



NATIONAL MARINE FISHERIES SERVICE

*Assurance of Animal Care and Use
Form*

IACUC Use Only

IACUC Number: SWPI2010-03

USDA Classification: C / D / E

(Circle One)

Date Received: 9-20-2010

Initial Review Date: 11-12-2011

IACUC Training Complete:

IACUC Recommendations: Approved: Not Approved:

Withhold Approval Pending Modification

Type of Submission: New

Modification

3-Year Renewal

IACUC Chair Signature: _____

Date: 2/22/11

Project Title: Ocean capture research of green and hawksbill turtles in the Hawaiian Islands.

Principal Investigator: George Balazs

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: Ocean capture research of green and hawksbill turtles in the Hawaiian Islands.

Department or Office: NOAA, NMFS, PIFSC, Marine Turtle Research Program

Principal Investigator: George Balazs, Program Leader

Mailing Address: 2570 Dole Street, Honolulu, HI 96822

Telephone: (808) 983-5733 **Fax:** (808) 983-2902 **Email:** George.Balazs@noaa.gov

Initial Submission **Renewal** **or Modification**

Funding Source: NMFS

Grant Title (if different from Project Title):

Anticipated Start Date: January 1, 2012 **Anticipated End Date:** December 31, 2017

Location Where Animals Will Be Housed or Study Site(s): Islands of Hawaii, Maui, Lanai, Molokai, Oahu, Kauai, and Midway Atoll

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.

Permit Type	Permit Number	Expiration Date
State of Hawaii, Department of Land & Natural Resources, Division of Forestry and Wildlife, Protected Wildlife Permit #	ED2011-03	Sept 30, 2011
NMFS Permit to Take Endangered and Threatened Species	1581	December 31, 2011

*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?

Green turtles (*Chelonia mydas*) are listed as a threatened species and hawksbills (*Eretmochelys imbricata*) as an endangered species under the US Endangered Species Act (ESA). NOAA Fisheries has the lead responsibility for the conservation and recovery of sea turtles in the marine environment. This long-term study of green and hawksbill turtles also directly responds to recommendations listed in the Recovery Plan for U.S. Pacific Populations of the Green Turtle and the Recovery Plan for U.S. Pacific Populations of the Hawksbill Turtle. It specifically addresses recovery action items 2.1.2 "Determine distribution, abundance, and status in the marine environment" and 2.1.6 "Study the impact of diseases on turtles" in both plans.

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

The purpose of this research is to conduct long-term monitoring of the status of green and hawksbill turtles in the Hawaiian Islands to determine their stock structure, abundance, size ranges, health/disease status, foraging ecology, habitat use, diving behavior, local movements, and migration routes. A primary goal is to integrate data from genetic analysis, flipper tagging, and satellite telemetry to identify nesting beach origins of turtles occurring in the Hawaiian Islands and contribute to the overall understanding of sea turtle stock structure in the Pacific Ocean. Further, we will compare current data with those collected in the Hawaiian Islands since 1973 to determine growth rates (Balazs and Chaloupka 2004, Chaloupka and Balazs, 2005) of juveniles and adults, assess changes in the health status of the population, and examine population abundance trends.

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

There are no non-animal alternatives available to study the life history of these two protected species (*Chelonia mydas* and *Eretmochelys imbricata*).

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 **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:

Green turtle (*Chelonia mydas*) – Hawaiian stock
Hawksbill turtle (*Eretmochelys imbricata*) – Hawaiian stock

These two species reside/forage in Hawaii’s nearshore waters and nest on beaches in the Main Hawaiian Islands (MHI – hawksbills and greens) and Northwestern Hawaiian Islands (NWHI - greens) and are protected under the ESA. Because of their protected status and the need to obtain information specific to each species, they are the most appropriate species to use in these studies.

