Standard Research Protocols for Nesting and Basking Marine Turtles in the Pacific Islands Region

These protocols apply to research involving:

Green Turtle (*Chelonia mydas*) Nesting and Basking in the Central North Pacific DPS (T), Central South Pacific DPS (E) and Central West Pacific DPS (E)

Hawksbill Turtle (*Eretmochelys imbricata*) Nesting on Pacific Ocean Islands under US Jurisdiction (E)

Olive Ridley Turtle (*Lepidochelys olivacea*) Nesting on Pacific Ocean Islands under US Jurisdiction (E)

Leatherback Turtles (*Dermochelys coriacea*) Nesting on Pacific Ocean Islands under US Jurisdiction (E)

USFWS Pacific Islands Fish and Wildlife Office

and

NOAA Pacific Islands Fisheries Science Center

2019

TABLE OF CONTENTS

DOCUMENT OVERVIEW AND AUTHORIZATIONS

This document covers the general protocols and activities related to terrestrial research on marine turtles in the Pacific Islands, including the Hawaiian Archipelago, Mariana Archipelago, American Samoa and associated islands, and the Fish and Widlife Refuges and Marine National Monuments in the Pacific.

Note that individuals or organizations granted permits are not necessarily authorized to conduct all of the activities listed in this document:

- Check boxes are included to the left of certain research activities.
- The permittee is only authorized to conduct that activity if the check box has been marked.
- If the checkbox has not been marked, the permittee is not authorized to conduct that activity.
- If there is no checkbox next to a guideline or activity, the permittee are required to adhere to directions stated therein.

A separate research proposal must be submitted for any research activities not covered in this protocol. Permittees must demonstrate training and experience to be authorized for select activities. If individuals want to conduct research on activities for which they do not have experience, they can contact the NOAA Pacific Islands Fisheries Science Center's (PIFSC) Marine Turtle Biology and Assessment Program (MTBAP) (nmfs.pic.MTBAP@noaa.gov) to learn how to receive the necessary training.

Copies of all raw data collected under this permit must be provided on an annual basis by January of the following calendar year to:

- USFWS Pacific Islands Fish and Wildlife Office (PIFWO) – Recovery Permit Coordinator, Email: FW1PIE_RecPermitAnnRpt@fws.gov
- NOAA's PIFSC MTBAP – Email: nmfs.pic.MTBAP@noaa.gov

1. GENERAL MONITORING PROTOCOLS

Permittees must always use care when handling live animals to minimize stress and injuries (Phelan and Eckert, 2006). In the event of an emergency, contact:

- HAWAIIAN ARCHIPELAGO and areas not specifically listed below - Hawai'i Marine Animal Response Hotline at (888) 256-9840
- GUAM – Guam Division of Aquatic and Wildlife Resources (671) 735-3955 ext. 6;
- COMMONWEALTH of the NORTHERN MARIANA ISLANDS – CNMI Division of Fish and Wildlife's Sea Turtle Program Stranding Hotline at (671) 287-8537 or (671) 472-7200
- AMERICAN SAMOA - American Samoa Department of Marine and Wildlife Resources at (684) 633-4465 or (684) 633-5102)

1.1. Nesting surveys and monitoring

1.1.1. Lights and clothing

1.1.1.1. Night surveys will be conducted using ambient light when possible. When necessary, a flashlight using red LED bulbs or fitted with a red filter can be

used (Witherington and Martin, 1996; Choi and Eckert, 2009). When feasible, night-vision goggles are recommended in lieu of red light.

1.1.1.2. Use of cell phones, tablets or any other light emitting electronic with the exception of GPS units, PIT scanners and other approved research electronics) is prohibited during night surveys.

1.1.1.3. Dark clothing is to be worn during night surveys (Chacon et al., 2008).

1.1.2. Nesting turtles

1.1.2.1. Marine turtles attempting to nest should not be approached or restrained for research activities prior to initiating oviposition (see section 1.1.3.2 and section 2 for exceptions).

☐ 1.1.2.2. Permittees may approach a nesting turtle to confirm the status of nesting activity phase (e.g., excavating nest chamber, laying eggs, etc.). These should consist of brief approaches by a single person approaching from the rear of the turtle (Yanez, Gaos, and Arauz 2006).

☐ 1.1.2.3. Permittees may approach a nesting turtle for workup (see section 1.2.2. for authorizations) once oviposition has initiated.

☐ 1.1.2.4. In situations where a turtle does not deposit eggs and is returning to the sea, the turtle can be briefly restrained for workup (see section 1.2.2.).

