National Sea Turtle Research Standard Methods Manual

Erin McMichael, Amy Hapeman, Brian Stacy, Lesley Stokes, Jeffrey Seminoff, Heather Haas, Summer Martin, Chris Sasso, Stacy Hargrove, and Karen Frutchey

U.S. Department of Commerce
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Cover Photo: Pacific green turtle off the coast of the Southwestern United States. Photo courtesy of Amy Baldwin-Granger.

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EXECUTIVE SUMMARY

All sea turtle species occurring in U.S. waters are protected under the Endangered Species Act of 1973 (ESA) and as such are afforded special conservation status and rely on continued management actions for their recovery. In U.S. waters, NOAA Fisheries is the federal agency responsible for the management, conservation, and recovery of sea turtles. Research, guided by sea turtle recovery plans, is an important part of this process as it provides sound scientific knowledge that increases our understanding of sea turtle biology and ecology, allowing for more targeted and successful management actions. However, sea turtles are notoriously difficult to study due to their cryptic, largely marine existence and complicated life history. Research protocols may vary according to research logistics, species, life history stage, and habitat, further complicating scientific research efforts and data comparisons across research studies. This document sources from regional methodologies employed in the U.S. to provide research protocols that can be implemented broadly where appropriate through a shared national vision of sea turtle research standard methods applicable to in-water activities. We provide an initial compendium of standard research methods, with standard methods defined as those that are routine and not likely to change substantially except for minor improvements based on the latest scientific advancements that are implemented regularly with known impacts. We provide general information on the purpose and description of each standard method and include relevant information on animal welfare, human training, and safety for each method and example protocols as appendices. The primary goal of this Technical Memorandum is to help streamline scientific research permit application reviews required under Section 10 of the ESA and internal Institutional Animal Care and Use Committee research approvals as required under the Animal Welfare Act. Ideally, the information contained herein contributes to developing standardized sampling and data collection protocols and methodologies listed as recovery actions in U.S. sea turtle recovery plans. We hope that this document also serves as a reference guide for researchers in other regions of the world. As such, we provide an initial framework for standardized research methods that may lead to the collection of comparable data throughout the many regions sea turtles inhabit.

1. INTRODUCTION

All sea turtle species in U.S. waters have special conservation status under the Endangered Species Act of 1973 (ESA). NOAA Fisheries, also known as National Marine Fisheries Service (NMFS), is the federal agency responsible for implementing in-water conservation and management actions that promote the recovery of these species. Scientific research is essential to the recovery process as it increases our understanding of sea turtle biology and ecology and allows for more effective conservation and adaptive management decisions.

Any in-water sea turtle research conducted in U.S. waters requires ESA Section 10 scientific research permits[1] that are issued by NOAA Fisheries' Office of Protected Resources (OPR) Permitting Division. The permitting process involves a thorough review of all proposed research activities and associated take. The Animal Welfare Act requires that all research and monitoring activities conducted by NOAA Fisheries' scientists must also be reviewed and approved by NMFS internal Institutional Animal Care and Use Committees (IACUCs) and that a Letter of Assurance must be issued prior to obtaining the requisite scientific research permits.

Under the ESA, in U.S. waters a take means to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, collect, or attempt to do any of the preceding of an ESA-listed species or its parts (50 CFR 222.102).

NOAA Fisheries' OPR issued an ESA Section 7 Programmatic Biological Opinion (BiOp) for OPR's Sea Turtle Permitting Program in December 2017 (NMFS, 2017) with amendments in 2019 (NMFS, 2019). This BiOp supported efforts to streamline ESA Section 7 consultations and thereby issuance of scientific research permits for commonly used sea turtle research methods and activities. At the project-specific consultation stage, a proposed activity is reviewed to determine if it can be implemented in accordance with the described research protocols and mitigation measures identified in the programmatic consultation. However, this Programmatic BiOp is broad in scope and does not include specific details for some methodologies as required during NOAA Fisheries internal IACUC research review and approval.

This Technical Memorandum (TM) has been developed to fulfill the needs of both NMFS IACUC and ESA Section 10(a)(1)(A) research permit application reviews by providing an initial compendium of many broadly accepted research protocols and best practices (hereafter referred to as standard methods) for sea turtle research. Recovery Plans for U.S. sea turtle populations and/or management units identify the development of sampling and data collection protocols and methodologies as important recovery actions (NMFS and USFWS, 2008). This TM also serves as a first step in identifying and describing such protocols.

[1] NOAA Fisheries ESA Scientific Research Enhancement Permits. [Available at https://www.fisheries.noaa.gov/permit/esa-scientific-research-and-enhancement-permits]

This *National Sea Turtle Research Standard Methods Manual* will facilitate NOAA Fisheries internal IACUC reviews and all ESA Section 10(a)(1)(A) permitting by enabling faster reviews and approval of NMFS sea turtle research permit applications in the U.S., as well as providing a standalone resource for researchers around the world. We define standard methods as those that are routine and not likely to change substantially other than minor improvements based on the latest scientific advancements and are implemented regularly with known impacts. We provide general information on the purpose and description of each standard method and include relevant information on animal welfare and safety and human training and safety for each method. Methods can be combined depending on research objectives. As many methods are not always executed in the exact same manner, we include example protocols as appendices. Throughout the document, information related to NMFS U.S. permit requirements and mitigation measures described by NMFS (2019) are contained in blue boxes. In addition to these standard methods, we provide concise descriptions of some procedures that have been permitted by NMFS but are not commonly used, as well as methods that are now considered infrequent or historical. We recognize that there are additional commonly used methods, especially outside of the U.S., that are not included in this TM.

We also recognize that additional details may need to be provided for NMFS IACUC and ESA Section 10 permit approval. In addition, researchers must be familiar with other applicable laws and regulations such as those governing the use of medications and veterinary medicine. For example, all research personnel must follow approved IACUC protocols for ongoing approved research. All new internal applications seeking NMFS IACUC approval should refer to this document and include other information required per NMFS Policy and Procedural Directives for Animal Use and Care and in accordance with Animal Welfare Act Regulations[2] and the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.[3]

This document sources from regional methodologies employed in the U.S. to provide research protocols that can be implemented broadly where appropriate through a shared national vision of sea turtle research standard methods applicable to in-water activities. Permit applicants will be able to cite this document when including these standard research methods within their permit applications. As current methods evolve and new methods are introduced, they will be reviewed by the working group and included as appropriate in later versions of this TM. Inclusion of a method will be based on the criteria outlined above regarding its potential impacts, use in the scientific community, and guidance from the Turtle Working Group that created this document. In addition, NOAA Fisheries will create and maintain a public website where updated or new standard NOAA Fisheries-approved methods will be posted.

Although our primary focus is identifying standard methods for in-water sea turtle research in U.S. waters, we hope that this document will also serve as a reference guide for researchers in other regions of the world. As such, we provide an initial framework for standardized research methods that may lead to the collection of comparable data throughout the many regions that sea turtles inhabit.

[2] Animal Welfare Act Regulations. [Available at https://www.aphis.usda.gov/media/document/17164/file]
[3] U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. [Available at https://olaw.nih.gov/policies-laws/gov-principles.htm]

2. EXPECT THE UNEXPECTED

2.1 Animal Emergencies

Conducting field research with live animals almost always involves some risk to the animal's health and welfare and to the field personnel involved. Researchers must be prepared for unexpected emergencies and have protocols in place that lessen the impacts of these emergencies (Manire et al., 2017). For example, NMFS (2019) requires that researchers have an experienced veterinarian on site or on-call for animal emergencies in the event that a sea turtle is found to have or develops a medical problem during research activities. Detailed information on NMFS requirements that ensure researchers are prepared for emergency situations are included in the blue boxes below.

NMFS permits require that researchers must have an experienced sea turtle veterinarian on call for emergencies, and that a permitted rehabilitation facility(ies) be identified should veterinary care be required on shore to treat a compromised turtle. Compromised turtles include animals that are stranded, obviously weak, lethargic, positively buoyant, emaciated, or that have severe injuries or other debilitating abnormalities. Prior to conducting research, field personnel must notify both the veterinarian and facility of the dates and times of the research to ensure their availability.

It is good practice to carry an animal first aid kit into the field. Sterile wound flush (saline), gauze, bandaging, blood-clotting aids, and extra disposable gloves come in handy if an injured animal is encountered or unexpected bleeding occurs during a procedure (e.g., from a biopsy or tagging site).

Sea turtles can become unresponsive during capture and handling, including showing signs of prolonged oxygen deprivation or aspiration of water and physiological effects of prolonged or excessive exertion, overheating, or cooling, or as a consequence of injury or pre-existing disease. Compromised turtles include animals that are obviously weak, lethargic, positively buoyant, emaciated, or that have severe injuries or other abnormalities resulting in debilitation. Procedures to promote recovery in compromised and/or unresponsive turtles are listed in the blue box below.

NMFS permits specify that If an animal exhibits any major abnormalities (including weakness, lethargy, or unresponsiveness) or is severely injured during capture or handling, or is found to be severely injured or otherwise compromised upon capture, researchers must forgo or cease activities that will further stress the animal (erring on the side of caution) and contact the on-call veterinarian as soon as possible.

The following procedures are used to promote recovery:

1. Seek veterinary assistance as soon as possible.
2. Place the turtle on its plastron so that the turtle is right side up and elevate its hindquarters at least 6 inches (approximately 15–30 degrees) to facilitate expelling of any inhaled water. The amount of the elevation depends on the size of the turtle; greater elevations are needed for larger turtles.
3. Periodically, rock the turtle gently left to right and right to left by holding the outer edge of the carapace and lifting one side about 3 inches then alternating to the other side.
4. While attempting recovery, protect the animal from the elements and maintain an ambient temperature that is seasonally appropriate for a healthy free-ranging sea turtle (generally between 60–85 °F). Do not place an unresponsive or lethargic turtle into the water or into a container holding water.
5. Continue recovery efforts as long as feasible or until death is confirmed by the onset of rigor mortis (muscle stiffness), decomposition, or detection of cardiac arrest (i.e., using Doppler, electrocardiogram (ECG), or ultrasonography). Otherwise, recovery may still be possible, and procedures must continue, as it can take turtles many hours to recover. All reasonable efforts must be made to retain the carcass of turtles that die during directed research for subsequent necropsy unless conditions compromise human health and safety.

After initiating the resuscitation techniques above or for all other animal medical concerns, one of the following options must be implemented (in order of preference):

a. Contact the designated veterinarian immediately for consultation. If necessary, transfer the animal to the veterinarian as soon as possible or to a permitted rehabilitation facility to receive veterinary care.
b. If the veterinarian or permitted rehabilitation facility cannot be reached, err on the side of caution and bring the animal to shore for medical evaluation and rehabilitation at a permitted rehabilitation facility, as soon as possible.
c. If the animal cannot be taken to a permitted rehabilitation facility due to logistical or safety constraints, allow it to recuperate as conditions dictate, and return the animal to the water.

Although research is needed to collect data on these protected species, their welfare and health should not be put at risk during any research activities. If unexpected emergencies occur, make every effort to ensure the well-being of all research animals.

2.2 Human Safety Considerations

Human safety is a top priority as field conditions can be unpredictable with a range of risks. Some safety considerations and best practices include:

- Planning field campaigns.
- Defining roles and responsibilities for all field personnel prior to any research activities.
- Evaluating field sites and conditions.
- Identifying hazards.
- Creating emergency protocols.
- Training field personnel on emergency protocols.
- Ensuring that field equipment is in working order.
- Keeping emergency equipment and supplies (e.g., first aid kits, navigation, communication devices, maps, water, weather protection) readily available during all field operations.
- Carrying extra supplies for emergencies (e.g., water, sunscreen, and food).
- Scheduling check-ins with the designated local contact (on land).

Researchers also face risks from sea turtles, ranging from exposure to zoonotic diseases to physical hazards. Risks are often defined by research goals, study animals, and study sites. In general, it is good practice for field personnel to wear disposable gloves whenever possible. Researchers should also be familiar with defensive turtle behavior and be prepared to react quickly when this behavior occurs. For example, researchers should be aware of the turtle's head/mouth, flippers, and claws to prevent and avoid injury from being hit, bitten, and/or clawed. Moreover, researchers should be trained and supervised by experienced personnel while in the field and while implementing research methods. It is important that each research team take appropriate safety precautions to mitigate risks associated with field research.

3. IN-WATER CAPTURE

3.1 Overview

Capture of sea turtles in the marine environment includes selective methods that are likely to capture only the target animals and non-selective methods (e.g., trawls and entanglement nets) that may also capture non-target species. In general, even non-selective methods can effectively target sea turtles depending on site selection and method of deployment. All capture methods have some inherent risks for people, sea turtles, and other animals in the marine environment.

3.1.a Minimizing Risks During Capture: Safety, Animal Welfare, and Biosecurity Considerations

Any capture of wildlife species exposes people to various levels of risk. To minimize this risk, capture techniques may only be attempted by experienced personnel or those being trained by and working under the direct oversight of experienced individuals. Personnel roles and responsibilities should also be carefully choreographed since an uncoordinated team can lead to failures and added stress to both humans and animals. There must also be plans for exigencies since the capture of study animals does not always go as planned.

Specific measures to mitigate risks for turtles during capture aim to prevent unintended injury and mortality and minimize harmful physiological effects of overexertion and prolonged submergence. An important overarching principle is to maintain awareness of a sea turtle's response to capture and signs of any condition that may result in greater risk. In the majority of instances, capture is only attempted for sea turtles that are behaving normally and do not have major, non-healed injuries or other debilitating conditions. If previously unapparent abnormalities are detected and are deemed by the research leader or veterinarian/technician to create a significant additional risk of complication, research activities are aborted and the animal released or taken to an authorized rehabilitation facility.

Sea turtles are regularly monitored throughout capture to ensure that they remain active and alert and maintain regular respiration and are provided with an appropriate ambient temperature. Additional specific monitoring requirements may be necessary under some circumstances (e.g., heart rate monitoring for leatherback turtles), particularly with longer capture intervals or invasive procedures. See Section 5.4 Monitoring Vital Parameters and Appendix B: Special Considerations for Leatherback Turtles.

