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

<u>SUBMITTAL VIA ELECTRONIC MAIL IS PREFERRED BUT NOT REQUIRED. FOR ADDITIONAL SUBMITTAL INSTRUCTIONS, SEE THE LAST PAGE.</u>

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: Annette W. Tagawa
Affiliation: State of Hawaii, Department of Land & Natural Resources, Division of Aquatic Resources

Permit Category: Research
Proposed Activity Dates: May 1st-Nov 1st 2012
Proposed Method of Entry (Vessel/Plane): Vessel and Plane
Proposed Locations: Midway and Kure

Estimated number of individuals (including Applicant) to be covered under this permit: 8
Estimated number of days in the Monument: 14

Description of proposed activities: (complete these sentences):

a.) The proposed activity would…

Develop a better biological understanding of the moi aka Pacific threadfin, Polydactylus sexfilis, in relation to 1) the genetic structure of these two species between the Monument and the Main Hawaiian Islands, 2) contributing to a broader understanding of Monument ecology and population dynamics of this species and 3) Determine whether stock enhancement activities within the Main Hawaiian Islands has altered the genetic population structure by utilizing Monument wild stocks as a comparison.

b.) To accomplish this activity we would ….

Collect small, non-lethal fin clip tissue samples from 50 P. sexfilis for DNA analysis to determine if these two populations possess significant genetic structure between the Main Hawaiian Islands and the Monument, in reference to parallel research activities conducted in the MHI.

c.) This activity would help the Monument by …

Providing Monument managers with baseline information on the population structure of P. sexfilis within the Monument and how it relates to the population structure of P. sexfilis within the Main Hawaiian Islands. This information will provide understanding of the Monument's open system and how these resources geographically relate to the moi resources in the MHI. All of this information will enhance management decisions for these culturally and ecologically important species for both the MHI and the Monument.

Other information or background:

The family Polynemidae consists of 38+ species known from the areas of French Polynesia to Mauritius, Seychelles, Kenya and many islands of the northern Indian Ocean. Only one species, Polydactylus sexfilis, is listed as occurring in the Hawaiian Archipelago. Known as the Pacific Threadfin or moi, this species is a very desirable food fish locally accounting for its high market value. It was so highly prized in Hawaiian culture that centuries ago, moi were strictly reserved for the ali'i or Hawaiian royalty. Juveniles (moi'lii) and adults inhabit the surf zone, reef faces and sandy areas
across the Hawaiian Islands. Due to the ecological and economic importance of P. sexfilis, concerns have been raised over population reductions around the Hawaiian Islands due to overfishing as well as varying indirect anthropogenic stresses (i.e., eutrophication, habitat change, the proliferation of invasive species, etc.). This has spurred efforts toward developing a better biological understanding of these species.

Most of the research occurring for the moi species in Hawaii focuses on the culturing aspects of this species due to its high desirability as a food fish. DAR will be implementing a statewide angler-based tagging program focusing on the moi species to gain a better understanding on the condition of the moi resources in the Main Hawaiian Islands. Our focus with the tag and release data will be to monitor size class distributions, abundance distribution and migration patterns statewide.

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Tagawa, Annette, W.

Title: Aquatic Biologist IV

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

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: N/A

3. Affiliation (institution/agency/organization directly related to the proposed project):
State of Hawaii
Department of Land & Natural Resources
Division of Aquatic Resources

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):

Paul Murakawa, Field Technician
Scott Santos, Researcher, Field Technician
Rodney Young, Field Technician
Neal Hazama, Field Technician
Vincent Goo, Field Technician
Tracy Tanaka, Field Technician
Thomas Iwai Jr., Field Technician
Clayward Tam, Field Technician

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:
Fishing and Fin Clip Collection
Fish capture, tagging and fin clip collection will be shore-based from Midway Atoll where collection activities will take place from the pier and various shoreline locations where access is allowed. Collection activities will also be conducted from small boat vessels if there are any small boats available for charter.

On Kure Atoll collection activities will also be shore-based where collection activities will take place from various shoreline locations. If we are able to secure a vessel for near shore based collection activities, then we will be conducting collection activities from small boat vessels as well. In the event that we need to spend a few days at Kure Atoll, camping equipment will be used to provide temporary lodging during our stay.