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:

Our current NMFS Permit #1581 for the non-lethal take of Threatened and Endangered Species allows for the annual ocean capture and tagging of 600 green turtles and 10 hawksbills. The same number of green turtles and an increase to 25 hawksbills is justified for the next 5-years on the basis of recent formal recommendations for increased data urgently necessary for sea turtle population assessments in the USA (National Research Council, 2010). Expanded knowledge of changes in demographic rates at all sea turtle life stages is critically needed. The majority of these life stages are immature stages (non-reproductive, non-nesting) hence only available to science by capture of individuals in the sea. Bolker et al. (2007) has also emphasized, whenever possible and practical, the quantitative goal for large sample sizes in ocean studies and understanding of complex and multi-faceted aspects of sea turtle life histories. The ultimate goal is reliable information for recovery and management. In the Hawaiian Islands 24 discrete long-term study locations have been established for ocean-capture tagging and monitoring, historically ranging from 5-30 years. However, not all sites can be sampled annually due to fluctuating logistical and agency funding limitations. In addition, the number of turtles that can be captured on an expedition (or in a given year) is always uncertain due to weather/ocean conditions, and behavioral foraging variations, hence availability of the turtles (Figures 1 and 2). Realizing these somewhat unpredictable limitations, the goal, concomitant with National Research Council (2010) and Bolker (2007), is to safely, humanely and effectively capture as many turtles as possible for quantitative analyses, such as trends in abundance and survival probability. For example, for two discrete study locations investigated in the Bahamas, Bjorndal et al. (2003, 2005) averaged 66 and 32 green turtles captured annually over 24 and 13 years, respectively. Hence, a continuation of 600 green turtles annually requested for 24 discrete locations throughout the 2400 km expanse of the Hawaiian Archipelago is justified both analytically and historically as scientifically appropriate and reasonable, given our capture performance and journal publication record over the past 5-year permit period. The requested increase to 25 hawksbills annually (from 10 annually) is prudent and justifiably consistent with the above on the basis that 1) increased research focused on Hawaiian hawksbills is planned over the next 5-year period, and 2) indicators that hawksbills are gradually becoming more numerous in the Hawaiian Islands perhaps, like green turtles, in favorable response to conservation actions implemented over the past 30 years.

In addition to the above justification, the requested sample sizes were determined based on the following logic. Nowadays, high quality publishable studies, such as for contaminants being pursued under project, contain 60 or more samples with at least 10-25 animals per group or category being tested. We have determined that for experimental designs with enough statistical

power, approximately 20 turtles are typically needed within each grouping or category for hypothesis driven research questions. Within a single collection year, samples will be collected for two major competing goals: specimen banking (long-term storage for future retrospective analysis), as well as a variety of real-time contaminant projects.

For specimen banking, at least 25 turtles per year from at least three sites (N=75) should be stored. Power analysis has shown that a sample size slightly smaller than this (N=60 total) is robust for detecting statistical differences of, for example, mercury in seabird eggs from different colonies in Alaska (Day et al. 2006). Mercury measurements are often less variable than organic pollutants, so to account for the decreased power for organic contaminant analysis, the samples size was increased to 25/year.

This leaves no fewer than 175 possible samples per year to undertake the second goal, real-time contaminant projects that can address important timely issues for sea turtles. The objectives of these projects cannot be predicted in detail right now and will change based on the staff available to analyze the samples, thus only the samples needed to accomplish the projects will be collected. Likely projects include 1) contaminant monitoring of turtles at two or more sites based on health status or nutrient inputs from different watersheds (20 turtles/site with fibropapillomas, 20 turtles/site without fibropapillomas x 2 sites; N=80), 2) contaminant monitoring of turtles between age and sex classes (20 turtles/age class & sex x 4 categories; N=80), 3) contaminant monitoring of turtles that have different behavioral habits (20 turtles/category of diurnal migratory and foraging habits x 3 categories; N=60). With the 250 turtle limit, only 2 of these 3 real-time projects could be undertaken in any one year.

