

All monitoring, and research projects involving marine mammals and marine turtles must be approved by the SWR/PIR Regional NMFS Institutional Animal Care and Use Committee prior to the commencement of the project. Principal Investigators (PI) are to submit the completed Assurance of Animal Care Form (hereafter Assurance Form) to the SWR/PIR IACUC Office. Assurance forms need to be submitted for IACUC review 4 weeks prior to submitting a permit application.

Please submit the Completed Assurance Form as an electronic file in Microsoft Word to Marisa Trego, SWR/PIR IACUC Coordinator, (Marisa.Trego@noaa.gov). Please check to ensure we have received the document. **Remember that you must still sign the declaration page.** This may be done in person or you may print off the declaration page, sign it, and send it via regular mail or by fax (858-546-7003). A specific IACUC number will be assigned to the Assurance Form. If you are unclear as to what is required to complete the Assurance Form, please contact Siri Hakala (858-546-7166) or Marisa Trego (858-546-7066).

YOUR ASSURANCE FORM WILL NOT BE APPROVED UNTIL COMPLETE.

The Assurance Form will be valid for **3 years after approval** contingent upon the IACUC receiving annual reports and that methods have not changed. As stipulated in the Animal Welfare Act, the Assurance Form may be renewed annually by the PI for a maximum of 2 renewals. You will receive an annual review form from the SWR/PIR IACUC for 2 years and on the third anniversary of this approved Assurance Form you will be notified of its termination. At this time you will need to submit a new Assurance Form for review.

A. Administrative Data

Project Title:

Permit for Scientific Research and to enhance the survival of Central and Western Pacific cetacean species under the Endangered Species Act, and to enhance the recovery of Central and Western Pacific cetacean species under the Marine Mammal Protection Act

Department or Office:

Cetacean Research Program, Pacific Islands Fisheries Science Center

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Initial Submission

Renewal

or Modification

Funding Source: NMFS

Grant Title (if different from Project Title):

Anticipated Start Date: May 1, 2011 **Anticipated End Date:** 5 years from date of permit issuance

Location Where Animals Will Be Housed or Study Site(s): U.S. waters of the Pacific Islands Region (PIR), including the Hawaiian archipelago, Johnston Atoll, Kingman Reef and Palmyra Atoll, Baker and Howland Islands, Jarvis Island, American Samoa, Wake Island, Guam, and the Commonwealth of the Northern Mariana Islands, and surrounding international waters.

Permits: Identify all relevant permits (Federal, State and other) necessary to conduct this project. Provide permit type(s), permit number(s), and expiration date(s). Please indicate if a permit application is pending a decision.

Marine National Sanctuaries/Monuments are located in the Northwestern Hawaiian Islands (Papahānaumokuākea Marine National Monument), the main Hawaiian Islands (Hawaiian Islands Humpback Whale National Marine Sanctuary), American Samoa (Rose Atoll Marine National Monument and Fagatele Bay National Marine Sanctuary), the Line Islands Marine National

Monument, and Marianas Trench Marine National Monument. In the past, the Pacific Islands Fisheries Science Center (PIFSC) has obtained access permits to conduct research in the Northwestern Hawaiian Islands from the U.S. Fish and Wildlife Service, State of Hawaii Department of Land and Natural Resources, the Hawaiian Islands National Refuge, and the Papahānaumokuākea Marine National Monument. Additionally, we have obtained research permits from the Government of American Samoa, and from the U.S. Fish and Wildlife Service for research at Johnston Atoll, Palmyra Atoll, and the Commonwealth of the Northern Marianas. In the future, when a specific project is planned for work in any of these designated areas we will apply for an access permit. We currently hold a Special Use permit from US Fish and Wildlife to conduct research on cetaceans at Palmyra Atoll.

Permit Type	Permit Number	Expiration Date
Papahānaumokuākea Marine National Monument	Pending	
Special-Use Permit- Palmyra Atoll	12533-10016	Dec 31, 2010 (renewed annually)
CITES	Pending	

*The NMFS policy intends to comply with the **Animal Welfare Act (AWA)** - Title 7 of U.S. Code §2131 et. seq. and implementing regulations and adhere to the principles of the **U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (USGP)** and follow the guidelines in the **National Research Council Guide for the Care and Use of Laboratory Animals**.*

B. Justifications

In accordance with USGP #2, "Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society."

1. Research Goals:

- a. What are the scientific issues addressed by the research? Specifically, how will this research improve human and animal health or advance knowledge?

We are seeking a permit from the National Marine Fisheries Service to conduct cetacean studies. The ultimate purpose of the research activities and data collection and analysis conducted by the PIFSC are for the protection, management, and recovery of protected resources to ensure populations remain at sustainable and healthy levels and the most effective fishing regulations and international treaties are implemented. Much of the scientific effort is directed at improving our ability to detect trends in cetacean populations in order to best predict and prevent detrimental effects of natural or man-caused environmental changes (e.g., fishing, habitat disturbance, global warming) on these populations.

- b. What are the specific goals of the animal studies described in this protocol?

