traps. If possible, whole lobsters will be captured underwater, brought to the surface for data collection, and safely released on the ocean bottom. If this proves difficult for a variety of reasons, a lobster appendage will be sampled underwater, without the additional data. This will affect some aspects of the study, but not the most important ones.

At all banks, we will avoid conducting dive operations near any historical or cultural area of significance. If an undocumented historical resource is found, we will move our dive operations to a new location and immediately notify monument staff of its location.

Divers have all undergone extensive training and have thousands of combined hours of experience underwater, enabling us to conduct research on particular organisms with negligible disturbance to the surrounding habit. Impacts on Monument resources have been negligible in the past. Most of the researchers who will be aiding in my sample collection are veterans of working in the Monument and fully aware of the best practices required to prevent damaging or disturbing any of the ecological resources other than targeted collection species. They have consistently demonstrated that they can and will implement these best practices.

Trapping:

The use of commercial lobster traps is widely regarded as one of the most efficient ways to sample lobster populations while minimizing the impact to the integrity of the ecosystem. For example, for 20 years commercial traps have been used to monitor lobster populations in the NWHI, and over 74,000 P. marginatus and S. squammosus (combined) were caught during that time period (Moffitt et al 2006). Traps will be most highly effective in catching these two species, but there have also been areas where S. haanii and P. antarcticus were captured in large numbers.

The traps are also highly target-species specific. Bycatch associated with lobster trapping is fairly low, making up only 27% of the trap catch in plastic traps from 1986 to 2003. Abundances of non-target species in traps have not shown any declines in the 20 year study, suggesting that traps are not having a negative impact on these species (Moffitt et al 2006).

I will trap in bedrock areas of low relief or sand bottom, both to minimize damage to habitat and maximize lobster catch in a short period of time. These habitats have extremely low abundance of coral, so trapping causes negligible damage to the habitat and biota where it is conducted. All areas of historical and/or cultural significance will be avoided during trapping operations.

Lobsters will be handled with extreme care to ensure their successful release and survival after a tissue sample is taken. Lobsters will be processed in a shaded area on the boat, and will be held in a tank with circulating seawater

both prior to and after data collection. Lobsters will be released directly back onto the seafloor using a specially designed release cage to ensure their survival after capture. These methods have proven effective over multiple years during both NMFS/PFSC lobster population monitoring and tagging cruises.

One major concern is that traps are occasionally lost and then continue to fish on the bottom in perpetuity, killing lobsters and other species unnecessarily. All possible efforts will be made to retrieve all traps that are set. Trap losses on previous cruises have been minimal. In addition, studies have shown that "ghost fishing" by lost gear does not occur in the NWHI (Parrish and Kazama 1992).

A final concern is that the invasive algae (Hypnea musciformis) may be spread via lobster traps. To ensure that this does not happen, none of the traps used in my study will have been used in the main Hawaiian Islands where Hypnea has been found. As an additional precaution, all traps will be visually inspected and all algae will be removed before transiting to another bank. Traps will be left in direct sunlight while transiting from one bank to another in order to allow desication to destroy any algae propagules that may remain. These methods have been used previously on NMFS/PFSC lobster tagging cruises with no documented spread of Hypnea.

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, all vessels from which research will be conducted have been outfitted with the approved mobile transceiver unit.

j. Demonstrate that there are no other factors that would make the issuance of a permit for the activity inappropriate.
There are no other factors that would make the issuance of a permit for the activity inappropriate. All proposed methods have been previously utilized in the Monument with no detectable adverse effects on any of the monument resources. The previous NWHI cruises involving personnel in the Toonen/Bowen lab group, the NMFS/PIFSC lobster tagging cruises led by Joseph O'Malley, and the annual NMFS/PIFSC lobster monitoring cruise have all been conducted with absolute scientific and ethical integrity by all individuals listed on this request. This compliance and respect for the Monument's resources will continue this year.