Although this is not an exhaustive list, good animal welfare and safety practices during capture include:

- Keeping all capture activities, including chase, submergence, and handling, as brief as possible to minimize the increased stress and associated physiological changes that accompany capture.
- Removing turtles from the net as quickly and as safely as possible.
- Aborting capture if sea turtles become visibly exhausted (e.g., evidenced by frequent surfacing intervals) or distressed (e.g., exhibit behaviors consistent with distress).
- Avoiding capture or sampling when the research leader or veterinarian/technician deems it a significant additional risk to injured turtles (unless otherwise stipulated, e.g., under specific authorized research objectives).
- Minimizing the risk of human-mediated transfer of transmissible diseases by disinfecting capture equipment, wearing disposable gloves whenever possible, and practicing good hand sanitation (thorough washing between the capture of each animal). See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

ESA Section 10(a)(1)(A) permits issued by NMFS OPR authorizing in-water sea turtle research and enhancement activities in U.S. waters require researchers to adhere to specific mitigation measures stipulated in the issued permit. Although this manual outlines many of these measures, additional actions may be required by NMFS Section 10 permits. These measures are designed to minimize and mitigate the impacts of capture to sea turtles and other species based on the best available science. As such, these measures may be revised as new information becomes available.

3.1.b Minimizing Impacts to Non-Target Species

Non-selective capture techniques pose a greater risk of interacting with non-target species, and specific attention should be given to any interactions with other protected species. Best practices to mitigate the risk of encountering other protected species are listed below in the blue box.

To mitigate the risk of encountering marine mammals or other protected species, NMFS permits require the following:

- Visually survey the study site for non-target species prior to and during net deployment.
- If non-target protected species are sighted near the netting site prior to or during net deployment, do not deploy nets (or immediately retrieve them) until the animals have left the area.
- For trawling, do not deploy nets or initiate trawling when researchers observe marine mammals, except dolphins or porpoises, within the vicinity of the study area. Allow marine mammals to leave or pass through the area safely before deploying nets.
- For seine and tangle nets, if marine mammals or other protected species enter the research area after the nets have been deployed, raise the lead line and/or float line and drop or slap the line on the water in an attempt to make marine mammals in the vicinity aware of the net.
- If marine mammals do not immediately leave the vicinity of the study area, retrieve the nets and re-deploy the nets once the area is clear of any signs of marine mammals.
- For trawls, avoid releasing fish in a manner that is easily accessible to dolphins.
- If a marine mammal or other protected species becomes entangled, immediately cease netting activities.
- If the animal is alive and uninjured, immediately free it from the net, cutting the net if necessary.

If a protected species is injured or killed, hold the injured animal or carcass and notify the appropriate agency or personnel. Follow the Terms and Conditions outlined in the NMFS permit, which include suspending permitted activities until the NMFS OPR Permitting Division has granted approval to continue.

3.2 Capture Methods

3.2.a Overview

Sea turtles are most commonly captured for research via hand capture (i.e., without nets) or with the use of a dip/scoop net, hoop net, encircle/strike net, entanglement net, pound nets, seine, or trawl net (NMFS, 2019). While there are additional ways in which turtles may be captured for research purposes and slight variations in capture techniques exist due to species, size, habitat type, environmental conditions, and vessel configurations, the sections below describe the most common capture methods.

3.2.b Selective Hand Capture

These capture methods involve one or more researchers getting in the water to capture the turtle by hand. There are slight variations in specific capture techniques depending on species, size, habitat type, environmental conditions, and vessel configurations.

3.2.b.1 Diving from a Vessel

Purpose: Capture of a single hard-shelled turtle.

Description:
Hand capture of sea turtles from a vessel usually requires a minimum of three people, the boat operator, a spotter, and a designated capture person (diver). The diver is positioned on the bow of the vessel, and all crew scan the water for turtles (Figure 1a). When a turtle is located, the vessel operator approaches it and maneuvers close enough for the diver to safely grasp it upon entering the water (Figure 1b). Turtles typically must be followed for some interval until their initial flight response diminishes and the vessel/diver can be maneuvered into an optimal position for attempted capture (Ehrhart and Ogren, 1999). Once the diver has a grasp of the turtle [e.g., the diver places one hand on the leading (cranial) edge of the carapace and the other hand on the trailing (caudal) edge], the turtle is tilted upward and guided to the surface (Figure 1c) to be taken onboard the research vessel (Figure 2). A second diver may assist with capture and surfacing for larger sea turtles and greater depths. See Appendix C for example protocols.

Typically, NMFS permits limit the number of hand capture attempts of an individual to three per day to limit chase-related exertion, associated physiological changes, and increased stress.

Figure 1. 1a.) Researchers positioned on the bow of the boat spot a turtle during vessel pursuit. Photo courtesy of NMFS Southeast Fisheries Science Center (SEFSC), NMFS Permit 21233. 1b.) A researcher then dives into water to hand-capture the turtle. Photo courtesy of Inwater Research Group (IRG), NMFS Permit 25696/21169 and Florida Fish and Wildlife Conservation Commission (FWC) Marine Turtle Permit (MTP) 125/204. 1c.) The researcher then brings the turtle to the research vessel so the turtle can be lifted on board. Note the position of the hands used to guide the turtle to the surface. Photo courtesy of NMFS SEFSC, NMFS Permit 21233.

Animal Welfare and Safety:

- Keep in-water chase activities as brief as possible to minimize the increased stress and associated physiological changes that accompany capture.
- During pursuit, the vessel operator maintains awareness of the turtle's location with the aid of the spotter and diver and must be careful to avoid passing over it or piloting the vessel in a manner that would place the animal in a potentially harmful situation (e.g., near a rotating propeller).
- In the event that the turtle's location is lost in proximity to the vessel or it nears the propeller, the operator must shift the engine into neutral until it is safe to re-engage.
- Once the diver is in the water, the vessel operator places the engine in neutral or otherwise maneuvers the vessel to avoid injury to the turtle.
- For circumstances in which capture personnel are both in the water and on board a vessel, sea turtles are boarded by crew lifting from above with the assistance and support of those in the water lifting from underneath (Figure 2). Avoid lifting by the extremities (distal to the elbow or knee).

Figure. 2. Lifting a turtle onto a research vessel using both in-water and vessel crew members. Note, the person in the boat is positioning their right hand to grab the base of the right flipper for lifting. Photo courtesy of NMFS Pacific Islands Fisheries Science Center (PIFSC), NMFS Permit 21260.

Human Training and Safety:

- Divers must not dive into the path of the vessel.
- If a shark is observed in the vicinity of a turtle, divers should not enter the water.
- Divers need to be aware of the water depth and distance between the turtle and the surface to avoid serious injury during capture.
- If a support vessel is used, once the diver is in the water, the vessel must not be maneuvered over the diver or put the diver at risk of being hit by the vessel or a rotating propeller. A spotter maintains visual contact with the diver until the turtle and diver are both safely on board the vessel.
- Divers should remain a safe distance from the vessel's rotating propeller(s) to avoid injury.
- When handling turtles, researchers must be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel.

3.2.b.2 Free-Diving/SCUBA

Purpose: Capture of a single hard-shelled turtle.

Description:
There are several circumstances when sea turtles can be approached and captured by hand while free-diving or using SCUBA (Ehrhart and Ogren, 1999). A second diver may be nearby in the water to serve as an observer and to assist if needed. In addition to catching turtles while free-swimming (Figure 3), divers also may be towed behind a vessel to facilitate underwater search. Once a sea

turtle is sighted, the diver signals to the boat operator and commences capture. The diver places one hand on the leading (cranial) edge of the carapace and the other hand on the trailing (caudal) edge, tilting the turtle upward and guiding it to the surface. The second diver may assist with capture and surfacing for larger sea turtles and greater depths. In some locations where the foraging habitat is accessible from land, there is no boat involved. The turtle may be placed in an inner tube with a bottom, or the snorkeler/diver may swim the turtle to shore while holding it with both hands.

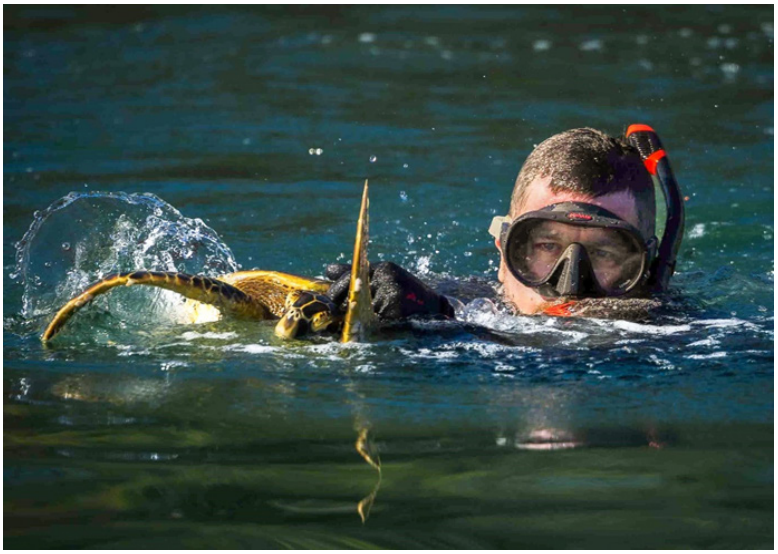

Figure 3. Capture of a hard-shelled turtle via free diving and/or snorkeling. Photo courtesy of NMFS PIFSC, NMFS Permit 21260.

Animal Welfare and Safety:

- Keep in-water chase activities and exertion as brief as possible to minimize the increased stress and associated physiological changes that accompany capture.
- If a support vessel is used, once the diver is in the water, do not maneuver the vessel over the diver or put the diver at risk of being hit by the vessel or a rotating propeller. A spotter maintains visual contact with the diver until the turtle and diver are both safely on board the vessel.

Human Training and Safety:

- If a shark is observed in the vicinity of a turtle, divers should not enter the water.
- A second diver may also be in the water to serve as an observer and to assist should any safety issues arise (e.g., blackout or injury).
- Divers need to be aware of the water depth and distance between the turtle and the surface to avoid serious injury during capture.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel.

3.2.c Selective Capture with Hand-Held Nets

3.2.c.1 Dip Nets

Purpose: Capture of a single hard-shelled turtle, often in shallow waters.

Description:
A dip net is usually a net affixed to a hoop that is attached to a handle. The hoop diameter varies depending on the species and life stage targeted but is generally large enough for targeted turtles to easily enter with the front flippers loosely held against the body (1 meter or smaller). The handle length depends on the capture platform but can be several meters. Various forms and modifications of dip nets exist for specific applications in terms of size and material. For example, collapsible hoop nets and small turtle hoists are sometimes used and have lines attached rather than a hard handle. Regardless of size and material, all function similarly in terms of the mechanism of capture. Dip nets can be used from a vessel, shore, and from structures such as jetties or piers. When used from a vessel, sea turtles are dip-netted at or near the sea surface at slow speed by placing the net behind the turtle and using the momentum of the vessel to move the turtle into the net or placing the net in front of the turtle and reversing the vessel (Witherington, 2002; Kubis et al., 2009; Figure 4). Once in the dip net, the turtle is carefully lifted out of the water and placed on the deck of the research vessel or other surface. See Appendix C for example protocols.

Figure. 4. Researchers using a dip net on the bow of the boat to capture a juvenile green turtle. A second researcher helps maneuver the net to bring the turtle safely on board the vessel. Photo courtesy of IRG, NMFS Permit 25696/21169 and FWC MTP 125/204.

In general, NMFS permits limit the number of dip net capture attempts of the same individual to three attempts per day to limit chase related exertion, associated physiological changes, and increased stress.

Animal Welfare and Safety:

- Keep in-water chase activities and exertion as brief as possible to minimize the increased stress and associated physiological changes that accompany capture.
- When dip netting from a vessel, the vessel operator must maintain awareness of the turtle's location with the aid of a spotter and be careful to avoid passing over it or piloting the vessel in a manner that would place the animal in a potentially harmful situation (e.g., near a rotating propeller).
- In the event that the turtle's location is lost in proximity to the vessel or it nears the propeller, the operator shifts the engine into neutral until it is safe to re-engage.

Human Training and Safety:

- Take precautions to ensure the safety of those handling dip nets as dip nets used from the bow of a moving vessel involve the risk of the researcher being tossed into the water or hazardous items in the environment.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel or to land.

3.2.c.2 Breakaway Hoop Net

Purpose: Capture of leatherback turtles and large hard-shelled turtles.

Description:

A breakaway hoop net is primarily used to capture leatherback sea turtles from a vessel (James et al., 2005a; James et al., 2005b; Benson et al., 2011; Figure 5) but may also be used to capture other sea turtle species. The basic configuration of these nets is similar to a dip net in terms of netting supported by a hoop with a handle. In U.S. waters, hoop nets are typically 1.5 meters in diameter with a 4-inch mesh (10-centimeter mesh; NMFS, 2019). However, instead of being permanently affixed to the hoop, the netting is attached by a mechanism that allows it to detach from the frame following deployment. A line threaded around the opening of the net is then pulled to close the opening and contain the turtle. The breakaway mechanism can be any device or material that appropriately detaches under tension (e.g., release clips used for fishing). A second line can be affixed to the hoop opposite the handle to help keep the hoop vertical in the water column during capture.

Leatherback turtles are most frequently sighted at the sea surface and are usually approached from behind during capture. Spotter aircraft are often used to locate turtles and advise the vessel during approach (Benson et al., 2011). The crew is often located at multiple positions on the vessel to help maneuver the net and assist with capture. Once a turtle is sighted and the crew is positioned along the vessel, the net is rapidly deployed into the water in front of the turtle, and the boat is quickly reversed until the turtle is securely in the net. Upon capture, the net detaches from the hoop, and the opening is cinched using the line to enclose the turtle. Once in the net, the turtle is brought onto a research platform, which may be a vessel modified for boarding or other platform (Figure 6). Wide straps (or equivalent) can be placed around the base of both front flippers to assist in bringing the turtle onboard (Figure 6). See James et al. (2005a; 2005b) and Benson et al. (2011) for successful field capture examples.