The research objectives are to determine the abundance, stock structure, distribution, movement patterns, and ecological relationships of cetaceans occurring in U.S. and international waters of the Pacific Islands Region (PIR). The studies are conducted through vessel surveys, aerial surveys, photo-identification (from vessels and small boats), biological sample collection, and tagging and telemetry studies. Cetacean abundance data will be used to set potential biological removal limits (PBRs) of allowable human-caused mortality under the Marine Mammal Protection Act (MMPA) and to monitor trends in abundance through time. Genetic and other analyses of biological

samples collected will be used to determine stock structure for the appropriate management of these species.

2. Explain why animal studies are preferred to **non-animal alternatives** in achieving these research goals.

Not Applicable

In accordance with the Animal Welfare Act – “...the principal investigator has provided written assurance that the activities do not unnecessarily duplicate previous experiments.”

3. Does this research **duplicate** previous experiments? YES NO
If YES, please explain why this duplication is necessary.
4. Do the animal procedures planned for this research involve only **simple field observation** with no impact on either the animals or their environment?
 YES NO

If **YES**, it is not necessary to complete the informational sections of this protocol form. Instead, fill answer the following:

- a. Use Appendix A to describe the study activities. Include all precautions to ensure no adverse impact on the study animals and their environment.
- b. Include species copies of any required permits.
- c. Sign this form under Section H
- d. Submit this package to the NMFS Regional IACUC Chair

If **NO**, the remainder of this form must be completed. Proceed to the next section.

In accordance with the USGP #3, “The animal selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results.”

5. List the **research species** (and stock) and describe why is the most appropriate species to use in these studies:

All of the species listed are those known to occur within the U.S. EEZ waters of Hawaii and the PIR. Because cetaceans have not been studied in a large portion of the PIR, stock status is often not known, and there may be additional cetacean species encountered and taken in the course

of these studies. Also, it is likely that stocks will be newly identified during this research effort. For several species, only Hawaiian stocks have been addressed to date in Stock Assessment Reports and for many of these stocks their status is unknown. For species known to occur within the EEZs of the PIR and surrounding high-seas waters, but for which there is not yet a SAR, we've indicated the stock as 'Range-wide'

Common name	Scientific name	Stocks
Rough-toothed dolphin	<i>Steno bredanensis</i>	Hawaii, American Samoa, Range-wide
Risso's dolphin	<i>Grampus griseus</i>	Hawaii, Range-wide
Common dolphin	<i>Delphinus delphis</i>	Range-wide
Common Bottlenose dolphin	<i>Tursiops truncatus</i>	Hawaii, Range-wide
Indo-Pacific Bottlenose dolphin	<i>Tursiops aduncus</i>	Range-wide
Pantropical spotted dolphin	<i>Stenella attenuata</i>	Hawaii, Range-wide
Spinner dolphin	<i>Stenella longirostris</i>	Hawaii, American Samoa, Range-wide
Striped dolphin	<i>Stenella coeruleoalba</i>	Hawaii, Range-wide
Fraser's dolphin	<i>Lagenodelphis hosei</i>	Hawaii, Range-wide
Melon-headed whale	<i>Peponocephala electra</i>	Hawaii, Range-wide
Pygmy killer whale	<i>Feresa attenuata</i>	Hawaii, Range-wide
False killer whale ¹	<i>Pseudorca crassidens</i>	Hawaii Pelagic, Hawaii Insular, Palmyra, American Samoa, Range-wide
Killer whale	<i>Orcinus orca</i>	Hawaii, Range-wide
Short-finned pilot whale	<i>Globicephala macrorhynchus</i>	Hawaii, Range-wide
Blainville's beaked whale	<i>Mesoplodon densirostris</i>	Hawaii, Range-wide
Cuvier's beaked whale	<i>Ziphius cavirostris</i>	Hawaii, Range-wide
Longman's beaked whale	<i>Indopacetus pacificus</i>	Hawaii, Range-wide
Unidentified beaked whale	<i>Mesoplodon sp.</i>	Range-wide
Pygmy sperm whale	<i>Kogia breviceps</i>	Hawaii, Range-wide
Dwarf sperm whale	<i>Kogia sima</i>	Hawaii, Range-wide
Sperm whale	<i>Physeter macrocephalus</i>	Hawaii, Range-wide
Blue whale	<i>Balaenoptera</i>	Hawaii, Range-wide

Fin whale	<i>musculus</i> <i>Balaenoptera</i> <i>physalus</i>	Hawaii, Range-wide
Sei whale	<i>Balaenoptera borealis</i>	Hawaii, Range-wide
Minke whale	<i>Balaenoptera</i> <i>acutorostrata</i>	Hawaii, Range-wide
Bryde's whale	<i>Balaenoptera edeni</i>	Hawaii, Range-wide
North Pacific right whale	<i>Eubalaena japonica</i>	Range-wide
Humpback whale	<i>Megaptera</i> <i>novaeangliae</i>	Central North Pacific, American Samoa, Range-wide

6. How many animals do you plan to use for the protocol? Please provide a justification for the numbers of animals used (e.g., statistical power, survey, etc). Complete the following table below to define the numbers(s) of animal(s) to be used in each **category and type procedure(s)**. All information must be consistent with the project plan and MMPA/ESA permit application. Use the following animal welfare categories:

Category (adapted from AWAR):

- B:** applies only to animals held captive in non research status (display, rehabilitation, brood stock, holding).
- C:** applies to little or momentary pain or discomfort e.g. blood sampling with a needle and syringe, morphometric measurements, lavage, suction-cup tagging, etc
- D:** applies to potential discomfort or pain which is relieved by the appropriate anesthetic or analgesic e.g. transmitter implantation under general or local anesthetic with analgesic effect, skin or blubber biopsy *under local anesthetics*, coring of dorsal ridge, etc.
- E:** applies to discomfort of pain which is not relieved (biopsy darting, dart-tagging etc.) thus requires written justification and full IACUC approval and documented in the annual report to APHIS (must consider the 3 R's)

In accordance with the AWA: "The principal investigator has considered alternative to procedures that may cause than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources (e.g. the Animal Welfare Information Center) used to determine that alternative were not available...."