8. Procedures/Methods:

The objective of my research is to survey 6 different lobster species across the Hawaiian archipelago to assess the level of connectivity among these isolated reef habitats. By investigating population relationships of both fished and unfished species, archipelago endemics and species that range across the Pacific Ocean, I intend to gain correlative insights into what drives lobster larval connectivity patterns in the Hawaiian archipelago. To achieve this goal, I will collect a small tissue sample from 50 different individuals of each lobster species listed in question 9. My virtually non-destructive sampling method maximizes the benefit of this research while minimizing the impact on Monument resources.

Collection

All species of lobsters will be hand-caught while scuba diving, or obtained using standard lobster traps. If necessary, lobsters may be speared with a Hawaiian three-prong spear while scuba diving. Carapace length and sex will be recorded for each lobster collected, and a leg will be taken for a tissue sample. GPS of sampling locations will be taken, and lobsters will then be returned to the wild if possible.

Scuba Diving:

Dive sites will be accessed using small vessels that are launched from the Hi'ialakai. Lobsters will captured by hand while diving in shallow reef-rubble habitat. Lobsters will be kept in a catch bag until the end of the dive. Upon surfacing, the carapace length and sex of each lobster will be recorded, and a small piece of the lobster's leg will be taken as a tissue sample. Once all lobsters have been sampled, they will be placed in a specially designed release cage and released on the seafloor, and a GPS point of the sample location will be recorded. This prevents the lobsters from being attacked by ulua or shark species. If a lobster cannot be obtained by hand capture, a lobster leg or a piece of the lobster's antenna will be taken while underwater. In this case, the tissue sample will be placed in an individual vial and the GPS location of the sample will be recorded. As a last resort, a three prong spear may be used to assist in capturing the lobster. This may cause the destructive sampling of a small number of lobsters.

Lobster trapping on board the NMFS/PIFSC lobster tagging cruise:

Sites of high lobster abundance and past tagging will be visited at Necker Island, Gardner Pinnacles, Maro Reef and Laysan Island. Specific trapping locations will be provided before the tagging vessel enters the monument. Five strings of 20 traps, each of which will be baited with 1 kg of Pacific chub mackerel Scomber japonicus, will be deployed each day and will soak overnight. The deck of the vessel is covered to keep lobsters out of direct sunlight to reduce the effects of exposure to light. Prior to data collection all lobsters are held in a tote with circulating water/spray. After data collection all lobsters are held in a specially designed release cage in a covered container of circulating sea water. All lobsters will be released on the seafloor in the immediate vicinity of capture via the release cage (cage is lowered to the seafloor, inverted and opened). Upon release the position will be recorded.

Lobster trapping on board the Hi'ialakai small boats:

A small number of traps may be deployed from the small boats and retrieved after an overnight soak. Traps will be deployed singly (not in strings) in rock rubble, pavement, or sand habitat between 5 and 40 meters depth. Traps will be baited with 1 kilogram of Pacific chub mackerel (Scomber japonicus). Traps will be hand-pulled each day, re-baited and re-set as long as the Hi'ialakai remains at the same bank. Lobsters will remain in the trap until the data is collected and the trap will be covered to limit exposure to the sunlight. As the data is collected for each lobster, it will be returned overboard. If this attracts too much attention from ulua and sharks as has happened while trapping from larger boats, a release cage will be used to release all lobsters at once on the seafloor to ensure their survival.

Lobster trapping on board the Hi'ialakai itself and/or the Oscar Elton Sette:

If possible, traps will be deployed directly from the Hi'ialakai/Sette. One string of 20 - 50 traps, each baited with 1kg of Pacific chub mackerel (Scomber japonicus) will fish overnight and be pulled the following day. Traps will be deployed in areas of previously identified high lobster abundance with

low bottom relief and consisting of rock rubble, pavement or sand substrate between 5 and 65 meters depth. Prior to data collection all lobsters will be held in a tote with circulating water/spray. After data collection all lobsters are held in a specially designed release cage in a covered container of circulating sea water. All lobsters will be released on the seafloor in the immediate vicinity of capture via the release cage (cage is lowered to the seafloor, inverted and opened).