All fish capture, tagging and fin clip collections will occur in the shallow waters around the Monument locations listed above.

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 purpose of this research is to provide managers with data on the genetic structure of P. sexfilis populations within Monument waters to evaluate population structures and their relationship to stock enhanced population structures within the MHI. We have the following specific goals and objectives:

1) Non-lethal collection of small samples of fin clips from 50 P. sexfilis for DNA analysis. Collection of fin clip samples will be from Midway and Kure Atolls. All fish will be collected with small mesh thrownet and/or hook and line and subsequently released after collection of fin clip material. We have empirical data from over 10 years of sampling and collecting moi from the shoreline that demonstrates all released fish exhibit vigorous behavior and resume active swimming activities immediately upon release.

2) Determine if there is any distinct genetic structure of P. sexfilis between the Monument and the MHI.

3) Obtain baseline information on the population dynamics and evolutionary history of P. sexfilis within the Monument.

Due to the ecological and economical importance of P. sexfilis, concerns have been raised over maintaining sustainable populations around all the Hawaiian Islands. All informational findings will serve to develop a better biological understanding of these species that will contribute toward management strategies for P. sexfilis within the Monument and the MHI.

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?
The activity will be conducted with adequate safeguards for the resources and ecological integrity of the Monument. We would be using non-lethal catch and release techniques (refer to Procedures/Methods in question #8) for the moi that have minimal impact on the resources and ecological integrity of the Monument. We would be collecting a maximum amount of 50 P. sexfilis fin clip samples that will be collected from Midway and Kure Atolls for DNA analysis. Samples will be transferred to DAR contracted researcher, Dr. Scott Santos, for genetic analysis. This project is a first effort to obtain biological and life history information for the moi resources within the Monument and the MHI for the purposes of conservation management strategies.

Research activities will be conducted outside the vicinity of any known historical locations and objects. In the event we find any historical objects or resources, we will seek to follow the recommended guidelines of any protocol that is available from the Monument Office.

We will attempt to conduct all research activities outside the vicinity of any known cultural resources at all times. We will seek appropriate guidance from Hawaiian Cultural Practitioner and DLNR Chairperson William J. Aila Jr. on how to mitigate potential impacts associated with our research activities

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?
The proposed activities will have minimal impact on the resources of the region. The collection of fin clips consists of non-lethal catch and release techniques. The size of each fin clip tissue sample to be collected would be no larger than 1 cm X 1 cm per fish. The moi will be able to easily regenerate this fin tissue within a short amount of time. We have empirical data from over 10 years of sampling and collecting moi from the shoreline with small mesh thrownets as well as hook and line which demonstrates that catch and release activities have not interfered with growth and behavior patterns over time.

The proposed activities are in direct support of the Monument Management Plan's priority management need 3.1 – Understanding and Interpreting the NWHI (through action plan 3.1.1 – Marine Conservation Science). This action plan specifies to "measure connectivity and genetic diversity of key species to enhance management decisions." Activities to support marine conservation science, including connectivity and genetic diversity surveys such as those to be carried out by the Applicant, are also addressed in the Monument Management Plan Environmental Assessment (December 2008) which resulted in FONSI. This EA summarizes that understanding the genetic diversity of species groups and how these populations change could be helpful to forecast, prepare for and mediate potential threats to populations within the Monument (PMNM MMP Vol. 2, p.171). Identification of genetic diversity and connectivity of reef fishes, such as those proposed, would enhance this understanding.

Research activities will be conducted outside the vicinity of any known historical locations and objects. In the event we find any historical objects or resources, we will seek to follow the recommended guidelines of any protocol that is available from the Monument Office.

We will attempt to conduct all research activities outside the vicinity of any known cultural resources at all times. We will seek appropriate guidance from a Hawaiian Cultural Practitioner on how to mitigate potential impacts associated with our research activities.