Figure 5. Series of steps involved in capturing leatherback turtles using a breakaway hoop net. Photos courtesy of NMFS Northeast Fisheries Science Center (NEFSC) and NMFS SEFSC, NMFS Permit 21233.

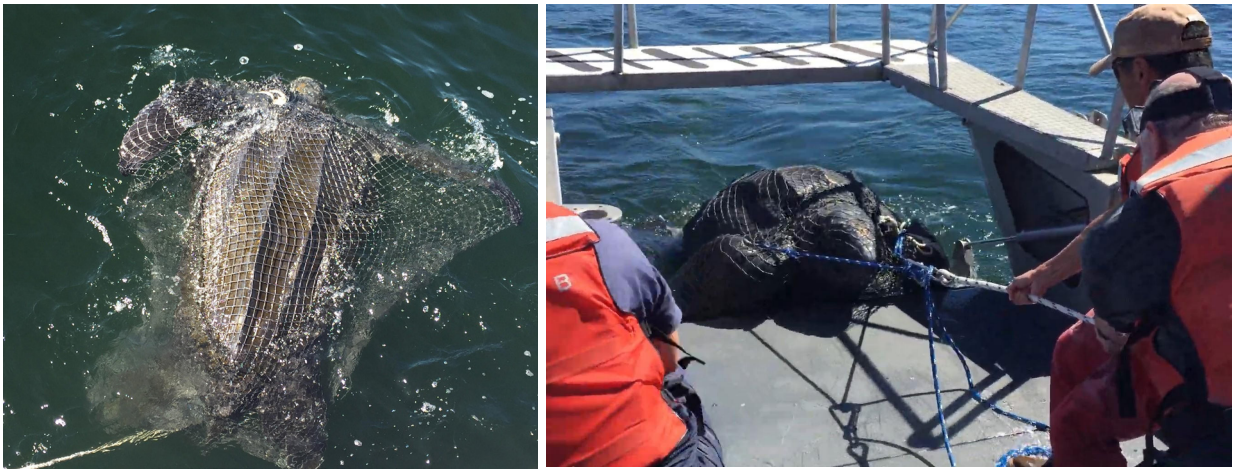

Figure 6. 6a.) Once a leatherback is securely in the net, the hoop is removed. 6b.) The turtle is then brought on board a modified vessel with a mechanical loading door. Photo courtesy of NMFS Southwest Fisheries Science Center (SWFSC), NMFS Permit 18238.

An important note about this capture method is that leatherbacks may exhibit defensive behavior in response to an approaching vessel or attempted netting (Figure 7). They will turn over onto their back, slap the water with their flippers, swim in fast erratic motions, and even make contact with the vessel. If this behavior occurs, it is generally best to abort capture and move away from the turtle as soon as the engine(s) can be safely engaged.

See Appendix C for example protocols.

Figure 7. Defensive behavior of leatherback turtles to avoid capture. 7a.) Leatherback on its back. 7b.) Leatherback slapping water with flippers. Photos courtesy of NMFS OPR (B. Stacy), NMFS Permit 21233.

Animal Welfare and Safety:

- Keep periods of capture and handling as brief as possible to minimize the turtle's stress and exertion.
- Only target leatherback sea turtles behaving normally with no evidence of recent external trauma.
- Design the research platform to safely and securely restrain the animal.
- Leatherbacks have relatively delicate skin; therefore, knotless netting is preferred to reduce abrasions during capture (NMFS, 2019).
- Use a hoop with a wide enough diameter to fit easily over the targeted turtle and keep the front flippers loosely held at the sides, which is essential for safe capture.
- Prior to boarding and while in the net, the turtle is monitored at all times to ensure that it can easily reach the surface to breathe.

NMFS permits require that all researchers limit the number of attempts to capture the same individual leatherback sea turtle to five per 24-hour period. If researchers are unsuccessful after the first three attempts, they must wait a minimum of four hours before making the final two attempts for the day.

For any opportunistic or targeted capture of leatherback turtles, researchers may only board leatherback turtles if they can be safely brought onto the vessel. NMFS permits specify that leatherbacks are not to be turned on their carapace and that researchers handle and support leatherbacks from underneath (NMFS, 2019). For directed research conducted on leatherback turtles, NMFS permits require that a designated medical observer is on each capture team that is specifically responsible for monitoring the turtle during all research activities. For additional leatherback requirements, see Appendix B: Special Considerations for Leatherback Turtles.

Human Training and Safety:

- Only personnel experienced with breakaway hoop net capture or those under the direct supervision of experienced personnel may perform this procedure.
- For leatherbacks, the powerful front flippers can cause significant injury and must be restrained at all times.
- Researchers must be mindful of entanglement hazards.
- If capturing large hard-shelled turtles, all researchers handling turtles must be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel.

3.2.c.3 Encircle Net/Strike Net (Focal Net)

Purpose: Capture of one or more hard-shelled turtles.

Description:
An encircle net or strike net is a net deployed from a research vessel in quick fashion to encircle a targeted sea turtle (or group of turtles) upon sighting (Figure 8). Although strike nets can vary in size, shape, depth, and materials, in U.S. waters, research projects typically use nets up to 300 meters long with a 36-centimeter stretch-mesh nylon webbing, 4-meter depth, braided polyfoam float line, and braided lead core line (Ehrhart and Ogren, 1999; Witzell and Schmid, 2004; NMFS, 2019).

When a turtle is sighted, the net is deployed with an anchor off the stern of the research vessel at relatively high speed. After the anchor and initial portion of the net are in the water, the vessel encircles the turtle as the net is belayed off the vessel. Once the boat circles back to the initial starting point, the two ends of the net are brought together and held closed. Upon entanglement, the turtle is removed from the net as soon as possible and brought on board the vessel. At times, a diver may enter the water to assist with disentanglement and transfer of the turtle to the vessel. If there is a second vessel involved, the secondary vessel may retrieve the turtle from the net as the original vessel hauls the net in.

See Appendix C for example protocols.

Figure 8. Capture of a juvenile green sea turtle by encircling it with a strike net. Photo courtesy of ©DavidM.Barron/www.oxygengroup.com and USGS Florida Cooperative Fish and Wildlife Research Unit (FL Coop Unit) at University of Florida (UF), NMFS Permit 1299 and FWC MTP 094.

Animal Welfare and Safety:

- Keep periods of capture and handling as brief as possible to minimize stress and exertion.
- Use nets with mesh size designed to minimize bycatch of non-sea turtle species.
- After encircling the turtle, disengage the vessel motor if the turtle is not visible to prevent possible injury to the turtle.
- If necessary, cut net material from animals to minimize any adverse effects of entanglement and to aid in their removal from the net.
- If a diver enters the water, do not maneuver the vessel over the diver or put the diver at risk of being hit by the vessel or a rotating propeller. A spotter maintains visual contact with the diver until the turtle and diver are both safely on board the vessel.

Human Training and Safety:

- Divers must be aware of the net's location in the water to avoid entanglement in the net.
- All researchers handling turtles must be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel or to land.

3.2.c.4 Cast Net

Purpose: Capture of one or more hard-shelled turtles.

Description:
A cast net or throw net is a circular net bordered by small weights and connected to a line for retrieval. Cast netting is usually a quick capture procedure used by turtle researchers, and nets vary in size from approximately 1 to 4 meters in diameter (NMFS, 2019). Cast nets are manually thrown from a vessel or from shore upon sighting a turtle at the surface. Typically, the net spreads into a circular shape once it is thrown and then sinks into the water column due to the weights that are attached. The line is quickly pulled to close the net, and the turtle is caught as the net is hauled back in.

Animal Welfare and Safety:

- Keep periods of capture and handling as brief as possible to minimize stress and exertion.
- Select a net size that is large enough to easily accommodate the size of the turtle being targeted (i.e., at least a meter or so larger than the carapace).
- Avoid use in bottom types and around structures/habitat that may snag the net and prevent timely retrieval.

Human Training and Safety:

- Only personnel experienced with throwing a cast net should use this technique.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel or to land.

3.2.d Non-Selective Capture Nets

3.2.d.1 Entanglement Net

Purpose: Capture of one or more hard-shelled turtles.

Description:
A large mesh entanglement net is a commonly used type of fixed gear for capturing turtles, typically in nearshore shallow waters. The nets are set at the surface and extend vertically through the water column. The top line floats at the surface, while the bottom line remains on or near the seafloor. Turtles swimming in the water column will become entangled in the net as they move through the area. When turtles become entangled, they are quickly removed from the net and boarded or brought to shore for processing (Ehrhart and Ogren, 1999; Seminoff et al., 2002b).

The top line of the net is often made of a braided line, which may have a foam core. Floats are attached to the top line. The bottom line is often a weighted continuous lead core line or a braided line with lead weights placed at regular distances. Anchors attached to both ends of the net keep it in position and prevent drifting of the net. The net mesh is usually tar-treated cotton line or monofilament line (80–100-pound test), and net mesh size is selected to minimize bycatch of non-turtle species but is not so large as to miss the smallest turtles potentially present in the study area. In U.S. waters, entanglement nets typically range in total length from 20 to 400 meters, with stretch mesh size ranging from 20 to 46 centimeters and net heights ranging from 1.5 to 8 meters, depending on the water depth in which the net is set (NMFS, 2019).

The netting area and the nets are continually visually monitored for the presence of turtles and other marine animals. When a sea turtle becomes entangled in the net, it most commonly is seen breaking the surface of the water and/or visibly causing movement of the surface floats and/or line. When this occurs, the net must be immediately and thoroughly checked and the animal removed from the net. However, there are instances when net movement is not visible despite an animal being caught in the net. Therefore, researchers must continuously monitor and physically check the net regardless of any observed movement. “Net checking” is defined as a thorough check of the net either by snorkeling along the net in clear water (entire net must be visible) or by pulling up on the top line such that the full depth of the net is viewed along the entire length (NMFS, 2019; Figure 9). In turbid waters, researchers raise the bottom (i.e., lead) line when checking for turtles. Net set (or soak) times may vary by location and project so long as the net is continually visually monitored and checked at required intervals (e.g., every 20 to 30 minutes) to ensure that turtles can reach the surface to breathe.

See Appendix D for example protocols.

Figure 9. Researchers "net checking" an entanglement net for captures. Photo courtesy of Ralph Pace and NMFS SWFSC, NMFS Permit 14510.

NMFS permits require that highly visible surface buoys or floats are attached to the float line of each net, spaced at intervals of 10 yards or less. Researchers must continuously monitor and physically check the net during deployment. The maximum time between physically checking any single point of the net ranges between 20–30 minutes. Nets must be checked every 30 minutes and more frequently if turtles or other organisms are being observed in the net. The nets must be checked every 20 minutes or less if water temperatures are ≤ 10 °C (50 °F) or ≥ 30 °C (86 °F).

Animal Welfare and Safety:

- Because entanglement nets are a non-selective gear type and there is potential for bycatch of other protected species (e.g., marine mammals), consult the mitigation measures described in Section 3.1.b.
- Ensure that the net heights are such that the netting extends throughout the water column and provides sufficient slack for entangled turtles of any size to reach the surface to breathe.

NMFS permits require that researchers plan for unexpected circumstances or demands of the research activities to the extent possible and have the ability and resources to meet the net checking requirements at all times. Contingencies for inclement weather must be in place.
For example:

- If an animal is highly entangled and requires extra time and effort to remove from the net, researchers must have sufficient staff and resources to continue checking the rest of the net at the same time.
- If inclement weather is predicted that would prevent meeting the net checking requirements, researchers must remove nets in advance of the weather event.

Human Training and Safety:

- When snorkelers check the net, there is risk of the snorkeler becoming entangled. All possible precautions must be taken to avoid entanglement as this can be extremely dangerous for both the researcher and turtles entangled in the net.
- If conditions create extensive danger to the snorkeler and/or turtle, snorkelers should not engage with the net but instead alert team members on the vessel to a turtle's capture location so that the vessel can then move in to disentangle the turtle and board the turtle.
- Snorkelers should not check the net or engage with the net in turbid waters due to limitations on sightability and increased danger due to entanglement.
- Researchers should avoid having gear or other articles attached to their person to avoid net snags and/or entanglement when checking the net from the vessel.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel or to land.

3.2.d.2 Pound Net

Purpose: Capture of one or more hard-shelled turtles.

Description:
Pound nets are stationary gear typically used in coastal bays and sounds. Pound nets direct fish and other marine life into enclosures or "pounds" by means of barriers called "leaders" (Figure 10). Most pound nets have a middle section or "heart" located in between the leader and the pound. As animals swim along the leader into the heart, they are directed into the pound by way of a mesh tunnel or corridor. Animals captured in the net are collected by gathering up the bottom of the pound, working from the tunnel wall to the back wall of the pound until the catch is concentrated in a trough at the back of the pound (Epperly et al., 2007a). Sea turtles are then removed by hand or dip net and placed into the research vessel. Nets are checked at various intervals. Net checking is defined as a thorough check of the net such that the full depth of the net and leader are visible along the entire length. In the U.S., pound nets that are used in sea turtle research studies are checked every 24 hours or less (NMFS, 2019). See Appendix D for example protocols.

Figure 10. Pound net used to capture turtles in coastal waters. The leaders (right side of the photo) guide animals into the pound section (left side of photo). Photo courtesy of NMFS SEFSC, NMFS Permit 21233.

While the exact design of pound nets used in turtle research (e.g., lead mesh size and length, pound area, and use of escape panels for small fish) can vary, NMFS permits require that researchers use a mesh size of 1¾-inch stretched mesh or less in the pound and heart to reduce sea turtle entanglement and mortality. Pound nets and leaders must be checked every 24 hours or less.

