

**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](mailto: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:** Simon Yung Wa SIN

**Affiliation:** Department of Organismic and Evolutionary Biology,  
Museum of Comparative Zoology,  
Harvard University

**Permit Category:** Research

**Proposed Activity Dates:** January 2016 - February 2016

**Proposed Method of Entry (Vessel/Plane):** Plane

**Proposed Locations:** Land-based habitat on Midway atoll

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

3

**Estimated number of days in the Monument:** 15

**Description of proposed activities:** (complete these sentences):

a.) The proposed activity would...

We will use the Black-footed albatross (*Phoebastria nigripes*) and Laysan albatross (*P. immutabilis*) as a model for investigating mate choice based on a diverse gene family called major histocompatibility complex (MHC) genes, which plays a crucial role in the adaptive immune system.

b.) To accomplish this activity we would ....

We would collect a small amount of blood from mated pairs of black-footed and Laysan albatross and from their chicks for genomic DNA extraction.

c.) This activity would help the Monument by ...

In addition to filling in an important gap in our understanding of albatross biology, this project would help the Monument determine proper translocation strategies for these two protected seabird species. With increasing risk of sea level rise and colony loss due to climate change, it is important to understand how these species select their mates based on their genetic constitution,

and how possessing different genotypes (e.g. MHC genes, genome-wide diversity) affects the offspring survival. The findings from this project would provide valuable information that would aid in decisions about which pairs to eventually translocate and how the birds maximize their fitness through optimal mate choice.

**Other information or background:**

## **Section A - Applicant Information**

### **1. Applicant**

Name (last, first, middle initial): SIN, Simon Yung Wa

Title: Postdoctoral fellow, Museum of Comparative Zoology, Harvard University

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

**2. Mailing address (street/P.O. box, city, state, country, zip):** Department of Organismic and Evolutionary Biology, [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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

**3. Affiliation (institution/agency/organization directly related to the proposed project):**  
Department of Organismic and Evolutionary Biology,  
Museum of Comparative Zoology,  
Harvard University

**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):**

Scott V. Edwards, Alexander Agassiz Professor of Zoology, Harvard University,  
[REDACTED]

Simon Yung Wa Sin, Postdoctoral fellow, Harvard University,  
[REDACTED]

Field Assistant, TBD – will complete in the Compliance Logistics Information Sheet

**Section B: Project Information**

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

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

**Ocean Based**

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

Location Description:

**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:***

We propose to use the Black-footed albatross (*Phoebastria nigripes*) and Laysan albatross (*P. immutabilis*) as models for investigating mate choice based on a diverse gene family called the major histocompatibility complex (MHC), which plays a crucial role in the adaptive immune system and response to pathogens. In some species MHC genes are a target by which individuals choose mates in order to optimize the fitness of their offspring, potentially mediated by olfactory cues. Mating with genetically dissimilar mates will increase offspring heterozygosity, and, because pathogen recognition is mediated by the sequence of individual MHC alleles, MHC heterozygotes might have an advantage over homozygotes when populations are exposed to pathogens. The MHC might therefore provide the basis on which individuals discriminate mating partners in order to increase the fitness of their offspring in response to disease.

Both Black-footed albatross and Laysan albatross belong to the order Procellariiformes, which are a highly olfactory group of birds and are known to use odor in a range of behaviors, including homing and individual recognition. Because body odor is potentially affected by the MHC genes, the strong olfactory sense makes the albatrosses a good model for MHC-based mate choice study. Their mating system, life-history characteristics and reproductive biology also make them well suited for investigating mate choice. They are socially monogamous, long-lived species that mate for life, with very high mate fidelity. They have low fecundity - all species lay only one egg annually or biannually. Both parents pay a large cost in raising the chick, and the rate of extra-pair paternity in many procellariiform seabirds is low. These characteristics emphasize the need for choosing a mate that is able to provide so called "indirect" genetic benefits, making them likely candidates for MHC-based mating preferences. We will also compare MHC similarity between mated pairs with the patterns observed in random markers across the genome to attempt to identify potential patterns of inbreeding or inbreeding avoidance, and to distinguish MHC-specific effects from genome-wide effects.

The findings from this proposed project would provide valuable information for translocation project of these two protected seabird species, which are facing increasing risks due to rising sea levels and loss of breeding habitat on low islands. It would be important to understand how these species choose their mates based on genetic markers, and how possessing different MHC genotypes and genome-wide diversity affects offspring survival.

**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. The activities use non-destructive and non-lethal techniques. The amount of blood (100 uL) collected from each individual is insignificant for birds of such large body size.

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 research consists of sampling small amounts of blood, with insignificant impact on the animals. Care will be taken when handling birds to minimize stress.

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 practicable alternative to conducting the activities in the Monument. Midway island is an important breeding site for these two species, and a large proportion of black-footed albatross population breed at this atoll. Midway presents really the only viable colony from which to sample these birds, since other colonies in the Monument have become more inaccessible during the main part of the albatross breeding season (e.g., Tern Island/French Frigate Shoals). The colonies of these species in Japan are much smaller than those in the Monument. It is therefore imperative to obtain the genetic information proposed in this study. Previous samples from these species were collected from random birds, not from mated pairs.

