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: Myra Finkelstein
Affiliation: University of California Santa Cruz

Permit Category: Research
Proposed Activity Dates: October 2008 to June 2011
Proposed Method of Entry (Vessel/Plane): Plane
Proposed Locations: Midway Atoll National Wildlife Refuge

Estimated number of individuals (including Applicant) to be covered under this permit:
5
Estimated number of days in the Monument: Total number of days ~420 (combined): Research technician 1: ~4 months per year on Midway Atoll during years 1-3 and ~2 weeks during year four. We would like this person to spend a proportion of their time (~50-75%) volunteering for the refuge while they are on island during years 1-3. Research technician 2: ~2 weeks per year during years 1-3 on Midway Atoll.

Description of proposed activities: (complete these sentences):

a.) The proposed activity would…
assess the relationship between contaminant exposure (e.g., PCBs, DDTs, mercury), indicators of health effects (hematology, immune, and endocrine function, egg shell thickness), and demography (survival, breeding probability, hatching/breeding success) in black-footed albatross in order to understand how contaminant exposure affects long-term population survival.

b.) To accomplish this activity we would ….
1) monitor a plot of ~100 black-footed albatross on Midway Atoll for survival and probability of breeding (the percent of banded birds that return to breed), hatching/breeding success (the number of eggs that hatch, the number of chicks that survive to fledge); 2) for a subset of these birds we will collect blood samples (2-5 mLs) to evaluate contaminant levels (PCBs, DDTs, mercury) and indicators of health effects (hematology, immune, and endocrine function, egg shell thickness); 3) construct population models to investigate the effects of contaminant exposure on the long-term population viability of black-footed albatross.

c.) This activity would help the Monument by …
providing important information on the long-term population-level effects of contaminant exposure in black-footed albatross, whose second largest breeding colony is located in the Monument (Midway Atoll) and is currently under review for possible listing under the US Endangered Species Act. The US Fish and Wildlife Service's 90-day finding on the listing petition (USFWS 2007) cited contaminants as one of the main threats that may warrant listing, yet there is no information on how contaminant exposure impacts black-footed albatross population health.

Other information or background:

Background:
Black-footed and Laysan albatrosses, which forage in the North Pacific, have the highest organochlorine concentrations of any albatross species studied (Guruge et al. 2001). Several studies have also described 2 to 5 times higher organochlorine concentrations in black-footed albatrosses compared to Laysan albatrosses (Jones et al. 1996, Finkelstein et al. 2006), which also bred on Midway Atoll. Finkelstein et al. (2006) recently suggested that the higher organochlorine and mercury body burdens in black-footed albatross are primarily due to regional differences in North Pacific foraging patterns and that the most probable route of contaminant exposure was contamination of food sources, a finding supported by Jones et al. (1996). In addition to the differences between species, Finkelstein et al. (2006) found that PCBs and DDE may be increasing in both albatross species, whose foraging ranges together encompass the entire North Pacific.

The black-footed albatross is currently under review for possible listing under the US Endangered Species Act, and the US Fish and Wildlife Service's 90-day finding on the listing petition (USFWS 2007) cited fisheries bycatch and contaminants as two of the main threats that may warrant listing. Exposure to contaminants such as those documented in black-footed and Laysan albatross (e.g., PCBs, DDTs, mercury) have been related to reproductive, immune, endocrine, and hematological disruption in many wild species (Fossi et al. 2001, Grasman & Fox 2001). Indeed, Finkelstein et al. (2007) recently determined organochlorine and mercury exposure are related to altered immune and hematological function in black-footed albatrosses. Despite these data, the effect of contaminant exposure on the long-term population viability of black-footed albatross, and indeed any pelagic seabird, remains unknown.