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 activities in the Monument. We are addressing questions regarding the biological and evolutionary life history aspects of one of the most desirable food fishes known within the Hawaiian culture which would be incomplete without Monument resources included as part of the investigation within the Hawaiian archipelago. This

information is directly relevant to the management integrity of both Monument and MHI resources, hence the study must be carried out within the Monument.

d. How does the end value of the activity outweigh its adverse impacts on Monument cultural, natural and historic resources, qualities, and ecological integrity?
The management value of data produced by our research activities outweighs the minor, transient impacts on Monument resources. The methods and procedures that we are proposing will have minimal impact on Monument resources, qualities and ecological integrity. No fish will be removed from the Monument. Fin clip tissues will be regenerated by the moi within a short period of time. We have empirical data from over 10 years of sampling and collecting moi from the shoreline with small mesh thrownets as well as hook and line which demonstrates that catch and release activities have not interfered with growth and behavior patterns over time. All information gained would be integrated with other projects to further understand the dynamics of Monument resources as a whole.

e. Explain how the duration of the activity is no longer than necessary to achieve its stated purpose.
We estimate that given no unforeseen circumstances, a total of 7 days of fieldwork would be a reasonable amount of time to complete the Monument portion of the fieldwork. This is necessary to obtain the desired sample size of fin clips needed to ensure the integrity of the genetic analysis. Once the DNA is identified, processed, and analyzed to ensure data integrity, there is no further need to collect any additional material within the Monument.

f. Provide information demonstrating that you are qualified to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.
The principal investigator has over 30 years of experience as an employee of the Division of Aquatic Resources and has spent over 20 years of this time as an Aquatic Biologist (see attached CV for details) and is well qualified to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.

All personnel included in this permit application have a combined total of over 100 years of extensive experience working in the marine science field as Aquatic Biologists and Fishery Technicians and as such are highly trained and well qualified to conduct the research activities requested in this permit.

Dr. Scott Santos has extensive experience in population genetics, resource conservation, genomic evolution and symbiosis biology in freshwater and marine aquatic organisms around the world including Hawaii.

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.
This research is part of one of many projects within the Division of Aquatic Resources that receives financial support through grants under the Federal Aid in Sport Fish Restoration Program. These resources are adequate to conduct and complete the proposed activities and mitigate any potential impacts resulting from its conduct.

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 methods and procedures that we are proposing are ideal for achieving our goals with minimal impacts to Monument resources, qualities and ecological integrity. No fish, animals, or organisms will be removed from the Monument as a result of our research. Fin clip tissues will be regenerated by the moi within a short period of time. We have empirical data from the DAR Moi Tagging Program as well as sampling activities from its previous Moi Stock Enhancement Program which both demonstrate that catch and release activities have not interfered with growth and behavior patterns for over 10 years time.

i. Has your vessel has been outfitted with a mobile transceiver unit approved by OLE and complies with the requirements of Presidential Proclamation 8031?
No vessels have been secured yet for the collection activities from small boat vessels. However, if we are able to obtain the use of any vessels, we assure that those vessels will be outfitted and meet all vessel requirements before entry into Monument waters.

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. We will seek appropriate guidance on how to mitigate potential cultural impacts associated with our research. However, if through the review process other factors arise, I and my team are more than happy to work with Agency personnel to mitigate any and all potential concerns.

8. Procedures/Methods:
Transportation by aircraft is recommended for Midway where activities will be conducted along the shoreline areas which will require some terrestrial access. Transportation to Kure, if available, will be by vessel, preferably if any of the NOAA research vessels are scheduled for destination to Midway and then to Kure. If there are no NOAA vessels scheduled for destination to Kure from Midway, then research activities will be limited and restricted to Midway.

Collection of P. sexfilis will be conducted from shore using a small mesh thrownet and/or pole, line and barbless hook with either an artificial lure or bait. "Artificial lure" refers to a fishing lure constructed by humans as an imitation of or substitute for natural bait or fish forage. "Artificial lure" includes, but is not limited to, artificial flies, spinners, spoons, poppers, plugs, jigs and plastic, rubber or other artificial imitations of natural bait. “Bait” refers to food used to entice fish as prey. Items to be used for bait include shrimp and squid.