The following table includes requested yearly takes for the collection of biopsy samples using a projectile dart, application of dart-satellite tags, and suction-cup attached tags. The total number equals the five-year total for the extent of the permit.

We have calculated our requested biopsy take numbers based on the likelihood of encountering a particular species and allowing a high enough number of takes to gather a satisfactory, successful, sample size to answer the questions we are mandated to ask. To conduct genetic stock structure analysis a sample size of 50 samples per strata is needed; sometimes there can be 5-10 genetic stocks within a species which could necessitate up to 500 successful samples. It is also important to collect a sample size that is relative to the population size to ensure we have sampled the genetic distribution as well as possible. In other cases, it is desirable to sample entire schools to answer questions about social structure. Furthermore it is desirable to continue to add to our genetics archive to continue to expand our geographic coverage of genetic samples across species, to detect trends in genetic distribution and in abundance over time.

We arrived at our tagging take requests based on the number of animals required for addressing questions of animal movement and behavior. Although statistical power analysis may suggest more tags are needed to address some specific questions, we have limited our request to the number of animals we hope to successfully tag, and then expanding that number to allow for a few malfunctioning tags (therefore needing to tag additional animals), and misses (resulting in a take, but where the animal did not receive a tag). See section IV-B2 of the attached NMFS permit application for current knowledge of target species for which takes are requested.

Species (Common Name)	Age/Sex	Category C (suction-cup tags)	Category E (biopsy)	Category E (dart tags)	Total # of animals needed for duration of project
Rough-toothed dolphin	Adult & subadult/ Male & female	40	200	20	1300
Risso's dolphin	Adult & subadult/ Male & female	40	100	40	900
Common dolphin	Adult & subadult/ Male & female	40	200	20	900

Common Bottlenose dolphin	Adult & subadult/ Male & female	40	400	40	2400
Indo-Pacific Bottlenose dolphin	Adult & subadult/ Male & female	40	400	40	2400
Pantropical spotted dolphin	Adult & subadult/ Male & female	40	1000	20	5400
Spinner dolphin	Adult & subadult/ Male & female	40	400	20	2400
Striped dolphin	Adult & subadult/ Male & female	40	100	20	900
Fraser's dolphin	Adult & subadult/ Male & female	40	100	20	900
Melon-headed whale	Adult & subadult/ Male & female	40	150	40	1350
Pygmy killer whale	Adult & subadult/ Male & female	40	150	40	1350
False killer whale	Adult & subadult/ Male & female	40	300	40	1900
Killer whale	Adult & subadult/ Male & female	25	100	25	750
Short-finned pilot whale	Adult & subadult/ Male & female	40	200	40	1400
Blainville's beaked whale	Adult & subadult/ Male & female	40	50	40	650
Cuvier's beaked whale	Adult & subadult/ Male & female	20	50	20	450
Longman's beaked whale	Adult & subadult/ Male &	10	30	10	250

	female				
Pygmy sperm whale	Adult & subadult/ Male & female	10	20	20	250
Dwarf sperm whale	Adult & subadult/ Male & female	40	20	20	400
Sperm whale	Adult & subadult/ Male & female	40	100	20	800
Blue whale	Adult & subadult/ Male & female	20	100	10	650
Fin whale	Adult & subadult/ Male & female	20	100	10	650
Sei whale	Adult & subadult/ Male & female	20	100	10	650
Minke whale	Adult & subadult/ Male & female	20	100	10	650
Bryde's whale	Adult & subadult/ Male & female	20	100	10	650
North Pacific right whale	Adult & subadult/ Male & female	5	25	5	175
Humpback whale	Adult & subadult/ Male & female	20	250	0	1350
Unidentified delphinid	Adult & subadult/ Male & female	0	100	0	1000
Unidentified rorqual	Adult & subadult/ Male & female	0	50	0	250
Unidentified beaked whale	Adult & subadult/ Male & female	20	25	0	550

7. If you have placed any animal numbers in **category E**, you must complete the following (use Appendix B if additional space is necessary)
- a. Explain why the pain or discomfort cannot be relieved and what procedure will be used to minimize discomfort.

For activities that involve biological sample collection or tagging of cetaceans, the methods used are considered the least likely to cause stress, pain or suffering primarily because they do not require capture of the animal. The actual biopsy or tagging events are short-lived and relatively non-invasive.