1.1.2.5. At all times other than those referenced above, permittees should remain out of sight of nesting turtles, including at least 30 feet (Pierre-nathoniel, 2006), or as far as possible if working on small beaches (i.e., where 30 feet is unavailable).

1.1.3. Hatchlings

1.1.3.1. Do not touch or interfere with hatchlings as they emerge from the nest or crawl to the ocean. Objects that present a major barrier to a hatchlings ability to reach the ocean may be cleared.

1.1.3.2. Keep a minimum distance of 10-feet away from hatchlings emerging from the nest and crawling from the beach to the water. See exceptions in sections 1.1.3.3. and 4.2.

☐ 1.1.3.3. Hatchlings that are in immediate danger from a threat (e.g., entrapment, depredation) can be moved the minimum distance necessary to avoid the threat and immediately allowed to continue their natural post-hatching activity.

1.1.4. Filming and photography

1.1.4.1. Use of artificial white light or flashes for filming or photos is strictly prohibited due to potential adverse impacts on both adults and hatchlings (Witherington and Martin, 1996).

1.1.4.2. A camera capable of infrared photo or videography can be used.

1.1.4.3. Any photos or filming must maintain a minimum distance of 10 feet from hatchlings and 30 feet from basking or nesting turtles.

☐ **1.2. Retention of nesting females and basking turtles**

1.2.1. *Start of retention:* For nesting turtles, flipper tagging, tissue sampling and electronic tag application shall be initiated after completion of successful nesting or

when a turtle abandons a nesting attempt (Balazs and Chaloupka, 2004; Jones et al., 2013). Basking turtles may be retained for the aforementioned purposes at any time.

1.2.2. *Data collection:* Nesting females (after completing oviposition) and basking turtles may be restrained for short periods of time (not to exceed 15 minutes), with the exception of section 1.2.3 to:

- ☐ 1.2.2.1. Record standard morphometric measurements (Eckert et al., 1999)
- ☐ 1.2.2.2. Check for Inconel flipper and passive integrated transponder (PIT) tags
- ☐ 1.2.2.3. Attach Inconel flipper tags (Eckert and Beggs, 2006)
- ☐ 1.2.2.4. Insert PIT tags (Eckert and Beggs, 2006)
- ☐ 1.2.2.5. Take biopsy tissue sample (Dutton et al., 1996)
- ☐ 1.2.2.6. Remove previously applied electronic tags (Mitchell, 2000a)

☐ 1.2.3. *Electronic tags:* Nesting female or basking turtles may be restrained for up to four hours to attach electronic tags (Mitchell, 2000b; Jones *et al.*, 2011; Martin, Gaos and Jones, 2018). See section 2.3 for details.

☐ **1.3. Handling of eggs**

1.3.1. *Clutches at risk*: Eggs that have a high probability (>80%) of inundation or erosion can be moved up to six hours after oviposition (LeBlanc et al., 2012), but care must be taken to maintain the axial orientation of each egg to avoid killing the embryos (Limpus, Baker and Miller, 1979).

1.3.2. *Sanitation:* All egg handling must be done with sanitary latex or nitrile gloves and hands should be clean of all chemical residues (e.g., sunscreen, insect repellent, etc.).

1.4. Fibropapilloma (FP) considerations

When tagging or sampling green turtles displaying FP tumors or lesions, the permittee must:

1.4.1. *Equipment:* Maintain a separate set of equipment for use on animals displaying FP tumors or lesions.

1.4.2. *Sanitation:* Clean all equipment (tagging supplies, calipers, tape measures, razor blades, etc.) using 70% alcohol (isopropyl or ethanol) or a surgical scrub (e.g. betadine scrub or chlorhexidine gluconate) prior to reuse.

2. MARKING AND TAGGING

2.1. Short-term turtle marking (shell painting and etching)

Shell painting and etching (hardshell species only) provides a temporary marking that allows researchers to distinguish between turtles on a beach and subsequently, while at sea.

☐ 2.1.1. *Etching and painting techniques:* An etching tool (e.g., Dremel Moto-Tool) with a "pear-shaped" bit can be used to place an etch or groove in the carapace (Hogarth, 2007). Non-toxic paint can be applied to the etching or directly to a non-etched shell (Hogarth, 2007).

☐ 2.1.2. *Basking turtles:* basking turtles may be approached when crawling up the beach or while basking in order to apply temporary paint or shell etchings.