There will be fishing teams consisting of two individuals with a maximum of two teams deployed at any one time. One team will utilize the small mesh thrownet while the other team will be utilizing 2 rod and reels. A maximum of one thrownet and 2 rod and reels will be used at any one time. However the primary method of collection will be with a small mesh thrownet measuring 8 ft. in radius with 1/4 inch square mesh. A second thrownet measuring 8 ft. in radius

with 3/8 inch square mesh will be brought along with 2 additional rod and reels that will stored away to serve as backups. The barbless hooks we will be using are the Maruto brand (or equivalent brand) type circle hook in the 16 to 22 size range which will target moi in the 6 to 24 inch fork length range. Duration of each proposed fishing attempt will be varied due to the unpredictable nature of the animal as well as other variables such as weather conditions, tide conditions, time of day, etc.

If the fish are captured by thrownet, several at a time will be contained in a bucket with a portable aerator until they can be processed one at a time. If the fish are captured by rod and reel, they will be processed immediately since the rod and reel can only capture one fish at a time. Each fish will be placed onto a cushioned surface pad onshore. The fish will be immobilized by a wet cloth being placed over the eyes to calm it down. Field technicians will then proceed to clip a small piece of the dorsal fin, measure the fork length and gently release the fish. This entire process can be completed within 5 minutes or less. All used hooks and scissors will be rinsed in a 10% bleach solution to sterilize equipment used for collection and processing of the next fish.

Bycatch potential will be minimum to none with regard to using a small mesh thrownet due to the very selective target of the specific species (moi) by sight. In addition, the small mesh size of the net is gentler on the fish and ensures that the fish will not be damaged as larger mesh sizes can cause injuries by ensnaring fish by their gill covers. In the event that a non-targeted species does enter the catch, it will be immediately retrieved from the net and released.

The pole, line and barbless hooks used as a secondary method of collection will be selected specifically for the size range of the targeted moi species. The barbless hook assures minimal physical impact along with ease of releasing the fish with minimal trauma as we have experienced with this sampling technique for over the last 10+ years. The use of artificial lures limits the amount of bycatch even further since these lures depend on sight rather than scent to attract fish. In addition, any fish smaller than or extremely larger than the targeted size range of fish will not be hooked or caught by the barbless hook. Furthermore, the type of baits used and how it is presented are specific to fishing techniques and methods used to target a specific type of fish which would be the moi in this case. In the event a non-targeted species is hooked, it will be released immediately upon retrieval.

All fin clips will be stored in 95% ethanol into small individually marked plastic vials. After being marked and catalogued, all samples will be transferred to DAR contracted researcher, Dr. Scott Santos, for processing and genetic analysis:

Task Description and Methodology
Two (2) major objectives will be pursued in testing the above hypothesis. These are:

1) Development and test specific DNA genetic markers in the form of microsatellites for the moi (P. sexfilis). Microsatellite loci, a category of DNA genetic markers commonly employed in human forensics, will be developed for this species in order to test the proposed hypothesis. The bulk of the proposal's budget and timeline are dedicated towards Obj. #1 since microsatellites

are typically specific for just the species they were developed for, thus necessitating independent allotments of effort. Identification of potential microsatellite loci for PCR primer development will be done following one (1) standard round of di-, tri- and tetra-nucleotide repeat enrichment followed by sequencing via either Roche 454 pyrosequencing or Illumina pair-end reads. Loci identification and primer design will be done via custom computation pipelines we have previously constructed at Auburn University. Primer pairs will be developed to these candidate loci and tested using PCR for their utility in addressing the proposed hypothesis.

2) Generation of microsatellite data for moi (P. sexfilis) sampled in the Main Hawaiian Islands as well as the NWHI. DNA samples from moi in the Main Hawaiian Islands have already been collected are currently archived in our freezer at the Aneunue Fisheries Research Center. These will be sent to the laboratory at Auburn University for DNA extraction. To this, novel individuals of moi (P. sexfilis) sampled in the NWHI will be included into the analyses. All samples of this species will be examined with the appropriate species-specific microsatellites developed under Obj #1. The final data set from this species will be analyzed using scientifically accepted methodologies and statistical approaches towards testing the proposed hypothesis.