For both biopsy and tagging activities, there are currently no alternatives that will yield the same quality of data in the same quantity to provide the research results to answer questions of population trends. Collections of sloughed skin generally do not yield high amounts of good quality DNA, or blubber for hormone analysis; identifying the individual the sloughed skin came from is often impossible, research questions would be limited by opportunistic data collection (very few samples of sloughed skin as opposed to biopsy samples) and the data set could be biased toward individuals who slough skin more easily or more frequently (e.g. males engaged in physical competition).

The only alternative to tagging would be photo identification studies which only yield information when the animal is resighted. This severely limits the data on geographic range (two or more isolated data points as opposed to a more continuous track).

When possible, attempts will be made to obtain photographs of tagged individuals to examine wound healing and modes of tag failures. Additionally, when working with coastal populations attempts will be made to monitor individuals' life history patterns through photo-identification.

- b. What informational methods and resources did you use to determine that (no-animal or non-painful) alternative were not appropriate for this research?
 - i. Include the databases that were searched (include keywords used).
 - ii. Include literature citations
 - iii. Include meetings with knowledgeable individuals (name, date)
 - iv. Include other methods/resources

Biopsy sampling has been used extensively worldwide and has become common and widely accepted method for obtaining tissue

samples, especially because the unequivocal value of molecular genetic tools and analyses has been recognized. The reactions of cetaceans to biopsy sampling have been studied for several species (see Lambertsen 1987, International Whaling Commission 1991, Brown *et al.* 1991, Weinrich *et al.* 1991, Weinrich *et al.* 1992, Clapham and Mattila 1993, Brown *et al.* 1994, Cockcroft 1994, Jahoda *et al.* 1996, Weller *et al.* 1997, Gauthier and Sears 1999, Hooker *et al.* 2001, Krützen *et al.* 2002, Jahoda *et al.* 2003, Best *et al.* 2005). Potential impacts from biopsy sampling and tagging may include behavioral disturbance, injury or infection. Disturbance may result from the biopsy itself or from the approach of the small boat. The most common reactions to biopsy sampling and tagging have been reported to include no reaction, a flinch or startle, or a tail flick and/or a rapid dive. In our experience, reactions by individuals of various species to biopsy sampling and tagging generally have been low-level and short-lived, with reactions ranging from no visible response to a “startled” reaction sometimes followed by an animal swimming away or diving. For bowriding dolphins sampled from the main research vessel, an individual animal will often continue to ride the bow after the biopsy sample has been collected. In our experience, individual animals are more likely to respond to the approach of the small boat than to the biopsy itself.

The potential for level A harassment exists in conjunction with biological sample collection (via biopsy) and dart-tagging activities; however, in the years that the SWFSC and the PIFSC have been collecting biopsy samples and deploying tags, no known instance of an injury to a marine mammal has occurred. There have been no documented cases of infection or injury to large whales resulting from biopsy sampling. Bearzi *et al.* (2000) reported on the death of a common dolphin following penetration of a biopsy dart and subsequent handling. The authors concluded that the biopsy dart did not produce a lethal wound, but that the biopsy darting and subsequent handling (perhaps in combination with potential pre-existing health conditions of the animal) produced physical and/or physiological consequences that were fatal to the animal. There is no evidence that the biopsy procedure or associated boat approaches, if conducted responsibly and by experienced individuals, has any significant impact on cetacean populations. It is clear from many years of work with this and other baleen whale species that biopsy approaches at worst result in temporary and minor disturbance in behavior of an individual, with no impact after the biopsy collection procedure is complete (Brown *et al.* 1994, Weinrich *et al.* 1991, Clapham and Mattila 1993, Best *et al.* 2005). Studies to date indicate no long-term consequences on survival, return rates or fecundity. Though this technique is not completely

devoid of risk, it is not likely to produce any long-term, deleterious effects on individual animals or populations of cetaceans.

The dart-tags described above have been safely and successfully deployed on beaked whales (Baird *et al.* 2008a, 2009), sperm whales (Schorr *et al.* 2007a), fin whales (Schorr *et al.* unpublished), pilot whales, melon-headed whales (Schorr *et al.* 2009) and false killer whales (Baird *et al.* 2008b, 2010).

See Appendix B for citations.

C. Research Procedures

1. General Procedures.

In accordance with the AWA, "Procedures that may cause more than momentary or slight pain or distress to the animals will a) be performed with appropriate sedatives, analgesics, or anesthetics unless withholding such agents is justified for scientific reasons in writing by the principal investigator and will continue for only the necessary period of time; b) involve in their planning, consultation with the attending veterinarian..., c) not include the use of paralytics without anesthesia..."*

**e.g. biopsy sampling, tagging etc.*

Photo-Identification activities are primarily conducted from small boats (5-10 m) with 120 hp to 150 hp four-stroke outboard engines either on an opportunistic basis during large vessel surveys or during small boat surveys off Hawaii, Palmyra, American Samoa, Guam, CNMI, or in international waters. When photographs are taken from boats with Digital Single Lens Reflex (DSLR) cameras and telephoto zoom lenses, the animals will be approached closely enough to optimize photographic quality (i.e., well-focused images, utilizing at least one half of the slide viewing area) while approaching from behind at a consistent speed and avoiding sudden changes in speed or direction. Distance for optimal approach varies with the species being photographed. Generally, large whales will be approached within approximately 15-20 m. Smaller animals, such as delphinids, will be approached within approximately 5-10 m. Photographs of bow-riding animals will also be taken on an opportunistic basis from large or small vessels and these animals will approach the vessel on their own in which case we will maintain a consistent speed to avoid startling any animals. These photographs will be used to estimate abundance, document movements, scarring rates and in some cases (e.g., spinner dolphins) estimate vital parameters such as survival and calving rates. Photo-identification studies are expected to be most useful for island-associated (or otherwise localized) stocks and migratory species exhibiting site fidelity. They are also used for stock identification. Photo-identification of adult and juvenile males and females will cease when clear photos have been obtained of all individuals present, or when excessive avoidance behavior is displayed by the group. If the opportunity