Rationale:
Midway Atoll National Wildlife Refuge is an ideal location to carry out the colony-based work for our project. M. Finkelstein conducted several years of field research that involved collecting blood samples from albatrosses on Midway (Finkelstein et al. 2003b, Finkelstein et al. 2006, Finkelstein et al. 2007), and thus is familiar with Midway's research facilities as well as the logistical constraints of working at this remote island location. S. Converse is also familiar with the field site as a member of a research team that designed the long-term population monitoring and analysis protocols for albatrosses on Midway Atoll and other locations in the Hawaiian Islands – protocols which have

been used by the US Fish and Wildlife Service over the past 3 years and will be used in the future.

Approach:
We will identify a sampling plot (~100 black-footed albatross) that does not interfere with ongoing monitoring efforts by USFWS and collect mark-recapture data, along with data on reproductive success, contaminant concentrations (e.g., DDT), and hematological parameters (hematocrit, WBC counts and differentials) every year over three breeding seasons. Mark-recapture data will be collected in a fourth season to obtain data adequate for estimating demographic rates (e.g., survival). On a subset (n=40) of monitored birds in the plot, we will evaluate immune, endocrine function and comprehensive contaminant (e.g., PCBs, DDTs, mercury) assessment, based on previously develop methods by M. Finkelstein (Finkelstein et al., 2003). Due to financial and logistical constraints, we can not conduct a comprehensive contaminant analysis and evaluate immune function for every bird monitored in the plot, but our previous work has demonstrated that a sampled population of n=26 black-footed albatross has a wide range of contaminant exposure (mercury = 3400-6400 ng/mL whole blood, PCBs = 77-460 ng/mL plasma, DDTs = 44-780 ng/mL plasma). Furthermore, past research on contaminant associations with immune function (Finkelstein et al., 2007) predicts that n=40 birds will provide sufficient statistical power to detect contaminant-associated effects if they exist.

We will then use demographic rates obtained through mark-recapture analyses (prediction of how many birds are alive based on re-sighting probabilities) on birds with known contaminant exposure as well as published fisheries bycatch estimates (Lewison & Crowder 2003, Véran et al. 2007) to build population-based models that allow a combined assessment of bycatch and contaminant exposure effects on black-footed albatross population growth and future stability.

Detailed methods of data collection and analysis:
1) Demographic parameters. Black-footed albatross mark-recapture sampling will consist of capturing breeding birds by hand in a defined sampling plot and marking birds with metal USGS and field-readable plastic bands. Subsequent samples will consist of reading field-readable bands. Albatross generally nest within 1 m of previous nests, but may move a small distance between years. In years 2-4, birds will again be sampled in the plot; also a buffer area around the plot will be searched and previously marked birds in these areas will be monitored, to avoid confounding mortality with movement outside the plot. Reproductive success (hatching and fledging success) will be evaluated through nest monitoring for every sampled bird.

Blood collection methods. Blood samples will be collected from banded adult black-footed albatrosses hand-caught from the monitored plot during the breeding season during years 1-3 of the proposed project. 2-5mLs of blood (as needed) will be collected from the cutaneous ulnar vein with a 21g Vacutainer winged collection kit attached to a pre-heparinized syringe. Following blood sampling, all birds will be immediately released. Whole blood will be processed (e.g., centrifuged for plasma collection) within 6 hours of collection according to established protocols.

Contaminant exposure.
Organic Analysis (e.g., PCBs, DDT, DDE). Plasma and egg/eggshell samples, collected and stored using established methods (Finkelstein et al. 2006) (~1mL), will be evaluated for organochlorines (PCBs, chlorinated pesticides) and PBDEs using gas chromatographic separation with electron capture and mass spectrometric detection (GC-ECD and GC-MS).

Mercury Analysis. All sample processing will be conducted under trace metal-clean HEPA filtered air (Class 100) laboratory conditions using clean techniques (Smith et al. 1992) and previously published methods (Finkelstein et al. 2003b). Samples will be analyzed using a Tekarn 2500 and 2600 cold vapor atomic fluorescence spectrometer.