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 genetic data and knowledge obtained after the study will be invaluable for helping guide eventual translocation strategies. Understanding how these birds choose mates and how they maximize genetic diversity in offspring is crucial to management of any

endangered species. This study will also provide some insight into the spatial distribution of MHC genotypes at the atoll and that knowledge might also guide translocation actions such as selection of animals to move.

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

A minimal amount of time will be spent on sample collection on each individual to minimised disturbance. The total time in the Monument is a function of the sample size required for both species. Our time in the Monument is designed to allow us to obtain a reasonable sample size for each species (150 birds per species).

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

Prof. Scott V. Edwards (included as additional persons to be covered by the permit) has extensive experience in conducting research in the Monument. He visited Tern Island in 1984 and 2002 and Midway island in 1994 to conduct research on seabirds and has extensive experience in taking blood samples from the Black-footed and Laysan albatrosses. His expertise would be very beneficial to the project and all personnel are trained by him. All personnel included in this permit application have extensive experience in the collection techniques used.

g. Provide information demonstrating that you have adequate financial resources available to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.

The applicants have been awarded funding from a Putnam Expedition Grant, Museum of Comparative Zoology, Harvard University, to cover the expenses of the principle investigator for this study (\$9280). Additional funds for Dr. Edwards will come from his Faculty Stipend fund through the Museum.

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 research consists of non-destructive and non-lethal sampling. Only small amounts of blood (100 uL) will be collected for each individual. The impact would be minimal on these two species of large-sized seabirds.

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

N/A

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 activities inappropriate.

### **8. Procedures/Methods:**

On Midway island, we will walk through the breeding colonies of Black-footed and Laysan albatross. We will identify mating pairs in the colony and sample blood from both individuals of each mating pair. If chicks are available at the time of colony visit, or if only one parent is available per pair, we will also sample blood from the chick. One researcher will hold the bird firmly on its back, either on the ground or on a support about 3 feet in height, and pull a pillow case over its head to keep it calm, while the other researcher will use a 22-gauge needle and syringe to take 100 uL of blood samples from the brachial vein, on the underside of the wrist. We will collect samples during the daytime. From a subset of birds (n=20) we will pluck two small feathers from the breast for potential work involving RNA, which is useful for helping to characterize MHC and other genes in the genome. Breast feathers will be stored non-cryogenically at room temperature, in a non-toxic solution called "RNA-later". If possible, the carcasses of already dead chicks (maximum n=5) may be salvaged for accession in to the collections of the Museum of Comparative Zoology.

**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:  
Black-footed albatross  
Laysan albatross

Scientific name:  
Phoebastria nigripes

**Phoebastria immutabilis**

# & size of specimens:

No more than 150 individuals will be sampled for each species. 100 uL of blood will be sampled for each individual.

No more than five skins of the carcasses of recently deceased chicks will be collected.

No more than 20 individuals will be sampled for feathers for each species.

Collection location:

Samples will be collected on the Midway island.

Whole Organism  Partial Organism

**9b. What will be done with the specimens after the project has ended?**

Blood and feather samples will be stored in freezers in Scott Edwards' laboratory at Harvard for genetic analysis. Carcasses will be deposited in the ornithology collection, Museum of Comparative Zoology, Harvard University as permitted under our Migratory Bird Treaty Act permit number MB105416-0.

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

N/A

• General site/location for collections:

See 9a. above.

• 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?

N/A

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

Blood samples will be stored in Queen's lysis buffer. Feather samples will be stored in RNAlater solution. Both solutions are non-toxic, non-cryogenic and completely safe for transport by air. Bird skin samples will be dried and prepared as voucher specimens. All samples will be transported by aircraft.

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

To the best of our knowledge, the proposed project is unique and does not duplicate any other current initiatives. Samples will be available to interested researchers as permitted by the Museum's USDA transport and import permits.

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

Blood samples will be collected by needles and syringes. Micro-centrifuge tubes will be used to store blood samples with Queen's lysis buffer. 15ml centrifuge tubes will be used to store feather samples with RNAlater solution. Dissection kit and stuffing materials will be used to process the skin samples.

**12b. List all Hazardous Materials you propose to take to and use within the Monument:**  
None.

**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:**

After fieldwork, the DNA extraction and analysis will take place from Mar 2016 - Aug 2016. Data analysis will be performed from Aug 2016 - Oct 2016. The proposed study will be written up and submitted to scientific journal by the end of 2016.

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

Walsh, H. E. and Edwards, S. V. (2005). Conservation genetics and Pacific fisheries bycatch: mitochondrial differentiation and population assignment in black-footed albatrosses (*Phoebastria nigripes*). *Conservation Genetics* 6:289-295.

Dierickx, E. G., Shultz, A. J., Sato, F., Hiraoka, T. and Edwards, S. V. (2015). Morphological and genomic comparisons of Hawaiian and Japanese black-footed albatrosses *Phoebastria nigripes* using double digest RADseq: implications for conservation. *Evolutionary Applications* 8: 662-678.

With knowledge of the penalties for false or incomplete statements, as provided by 18 U.S.C. 1001, and for perjury, as provided by 18 U.S.C. 1621, I hereby certify to the best of my abilities under penalty of perjury of that the information I have provided on this application form is true and correct. I agree that the Co-Trustees may post this application in its entirety on the Internet. I understand that the Co-Trustees will consider deleting all information that I have identified as "confidential" prior to posting the application.

Signature  Date 31 August 2015

**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