arises, females accompanied by calves may be approached for photo-identification, but efforts will cease immediately if there is any evidence that the activity may be interfering with pair bonding, nursing, reproduction, feeding or other vital functions.

Biological Sample Collection will occur opportunistically during small-boat and large-vessel surveys, from either vessel, using biopsy sampling (skin/blubber collected by projectile dart), collection of sloughed skin or feces using a net or sieve, or collection of sloughed skin attached to a recovered tag. Projectile biopsies will be collected using either a crossbow, adjustable-pressure modified air-gun, or pole.

During any single encounter, no more than five biopsy sample attempts per individual will be made. Success rate of biopsy darting may vary and depending on the combination of biopsier, weather, distance-to-animal and boat driver could be lower than 20%. It is rare that an animal would be targeted for biopsy more than twice during one encounter, but we conservatively request five sample attempts to allow for occasional low success rates. If signs of harassment such as rapid changes in direction, prolonged diving and other behaviors are observed from an individual or a group, the biopsy activities will be discontinued on that individual or group. The animals to be sampled will either approach the vessel on their own, be approached by the main research vessel during normal survey operations, or be approached by a small boat. The projectile biopsy sample will be collected from animals within approximately 5 to 30m of the bow of the vessel or small boat (Palsbøll *et al.* 1991).

Tethered biopsy darts will be used if animals are bow riding on the large vessel. We have two basic configurations for tethering biopsy darts. The one we use most often is for bow-riding dolphins. For that, we use a length of line-gun line – we tie one end to a handrail on the ship and the other end to the dart. The line is just long enough to go straight down to the water surface and back up. We tie a metal washer to the lower end to keep the line somewhat taught in case of wind. Most of the time when we shoot we hit the dolphin on the back close to the dorsal fin. When we hit (most of the time), the dart bounces up and back or away from the dolphin. Occasionally we miss and the dart goes down along side the dolphin and passes behind it; we retrieve the dart with the tether and repeat. The SWFSC has biopsied thousands of dolphins from 15 or more species this way with no entanglements. Quite often dolphins we biopsy this way do not even leave the bow, or if they do we often see them again a short time later. The other tethering situation involves using spooled line with the spool attached to the crossbow and the other end of the line attached to the dart. This set-up is most often used when we are attempting to sample large whales from a ship where dart retrieving is unfeasible. The line is light enough that it would be easily snapped by a large whale were it to become entangled, but we have never seen an entanglement using this method either. In general, except for bowriders, we

prefer not to use tethered systems because the trajectory of a tethered dart is more easily affected by the wind but it can be useful at times.

For small cetaceans, the tissue sampled is a small plug of skin and blubber, approximately 7mm in diameter and 20mm long. It is collected from the area behind the blowhole and in front of the dorsal fin. The depth of the biopsy tip is controlled by a cushioned stop (25mm in diameter) of neoprene vacuum hose encircling the biopsy head. For large cetaceans, small samples (<1 gram) will be obtained from free-ranging individuals using a biopsy dart with a stainless steel tip measuring approximately 4 cm in length with an external diameter of 9mm and is fitted with a 2.5 cm stop to ensure recoil and prevent deeper penetration (so that only 1.5cm of the tip is available to penetrate the animal). Between sample periods, the biopsy tips are thoroughly cleaned and sterilized with bleach. Biological samples may be collected from adults and juveniles. No biological samples will be taken from calves.

The samples will initially be stored on ice, and then as soon as they are processed they will be stored in a cryovial and either stored immediately in a -80°C freezer, frozen in a cryovial with 90% ethanol in a -20°C until a -80°C freezer is available, or frozen in a cryovial which is placed in liquid nitrogen until a freezer is available or stored in DMSO. Labels with the field id will be put both on the outside of the vial and inside with the sample. The samples will then either be stored in the PIFSC genetics freezer, or sent to Southwest Fisheries Science Center for entry into their archive. If the samples are to be shipped they will be sent overnight in Styrofoam packaging with dry ice to keep the samples frozen.

Tagging will be conducted during both large vessel surveys and coastal small boat surveys. The types of tags that will be employed are: VHF transmitters, time depth recorder (TDR) tags, acoustic recording tags (b-prodes and DTAGs), GPS-location tags, and satellite tags. The choice of tag or tags will depend on the primary research question being addressed: Suction cup attached TDR, GPS, and acoustic recording tags will be used to study diving and foraging behavior as well as measure the sounds produced by the animals during different behavioral states and in the presence of anthropogenic noise sources. Suction-cup attached tags detach from the animals within 72 hours. Satellite tags, which help determine movement patterns over a period of weeks to months (depending on type of tag used) will be used to assess residency or non-residency, provide insight into areas of high-importance for foraging, and provide details necessary to better formulate appropriate management and conservation practices. Satellite tags, and satellite-linked TDR tags will be deployed as dart-tags, attached externally to the animal by barbed posts (see Andrews et al. 2008, Schorr et al. 2009).