Animal Welfare and Safety:

- Because pound nets are a non-selective gear type and there is potential for bycatch of other protected species (e.g., marine mammals), consult the mitigation measures outlined in Section 3.1.b.

Human Training and Safety:

- Because of the complexity and weight of pound nets, researchers must receive extensive training with experienced individuals to learn to safely operate this type of fishing gear prior to independent implementation.
- When fishing a pound net, researchers may be exposed to bycatch which may include fish, rays with spines, and other potentially hazardous animals. Care must be taken when handling these animals to avoid injury.
- When handling turtles, researchers must be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel or to land.

3.2.d.3 Seine Net

Purpose: Capture of one or more hard-shelled turtles.

Description:
Seine nets are used to capture sea turtles in close proximity to the shoreline. These nets are often considered small mesh gill nets that have double float and lead lines and are deployed approximately 100 to 200 meters offshore in a straight or slightly curved line running parallel to the shoreline. In U.S. waters, a typical haul seine net is up to 7 meters high/deep, up to 366 meters long, with a stretched mesh size of 20–30 centimeters (NMFS, 2019).

One end of the net is anchored in shallow water, on shore, or held by a vessel, while the other end is carried out to sea by another vessel. Once the seine is set, each boat at the ends of the net pulls it toward the beach at a speed of about 2 to 3 knots (NMFS, 2019). When the ends of the net are close to the beach, the net is pulled onto the beach by hand. Researchers on the boats may assist by doubling back out along each side of the net, attaching to the float line about 20 meters from the beach, and pulling the net toward the beach.

NMFS permits limit net pulls to 30 minutes or less.

In some cases, entanglement nets (described above) can be used as a set net placed over a mud bottom substrate and/or deployed in the manner of a seine net from the vessel or on shore. When using an entanglement net as a seine net, one side of the net is set with an anchor, and the other side is deployed from the vessel in a circular fashion and slowly pulled into the vessel or onto shore within a few minutes of deployment.

Animal Welfare and Safety:

- Because seine nets are a non-selective gear type and there is potential for bycatch of other protected species (e.g., marine mammals), refer to the mitigation measures outlined in Section 3.1.b.
- Because the net setting and retrieval process is rapid, quickly bring any turtle that becomes entangled in the net to shallow enough water to reach the surface and breathe.
- Disentangle and retrieve turtles as quickly as possible.

Human Training and Safety:

- When fishing a seine net, researchers may be exposed to bycatch, which may include fish, rays with spines, and other potentially hazardous animals. Care must be taken when handling these animals to avoid injury.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel or to land.

3.2.d.4 Trawl Net

Purpose: Capture any species and/or number of turtles.

Description:
Trawl nets are nets towed behind vessels to capture animals on the seafloor or within the water column. There are many different types of trawl nets and deployment configurations (e.g., paired otter trawl, skimmer trawl). Turtles and other marine life are captured in the cod end, which often consists of 4-inch stretched mesh to reduce non-target bycatch (Figure 10). Trawls nets are brought on board using winches, and turtles are removed carefully from the net and safely placed on deck. See Appendix D for example protocols.

Figure 11. 11a.) The trawl net begins to surface as it is retrieved at the end of a tow. 11b.) As the net is pulled in, turtles caught in the cod end become visible to those aboard the vessel. 11c.) The net continues to be retrieved until the turtle is brought on board the vessel and safely removed from the net. Photos courtesy of NMFS SEFSC, NMFS Permit 21233.

Because of the increased risk of mortality or serious injury associated with this gear, NMFS permits require that nets are towed for 30 minutes (bottom time) or less and in waters no deeper than 20 meters.

Animal Welfare and Safety:

- Because trawl nets are a non-selective gear type and there is potential for bycatch of other protected species (e.g., marine mammals), consult the mitigation measures outlined above in Section 3.1.b.
- Closely monitor animals within the gear during hauling to prevent injury of exposed or entrapped appendages.
- Do not drop turtles onto the deck when opening the cod end; the turtles must be resting on the deck before the cod end is opened.

Human Training and Safety:

- Be cautious around cables and lines under tension, unsecured trawl doors, and lines and netting underfoot.
- Researchers may be exposed to bycatch when nets are emptied on board the vessel. Bycatch may include fish, rays with spines, and other potentially hazardous animals. Care must be taken when handling these animals to avoid injury.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while bringing the turtle on board the vessel.

4. HANDLING, HOLDING CONDITIONS, AND RELEASE

4.1 Overview

Once a sea turtle is successfully captured, it is brought on board a research vessel or brought to land. Some research vessels have either robust lifting systems or use vessels with a low freeboard, cut-out in the gunwale, modified bow or stern, doors, or similar modifications/configurations to reduce the lift height required for boarding, especially for larger animals (e.g., leatherback turtles; see Appendix B: Special Considerations for Leatherback Turtles). If an animal is deemed appropriate for research activities, field crews handle turtles in various ways to collect a variety of data based on research priorities. The section below provides information on general handling, holding times and conditions, transportation to laboratory facilities, and release.

4.2 Minimizing Risks During Handling and Holding: Safety, Animal Welfare, and Biosecurity Considerations

Good practices in wildlife research include minimizing the risk of human-mediated transfer of transmissible diseases by disinfecting equipment and surfaces in contact with animals and avoiding translocation of animals and infectious materials. Researchers should wear disposable gloves whenever possible, especially if there is any risk of contact with breaches in the skin (e.g., biopsies, administering hemostasis), or to minimize opportunities for the contamination of sterile instruments. For circumstances where disposable gloves are impractical, good hand sanitation (thorough washing) between animals is recommended.

For sea turtles, fibropapillomatosis (FP) is perhaps the most well-known transmissible disease (Stacy et al., 2018; Figure 11), and steps must be taken to minimize and prevent the transmission of FP, especially to geographic regions where the disease is not known to occur. Although most sanitation and biosecurity practices for sea turtle research have been developed around FP, most of these measures are general good practices to reduce risks associated with other potential pathogens as well.

NMFS permits require that all measures possible must be exercised to minimize exposure and cross-contamination between affected turtles and those without apparent disease, including the following:

- Use designated equipment (e.g., tagging pliers, measuring tape, bins) for turtles with and without FP and thoroughly disinfect reusable equipment and surfaces between animals.
- After working at sites where FP is known to occur and before using equipment in areas where FP is not known to be present, is considered uncommon, or where there is limited or no information on FP prevalence, disinfect nets using a broadcidal solution and the product-recommended contact time or by thoroughly drying nets in sunlight to inactivate FP-associated herpesvirus.
- Appropriate disinfectants for tools and surfaces include 70 percent isopropyl alcohol, 10 percent bleach, and other virucidal solutions with proven efficacy against herpesviruses (NMFS, 2019).

Figure 12. Examples of external FP tumors. External tumors can form anywhere on the soft tissues of a sea turtle's body and sometimes on the plastron or carapace. Common external locations include the axillary and inguinal regions (where flippers attach to the body), neck and chin area, eyes, flippers and shoulder region, tail, and around the cloaca. Tumors can be rough and cauliflower-like and/or smooth. Photos courtesy of IRG, NMFS Permit 25696/ 21169 and FWC MTP 125/204.

4.3 Handling, Holding Conditions, and Release

Purpose: Required in due course of data and sample collection.

Description:

Researchers conduct an initial assessment of the animal's behavior and general condition (e.g., whether it is in discomfort, distress, or pain) to identify any abnormalities of concern. If the turtle is appropriate for further study, the turtle is then placed in a safe, shaded location (Figure 12) or restrained in a manner to ensure its safety, and ongoing monitoring begins. Hard-shelled sea turtles are often placed on a padded surface, prevented from accessing areas that may lead to injury (e.g., by using plastic tubs, barriers, or elevated restraint platforms), and protected from other sea turtles, as necessary (Figure 13). Sea turtles are monitored throughout handling to ensure that they remain alert and responsive to stimuli, maintain regular respiration, and are provided with an appropriate ambient temperature. Additional specific monitoring requirements

may be necessary under some circumstances (e.g., heart rate monitoring for leatherback turtles), particularly with longer capture intervals or invasive procedures. See Appendix B: Special Consideration for Leatherback Turtles for more information on monitoring leatherback turtles.

See Appendix E for example protocols.

NMFS permits for any opportunistic or directed capture of leatherback turtles require that researchers handle and support leatherback turtles from underneath and do not turn leatherback turtles on their carapace. Additional NMFS permit requirements for dedicated leatherback research are included in Appendix B: Special Considerations for Leatherback Turtles.

Figure 13. Various holding conditions during work-up. 13a.) Holding turtles on land during work-up in separate "turtle boxes" in the shade. Photo courtesy of NMFS SWFSC, NMFS Permit 18238. 13b.) Holding a turtle on board a vessel in a designated area free from debris and in the shade. Photo courtesy of NMFS SEFSC, NMFS Permit 21233. 13c.) Holding a green turtle on a tire with a damp towel over its head to limit movement during research. Photo courtesy of NMFS PIFSC, NMFS Permit 21260.

The following are NMFS permit handling and holding requirements:

- If an animal is or becomes highly stressed, injured, or unresponsive during capture or handling, or is found to be compromised or injured upon capture, researchers must forgo or cease activities that will further stress the animal (erring on the side of caution).
- Handling time for research procedures varies based on the activities; however, most are released within 1 hour, and almost all will be released within a few hours.
- NMFS permit applicants may request to temporarily transport and hold sea turtles for up to 36 hours from time of capture to release in a U.S. Fish and Wildlife Service (USFWS)-approved facility to accommodate some methods (e.g., fecal sampling, imaging techniques).
- For the transport, maintenance, and care of turtles temporarily held in a facility, researchers must follow the "Standard Conditions for Care and Maintenance of Captive Sea Turtles" issued by the USFWS[4] (USFWS, 2019) and, if in the State of Florida, the Florida Fish and Wildlife Conservation Commission (FFWCC) Marine Turtle Conservation Handbook,[5] Section 4, "Holding Turtles in Captivity" (FFWCC, 2016).
- NMFS permits require that all measures possible must be exercised to minimize exposure and cross-contamination between affected turtles and those without apparent disease (e.g., FP), including the use of disposable gloves when possible.
- To prevent injury during release, NMFS permits require that turtles are lowered as close to the water's surface as possible. Researchers must carefully monitor the newly released turtles' abilities to swim and dive in a normal manner. If a turtle is not behaving normally upon release, recapture the turtle, if safely feasible, and contact the on-call veterinarian. Release should occur as close to the capture site as possible to minimize disturbance of the animal's behavioral ecology. Exceptions within short distances are acceptable to avoid recapture if necessary.
- Researchers must protect sea turtles from temperature extremes [the ideal temperature for holding turtles out of the water ranges from 70 °F (21.1 °C) to 80 °F (26.7 °C) and keep animals moist when the temperature is at or greater than 75 °F (23.9 °C).

Animal Welfare and Safety:

- During lifting, large turtles must be supported from both the body and base of the flippers (proximal to the elbow/knee) using multiple points of contact and avoiding strain on the neck, joints, and the distal flippers to minimize injury.
- The location of shore-based activities must be as close to the capture area as possible to minimize the duration of transport and associated stress.
- Turtles are kept separately and constantly monitored (especially those prone to aggression) to prevent injury (Figure 13).

[4] USFWS Standard Conditions for Care and Maintenance of Captive Sea Turtles. [Available at https://www.fws.gov/media/standard-conditions-care-and-maintenance-captive-sea-turtles]

[5] Florida Fish and Wildlife Conservation Commission Marine Turtle Conservation Handbook. [Available at https://myfwc.com/media/3133/fwc-mtconservationhandbook.pdf]

- Turtles can be placed on top of padding (e.g., tires, cushions) within a confined area (e.g., plastic tub) or surrounded by non-abrasive barriers to limit movement and prevent injury and disease transmission (Figure 13).
- Each container, tire, divider, etc. must be thoroughly cleaned and disinfected between each use. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Enclosed spaces and containers must allow good airflow.
- The area surrounding the turtle must be free of materials that could be accidentally ingested or that could injure the turtle during holding (Figure 13).
- A clean, damp, or wet towel may be placed over the eyes, which can sometimes help keep turtles calm and prevent injury from struggling or excessive movement (Figure 13).
- Hard-shelled sea turtles can be turned on their backs for examination and brief procedures. Time on their backs must be as brief as possible to limit additional stress and compression of internal organs, especially after capture.
- Extra care must be exercised when handling, sampling, and releasing leatherback sea turtles. Leatherback sea turtles have more friable skin and softer bones and are more susceptible to external trauma. See Appendix B: Special Considerations for Leatherback Turtles.
- Turtles should be lowered onto the water's surface and then released (Figure 14).