Figure 1. Green turtle captures in the Main Hawaiian Islands
1980 - 2009

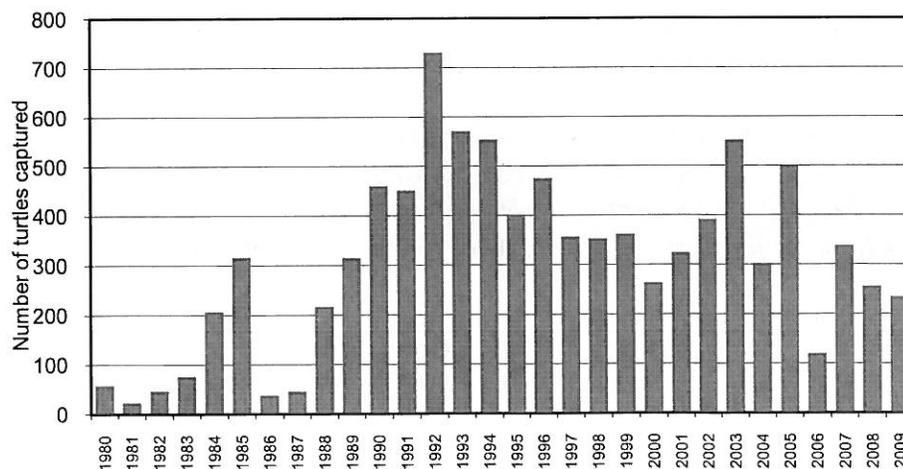
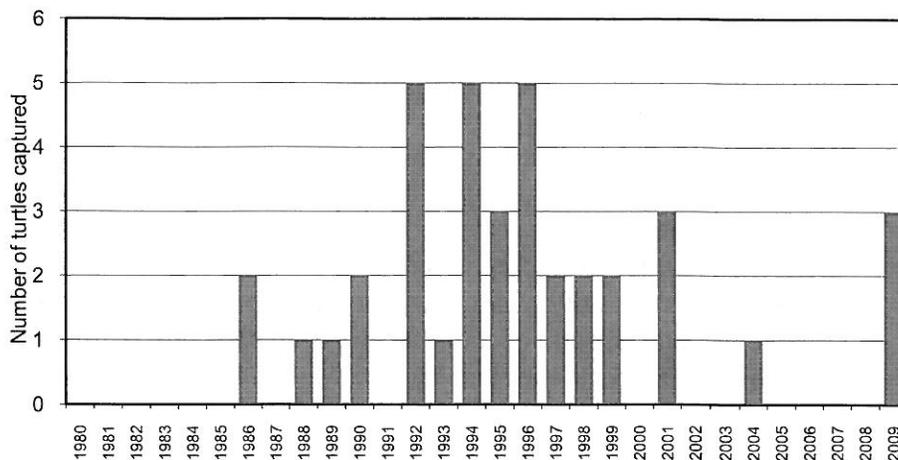


Figure 2. Hawksbill turtle captures in the Main Hawaiian Islands
1980 - 2009



Category (adapted from AWA):

- 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...."

	Green turtle juvenile, sub-adult, adult	Hawksbill turtle juvenile, sub-adult, adult
Category C (capture, measure, weigh, PIT/flipper tag, release)	≤50/yr	≤5/yr
Category C (shell-etching)	≤50/yr	≤5/yr

Category C (scute scraping)	≤50/yr	≤5/yr
Category C (blood sample)	≤50/yr	≤5/yr
Category C (lavage)	≤50/yr	0
Category C (transmitter attachment)	≤0/yr	≤/yr
Category D (≤6mm skin biopsy)	≤50/yr	≤5/yr
Total # of animals needed for duration of project	≤00/yr	≤5/yr

7. If you have placed any animal numbers in **category E**, you must complete the following (use Appendix B if additional space is necessary)
- Explain why the pain or discomfort cannot be relieved and what procedure will be used to minimize discomfort.
 - What informational methods and resources did you use to determine that (no-animal or non-painful) alternative were not appropriate for this research?

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.*

2. Anesthetics and Analgesics: lidocaine

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
≤6mm skin biopsy	lidocaine	1 mL injection or applied topically as a cream for 2-3 minutes prior to sampling

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.

All individuals included in this protocol have been trained to capture and handle/restrain marine turtles and insert PIT tags into flippers. Standard operating procedures and approved methodologies described in permit 1581 are implemented to minimize stress and pain to the animals. Skin biopsies, blood samples, and diet samples (i.e. lavage) are only taken by the PI, CIs, or DVMs listed below. CVs for the PI and CIs on permit 1581 are attached.

The number of years of experience handling and tagging marine turtles is provided for each person in parentheses after their name.

George Balazs, PI (30)
Stacy Hargrove, CI (13)
Marc Rice, CI (23)
Shawn Murakawa (15)
Shandell Brunson (5)

Tyler Bogardus (4)
Wendy Marks (2)
Thierry Work, DVM (15)
Robert Morris, DVM (18)

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.