Approaches will be similar to those used during biopsy activities. During any single encounter, no more than five tag deployment attempts per individual will be made. Tagging activities may be conducted on adult and juvenile males and

females, though only one dart tag will be attached per animal at a time (we may suction-cup tag an individual who already has a satellite tag). Individuals may be re-tagged after attachment of a first tag has failed, but only up to three tags per tag-type per year will be placed on the same individual. No tagging attempts will be made on dependent calves; however, we are requesting to tag mothers accompanying calves. The minimum age of large whales we are requesting to tag would be six-months (this age was chosen to correspond with when the calves are usually weaned). For medium sized cetaceans and smaller, the minimum age would be one year. All tags will be deployed with a crossbow, adjustable-pressure modified air-gun, or pole. Tags will be applied either to the dorsal fin, or to the dorsal surface of the whales. Please note that dimensions and weights of tags given here may differ to those actually used, as advancements in technology may lead to smaller and more effective tags. Tagging equipment is constantly being improved in terms of size and weight, and the PIFSC continues to update its tagging equipment as newer models become available. Careful consideration of the primary research objective will be given before finalizing the tag package and deployment system to ensure that the smallest, lightest package is deployed.

Deployment of suction-cup tags will include approach from behind using a small boat to within ~1–8 m. The tag is attached to the whale using a pole or by crossbow. An attachment pole is used with a specially designed bracket to hold the tag in place yet allow it to detach from the pole after becoming attached to the whale. When using the crossbow, the tag is deployed with a modified crossbow-bolt, tipped with a rubber suction cup attached to the tag. Once tagged, the whale will be photographed for individual ID. The tagged whale's position and behavior relative to the ship will be monitored at several hundred meters distance using reticle binoculars and GPS. Whale reaction to tagging is generally short-term with resumption of previous behavior within one dive cycle. Skin will be collected from the inner surface of the suction cup or tagging apparatus to allow for the evaluation of sex-based differences in behavioral reaction.

When pole attachment techniques are used, the pole will be 3-8 meters in length, potentially necessitating approach to closer than 4 m to attach the tag. If attachment is by crossbow, attachments will be made from distances of approximately 6-8 m. In an encounter to place a tag, each individual whale will be approached no more than three times. We will attempt to place the tags on the back of the whale mid-way between the blowhole and the dorsal fin. Because attachment is by suction-cup the tags will not penetrate the skin.

When a tag is successfully attached, we will track the tagged animal using the small boat (if possible) or the larger support vessel. Suction cup tag attachments vary in duration from a few minutes to at most several days. The tag radio transmitter will be used to indicate when the animal is at the surface, and also the direction to the animal, using a directional radio antenna. During tracking we will

maintain a distance of 100 m or more between the tracking vessel and the tagged animal. We would like to minimize the impact of the tracking vessel on the tagged whale, and we do not anticipate closely approaching and/or harassing the tagged animal while the tag is in place. After the tag detaches from the animal we will recover it with the tracking vessels, using the VHF radio signal as a guide. Suction cups are used to attach the tag to the animal.

Small satellite “dart-tags” will be attached externally to the dorsal fin or dorsal surface of delphinids and large whales. The tag package will be based on a design that has been used successfully on false killer whales, pilot whales, beaked whales, melon-headed whales and other odontocetes in Hawaiian waters (e.g. Baird *et al* 2009, Schorr *et al* 2008). This tag is small (6.3 cm long by 3.0 cm wide by 1.9 cm tall) and lightweight (mass = 40 grams). The tag will be held in place on the external surface of the whale using two barbed darts. In the current design these are 4.2mm in diameter and penetrate 6.5 cm. These dimensions may vary depending on the target species. These tags have great utility for movement data over periods of weeks to months, in an externally-mounted package. These low profile dart-tags will be remotely attached using an adjustable-pressure modified air-gun or crossbow equipped with a 150 lb. draw limb.

Whales exhibiting aerial behaviors will not be approached. This will be done for the safety of the researchers as well as to minimize any adverse impacts to the individual whales from the proposed activities. No known unintended mortality has arisen from these activities when conducted by the SWFSC/PIFSC under past permits and none is expected in the future.

2. Anesthetics and Analgesics:

If anesthetics or analgesics are to be used, please provide the following information: procedure, anesthetic, dose and method of administration

Procedure	Anesthetic	Dose & Method of Administration

In accordance with AWA: "Activities that involve surgery include appropriate provision for pre-operative and post-operative care of the animal in accordance with established veterinary medical and nursing practices. All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques."

3. **Surgical Procedures** – Is surgery to be performed? YES NO

a. If YES, list surgery location/room or field site:

b. If YES,

i. is it a terminal procedure? YES NO

ii. is it a survival procedure? YES NO

c. If YES, then describe the surgical procedure to be performed in Appendix B. Be sure to include the protocol to be followed to ensure asepsis.

d. If aseptic procedures are not to be performed, use this space below to justify why not and describe the procedure of choice.

e. Describe the post-operative care (both immediate and long-term).