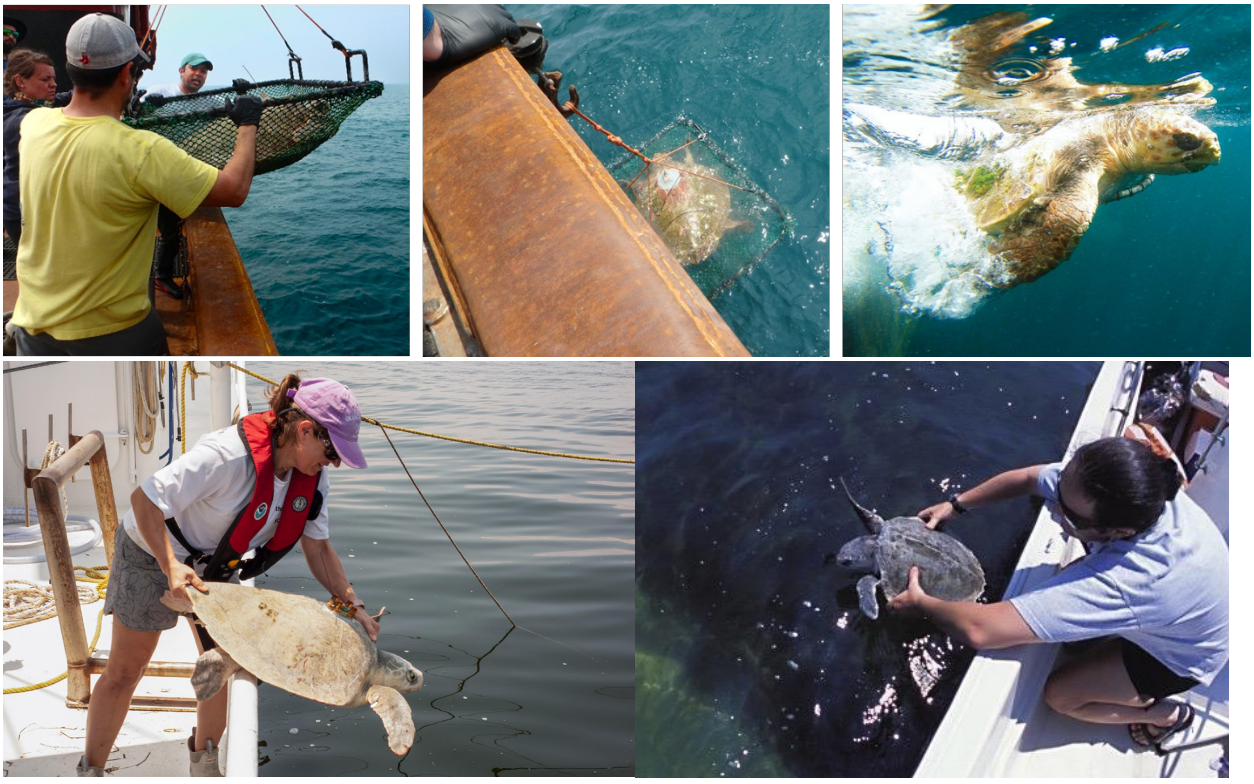

Figure 14. Turtles can be released manually or mechanically. 14a.–14c.) Turtles are lowered onto the water using a mechanical apparatus. Photo courtesy of Coonamessett Farm Foundation and NMFS NEFSC, NMFS Permit 18526. 14d. and 14e.) Turtles are manually brought as close to the water's surface as possible and then released. Photos courtesy of NMFS SEFSC, NMFS Permit 21233 and FL Coop Unit at UF and © David M. Barron/ www.oxygengroup.com, NMFS Permit 1299 and FWC MTP 094.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while handling turtles.

Refer to Appendix B: Special Consideration for Leatherback Turtles for NMFS permit training requirements for dedicated leatherback research activities.

5. EXAMINATION, MORPHOMETRICS, AND MONITORING

5.1 Overview

Upon capture, each turtle is visually assessed (i.e., looking for signs of illness, injury, entanglement, epibiont load, behavior, etc.) to determine its general state of health and suitability for research procedures (Herbst and Jacobson, 2003). For example, the mouth (oral cavity), eyes, and vent (cloaca) may be examined for signs of injury, ingestion of hooks or marine debris (e.g., plastics, tar balls), and disease. Additional information on oral cavity assessments can be found in Section 11.2. All turtles are assessed for the presence of FP tumors. Turtles are also inspected for the presence and/or evidence of tags and/or previous markings. This section outlines physical assessments, data collection, and monitoring of turtles during post-capture processing.

5.2 Photographs

Purpose: Document condition, physical characteristics, tags, distinguishing marks, injuries, or other abnormalities.

Description:
A variety of photographs may be taken of each animal captured including full body (e.g., head to tail), head, carapace, plastron, injuries, and any abnormalities (Figure 15). Photographs are often taken with an identification placard and an object of known size for scale reference (e.g., ruler). See Appendix F for example protocols.

Figure 15. Examples of photographs taken with placards. Photos courtesy of FL Coop Unit at UF, NMFS Permit 1299 and FWC MTP 094.

Animal Welfare and Safety:

- Although hard-shelled sea turtles can be turned on their backs for examination and brief procedures, hard-shelled turtles should not be left on their carapace for long periods of time especially after a capture event because of additional stress and poor ventilation due to compression of the lungs.
- Leatherbacks must not be turned over onto their carapace. See Appendix B: Special Considerations for Leatherback Turtles.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed.

5.3 Morphometrics

5.3.a Body Measurements

Purpose: Collect morphometric data related to life stage, sexual maturation, and body condition. With subsequent recapture, these data can also be used to study growth, ontogenetic shifts/recruitment, dispersal, and remigration (Chaloupka and Musick, 1997).

Description:

Turtles may be measured using a flexible measuring tape or calipers for curved and straight measurements, respectively (Figure 14). Depending on the program, historical data, species, and research questions, various carapace lengths (e.g., straight, curved) are collected based on key anatomical landmarks: the nuchal notch, pygal notch, and cranial and caudal-most projections. Additional body measurements may be taken as needed (e.g., straight and curved carapace width, maximum head length and width, plastron length, tail length, plastron-to-vent length, vent-to-tip length, body depth, and circumference), following protocols outlined in Bolten (1999) and Wyneken (2001). Because of variation from respiration, body depth can be measured three times after the turtle inhales and then averaged (Wyneken, 2001). See Appendix F for example protocols.

Figure 16. 16a.) Measuring straight carapace length using calipers. Photo courtesy of NMFS PIFSC, NMFS Permit 21260. 16b.) Measuring curved carapace width with flexible tape measure. Photo courtesy of IRG, NMFS Permit 25696/21169 and FWC MTP 125/204.

Animal Welfare and Safety:

- Place turtles on padded surfaces to help avoid injury while measurements are taken.
- Clean and disinfect all equipment (e.g., tagging equipment, tape measures, etc.) and surfaces that come in contact with each turtle. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed.

5.3.b Weight

Purpose: Measure the body mass of captured turtles as related to body condition, reproduction, and general health.

Description:
Sea turtles may be weighed using various types of scales (e.g., platform, suspension), depending on the turtle's size. In all instances, animals are supported using containers, specially designed harnesses, or netting that allow safe lifting practices that provide adequate support of the body during weighing (Figure 17). Larger turtles can be weighed using a portable tripod with a pulley system to lift the turtle (Bolten, 1999). All scales should be periodically calibrated to ensure accuracy. See Appendix F for example protocols.

Figure 17. 17a.) Weighing a leatherback turtle using a harness. Photo courtesy of NMFS SWFSC, NMFS Permit 18238. 17b.) Weighing a hard-shelled turtle using a rope harness and spring scale. Photo courtesy of NMFS SWFSC, NMFS Permit 18238. 17c.) Weighing a hard-shelled juvenile turtle on a vessel using a cargo net and spring scale. Photo courtesy of IRG, NMFS Permit 25696/21169 and FWC MTP 125/204.

Animal Welfare and Safety:

- All weighing devices must provide adequate support to the turtle's body to prevent injury.
- Care must be taken to ensure that turtles do not struggle and fall off of or out of weighing devices, especially depending on sea state.
- Equipment that comes into contact with animals must be cleaned and disinfected after each use. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:

- Weighing large turtles by non-mechanical means can present significant risk of physical injury and should always follow safety best practices.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during weighing.

5.4 Monitoring Vital Parameters

5.4.a Overview

Vital parameters may be measured as part of specific research objectives (e.g., physiological studies) or as a means of monitoring animals that appear more sensitive to the stress of capture or as deemed prudent based on the discretion of an attending veterinarian or lead researcher. These are rapid, non-invasive or minimally invasive procedures that measure health parameters across tissue membranes or surfaces. Manire et al. (2017) recommend that each research team should include a designated turtle monitor who is a veterinarian, veterinary technician, or a skilled animal care specialist with specific training in monitoring vital rates. Moreover, this individual should not have any other duties that distract from monitoring (NMFS, 2019).

5.4.b Heart Rate

Purpose: Monitor the heart rate of turtles as a physiological parameter or to assess clinical status.

Description:
Heart rate can be monitored using a Doppler blood flow detector, electrocardiogram, or ultrasonography probe. For reference, the normal heartbeat of a turtle at 24 °C (75 °F) ranges from 30–60 beats per minute but can vary substantially depending on species, size, activity, and stress levels (Manire et al., 2017).

- For Doppler flow detectors and visual ultrasonography, ultrasound gel is applied to the skin. A hand-held instrument (transducer) is then passed lightly over the skin to locate pulsatile flow within blood vessels or the heart (Figure 18).
- Electrocardiograms involve the placement of electrical adhesive, leads, clips, or probes in various configurations on the skin to measure electrical activity of the heart.
- A pulse oximeter is a medical device that indirectly measures the amount of oxygen in a patient's blood (as opposed to measuring oxygen saturation directly through a blood sample). Although this device is inaccurate for measuring blood oxygen levels in sea turtles, it can be used as an alternative measure of a sea turtle's heart rate (NMFS, 2019). Pulse oximetry probes come in various configurations that can be applied to the skin or inserted into the cloaca.

See Appendix F for example protocols.

Figure 18. Monitoring heart rate using a doppler flow detector. Photo courtesy of NMFS SEFSC, USFWS Permit TE676379.

Animal Welfare and Safety:

- Pulse oximetry probes must not be inserted into the cloaca if resistance is encountered.
- All equipment must be disinfected in between turtles. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:

- Those responsible for monitoring should be familiar with the use of these instruments in sea turtles and the normal parameter thresholds.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during monitoring.

5.4.c Respiration

Purpose: Monitoring the respiration of turtles as a physiological parameter or to assess clinical status.

Description:

Turtle respiration rate is monitored by visual and auditory detection of inhalation and exhalation. Respiratory rates vary with temperature and activity level. Manire et al. (2017) recommend that the respiratory rate, interval, and quality of breaths (shallow vs. deep) should be noted every 15 minutes. During in-water capture and processing, hypoventilation and prolonged periods of apnea may be an early sign of distress (Manire et al., 2017).

Animal Welfare and Safety:

- A designated turtle monitor may be included in the research team. This individual should focus on monitoring the turtle and have no other duties (Manire et al., 2017; NMFS, 2019).

Human Training and Safety:

- Those responsible for monitoring should be familiar with normal respiratory sounds and rates in sea turtles.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while monitoring respiration.

5.4.d. Temperature

Purpose: Measure body temperature as a physiological parameter or to monitor for hyperthermia or hypothermia during the course of research activities.

Description:
The temperature of sea turtles is often a representation of the temperature in their recent environment. Body temperature is measured in turtles using either a thermometer probe inserted into the cloaca or externally using an infrared thermometer (typically the neck/shoulder regions or prefemoral areas). Cloacal thermometers consist of a flexible thermistor probe (or equivalent) that is lubricated and gently inserted into the cloaca to the level of the caudal plastron or until resistance is encountered. See Appendix F for example protocols.

Animal Welfare and Safety:

- Cloacal thermometers must not be inserted if resistance is encountered.
- All equipment must be cleaned in between use on each turtle. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed while collecting temperature data.

6. IDENTIFICATION TAGGING AND CARAPACE MARKING

6.1 Overview

Tagging and marking are crucial methods used in sea turtle research, which allow for identification of individual turtles. Once tagged or marked, these individuals can be monitored during recapture events, and additional information can be collected on that individual's body condition, growth, maturation, habitat use, movement, and dispersal. Several tagging and marking techniques have been developed and used with varying degrees of success. For long-lived, wide ranging animals like sea turtles, tag retention is of utmost importance and allows monitoring of the individual over time. Tagging varies based on tag function, location, method, technology, and research goals. Here, we

provide an overview of standard identification tagging and marking commonly used during sea turtle research.

NMFS permits require that all measures possible must be exercised to minimize exposure and cross-contamination between affected turtles and those without apparent disease, including the use of disposable gloves, a separate set of equipment for processing turtles with FP, and thorough disinfection of equipment and surfaces. Appropriate disinfectants for equipment used with FP turtles include 70 percent isopropyl alcohol, 10 percent bleach, and other virucidal solutions with proven efficacy against herpesviruses.

6.2. Identification Tagging

6.2.a Overview

Tagging methods have evolved over the years, and although additional tagging methods have been used in the past (see Section 11.3), this section focuses on current tagging methods that are considered standard. Typically, tagging methods have minimal effects on individual turtles when conducted according to established protocols (NMFS and USFWS, 2008).

6.2.b Metal Flipper Tagging

Purpose: Long-term identification of individual turtles using visible external tags.

Description:
Metal (Inconel) flipper tags are likely the most common method for long-term identification of individual turtles. The typical application method for metal flipper tags is described by Balazs (1999). 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 other side (Figure 19). Proper seating of the tag into the pliers is essential to ensure a secure crimp (Figure 20). Tag retention for these tags varies; although some tags are retained for 30 years or more, some loss occurs after 2–4 years. Tags can be torn out of the flipper over time due to environmental factors and fouling with debris. Standard Inconel tags are only used in sea turtles over 30 centimeters in carapace length. Smaller metal (Monel) tags (1005 series or similar) are used by some researchers for temporary tagging of turtles that are 20–30 centimeters. These tags are intended to be replaced by standard tags if the turtle is re-encountered at a larger size.

Figure 19. Attaching metal flipper tags to different locations in hard-shelled turtles using a specialized applicator. 20a). Attaching a metal tag between the scales on the trailing edge of a front flipper of a loggerhead turtle. Photo courtesy of NMFS SEFSC, NMFS Permit 21233. 20b.) Attaching a metal tag through a scale on the trailing edge of the front flipper of a different loggerhead turtle. Photo courtesy of IRG, NMFS Permit 25696/21169 and FWC MTP 125/204.

Figure 20. A crimped Inconel metal flipper tag. The piercing side of the tag is locked into the receiver portion to ensure secure tag attachment and minimize tag loss. Photo courtesy of IRG, NMFS Permit 25696/21169 and FWC MTP 125/204.