Request of Monument staff:

I greatly appreciate the offer of assistance from Monument staff to collect samples in association with my proposed activity in my presence or absence. Although I have no expectation of assistance, I would be extremely grateful to any staff member that would be willing to collect samples for me while (s)he is in the Monument. Samples could be collected during any diving trip in the Monument if there is time after the assigned diving tasks with little additional time investment. I would be happy to provide the vials, EtOH, bone shears, and other tools necessary for sample collection and preservation. I would also be happy to work out a compensation plan on an individual basis for anyone that would be willing to help me collect tissue samples.

Sample processing and genetic markers

Tissue samples from each lobster will be stored at room temperature in >70% ethanol (EtOH) until processed. DNA will be extracted from each sample using either a DNeasy kit (Qiagen, Vaencia, CA, USA) or a Puregene DNA Isolation Kit (Gentra, Minneapolis, MN, USA) according to the manufacturer's protocols.

Both mitochondrial DNA (control region) and ideally 10 microsatellite markers will be used to assess population relationships. Microsatellites are regions of non-coding, repetitive DNA sequences that are ideal for population genetics studies because of their high mutation rates. This results in differences in microsatellite fragment lengths among even closely related populations (Jarne and Lagoda 1996).

A 506 base pair region of the control region of the mitochondria will provide direct sequence data to corroborate the microsatellite analysis. Utilizing both microsatellites and mitochondrial DNA will provide a robust picture of population relationships.

Once all markers are optimized, each DNA sample will be amplified at all of the microsatellite loci and at the mitochondrial control region (mtCRL) using the Polymerase Chain Reaction. Microsatellite products will be genotyped by an automated DNA sequencer at the Hawaii Institute of Marine Biology (ABI 3100). Samples will be sequenced at the mtCRL region using an ABI XL3730 capillary sequencer at the ASGPB sequencing facility in Snyder Hall, University of Hawaii, Manoa.

Analysis

If enough microsatellite loci can be developed and Fst > 0.01 as in a previous spiny lobster study (Perez-Enriquez 2001), I will utilize statistical assignment tests to fully understand population relationships. These tests use a "DNA fingerprint" to statistically trace each individual to the population with the gene frequencies most likely to produce that individual (Wilson & Rannala 2003). This technique should reveal specific source and sink populations. In addition, I will examine spatial gene structure using a hierarchical Analysis of Molecular Variance (AMOVA) using Arlequin v. 3.1 (Excoffier et al 2005). I will use IBDS to test if there is a positive correlation between genetic differentiation between populations and their distance from one another (Jensen et al 2005). With the sequence data, I will create haplotype networks using TCS v. 1.21 in order to infer evolutionary relationships between specific genotypes (Clement et al 2000). I will also be able to detect the any directionality in gene flow using Migrate 2.3 (Beerli, 2004), which will provide an indication of the source-sink dynamics of these metapopulations.

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:
Please see attached species list

Scientific name:
Please see attached species list

& size of specimens:
50 samples per species, per bank for all species listed in attached sheet and for all banks checked off above. All sizes of lobsters that can be obtained through trapping and/or diving will be considered for samples, since organisms will be released alive after a small dactyl is removed, and lobsters readily replace lost dactyls within one molt cycle (Mykles 2001).

Collection location:
Sampling will take place between 5 and 65 meters depth at all banks checked off in question 5a. Due to the nature of the research, I will not know the precise locations where samples will be collected at each bank until sampling commences.

☐ Whole Organism ☒ Partial Organism

9b. What will be done with the specimens after the project has ended?
Whenever possible organisms will be returned to the wild once a small tissue sample has been collected. All tissue samples will remain stored in ethanol at HIMB for any future research needs so duplicate samples do not need to be collected.