Biological markers of contaminant exposure.
Hematology (hematocrit, WBC counts and differentials) values will be assessed to identify diseased individuals, provide important baseline data on the health status of each bird sampled, as well as examine relationships between hematology variables with both demography and contaminant exposure. Hematocrit will be determined by the microhematocrit method, and total WBC counts will be determined with the Unopette prepared dilutent (Becton Dickinson, Franklin Lakes, NJ) within 3 hours of sample collection. Thin blood smears will be prepared within 5 minutes of sample collection. Slides will be sent to a commercial diagnostic laboratory (ANTECH diagnostics, Irvine, CA) where they will be stained with Wright/Giemsa, evaluated for RBC morphology, presence of thrombocytes, hemoparasites, and a WBC differential will be preformed.

Egg shell thickness: We will, using accepted USFWS protocols, collect up to 30 abandoned/failed eggs per year in the monitored plot, buffer, and surrounding areas to measure egg shell thickness and DDE concentrations.

Immune Function - Mitogen-induced lymphocyte proliferation. Immune function, and specifically mitogen-induced lymphocyte proliferation, is recognized as a sensitive biological marker for contaminant exposure effects in wildlife (Keller et al. 2000, Finkelstein et al. 2003a) including black-footed albatross (Finkelstein et al. 2007). In order to evaluate lymphocyte proliferation, cryopreserved white blood cells will be thawed and evaluated for mitogen-induced T lymphocyte proliferation according to previously published methods (Finkelstein et al. 2003a, Finkelstein et al. 2007). Briefly, for mitogen-induced T lymphocyte proliferation, 100µl of 3.5x106 live lymphocytes/mL will be distributed per well in triplicate wells in 96 well plates with either concavalin A mitogen (Con A, Sigma, St. Louis, MO) (2.5 and 5 µg/well) or no mitogen (non-stimulated). Plates will be incubated for 48 hours (41˚C, 5% CO2) after which the 5-bromo-2'-deoxyuridine (BrdU, Boehringer Manneheim, Indianapolis, IN) labeling agent is added to each well and the plates incubated for an additional 22 hours. Cells will be harvested and BrdU label absorbance and measured on an ELISA plate reader (Bio-RAD model 3550-UV).

Endocrine Function. Xenoestrogens are compounds that have structural similarities to estrogen and are therefore capable of binding to estrogen receptors, potentially invoking a biological response through endocrine disruption (Witorsch 2002). Some well-known xenoestrogens are persistent organic pollutants such as DDTs and PCBs (Colborn et al. 1993).

A) Vitellogenin (VTG) Induction. VTG is a precursor protein for egg production that is synthesized in the liver of egg-producing vertebrates and circulates in the blood stream where it is taken up by oocytes to be cleaved into nutritional proteins for egg development. Male and juvenile oviparous animals have the genetic information to produce VTG, however, physiological levels of estrogen are normally only sufficient to stimulate VTG synthesis in egg-laying females. Upon exposure to estrogen, or estrogen-like compounds (xenoestrogens), VTG can be detectable in blood in male oviparous species, indicating endocrine disruption. VTG induction will be examined in plasma samples from male seabirds (determined through genetic gender identification) to assess if male albatrosses are experiencing contaminant-induced endocrine disruption.
B) Thyroid Hormone Levels. Altered plasma thyroid hormone levels have been shown to be correlated with organochlorine concentrations in Arctic glaucous gulls (Larus hyperboreus) (Verreault et al. 2004). Thyroid levels (T3 and T4) will be assessed according to the methods of (Verreault et al. 2004). Briefly, plasma samples (~1 mL) will be isolated from whole blood via centrifugation, transferred to cryogenic vials and stored in liquid nitrogen until analysis. Commercially available radioimmunoassays (Coat-A-Count; Diagnostic Products Corporation Inc., Los Angeles, CA, USA) will be used to determine the plasma concentration of total and free T3 and T4 levels.