Prior to applying new tags, each turtle is first inspected for existing flipper tags or signs of previously attached flipper tags. If at least two existing tags are found and are in good condition, no additional flipper tags are applied. If tags are damaged, detaching, or deteriorating, they may be removed and new ones applied at a nearby/alternative site. In most instances, the tag can be removed using two pairs of pliers to uncrimp the tip, but wire or bolt cutters may be necessary. If a previously applied tag is removed, the identification number is recorded, and the tag reported to the original tagging project, and, in the eastern U.S., to the Cooperative Marine Turtle Tagging

Project (CMTTP) at the Archie Carr Center for Sea Turtle Research (ACCSTR).[6] Other areas report their tags to the appropriate organization that maintains the corresponding regional metal flipper tag database.

The specific location of tag placement depends on the tagging program and species. The most commonly used locations in hard-shelled turtles are the proximal trailing (caudal) edge of front or rear flippers. In the front flippers, tags are placed either through or in between the largest, proximal-most scales, which most often reflects the training and preferences of the individual researcher or program (Figure19 and 21). In leatherbacks, metal flipper tags are often applied in the skin webbing between the tail and the hind flippers (Figure 21 and 22).

Figure 21. Various attachment sites for metal flipper tags. 21a.-12b.) Metal flipper tags attached to the trailing edge of the front flippers of hard-shelled turtles. Photos courtesy of FL Coop Unit at UF, NMFS Permit 1299 and FWC MTP 094, and IRG, NMFS Permit 25696/21169 and FWC MTP 125/204. 21c.) Metal flipper tag attached to right rear flipper of a hard-shelled turtle. Photo courtesy of NMFS PIFSC, NMFS Permit 21260. 21d.) Metal flipper tag attached to a leatherback turtle between the tail and right rear flipper. Photo courtesy of IRG, NMFS Permit 25696/21169 and FWC MTP 125/204.

[6] Cooperative Marine Turtle Tagging Project at the Archie Carr Center for Sea Turtle Research. [Available at https://accstr.ufl.edu/resources/tagging-program-cmttp/]

It is common practice to apply two flipper tags (one on the right and one on the left; FIgure 22), although other combinations are sometimes used. Double tagging minimizes the probability of complete tag loss. A single tag may be applied if a Passive Integrated Transponder (PIT) tag is also inserted or if a flipper is missing or has any other abnormality that would pose increased risks from tagging. If the tagging site has been injured or is unsuitable for tag application, an alternate site along the trailing edge of the flipper can be used. Metal flipper tagging is only expected to cause minimal and temporary discomfort with healing occurring within a short period of time (NMFS, 2019). See Appendix G for example protocols.

Figure 22. Double tagging with metal Inconel flipper tags. 22a.) A hard-shelled turtle (Kemp's ridley) tagged in each front flipper with metal Inconel flipper tags. Photo courtesy of FL Coop Unit at UF, NMFS Permit 1299 and FWC MTP 094. 22b.) A leatherback turtle double tagged with metal Inconel flipper tags attached to the skin webbing between the tail and both the left and right rear flippers. Photo courtesy of IRG, NMFS Permit 25696/21169 and FWC MTP 125/204.

NMFS permits require researchers to clean the flipper tag application site and then scrub it with a medical disinfectant solution (e.g., Betadine®, chlorhexidine) followed by 70 percent alcohol before the tag pierces the animal's skin. Researchers must also exercise all measures possible to minimize exposure and cross-contamination between turtles with any signs of disease (e.g., FP) and those without apparent disease, including the use of disposable gloves and thorough disinfection of equipment and surfaces.

NMFS requires that turtles greater than 30 centimeters straight carapace length (SCL) must be tagged with standard 681 flipper tags. Smaller tags (e.g., 1005 Monel series or similar) must be used for turtles 20–30 centimeters SCL. Turtles less than 20 centimeters SCL (nuchal notch to pygal tip) must not be tagged. Only one tag is applied to a flipper, with a maximum of two tags per turtle.

Animal Welfare and Safety:

- Prior to use, all tags and applicators must be cleaned and disinfected before first use and between animals. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Gloves must be worn when handling turtles with any signs of disease (e.g., FP). Refer to Section 4.2 for more information on handling turtles with FP. Also see Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- To accommodate future growth in young turtles, flipper tags are attached with additional space between the edge of the flipper and the curve of the tag.
- To avoid injury and minimize tag loss, researchers should ensure that the piercing tab of the tag is within the receiver portion and is securely folded (crimped; Figure 20).
- If any bleeding occurs after the tag has been injected, a swab soaked in povidone-iodine can be held to the injection site until the bleeding stops.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during tag application.

6.2.c Passive Integrated Transponder Tagging

Purpose: Long-term identification of individual turtles using small microchips that are inserted internally and emit a unique code when read.

Description:

PIT tags are small rice-sized microchips that emit a unique code when activated by a PIT tag reader. PIT tags are internally injected and are used because of their long retention time; they are not subject to environmental conditions that may cause tag loss (Parmenter, 1993; McDonald and Dutton, 1996; Balazs and Chaloupka, 2004). PIT tags can provide life-long identification, making them vital to sea turtle research. Tags are designed to be small, physiologically inert, and not hinder movement or cause chafing. In addition, currently available PIT tags are designed with a glass or plastic coating that promotes the growth of muscle fibers to heal and hold the PIT tag in place when injected into muscle (NMFS, 2019). Previous studies have not reported any negative impacts to sea turtles (Balazs, 1999; NMFS-SEFSC, 2008; Stapleton and Eckert, 2008). PIT tags are typically used in combination with metal flipper tags because not all individuals who may encounter a tagged turtle will have access to a PIT tag reader (e.g., the public encountering a stranded turtle).

PIT tagging is only expected to cause minimal and temporary discomfort with healing occurring within a short period of time (NMFS, 2019). Because a foreign body (the tag) is being injected into the body, it is inherently prone to infection. Thus, cleanliness is especially important when implanting PIT tags. For many years, the standard size of a PIT tag applied in sea turtles was a 12.5-millimeter tag inserted using a 12-gauge needle, which is relatively large and prone to causing bleeding at some sites. A smaller 10.3-millimeter PIT tag has now gained more widespread use in hard-shelled species and only requires a 16-gauge needle, which is anticipated to be less painful and less likely to cause bleeding. These smaller tags are required by NMFS for

tagging sea turtles under 50-centimeter carapace length but are preferred in hard-shelled species of any size, as detectability is equivalent to the size of the tags (Foley et al., 2021).

Prior to applying any new PIT tags, NMFS requires that researchers check all turtles for existing PIT tags. If existing PIT tags are found, tag information is reported to the appropriate regional organization that manages a tag inventory, such as the CMTTP at the ACCSTR.[7] Researchers must have PIT tag readers capable of reading 125, 128, 134.2, and 400 kilohertz tags.

As with metal flipper tags, the locations where PIT tags are injected depend on the program and researcher, as well as the species and life stage. In hard-shelled species, turtles are tagged in the front flipper either proximally within the triceps muscle complex, more distally in a fleshy area near the wrist, or along the flipper blade (Figure 23). Some programs preferentially tag in the rear flippers. Although the triceps location (Wyneken et al., 2010) is reported to have slightly less propensity for migration, the distance of tag movement was insignificant relative to the functionality of tag readers, and the detection rates are similarly high among sites (Foley et al., 2021). In leatherbacks, PIT tags are typically embedded into the heavy musculature of the dorsal shoulder region.

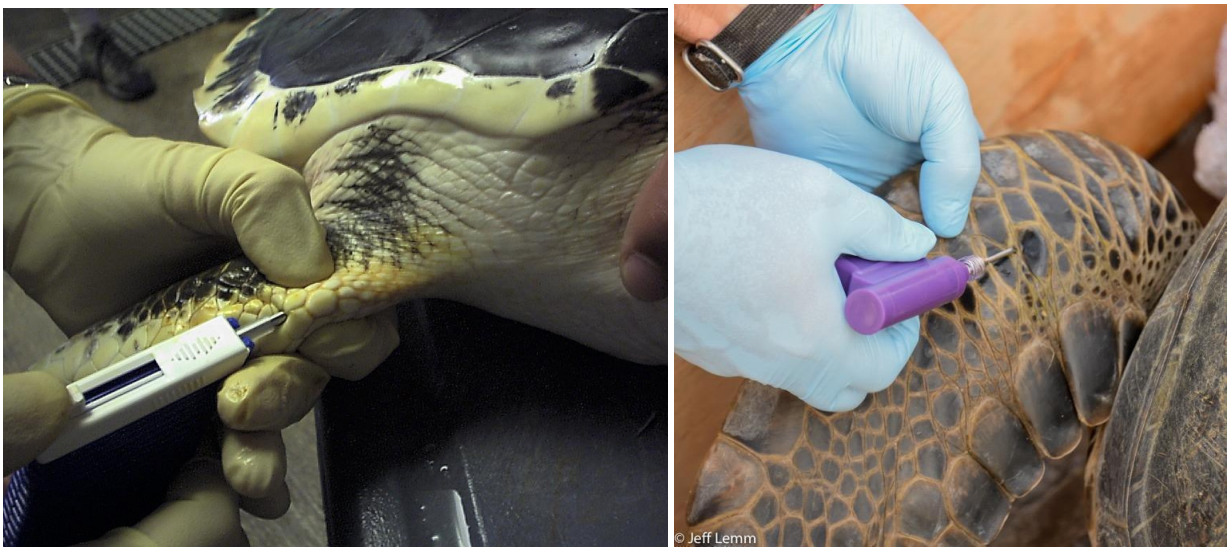

Figure 23. PIT tag application to two different sites of the front flipper. 23a.) PIT tag insertion into triceps superficialis muscle in the front flipper of a Kemp's ridley turtle. Photo courtesy of NMFS SEFSC, USFWS Permit TE676379. 23b.) PIT tag insertion into the soft, fleshy area dorsal to the wrist bones of the front flipper at a seam between the scales of a green turtle. Photo courtesy of NMFS SWFSC, NMFS Permit 18238. Note that PIT tags are often inserted 2 centimeters caudal to the tagging site shown in 23b. as it causes bleeding less frequently.

[7]Cooperative Marine Turtle Tagging Program at the Archie Carr Center for Sea Turtle Research. [Available at http://accstr.ufl.edu/resources/report-a-tag/]

Prior to inserting new tags, each turtle is checked for an existing PIT tag by slowly passing the tag reader just over or gently touching the skin. A common source of failure to detect existing tags is the use of a faulty reader or one with a low battery, particularly older models. Always verify that the reader is working using a test tag prior to scanning a turtle. It is important to slowly move the reader over areas multiple times, allowing it to cycle through different tag frequencies to avoid missing a tag, although this is less of an issue in newer models. The button on the scanner needs to be continuously depressed throughout the scanning process, and the screen may display "WORKING" or a similar term (depending on the type of scanner) when functioning properly. Most PIT tag scanners used today have a radiofrequency of 125 to 134.2 kilohertz (Manire et al., 2017). While some brands are encrypted and can only be read by certain scanners, universal scanners read multiple codes and frequencies.

For hard-shelled turtles, researchers scan the dorsal surface of both front flippers, the shoulder and neck areas, and rear flippers with the PIT tag reader. Researchers should also attempt to scan the ventral surfaces, especially all four flippers and the neck, given the multiple possible tagging locations in use. Small turtles can be turned over for access to ventral surfaces. For leatherbacks, researchers scan the dorsal musculature of both forelimbs, the shoulder region, and the top of the neck. It may be necessary to press the reader into the skin of leatherbacks to detect deep tags, particularly with older model readers.

See Appendix G for example protocols.

NMFS permits require that researchers clean and disinfect all tag applicators. The injector handle between animals must be sterilized if it has been exposed to fluids from another animal. Researchers must also clean the application site and then scrub it with two replicates of a medical disinfectant solution (e.g., Betadine®, chlorhexidine) followed by 70 percent alcohol (disinfectant/alcohol/disinfectant/alcohol) before the applicator pierces the animal's skin. A new, sterile needle must be used for each PIT tag application.

If the triceps site is used, the tag is injected into the thickest part of the triceps superficialis muscle and must occupy no more than an estimated 20 percent of the muscle's total volume and length. To determine eligibility, pinch the muscle forward and assess the tag size relative to the muscle size. Researchers may use alternative sites provided the muscle has sufficient mass to accommodate the PIT tag (<20 percent) and PIT tagging poses minimal risk of injury to vital structures or other anatomical features.

Animal Welfare and Safety:

- As with all invasive procedures that involve piercing the skin, PIT tagging must adhere to aseptic techniques. All equipment must be cleaned in between use on each turtle. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- If any bleeding occurs after the tag has been injected, a swab soaked in povidone-iodine can be held to the injection site until the bleeding stops.

NMFS does not authorize PIT tagging of turtles less than 16 centimeters SCL. PIT tagging turtles between 16 and 30 centimeters SCL requires that researchers have specialized experience with tagging smaller turtles. Turtles between 16 and 50 centimeters SCL must be tagged with a 10-millimeter PIT tag and a 16-gauge injector. It is anticipated that researchers will adopt the use of 10-millimeter tags in all hard-shelled species once previously purchased stocks of larger tags are depleted.

Human Training and Safety:

- PIT tagging smaller turtles requires specialized experience (see the blue box above).
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during tag application.

6.3 Carapace Marking

6.3.a Overview

Similar to the above section on tagging, this section focuses on current carapace marking techniques that are considered standard. Historical carapace marking techniques (e.g., living tags) are described in Section 11.3. It is not expected that individual turtles would experience more than short-term stress as a result of these activities, and no injury is expected (NMFS, 2019). Turtles are marked as quickly as possible to minimize stress.

6.3.b Paint

Purpose: Temporary (within season) marking of previously sampled turtles for capture-mark-recapture purposes and to avoid any unnecessary repeated capture.

Description:
The carapace of hard-shelled sea turtles may be temporarily marked for easy identification of individuals from a distance. Carapace painting is most often used within a short time frame (e.g., one field season) as the paint wears off quickly (Figure 24). The ability to identify turtles in this manner enhances data collection and limits the need for recapture and associated disturbance and stress. Non-toxic paint is used to mark the carapace shell in various ways.