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2.1.3. *Nesting turtles:* Nesting turtles can be temporarily marked using paint or shell etching once completing oviposition. In situations where a turtle does not deposit eggs and is returning to the sea (i.e., false crawl), the turtle can be briefly restrained for workup (see section 1.2.2)(Hogarth, 2007)

2.2. Long-term turtle marking (Inconel and PIT tags)

☐ 2.2.1. *Previous tags:* All marine turtles should be examined for existing tags. If existing tags are found, the tag identification code (and tag location) should be recorded.

☐ *2.2.2. Inconel flipper tags*

2.2.2.1. *Tag preparation:* Prior to use, the oily residue from the manufacturing of the Inconel should be removed by cleaning the tags in hot soapy water and rinsing, or soaking in alcohol (Eckert and Beggs, 2006).

2.2.2.2. *Sanitation:* Identify where the tag will be applied and clean the area with 70% alcohol (isopropyl or ethanol) or a surgical scrub (e.g. betadine scrub or chlorhexidine gluconate).

2.2.2.3. *Tag placement:* Inconel flipper tags can be applied along the trailing edge of both front or rear flippers. The placement of tags varies (Van Dam and Diez, 1999) and can follow historical placement of individual projects. Tags on front flippers can be attached adjacent to or through the first or second large scales. On the hind flippers, the tagging site is adjacent to or through the first large scale. In general, placing a tag closer to the axilla will minimize tag loss (Eckert and Beggs, 2006). Attach Inconel tags so they extend from the edge of the flipper by approximately 25% the length of the tag (Van Dam and Diez, 1999; Eckert and Beggs, 2006). Ensure the tag is placed with the tag number facing upwards.

2.2.2.4. *Tag securement:* Once the tag has been attached, turn the flipper over and examine the bottom of the tag to confirm that the tag has penetrated and that the tip (tine) is completely bent over and secure. An Inconel tag that is not secure can often be re-crimped with the tagging pliers. If this fails, remove the tag carefully and try again with a new tag, using the same puncture hole if possible (Eckert and Beggs, 2006).

☐ *2.2.3. PIT tags*

2.2.3.1. *Sanitation:* Identify where the tag will be applied and clean the area with 70% alcohol (isopropyl or ethanol) or a surgical scrub (e.g. betadine scrub or chlorhexidine gluconate).

2.2.3.2. *Tag placement:* PIT tags can be injected to both rear or front flippers, or in the shoulder areas (Eckert and Beggs, 2006). The syringe containing the PIT tag should be inserted at a seam between scales so the tag is placed between phalanges.

2.2.3.3. *PIT insertion and sanitation:* Insert the needle at an acute angle parallel with the skin of the flipper, with the needle directed proximally (toward the turtle) and the terminal opening of the needle should face upward). Once the plunger has been used to insert the tag and the needle has been withdrawn, place a piece of cotton or gauze with 70% alcohol (isopropyl or ethanol) or a

surgical scrub (e.g. betadine scrub or chlorhexidine gluconate) over the needle entry point and maintain pressure for approximately one minute, or longer if bleeding occurs.

2.2.3.4. *Tag confirmation:* Swipe the PIT tag reader over the tagged flipper to confirm the tag is working and the tag number has been properly recorded.

☐ **2.3. Electronic tags (satellite telemetry, radio/sonic, archival)**

Satellite telemetry, radio/sonic and archival tags may only be attached to the carapace of a turtle. See section 1.2 for information on restraining times.

2.3.1.1. *Conditions:* During the time that a nesting turtle is held for electronic tag attachment, it should be kept in shaded area to avoid overheating and a damp cloth should be placed over its eyes to reduce stress.

2.3.1.2. *Methods:* Follow established electronic tag attachment methods (e.g., (Mitchell, 2000a; Jones *et al.*, 2018)

2.3.1.3. *Lighting:* White light is permitted during sat tag application.

3. BIOLOGICAL SAMPLING

All biological samples collected via this permit are to be archived at the Pacific Islands Fisheries Science Center (PIFSC).

☐ **3.1. Skin sampling**

3.1.1. *Method:* A new biopsy punch or razor blade should be used to remove a superficial tissue (~6mm diameter) from the shoulder/neck area or from the hind flippers (Dutton et al., 1996).

3.1.2. *Number of samples:* Two skin samples can be collected from each turtle.

3.1.3. *Sanitation:* Thoroughly clean the tissue surface with 70% alcohol (isopropyl or ethanol) or a surgical scrub (e.g. betadine scrub or chlorhexidine gluconate) prior to and after sampling.

☐ **3.2. Blood sampling**

3.2.1. *Method:* A blood sample (≤ 5mL/kg) can be collected by inserting a sterile needle into the venous sinus on the lateral dorsal region of the neck (Bentley and Dunbar-Cooper, 1980; Owens and Ruiz, 1980).