9c. Will the organisms be kept alive after collection? ☐ Yes ☒ No

• General site/location for collections:

• Is it an open or closed system? ☐ Open ☐ Closed

• Is there an outfall? ☐ Yes ☐ No

• Will these organisms be housed with other organisms? If so, what are the other organisms?

• Will organisms be released?

10. If applicable, how will the collected samples or specimens be transported out of the Monument?

Tissue samples will be transported out of the Monument in 5 ml plastic vials that each contain the tissue from one individual lobster and 95% EtOH. The vials will be contained in boxes on the vessel that was utilized to collect the samples (i.e. Hi'ialakai). Samples will remain at room temperature throughout the duration of the cruise.

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

I will keep an electronic database of all samples that have been collected at each bank for each sampling trip. Required sample numbers will be updated through out each trip and other collecting vessels will be notified to prevent over-sampling at any one bank. My database will be combined with the overall HIMB tissue database and tissue bank when it is created to prevent re-sampling in the future (except at the banks where temporal studies are being conducted). All leftover tissue samples will be made available in storage at HIMB for other researchers to utilize in future studies.

In addition, collaborative efforts have been established to utilize one individual lobster for multiple studies. At Maro Reef, Necker Island, Laysan Island and Gardener Pinnacles, 25 of the tissue samples I require for Panulirus marginatus and 25 of the tissue samples I require for Scyllarides squammosus will be provided by lobsters that have been removed from those banks for nutritional studies conducted by Joseph O'Malley on the NMFS/PIFSC lobster tagging cruise. This increases the usage of tissue samples that have already been collected, and prevents the removal of legs from additional lobsters.

This research is part of a larger project that includes the Main Hawaiian Islands. A permit for collecting these species of lobsters in the Main Hawaiian Islands using similar methods has recently been submitted to the Hawaii Division of Aquatic Resources. I will keep Monument staff informed of the progress of this permit.

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

The varying habitat of many of the lobster species necessitates a multi-method approach to capturing them. I will utilize both lobster traps and SCUBA diving gear to collect samples:

Trapping:

Lobsters will be fished using standard commercial shellfish traps made by Fathoms Plus® that were used by the Hawaiian commercial lobster fleet. All traps will be baited with 1 kilogram of Pacific chub mackerel (Scomber

japonicus) and will soak overnight and be pulled each day. Traps will be fished in one of two ways depending on the vessel/consent of the vessel captain: some traps will be fished from the small boats that are launched from the Hi'ialakai for diving operations. These traps will be set as single traps in 5 to 40 meters of water and will be pulled each day by hand. Other traps will be fished in strings of 20 - 50 directly from the Hi'ialakai or Oscar Elton Sette. Traps will be pulled using a winch and davit. On the NMFS/PIFSC lobster tagging cruise, 5 strings of 20 traps will be set daily. Please see Joe O'Malley's permit application for more information on equipment used on the NMFS/PIFSC lobster tagging cruise.

Scuba Diving:
Lobsters will also be fished by hand while scuba diving, and may be captured with a Hawaiian three-prong spear if necessary. For this type of sampling, standard SCUBA equipment will be necessary: regulator, buoyancy compensator, air tank, wetsuit, fins, mask, gloves, catch bag, etc.

Sampling:
All lobsters will be measured using a digital calipers, and a dactyl will be removed using medical bone shears. Tissue samples will be stored in 5ml plastic vials containing 95% EtOH.

All gear will be decontaminated using standard best practices between each atoll along the cruise.

A detailed list of all gear to be brought on each cruise will be provided at least 30 days prior to the vessel entering the monument.