4. **Euthanasia** - Will the animals be terminated at the end of the research?

YES NO

If YES, provide the method of euthanasia:

Please consult NMFS Research Protocol Guidelines (TBD) for acceptable practices. (AVMA Guidelines, AAZV Guidelines, etc)

In accordance with the AWA, "Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures."

5. Please describe below the **training and qualifications** of yourself and other individuals who are included in this protocol. In particular, please be very specific about the hands-on training of those individuals performing procedures which may produce animal discomfort (i.e., restraint, injections, blood collection, surgery, tagging, biopsy, tooth extraction, urine, fecal, gastric, milk, semen, sample collection, euthanasia, etc.). Use Appendix C to further describe training and experience.

The individuals listed for inclusion in this protocol (see Appendix C) have experience with tagging and/or biopsy of wild cetaceans. The persons listed have performed these duties in the field over several field seasons,

and have performed tagging and/or biopsy sampling during other NMFS permitted activities. All individuals are trained to collect biopsy samples from the dorsal surface of the animal, avoiding the blowhole and head. Individual animals are not biopsied more than once in an encounter and moms with attending calves are avoided. Proper cross-bow and rifle handling is practiced on all NMFS surveys.

Greg Schorr, Daniel Webster, and Allan Ligon have specific experience with the application of projectile satellite tags and have shown high success rate of attached tags, and shown tag attachment wounds healed within several months post-tagging. Nearly all satellite-tagged individuals have been re-sighted in subsequent surveys.

D. Husbandry Practices (research facility or rehabilitation facility)

If the animals are maintained at a research facility, an APHIS license is required and the facility must comply with AWA. If the animals are maintained at a rehabilitation facility, it must meet the NMFS Rehabilitation Facility Standards.

1. Will the research require holding the animals in temporary or long term captivity (this includes rehabilitation)? YES NO
2. If YES, describe the husbandry practices that will be used.
3. If YES, describe procedures for disposition of dead animals, including whether or not a necropsy will be performed.
4. Will the animals be removed from the facility? YES NO
 - a. If YES, for how long?
 - b. If YES, to where?
 - c. If YES, will they be returned to the facility? YES NO
 - d. If NO, why not?

Note - If removal will be greater than 24 hours, a variance request may be required.

E. Environmental Safety

1. Are infectious agents to be used or potential exposure? YES NO
If YES, the agent(s) is.....
If YES, is the agent infectious to humans? YES NO
2. Are chemical hazards to be used? YES NO
If YES, the chemical hazard is.....

3. Are radioisotopes to be used? YES NO
 If YES, the radioisotope is.....

4. Are other biohazards of concern like exposure to zoonotic agents?
 YES NO
 IF YES, the biohazard(s) is.....

Note - If any of the above questions are answered YES, all procedures must comply with NMFS Environmental Safety requirements (TBD).

F. NMFS Training on Animal Care and Use (TBD)

Have you completed the NMFS Vertebrate Animal Care and Use Training Program?

YES NO

If YES, give data of Training Program session....

If NO, you must complete this Training Program within 1 calendar year of the date of approval of this protocol and submit certification thereof to the Chair of the IACUC. This Program covers the composition and function of the IACUC, historical background, NMFS policy on animal care and use, animal welfare concerns, protocol submission, and occupational health and safety. Failure to complete this program within 1 calendar year could result in suspension of the project by the IACUC.

G. Occupational Health and Safety

List all the names and telephone numbers of personnel, including yourself, associated with this project and identified in this protocol who will work with animals or animal tissue. Check the appropriate box to indicate whether or not each individual has completed the NMFS Animal Care and Use Training Program. Also, check the appropriate box to indicate if each individual has fulfilled requirements for vaccination and/or testing.

NMFS Animal Care and Use Training	Vaccination/Testing	Name	Phone	Email
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Erin Oleson	808-944-2172	Erin.oleson@noaa.gov
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Marie Hill	808-944-2179	Marie.hill@noaa.gov

<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	Robin Baird	425-879-0360	RWBaird@cascadiaresearch.org
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	Frank Parrish	808-944-2181	Frank.parrish@noaa.gov
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	David Johnston	252-504-7593	David.johnston@duke.edu
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	David Mattila	808-281-6234	David.mattila@noaa.gov
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	James Barlow	808-285-6996	James.barlow@noaa.gov
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	Chad Yoshinaga	808-983-3712	Chad.yoshinaga@noaa.gov
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	Allan Ligon	808-268-0606	aligon@aol.com
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	Greg Schorr	206-931-4638	Gschorr@cascadiaresearch.org
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	Daniel Webster	406-599-1788	daniel_webster@mac.com
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	Charles Littnan	808-944-2175	Charles.littnan@noaa.gov
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	Mark Deakos	808-280-6448	deakos@hawaii.edu
<input type="checkbox"/> Yes X No	<input type="checkbox"/> Yes X No	Sarah Courbis	503-975-7010	sarahc@rogue.com

H. Assurance

I attest to the accuracy and completeness of the information provided. I promise to conduct this work with animals in accordance with the protocol as approved by the NMFS IACUC under the NMFS Animal Care and Use Policy. I will not make any substantive changes in the above protocol without first obtaining the approval of the NMFS IACUC, and I will not use any procedures which are not included in this form.