The following personnel have completed the University of Hawaii IACUC training and are certified under protocols #97-051-13 and #97-052-13.

George Balazs
Stacy Hargrove
Shawn Murakawa

Shandell Brunson
Tyler Bogardus
Wendy Marks

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	George Balazs	(808) 983-5733	George.Balazs@noaa.gov
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Stacy Hargrove	(808) 983-5734	Stacy.Hargrove@noaa.gov
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Marc Rice	(808) 881-4200	mrice@hpa.edu
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Shawn Murakawa	(808) 983-5731	Shawn.Murakawa@noaa.gov
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Shandell Brunson	(808) 983-5744	Shandell.Brunson@noaa.gov
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Tyler Bogardus	(808) 983-5747	Tyler.Bogardus@noaa.gov
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Wendy Marks	(808) 983-5732	Wendy.Marks@noaa.gov
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Robert Morris,	(808) 262-9621	morrisr005@hawaii.rr.com

		DVM		
<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Thierry Work, DVM	(808) 792-9520	Thierry_Work@usgs.gov

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:** _____

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 _____

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.

No more than 600 green turtles and 25 hawksbills will be captured by various methods including: hand/scoop net capture in shallow coastal and reef waters, hand capture/snorkeling, hand capture while diving from a slow moving boat, entanglement net capture, scoop net capture, and bullpen net capture (Balazs et al. 1987, Balazs et al. 1998). All of these methods have been successfully and safely employed to study and tag green turtles in coastal waters of the Hawaiian Islands. Turtles will be released at or very close to the capture site.

The turtle's skin and shell will be cleaned with an antiseptic (alcohol, betadine, etc.) prior to the application or attachment of tags.

All turtles are tagged with Passive Integrated Transponder (PIT) tags - small (14 mm length x 2 mm diam) electromagnetically-coded glass-encased "microchips" - Destron Tx 1406L. The tags are individually packaged in a disposable presterilized needle applicator to eliminate the possibility of cross contamination. PIT tags are injected subcutaneously into each rear flipper. They are read with a scanner, and are designed to last the life of the turtle. Preliminary PIT tag retention studies have shown extremely high retention (Parmenter 1993, McDonald and Dutton 1996, Balazs and Chaloupka 2004).

Turtles may be tagged with metal Inconel tags (Style 681, National Band and Tag Company) using the standard technique described in the Marine Turtle Specialist Group Manual on Research Techniques (Eckert et al. 1999). The tag will be attached to the trailing edge of a hind flipper. The applicator is similar to that used to ear-tag livestock; the pointed end of the tag goes through the flipper and connects on the underside. Tag retention for these tags varies; although some tags are retained for 30 years or more, some loss occurs after 2 - 4 years.

No more than 50 green turtles and 5 hawksbills may have biotelemetry packages attached to their carapaces. Satellite tags may be attached alone or as a combination of the following: 1) Telonics TAM 4510 (the largest satellite transmitter to be used), dimensions = 15.0 x 7.3 x 3.9 cm, 435 g; 2) Telonics TAM 4410, dimensions = 11.8 x 6.5 x 3.2 cm, 248 g; 3) Telonics TAM 2639, dimensions = 3.2 x 6.6 x 3.0 cm, 90 g; 4) Wildlife Computers 'Splash' Tag, variable dimensions based on configuration; and 5) Wildlife Computers 'Spot 5' Tag, variable dimensions based on configuration. The Telonics TAM-4xxx series utilizes a new hydrodynamic design. The TAM 2639 is a

location only tag. The TAM 4510, TAM 4410, Splash, and Spot-5 tags record location and depth data. Attaching two miniature satellite transmitters on the same turtle provides valuable data relating to tag failure and animal mortality. Archival tags may be attached to the carapace independently or in conjunction with a location only single satellite tag and/or acoustic tag. Wildlife Computers Mk9 Time-Depth Recorders (dimensions = 6.7 x 1.7 mm x 1.7 cm) and Lotek LAT 1500 Time, Temperature and Depth Recorders (dimensions = 11 x 35 mm) are used. Acoustic tags manufactured by Sonotronics and Vemco were used in previous habitat use studies in Kaneohe Bay (Brill et al. 1995). Currently, Vemco model V16-1L-R64K acoustic tags are being used in a habitat use study of Kailua Bay, on the island of Oahu.