Figure 24. Juvenile green turtle carapace marked with non-toxic paint, making it easier for resighting within a field season. Photo courtesy of NMFS SEFSC, NMFS Permit 21233.

NMFS permits require the use of non-toxic paints or markers that do not generate heat or contain xylene or toluene. Markings must be easily legible using the least amount of paint or marker necessary to re-identify the animal.

Animal Welfare and Safety:

- Paint turtles with non-toxic paint (e.g., paint sticks used for livestock). Potentially harmful or toxic paints include xylene- or toluene-based paints, those containing tributyltin and cyanide or copper cyanide, reflective paints, or paints with exothermic set-up reactions (NMFS, 2019).
- Allow for adequate ventilation when applying paint to prevent turtles from exposure to paint fumes.

Human Training and Safety:

- Allow for adequate ventilation to avoid breathing in fumes when applying paint.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during paint application.

6.3.c Etching

Purpose: Increase the long-term visibility of previously tagged turtles for capture-mark-recapture purposes and to avoid any unnecessary repeated captures.

Description:
Etching is a simple and durable carapace marking method to individually recognize turtles from a distance. The ability to identify turtles in this manner enhances data collection and sharply reduces the level of disturbance during encounters after the initial flipper tagging. Etching tools (e.g., Dremel® rotary tools with a "pear-shaped" bit) are used to place an etch (i.e., a shallow groove) in the carapace of hard-shelled sea turtles. The etch or groove is only made in the keratin layers of the scute. If desired, non-toxic paint can then be applied to the etched grooves in some instances to improve the visibility of animals post-capture (Balazs, 1995). See Appendix H for example protocols.

Figure 25. Hard-shelled carapace marked via etching. Photo courtesy of NMFS PIFSC, NMFS Permit 21260.

Animal Welfare and Safety:

- Disinfect bits prior to use. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Only place an etch or groove in the keratin layers of the scute so as to not injure underlying living tissue.
- Ensure that the etching is approximately 1–2 millimeters deep and 2 centimeters high (NMFS, 2019).
- Do not etch turtles with scutes that are too thin to be etched without risk of injury.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during etching.

6.3.d Plastron Delineation

Purpose: Characterize reproduction-related softening of the plastron in mature males.

Description:
For studies related to reproductive activity, the plastron of males may be marked using a minimally invasive protocol based on Blanvillain et al. (2008). Male sea turtles are placed in a recumbent position (carapace down), and the plastron is delineated using a non-toxic marker. A line can also be drawn around the area of softness to highlight this area for photo documentation and comparison with other parameters. See Appendix H for example protocols.

Animal Welfare and Safety:
- Use only non-toxic markers.
- All equipment must be cleaned prior to use and in between each turtle. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Time on their backs must be as brief as possible to limit additional stress and compression of internal organs.

Human Training and Safety:
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during marking.

7. BIOLOGICAL SAMPLING

7.1 Overview

Biological sampling encompasses a variety of research methods used to collect data on important life history characteristics such as sex, age, disease, health, diet, foraging behavior, and genetics (FitzSimmons et al., 1999; Wibbels, 1999; Herbst and Jacobson, 2003; NMFS, 2019). Samples most often collected from live turtles include skin and other soft tissues, scute, blood, feces, urine, and diet items. Most of these are accessed externally from body orifices or cutaneous incisions. Internal tissues may be accessed via laparoscopy which is used to identify the sex and reproductive status of an individual turtle by examining reproductive organs and ducts (Wood et al., 1983; Wibbels et al., 1999; Wyneken et al., 2007). One or more of these sampling techniques may be performed on a given individual. This section outlines the biological sampling techniques that are considered standard methods and provides guidelines for consistent and safe sample collection. The methods below are primarily listed from the least invasive to the most invasive. As with other procedures, the number of samples and volume should be the minimum required to fulfill research objectives, especially for invasive procedures.

For most of these procedures, researchers should be prepared to provide first aid in the event of bleeding and, for the most invasive (i.e., internal procedures), immediate access to veterinary intervention if needed. These preparations include having sterile gauze, bandaging, and blood clotting agents on hand for ready use. Cleanliness and pain management should be carefully considered for any procedure to minimize pain and discomfort and avoid potential complications from procedures.

7.2 Swabs

Purpose: Multiple uses including nucleic acid recovery (DNA, RNA), cytology, and characterization of microbiota.

Description:
Swab sampling collects surface or naturally exfoliating materials and does not injure the skin or mucosal surface. The specific type of swab, gauze, or similar material/instrument depends on the research objective. Swabs can be collected from a variety of locations. For example, nasal swabs are used to collect mucus with a shallow insertion of the swab into each nare and can be used to detect respiratory infections. Swabs collected from the cloaca can be used to study microbiota and parasites. Collection materials should be kept clean and free of contamination (e.g., sealed) prior to use. Because of the non-invasive nature of swabs, turtles are not expected to experience any additional stress than that of capture, tagging, and handling (NMFS, 2019). See Appendix I for example protocols.

Animal Welfare and Safety:

- During oral swabbing, a bite block or speculum is used to hold the mouth open for safe collection (see Section 11.2 for photos of bite block examples).
- For cloacal swabs, the swab is not inserted beyond the point of any resistance to avoid accidental perforation.
- Palpebral swabs are collected with care so as to not abrade the sensitive cornea.

Human Training and Safety:

- During oral swabbing, a bite block or speculum is used to hold the mouth open so that the turtle is not able to bite down on the researcher or ingest any foreign material during sampling.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

7.3 Epibiont Collection

Purpose: Multiple uses related to biodiversity habitat use, activity level, and health assessment.

Description:
Numerous organisms occur as epibiota on sea turtles (FIgure 26) and may be collected by researchers through various physical means, such as with the use of forceps, scraping devices, and swabs. For example, barnacles can be carefully pried off from the turtles' carapace, taking care not to remove the underlying scute (Figure 26). For epibiota present in areas other than the carapace, pry up the edge of the specimen, and pull the entire organism away from the epithelium. Other epibiota may be more easily removed. Over 150 species have been reported on the carapace of loggerhead and hawksbill turtles (Frick et al., 1998; Frick and Pfaller, 2013). See Appendix I for example protocols.

Figure 26. 26a.) Example of epibiota that can be present on the carapace of a hard-shelled sea turtle. Photo courtesy of FL Coop Unit at UF, NMFS Permit 1299 and FWC MTP 094. 26b.) Collecting epibiota from the carapace of a loggerhead turtle. Photo courtesy of NMFS SEFSC, NMFS Permit 21233.

Animal Welfare and Safety:

- Perform all collection procedures, including detachment of barnacles, in a manner that avoids injury to the underlying skin, carapace, or plastron.
- Do not attempt to remove boring barnacles embedded in the soft tissue or carapace of live turtles as the resulting wounds tend to require veterinary care.
- Be prepared to apply first aid if bleeding or injury occurs during collection.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

7.4 Keratin/Scute Sampling

Purpose: Stable isotope and contaminant analysis.

Description:
Keratin forms the non-living outer layer of the skin that provides protection from the environment and prevents fluid loss. It varies in thickness from relatively thin over highly mobile areas of the body to the thick outer layers of the carapace scutes. It is primarily used in sea turtle research for stable isotope analysis and to study exposure to heavy metals and other contaminants. Stable isotope analysis can provide crucial information on diet, habitat use, and trophic levels. Keratin samples can be collected as punches or cores using a biopsy tool or as scrapings collected by shaving off layers of keratin and may be subsampled for analysis in a laboratory setting (Day et al., 2005; Reich et al., 2007; NMFS-SEFSC, 2008; Vander Zanden et al., 2014; Figure 27). Typically, up to four 0.2–0.5-gram punches or cores of scute material are collected from individual turtles (Reich et al., 2007). Total keratin shavings collected from an individual turtle are usually 1 gram or less (Keller et al., 2014).

A specific site of collection is generally selected by the researcher based on specific objectives of analysis and to maintain comparability among samples. An important distinction is that true keratin sampling does not damage the underlying living layers of the skin, which would make it a biopsy (as covered below). For example, keratin scrapings can be collected from the radial edge of the post marginal scutes where the dorsal and ventral surfaces form a thin edge and the keratin and underlying tissue can be discriminated (Day et al., 2005). If the sampling site bleeds or exposes the white dermis (connective tissue beneath the epidermis), the skin has been breached as occurs during biopsy. Because of the non-invasive nature of keratin sampling, it is not expected that individual turtles would experience more than short-term stress from handling during this procedure (NMFS, 2019). See Appendix I for example protocols.

Figure 27. Keratin sampling from scutes using a scraping tool. Photo courtesy of Ralph Pace and NMFS SWFSC, NMFS Permit 16803.

Animal Welfare and Safety:

- Do not collect samples from areas with a visibly thin keratin layer to avoid penetrating the keratin layer.
- Do not reuse disposable biopsy punches or other single-use items. All reusable biopsy equipment must be sterilized prior to use and between each turtle. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:

- Researchers must be careful and avoid being injured by struggling turtles or shaving equipment.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

7.5. Fecal and Urine Sampling

7.5.a Voided and/or Digitally Extracted Sampling

Purpose: Multiple uses including dietary studies, parasitological examination, biotoxin exposure, and health assessment.

Description: Feces and urine may be voluntarily voided following capture. Urine is particularly challenging to collect from sea turtles and requires researchers to be prepared for sampling if the bladder is emptied. Feces can also be extracted by digital manipulation or using a veterinary fecal collection loop or swab. In hard-shelled species, this is most easily done with the turtle placed onto its back. In addition, turtles can be temporarily held in a suitable empty container or pool (e.g., abiding with standard conditions and parameters) until defecation occurs.

NMFS permit applicants may request to temporarily transport and hold sea turtles for up to 36 hours from the time of capture to release in a USFWS-approved facility to accommodate methods such as collection of voided fecal samples.

For the transport, maintenance, and care of turtles temporarily held in a facility, researchers must follow the "Standard Conditions for Care and Maintenance of Captive Sea Turtles" issued by USFWS[8] (USFWS, 2019) and, if in the State of Florida, the FFWCC Marine Turtle Conservation Handbook,[9] Section 4, "Holding Turtles in Captivity" (FFWCC, 2016)

[8] USFWS Standard Conditions for Care and Maintenance of Captive Sea Turtles. [Available at https://www.fws.gov/media/standard-conditions-care-and-maintenance-captive-sea-turtles]

[9] Florida Fish and Wildlife Conservation Commission Marine Turtle Conservation Handbook. [Available at https://myfwc.com/media/3133/fwc-mtconservationhandbook.pdf]

No injury or lasting effects are expected from this procedure. Individual turtles may experience short-term stresses and possibly some minor discomfort as a result of these activities (NMFS, 2019). See Appendix I for example protocols.

NMFS-permitted researchers may only attempt to digitally extract feces from turtles >50 centimeters SCL.

Animal Welfare and Safety:

- All hard-shelled turtles turned onto their carapace must be placed on padded surfaces to avoid injury. Time on their backs must be as brief as possible to limit additional stress and compression of internal organs.
- Objects inserted into the cloaca must not be forced beyond the point of any resistance to avoid accidental perforation.
- Gloves must be worn if digitally extracting samples. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Medical lubricant must be used for gloves and fecal loops.

Human Training and Safety:

- Researchers must wear gloves when digitally extracting samples and should be cautious as feces may include sharp objects such as invertebrate shells, spines, or fish bones
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

7.5.b Cloacal Lavage

Purpose: Fecal and residual matter are extracted from the cloaca for diet and health studies.

Description:
Cloacal lavage has the potential to yield feces, parasites, or smaller/liquid samples that may be retained in the cloaca that are otherwise difficult to obtain. In hard-shelled turtles (>45 centimeters SCL), a single clean, flexible tube (catheter) is inserted into the cloaca after a lubricant is applied to the tubing. A syringe is used to push saline solution into the tubing to flush the cloaca. Typically, a small amount of solution is needed (e.g., 5–20 milliliters). Feces and/or other matter is collected in a catch basin as it exits the cloaca.

Animal Welfare and Safety:

- All overturned turtles must be placed on padded surfaces to avoid injury. Time on their backs must be as brief as possible to limit additional stress and compression of internal organs.
- All equipment must be disinfected. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

NMFS permit conditions require that a new or thoroughly disinfected tube/catheter is used between turtles. The catheter/tube must be lubricated prior to insertion. The catheter/tube is not inserted further once resistance is encountered.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

7.6 Biopsies and Tissue Sampling

7.6.a Overview

Biopsies or the excision of tissue samples from a live animal are routinely collected for a variety of research purposes including analyses related to life history, genetics, sex, behavior, diet, and health. By far, the most frequently collected biopsies in the course of sea turtle research are skin biopsies for genetic studies and stable isotope analysis. Multiple other tissues may be used for stable isotope studies as well to gain insight into the diet, foraging behavior, and potential distribution patterns. Other types of biopsies are collected far less frequently, and examples include bone biopsies for aging studies (e.g., skeletochronology; Avens and Snover, 2013), biopsies of fat and liver to detect contaminants, and muscle biopsies for biochemical and metabolic studies (Southwood et al., 2006). In addition, biopsies of abnormal tissues for diagnostic purposes (e.g., histopathology) are a routine aspect of veterinary assessment of individuals and diseases such as fibropapillomatosis. For more information on biopsies, see Jacobson (1999).

7.6.b Skin Biopsy

Purpose: Multiple uses including genetic analyses, isotopic analyses, and disease studies.

Description:
Skin biopsies typically are collected from specific areas of the body, depending on their intended use (Figure 28). For example, samples for genetic analysis are most often collected from the edges of the flipper webbing for hard-shelled species. Skin overlying the shoulder areas or specific areas of the shell may be targeted for isotopic analysis, and specific types of skin lesions (e.g., fibropapillomas) may be sampled for disease studies. Sterile biopsy punch, forceps, surgical scissors, or a scalpel blade may be used to extract and trim the sample (Herbst and Jacobson, 2003). Sterile biopsy punches come in multiple diameters from 1 to 8 millimeters. In the U.S., skin biopsies collected from sea turtles are usually 6 millimeters or less (NMFS, 2019). Skin sampling is likely to have minimal effect on individual turtles when conducted according to established protocols (NMFS and USFWS, 2008).