12b. List all Hazardous Materials you propose to take to and use within the Monument:
I will use 95% ethanol (EtOH), MSDS attached, as a tissue preservative for all of my samples. EtOH is commonly sold for human consumption, so it should not pose a significant health or environmental risk. For each cruise, the EtOH will enter and exit the Monument on the vessel that it is contained on. For the two Hi'ialakai cruises, I will share EtOH with other researchers that need this chemical for preservation purposes. The amount brought on board will depend on all researchers' needs. My personal needs will also depend on how many and which banks each cruise will go to and the number of samples that are needed from that bank. Monument staff will be informed of the amount

of EtOH to be brought on each cruise prior to entering the Monument. The vessel to be used for the NMFS/PIFSC lobster tagging cruise has not been identified yet. EtOH will be stored in sealed containers in the safest possible manner on that cruise. EtOH will be contained in sealed containers inside a flame-proof cabinet on the Hi'ialakai and Oscar Elton Sette.

13. Describe any fixed installations and instrumentation proposed to be set in the Monument:
I do not intend to install any fixed instrumentation in the Monument.

14. Provide a time line for sample analysis, data analysis, write-up and publication of information:
January - June 2008: Optimization of primers for Panulirus marginatus and Panulirus penicillatus.
June - September 2008: Collection of lobster samples from the NWHI
September - December 2008: Extraction of spiny lobster samples collected during the summer, and initial analysis with chosen microsatellite and mitochondrial DNA primers.
January - June 2009: Finish data analysis for Panulirus marginatus (assuming all samples are collected in 2008. Continue work on Panulirus penicillatus samples (I don't think these sample collections will be completed in one year). Development of microsatellite DNA primers for slipper lobster species.
June - September 2009: Further sample collection if needed/possible
September 2009 - December 2009: Publication of Panulirus marginatus results. Finish analysis of Panulirus penicillatus results. Continue extractions and microsatellite development for slipper lobsters
January 2010 - December 2010: Publish Panulirus penicillatus results. Continue microsatellite development for slipper lobster species and begin preliminary analyses of slipper lobster data.
2011 - Continue analysis and begin publication of slipper lobster data.

Note: I have already initiated the design and optimization of primers for Panulirus marginatus, and initial results suggest that some primers (including some already designed for Panulirus argus) may work in multiple species of Panulirids. Therefore, I am fairly confident that the marker development for Panulirus marginatus and Panulirus penicillatus can proceed at the same time in a fairly rapid manor. The current bottleneck is obtaining the samples to analyze with these markers.

However, no population level genetic research has been conducted on slipper lobster species anywhere in the world, and only three mitochondrial DNA markers have been developed for a species of slipper lobster. These three markers (18s, 16s, 28s) are highly conserved across taxa and will not be useful for the questions I am asking. The lack of previous work on this family of invertebrates makes the time that it will take to develop markers for these species difficult to determine. I am confident it can be achieved successfully, but cannot give specific dates.

As always in science, these time frames are estimates, and data analysis may be finished earlier or later than predicted. In order to provide Monument staff with the most up to date information, I plan to provide biannual updates of my research progress. This frequent communication will allow Monument staff to be privy to information before it is published so they can make timely management decisions if necessary. It will also allow them to have a more direct impact on my research direction (e.g. if they need information on one species more quickly than another, I can tailor my research to prioritize the specific species they are interested in).

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

I have not yet published anything directly related to the proposed project because I do not yet have the samples to conduct the needed analysis. Below I list publications on a congeneric species of lobster that demonstrate my ability to conduct the proposed research and publish my results in a timely fashion:

Iacchei, M.J., P.W. Robinson, and K.A. Miller. 2005. Direct impacts of commercial and recreational fishing on spiny lobster populations at Santa Catalina Island, California, United States. New Zealand Journal of Marine and Freshwater Research, 39: 1201–1214.

Iacchei, M.J., P.W. Robinson, J. O'Malley, K.A. Miller. Can lobsters sense reserve borders?: adult Panulirus interruptus movement and implications for marine reserve design. In prep.

Ben-Horin, T., M. Iacchei, K.A. Selkoe, R.J. Toonen. Characterization of eight polymorphic microsatellite loci for the California spiny lobster, Panulirus Interruptus. Molecular Ecology Resources, in prep.

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.

02-01-08

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