Transmitters (satellite, sonic and TDR) will be attached to the carapace with thin coats of fiberglass resin as described in Balazs et al. (1996). The attachment area on the carapace will be lightly sanded to remove algae and cleaned with alcohol. A non-toxic elastomer compound will be used to "cushion" the transmitter and hold it in place during the attachment procedure. A thin coat of laminating resin will be applied to the carapace and transmitter and 4-6 strips of fiberglass cloth will be pasted over the transmitter to attach it. This technique has been widely used and is an accepted safe and effective method for transmitter attachment (Balazs et al. 1996). The turtles will be held on the shore (adjacent to capture location) for 3-12 hours until resin has cured and then released back into the water at the point of capture. Turtles are held in a certified large animal carrier and kept in the shade (natural or canvas tarp) during transmitter attachment.

All blood, tissue, and diet sampling will be performed by trained personnel. We will take a blood sample (approximately 10cc, $N \leq 250$) by inserting a sterile needle, attached to a vacuum syringe, into the venous sinus on the lateral dorsal region of the neck, using the technique described in Bentley and Dunbar-Cooper (1980) and Owens and Ruiz (1980). These samples will be analyzed by Dr. Peter Dutton at the National Marine Fisheries Service Southwest Fisheries Science Center for genetics studies and/or by Dr. Robert Morris, DVM for health assessments.

In addition to blood, we will collect a skin sample (6 mm disc) from each newly tagged turtle ($N \leq 250$) using a new sterile biopsy punch. The area will be cleansed with an antiseptic prior to tissue sampling. Tumor samples may be taken in addition to a skin sample if necessary using the same procedure; however, tumor samples are not taken from every turtle with FP. Skin samples are preserved and archived for future genetic analysis, disease related studies, and foraging ecology studies using stable isotope analysis.

We will lavage some animals ($N \leq 250$) immediately after capture in order to collect food samples for diet analysis (Balazs 1980, Legler 1977, Forbes and Limpus 1993, Seminoff et al. 2002). This procedure involves inserting a length of 3/4 inch diameter soft plastic tubing down the esophagus to the "pre-stomach," and flushing it with water poured into the tubing. Contents are caught in a mesh bag. The procedure takes 5 - 10 minutes, and if done properly poses no risk to the animal.

Appendix C

Training and Experience description(s)

George Balazs (PI) has more than 35 years of sea turtle handling and research experience. Stacy Hargrove (CI) has more than 13 years of sea turtle handling and research experience. Marc Rice (CI) has more than 23 years of sea turtle handling and research experience. These individuals are fully trained and competent in capturing, handling, biological sampling (blood sampling, tissue biopsy, and lavage), and tagging (PIT, flipper, shell-etching, and biotelemetry tag attachment to carapace) marine turtles. Support staff (Shawn Murakawa, Shandell Brunson, Tiffany Hooper, Tyler Bogardus, and Wendy Marks) are fully trained and competent in capturing, handling, and tagging marine turtles. Veterinarians Drs. Thierry Work and Robert Morris each have been working with sea turtles for more than 18 years and are fully competent in all aspects of health examinations including biological sampling and treatment of injuries and illness.

CVs for George Balazs, Stacy Hargrove, and Marc Rice are attached.

References

- Balazs, G.H. 1980. Synopsis of biological data on the green turtle in the Hawaiian Islands. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SWFC-7 and University of Hawaii Sea Grant Cooperative Report UNIH-SEAGRANT CR-81-02, 141 p.
- Balazs, G. H., R. G. Forsyth, A. K. H. Kam. 1987. Preliminary assessment of the habitat utilization by Hawaiian green turtles in their resident foraging pastures. NOAA Tech Memo NMFS-SWFSC 71: 1-107.
- Balazs, G. H., R. K. Miya, S. C. Beaver. 1996. Procedures to attach a satellite transmitter to the carapace of an adult green turtle, *Chelonia mydas*. Proceedings of the 15th Annual Workshop on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFSC-387, 355p.
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