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:
1)Moi, Pacific Threadfin

Scientific name:
1) Polydactylus sexfilis

& size of specimens:
1) 50 P. sexfilis fin clips @ no larger than 1cm X 1cm per specimen

Collection location:
Within waters surrounding Midway Atoll and Kure Atoll. Ideally we are targeting for 25 samples from Midway and 25 samples from Kure. In the event that logistics does not allow for transport to Kure, then all 50 samples would be collected from Midway.

☐ Whole Organism ☒ Partial Organism

9b. What will be done with the specimens after the project has ended?
The fin clips will be used in its entirety for the proposed research. In the event that there are any samples or tissue leftover, the samples will be stored and archived in the freezer in the Santos Laboratory at Auburn University for future retrieval.

Deliverables
Deliverables will include a final detailed report incorporating all aspects of the proposed work. From this, it is envisioned that one scientifically peer-reviewed publication (similar to that mentioned above) will result from this work, leading to a broader dissemination of the data generated under this proposal.

9c. Will the organisms be kept alive after collection? ☐ **Yes** ☐ **No**
N/A - no collection of live animals.

• General site/location for collections:
See 9a. Collection location.

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

• Is there an outfall? ☐ Yes ☐ No
N/A

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

• Will organisms be released?
All fish will be released.

10. If applicable, how will the collected samples or specimens be transported out of the Monument?
Fin clips will be stored in 95% ethanol into small individually marked plastic vials and transported back to Honolulu. In Honolulu, specimens will be recorded and catalogued into a database file. Specimens will then be packed and shipped to Auburn University in Alabama for laboratory processing and analysis. Fin clips will be stored in 95% ethanol into small individually marked plastic vials and transported back to Honolulu. In Honolulu, specimens will be recorded and catalogued into a database file. Specimens will then be packed and shipped to Auburn University in Alabama for laboratory processing and analysis. Specimens will be received by:

Dr. Scott R. Santos
Department of Biological Sciences,
Cell and Molecular Biosciences Peak Program &
Molette Biology Laboratory for Environmental and Climate Change Studies
Auburn University

[redacted]

11. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research:
We are requesting the minimal number of fin clip samples needed to confirm any findings brought up during the genetic analysis. Once this is confirmed, there will be no need to collect any further specimens/samples from the Monument. At present, we are only aware of one project that looked at cultured and wild stock moi fin clips on Oahu. Other than this we are unaware of any other duplicate research efforts regarding genetic work with P. sexfilis. Our research is more widespread than the one previously mentioned focusing on the status of the moi resources statewide. In addition, we have also met with Dr. Randall Kosaki, the Monument's Research Deputy to ensure that this project complements current research efforts and does not duplicate any other research efforts. If any concerns arise, we will be more than happy to address potential collaborative efforts.

12a. List all specialized gear and materials to be used in this activity:
Materials include 2 small mesh thrownets (one of which will used for back up), 4 rods and reels (two of which will be used for back ups), barbless hooks, cushioned pads, scissors, tweezers, dart tags, tag applicators, and small plastic vials.

12b. List all Hazardous Materials you propose to take to and use within the Monument:
1) 95% ethanol solution to preservative fin clip tissues for DNA analysis
2) 10% bleach solution for sterilizing scissors and tag applicators between usage

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:
Timeline
2012-2013 – Development of microsatellites for moi (Polydactylus sexfilis)

2012 - Collection of data from moi (Polydactylus sexfilis) individuals sampled in the NWHI

2012-2014 – Collection of data from moi (Polydactylus sexfilis) individuals sampled in the Main Hawaiian Islands

2014-2015 – Analyses of data from moi (Polydactylus sexfilis) in the high Hawaiian Islands and NWHI; preparation of final report and manuscripts.

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

Iwai, G. 2010. Identification of MitochodrialATP-synthase 8/6 gene DNA Sequence and Single Nucleotide Polymorphisms (SNPs) in Wild and Cultured Pacific Threadfin (Polydactylus sexfilis). M.S. Thesis in Animal Sciences. University of Hawaii College of Tropical Animal Sciences & Human Nutrition. 28 pp.1.