3.2.2. *Number of samples:* No more than two attempts will be made per dorsal sinus (4 attempts total per turtle) and a new needle will be used between blood sampling attempts.

3.2.3. *Sanitation:* Thoroughly clean the tissue surface with 70% alcohol (isopropyl or ethanol) or a surgical scrub (e.g. betadine scrub or chlorhexidine gluconate) prior to and after sampling.

☐ **3.3. Scute sampling**

3.3.1. *Method:* Scrapings (<0.5mm) can be collected from the central or posterior lateral scutes of hard-shelled turtles to collect keratin (Cardona, Aguilar and Pazos, 2009).

3.3.2. *Precautions:* Avoid (1) portions of the scute that have had previous injury, (2) scute seams, and (3) scraping too deeply.

☐ **3.4. Fibropapilloma sampling**
Tumor biopsies can be taken using the same biopsy procedure as described for skin sampling (see section 3.1)

☐ **3.5. Oral swabs**
A clean cotton swab can be rubbed inside the mouth to collect saliva samples.

☐ **3.6. Cloaca Swab**
A clean cotton swab can be moistened with saline solution and gently inserted into the cloaca of adult turtles to collect cloacal samples

3.7. Urine and/or fecal collection
Place a plastic conical tube or bag on or near the cloaca/penis when the animal is seen urinating or defecating (Amorocho and Reina, 2007).

☐ **4. NEST EXCAVATIONS**
Nests may be excavated to collect data on outcome and to rescue any trapped hatchlings.

4.1. Excavation dates/times
4.1.1. *Observed hatches:* Excavations will not be conducted until at least 24 hours after a hatchling emergence has taken place.
☐ 4.1.2. *Unobserved hatches:* Nests that have been previously marked and continuously monitored, or that appear to have previously hatched (i.e., sand depression, scattered eggs shells, etc.), the permittee is authorized to excavate them after a certain time period. However, incubation periods can vary within and among years and species. Hawksbill incubation periods in Pacific Islands average 63 days, but range from 49 to 91 days. Green turtle incubation periods in Pacific Islands average of 70 days, but range from 54 to 91 days. Given this context, whenever possible the permittee should use the incubation period of other nests that have been monitored and successfully hatched during a similar time period as a proxy for when the unhatched nest can be excavated. If such information is not available, the permittee must wait a minimum of 70 days and 80 days before attempting to excavate a hawksbill or green turtle nest, respectively. At that time the permittee should carefully dig up the sand until reaching the first few eggs and examine their condition. If they are sealed and white, indicating the eggs are potentially still incubating, they should be recovered and given an additional week prior to repeating the process. Nests can be excavated without reservation at 92 days for hawksbills and 100 days for green turtles.

4.2. Live hatchlings
All hatchlings found to be trapped in their nest by natural or manmade impediments should be held in a covered container (i.e., remain in the dark) until excavation of that nest has been completed. Upon completion of nest excavation, hatchlings should be immediately placed approximately two meters above the high tide line and allowed to crawl to the ocean on their own.

4.3. Samples

Unhatched eggs and dead hatchlings will be sent to the PIFSC for archiving and analysis unless otherwise specified. The contents from each nest must be placed in an individual bag (i.e., contents from two or more nests should never be placed in the same bag). Each bag should be labelled with the following information (at minimum):

4.3.1. Date of excavation
4.3.2. Location of nest
4.3.3. Name of person conducting the excavation.
4.3.4. Any other information associated with the nest (i.e., nest monitoring data sheet, nest excavation data sheet.

☐ **4.4. Egg categories**

For nests being inventoried in the field, data should be recorded on the number of:

4.4.1. Empty egg shells (only count those consisting of >50% of shell)
4.4.2. Dead hatchlings found in the nest
4.4.3. Live hatchlings found in the nest.
☐ 4.4.4. In cases where the permittee has been authorized to open whole eggs found in the nest instead of sending them to PIFSC, the contents of eggs can be categorized as follows:
  4.4.4.1. Undeveloped – no signs of embryonic development.
  4.4.4.2. Developed – signs of embryonic development
  4.4.4.3. Pipped – eggs that have holes and often contain ants, larvae, etc.
4.4.5. Nest predation: Note if there are any obvious signs of nest predation (e.g., mongoose, rats, etc.).

4.5. Nest remnants

Unless otherwise permitted, all remnants of the excavated nests that are not being submitted to PIFSC will be placed in the original nest chamber or reburied along the beach in order to maintain natural beach nourishment regimes (Lutz, Musick and Wyneken, 2003).

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