Principal Investigator/Applicant:  Date: 6/11/10

I have reviewed the research protocol outlined on this form and hereby transmit it to the NMFS IACUC for review

Center or Regional Director: _____ Date: _____

Appendix A

Observational Study Description(s) from page _____

Appendix B

Research Procedures Description(s) from page 14

Describe the animal procedures that are to be performed and the necessity in fulfilling the goals and objectives of the project. Be sure to be specific about any procedures which may impact the health and comfort of the study animals (e.g., frequency of performance of any procedures, methods of restraint, blood sample volumes, etc.). Please provide a justification for the animal numbers used.

Citations for Section B- Justifications (7b):

- Baird, R.W., D.L. Webster, G.S. Schorr, D.J. McSweeney and J. Barlow. 2008a. Diel variation in beaked whale diving behavior. *Mar. Mamm. Sci.* 24:630-642.
- Baird, R.W., A.M. Gorgone, D.J. McSweeney, D.L. Webster, D.R. Salden, M.H. Deakos, A.D. Ligon, G.S. Schorr, J. Barlow and S.D. Mahaffy. 2008b. False killer whales (*Pseudorca crassidens*) around the main Hawaiian Islands: long-term site fidelity, inter-island movements, and association patterns. *Mar. Mamm. Sci.* 24:591-612.
- Baird, R.W., G.S. Schorr, D.L. Webster, S.D. Mahaffy, D.J. McSweeney, M.B. Hanson, and R.D. Andrews. 2009. Movements of satellite-tagged Cuvier's and Bainville's beaked whales in Hawai'i: evidence for an offshore population of Blainville's beaked whales. Report prepared under Contract No. AB133F-08-SE-4534 from the Southwest Fisheries Science Center, National Marine Fisheries Service, La Jolla, California.
- Baird, R.W., G.S. Schorr, D.L. Webster, D.J. McSweeney, M.B. Hanson and R.D. Andrews. 2010. Movements and habitat use of satellite-tagged false killer whales around the main Hawaiian Islands. *Endang. Species Res.* 10:107-121.
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- Brown, M.W., S.D. Kraus, and D.E. Gaskin. 1991. Reaction of North Atlantic right whales (*Eubalaena glacialis*) to skin biopsy samples for genetic and pollutant analysis. *Rep. Int. Whal. Commn. Spec. Iss.* 13:81089.

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- Clapham, P.J. and D.K. Mattila. 1993. Reactions of humpback whales to skin biopsy sampling on a West Indies breeding ground. *Mar. Mamm. Sci.* 9(4):382-391.
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- Gauthier, J. and R. Sears. 1999. Behavioral response of four species of balaenopterid whales to biopsy sampling. *Mar. Mamm. Sci.* 15(1):85-101.
- Hooker, S.K., R.W. Baird, S. Al-Omar, S.Gowans, and H. Whitehead. 2001. Behavioral reactions of northern bottlenose whales (*Hyperoodon ampullatus*) to biopsy darting and tag attachment procedures. *Fish. Bull.* 99(2):303-308.
- International Whaling Commission. 1991. Report of the *ad-hoc* working group on the effect of biopsy sampling on individual cetaceans. *Rep. Int. Whal. Commn., Spec. Iss.* 13:23-27.
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- Krützen, M., L.M. Barre, L.M. Möller, M. R. Heithaus, C. Simms, and W.B. Sherwin. 2002. A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Mar. Mamm. Sci.* 18:863-878.
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- Schorr, G.S., R.W. Baird, D.L. Webster, D.J. McSweeney, M.B. Hanson, R.D. Andrews and J. Barlow. 2007b. Spatial distribution of Blainville's beaked whales, Cuvier's beaked whales, and short-finned pilot whales in Hawai'i using dorsal fin-attached satellite and VHF tags: implications for management and conservation. Talk presented at the 17th Biennial Conference on the Biology of Marine Mammals, Cape Town, South Africa, November-December 2007.
- Schorr, G.S., R.W. Baird, M.B. Hanson, D.L. Webster, D.J. McSweeney and R.D. Andrews. 2009. Movements of satellite-tagged Blainville's beaked whales off the island of Hawai'i. *Endang. Species Res.* 10:203-213.
- Weinrich, M.T., R.H. Lambertsen, C.S. Baker, M.R. Schilling and C.R. Belt. 1991. Behavioral responses of humpback whales (*Megaptera novaeangliae*) in the Southern Gulf of Maine to biopsy sampling. *Rep. Int. Whal. Commn. Spec. Iss.* 13:81-89.
- Weinrich, M.T., R.H. Lambertsen, C.R. Belt, M.R. Schilling, J.H. Iken and S.E. Syrjala. 1992. Behavioral responses of humpback whales (*Megaptera novaeangliae*) to biopsy procedures. *Fish. Bull.* 90: 588-598.
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Appendix C

Training and Experience description(s)

CVs are attached for the following individuals:

Erin Oleson
Jason Baker
James Barlow
Charles Littnan
Allan Ligon
Greg Schorr
Chad Yoshinaga
Sarah Courbis
Frank Parrish
Marie Hill
Robin Baird
David Johnston
David Mattila
Daniel Webster
Mark Deakos