Wang, H., T. Iwai Jr., B. Zhao, C-S, Lee, and J. Yang. 2010. Identification of microsatellite DNA markers for Pacific threadfin (Polydactylus sexfilis) parentage assignment. J. World Aquaculture Society, 41:640-647.

Iwai, T. Jr., N. Hazama, R. Young, P. Murakawa, G. Cambra, J. Leonard, M. Apana, V. Goo, and T. Tanaka. 2007. Moi (Polydactylus sexfilis) stock enhancement: Developing a sustainable moi fisheries in Hawaii. Department of Land & Natural Resources, Division of Aquatic Resources, Anuenue Fisheries Research Center, 1039 Sand Island Parkway, Honolulu, HI 96819. (Presentation at the Hawaiian Conservation Conference on July 25-27, 2007)

Iwai, T. Jr., N. Hazama, R. Young, and J. Leonard. 2007. The impact of recreational fishing within the Waikiki-Diamond Head Fisheries Management Area, Oahu, Hawaii. Department of Land & Natural Resources, Division of Aquatic Resources, Anuenue Fisheries Research Center, 1039 Sand Island Parkway, Honolulu, HI 96819. (Presentation at the Hawaiian Conservation Conference on July 25-27, 2007)

Iwai, T. Jr., P. Banis, N. Hazama, R. Young, C. Campos, S. Alexander, and J. Leonard. 2001. Preliminary investigation into the enhancement of the Pacific threadfin, Moi (Polydactylus sexfilis) population within the Waikiki-Diamond Head Marine Reserve, Oahu, Hawaii. Department of Land & Natural Resources, Division of Aquatic Resources, Anuenue Fisheries Research Center, 1039 Sand Island Parkway, Honolulu, HI 96819. 20 pp.

Ostrowski, A.C., T. Iwai Jr., S. Monahan, S. Unger, D. Dagdagan, P. Murakawa, A. Schivell, and C. Pigao. 1996. Nursery production technology for Pacific threadfin (Polydactylus sexfilis). Aquaculture, 139:19-29.

Leber, K., S. Arce, R. Nishimoto, and T. Iwai Jr. 1995. Developing Marine Stock Enhancement Technology in Hawaii: Progress and Application. Invited Symposium Paper for Special Session: Marine Stock Enhancement: A Global Outlook. World Aquaculture '95. San Diego, California, USA.

Weese, D.A., Y. Fujita, M. Hidaka, S.R. Santos. The long and short of it: Genetic variation and population structure of the anchialine atyid shrimp Caridina rubella on Miyako-jima, Japan. Journal of Crustacean Biology. in press.

Kocot, K.M., J.T. Cannon, C. Todt, M.R. Citarella, A.B. Kohn, A.Meyer, S.R. Santos, C. Schander, L.L. Moroz, B. Lieb, K.M. Halanych. 2011. Phylogenomics reveals deep molluscan relationships. Nature. 477:452-457.

Stephens, J.D., S.R. Santos, D.R. Folkerts. 2011. Genetic differentiation, structure, and a transition zone among populations of the pitcher plant moth Exyra semicrocea: Implications for conservation. PLoS One. 6:e22658.

Roopin, M., D.J. Thornhill, S.R. Santos, N.E. Chadwick. 2011. Ammonia flux, physiological parameters, and Symbiodinium diversity in the anemonefish symbiosis on Red Sea coral reefs. Symbiosis. 53:63-74.

Santos, S.R., D.A. Weese. 2011. Invited Contribution: Rocks and Clocks: Linking geologic history and rates of genetic differentiation in anchialine organisms. Hydrobiologia. 677:54-63.

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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.

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Papahānaumokuākea Marine National Monument Permit Coordinator
6600 Kalaniana'ole Hwy. # 300
Honolulu, HI 96825
FAX: (808) 397-2662

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☐ Electronic and Hard Copy of Application with Signature
☐ Statement of information you wish to be kept confidential
☐ Material Safety Data Sheets for Hazardous Materials