Figure 28. Skin sampling with a biopsy punch. Photo courtesy of NMFS SWFSC, NMFS Permit 18238.

NMFS permit conditions require researchers to follow a veterinary-approved pain management protocol when collecting skin samples that are larger than a 6-millimeter biopsy punch. Researchers must use sterile techniques during all skin and fat sampling. For procedures conducted on vessels or under other field conditions, researchers must use designated surgery areas kept as clean as possible (e.g., use of disposable surgical drapes) to minimize risk of contamination.

There are instances when sea turtles cannot be safely boarded onto a vessel but may be sampled using a biopsy pole. Samples are collected from anywhere on the limbs or neck (avoiding the head) that are most safely accessible to the researcher. Samples from leatherbacks are collected via shallow carapacial scrapes. Common sampling gear includes a sterile stainless-steel corer attached to a sectional anodized aluminum pole.

Biopsies of skin, especially those collected using biopsy punches, are typically left to heal by second intention (i.e., without surgical closure). Researchers have examined hard-shelled turtles re-captured two to three weeks after biopsy and noted that the sample collection site was almost completely healed (NMFS, 2017). As long as these methods are conducted properly, turtles should not experience any additional stress or discomfort beyond what was experienced during the capture, collection of measurements, and flipper tagging (NMFS, 2019).

See Appendix J for example protocols.

For turtles brought on board the vessel for sampling, NMFS permits only allow for tissue sampling from the limbs, neck, carapace, or shoulder region. Researchers must avoid sensitive areas. For small skin biopsy samples (i.e., 6-millimeter diameter or smaller) use aseptic techniques at all times. At a minimum, thoroughly swab the tissue surface with a medication disinfectant solution (e.g., Betadine®, chlorhexidine) followed by 70 percent alcohol before sampling. Researchers may use two applications of alcohol if disinfectants may interfere with analyses.
Keep the procedure area and your hands clean.

For turtles sampled remotely using a pole biopsy (also 6 millimeter diameter or smaller) or for leatherbacks via shallow carapacial scrapes, samples must be taken in the location most safely and easily accessed. Researchers may sample from anywhere on the limbs or neck, avoiding the head.

Animal Welfare and Safety:

- Gloves should be worn to protect the biopsy site.
- The biopsy site must be cleansed using a disinfectant (e.g., 10 percent povidone-iodine or chlorhexidine) and rinsed with 70 percent isopropyl alcohol. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity. If disinfectants will interfere with the intended analysis, at minimum the site must be thoroughly cleaned using alcohol.
- If warranted, a blood clotting agent, such as ferric subsulfate or Clotisol®, or a cyanoacrylate tissue glue, such as Nexaban® can be used for hemostasis.
- Analgesia (i.e., use of local anesthetics) under the direction of a veterinarian is encouraged when possible for biopsies of any size. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

NMFS permit conditions require that a new, sterile biopsy punch, scissors, or surgical blade is used on each sample to avoid cross-contamination. The tissue sampling surface must be cleaned and disinfected following Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

NMFS permits limit sampling events to no more than twice per 12-month period unless additional sampling is defined in a veterinarian-approved protocol.

Human Training and Safety:

- Gloves must be worn to protect the hand that is holding the flipper or sampling surface.
- Sampling must be performed or overseen by experienced personnel.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

7.6.c Other Tissue Biopsies (Percutaneous)

Purpose: Multiple uses including analyses related physiology, diet, nutrition, reproduction, and health assessment.

Description:
This section refers to tissues that are biopsied through skin incision but without laparoscopy, such as skeletal muscle, fat, and bone. In contrast to most skin biopsies collected from sea turtles for research purposes, access to other soft tissues is inherently more invasive and requires a surgical procedure. Therefore, these biopsies must follow a veterinarian-approved protocol and are only performed by a veterinarian or, more rarely, under direct veterinary supervision. Protocols should consider aseptic preparation of the biopsy site, need for sedation or anesthesia, other pain management, any indicated antimicrobial prophylaxis, and procedure details. Procedures may occur in a facility or under field conditions provided that an appropriate designated surgery area is used. In general, the collection site is cleaned with a surgical scrub prior to sampling. The researcher wears sterile gloves to avoid contamination. Sterile equipment (e.g., biopsy punch, scalpel) is used to collect the sample after a small surgical incision is made. The incision site is closed using sutures or other methods to encourage healing by primary intention. No lasting effects or complications are expected from these procedures, although individual turtles may experience short-term stresses and possibly some minor discomfort (NMFS, 2019). See Appendix J for example protocols.

Animal Welfare and Safety:
- Researchers must follow a veterinarian-approved protocol.
- Percutaneous biopsies must be performed only by or under direct veterinary supervision.
- Procedures must follow provisions in Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:
- Only a veterinarian or other highly trained individual may conduct this procedure.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

7.7 Blood Sampling

Purpose: Multiple purposes including genetic analysis, sex determination, physiological studies, health assessment, clinical evaluation, molecular diagnostics, toxicant detection, and stable isotope analysis.

Description:
Blood sampling is a minimally invasive method to study a variety of sea turtle biological and health-related parameters (e.g., hematology, blood chemistry, population genetics, isotopic analyses, biotoxins, and contaminants). Blood collection is considered a routine procedure and is easily

collected from one of multiple commonly used venipuncture locations (Stacy and Innis, 2017). The most commonly used sites for blood collection in hard-shelled turtles are the large paired dorsal cervical sinuses (external jugular veins), which are accessible from the dorsal or dorsolateral aspects of the neck (Owens, 1999; Wyneken et al., 2006; Figure 29). In leatherbacks, blood can also be collected from popliteal sinuses (venous plexi) located in the proximal rear flippers, interdigitary vessels, or the coccygeal (dorsal tail) vein (Wallace and George, 2007; Stacy and Innis, 2017; Figure 30a). Blood sampling is likely to have minimal effect on individual turtles when conducted according to established protocols (NMFS and USFWS, 2008). Study animals may experience a short-term stress response in association with handling, restraint, and the pain associated with blood sampling (NMFS, 2019).

Important considerations regarding blood sampling from sea turtles are disinfection of the venipuncture site, the number of attempts allowed, the volume to be collected, needle size, and any pertinent consideration related to serial collection, anticoagulant use, and blood processing and storage. In general, blood volume in reptiles is approximately 5–8 percent of the body weight, and 10 percent of the volume can be safely collected from clinically stable animals (Jacobson, 1993). This is far more volume than is typically needed, especially from larger individuals. The amount of blood collected should be the least amount necessary to accomplish research objectives. The conservative threshold of 3 milliliters per kilogram is usually adequate for most studies. The needle size is dependent on the size of the animal. For example, a 21 or 22 gauge, 1- to 1.5-inch needle is adequate for hard-shelled adults (Owens and Ruiz, 1980); a 23 gauge, 0.5-inch needle is appropriate for smaller animals; longer spinal needles are needed to reach the cervical sinuses of leatherbacks (NMFS, 2019).

During blood sampling, lateral movement of the needle and unnecessarily deep insertion are avoided to prevent injury. The needle is retracted to the level of the subcutis prior to redirection.

See Appendix K for example protocols.

Figure 29. Collecting blood from the ventral dorsal cervical sinus of hard-shelled turtles. 29a.) Photo courtesy of NMFS PIFSC, NMFS Permit 21260. 29b.) Photo courtesy of NMFS NEFSC and SEFSC, NMFS Permit 1576.

Figure 30. Collecting blood from leatherback turtles in two different locations. 30a.) Collecting blood from the rear flipper. 30b.) Collecting blood from the dorsal cervical sinus. Photos courtesy of NMFS NEFSC and SEFSC, NMFS Permit 21233.

For blood sampling, NMFS permits:

- Only allow experienced personnel to perform or supervise blood sample collection.
- Require that new disposable needles are used on each animal and that needles are changed immediately if they contact other surfaces or otherwise become contaminated or damaged. Researchers must thoroughly swab blood collection sites with a medical disinfectant solution (e.g., Betadine®, chlorhexidine) followed by 70 percent alcohol before sampling. Researchers may use two applications of alcohol if disinfectant solutions may affect intended analysis. If an alternative disinfecting method is needed, researchers must consult with OPR's Permitting Office.
- Do not authorize blood sampling on any turtle that cannot be adequately immobilized or when conditions on the boat/holding platform preclude the safety and health of the turtle.
- Require researchers to limit attempts (needle insertions) to extract blood from the neck to a total of four, two on either side.
- Only allow an individual needle to be used for one or two attempts before replacing it.
- Require researchers to follow best practices, including retracting the needle to the level of the subcutis prior to redirection to avoid lacerating vessels and causing other unnecessary soft tissue injury and immediately removing the needle if the animal begins to move.

Animal Welfare and Safety:

- Turtles must be adequately restrained during collection to prevent movement. If necessary, the needle is removed if the animal begins to move.
- Equipment and collection sites must be thoroughly disinfected. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Blood must not be collected should conditions on the boat preclude the safety and health of the turtle.

NMFS permits require that:

- Researchers limit the amount of blood withdrawn to the minimal volume necessary to complete permitted activities. Researchers must not collect more than 3 milliliters per 1 kilogram of animal per sample.
- Researchers cannot exceed the cumulative maximum safe limit described above from a single turtle within a 45-day period. If researchers take more than 50 percent of the maximum safe limit in a single event or cumulatively from repeated sampling events from a single turtle within a 45-day period that turtle must not be re-sampled for 3 months from the last blood sampling event.
- Researchers must, to the maximum extent practicable, attempt to determine if any of the turtles they blood sample may have been sampled within the past 3 months or will be sampled within the next 3 months by other researchers. The Permit Holder must make efforts to contact other researchers working in the area that could capture the same turtles to ensure that none of the above limits is exceeded.

Human Training and Safety:

- Sampling may only occur if directly taken by or supervised by experienced personnel.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

7.8 Esophageal or Gastric Lavage

Purpose: Collect ingested contents of the esophagus and stomach from hard-shelled turtles for diet and foraging related studies.

Description:

Lavage of the esophagus and stomach has been successfully used in green, hawksbill, olive ridley and loggerhead turtles ranging in size from 25- to 115-centimeter curved carapace length (CCL) without any apparent ill effects (Fuentes et al., 2006; Hart et al., 2013; NMFS, 2019). Standard procedures generally follow those described by Forbes (1999) and Seminoff et al. (2002a). One or two clear, flexible vinyl tubes are used for the lavage procedure. If one tube is used, it serves to both introduce water and collect material. If two tubes are used, one is used to introduce water for lavage and the other for retrieval. Tube length is compared with the turtle to identify the length necessary to reach the opening into the stomach. Turtles are often restrained by placing them

briefly (usually no longer than 3 minutes) in an inverted position on a cushioned surface so that the head is below the body and gravity assists with the collection of contents. Researchers may restrain the flippers and hold the head so that the neck and esophagus remain in line with the body. The turtle is prompted to open its mouth by touching the beak on either side or by grasping the skin of the gular region and gently pulling the jaw open. A padded and disinfected bite block, speculum, or other mouth gag is placed in the mouth to keep it open.

The tubes are lubricated with vegetable oil or non-toxic water-based gel and inserted into the esophagus to the pre-measured depth. Water is pumped into the esophagus at a rate of 10–25 psi and 9 liters/minute (Forbes, 1999) to flush out food particles. The forthcoming fluid and any ingesta are discharged through the retrieval tube. Typically, the lavage continues for approximately 30–45 seconds as the tube is passed up and down the length of the esophagus. The fluid and ingesta can then be strained to collect any food items or particles. The tubes are then removed, followed by the bite block or speculum, and the head is elevated to prevent aspiration.

See Appendix K for example protocols.

Animal Welfare and Safety:

- Use caution when placing tubes to ensure that they do not enter the glottis.
- Place any overturned turtles on padded surfaces to avoid injury. Time on their backs must be as brief as possible to limit additional stress and compression of internal organs.
- Ensure that tubes are not inserted beyond the point of any resistance.
- Disinfect all equipment and surfaces between uses. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

NMFS permits require that experienced personnel directly perform or supervise lavage activities. Water can only be pumped for 3 minutes to wash out any contents. Once the samples have been collected, the water is turned off, and researchers must allow water and food to drain until all flow from the esophagus has stopped. The posterior of the turtle is lightly elevated to assist in drainage. Researchers must thoroughly clean and disinfect equipment after each use. Lavage must not be conducted on compromised animals.

Human Training and Safety:

- Only experienced personnel or those under the direct supervision of experienced personnel may perform this procedure.
- Researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

7.9 Laparoscopy and Associated Internal Tissue Sampling

Purpose: Visualization of the body (coelomic) cavity primarily for sex determination and reproduction studies.

Description:
Laparoscopy is a surgical procedure that uses a fiberoptic camera and miniature instruments to visualize and sample visceral organs through a small surgical incision made through the body wall. It is primarily used in sea turtle research to determine the sex of immature individuals, characterize stage of maturation and the reproductive cycle, and, less commonly, as a means for collecting biopsies of internal organs (Wibbels et al., 1990; Owens, 1999; Wyneken et al., 2003). Aside from research, it is commonly used for diagnostic purposes in sea turtles during rehabilitation.

Typically, turtles are restrained for laparoscopy either on their carapace in an inverted position or on their side (in lateral recumbency) with the side where laparoscopic entry will occur facing up. Sedation is not required but is recommended if conditions and personnel expertise allow. Following surgical disinfection of the site, a small incision is made at the port of entry, typically within the prefemoral area. A trocar (a surgical instrument with a three-sided cutting point enclosed in a tube, used for withdrawing fluid from a body cavity) is used to penetrate the body cavity and insert a cannula for passage of the sterilized endoscope. If the introduction of gas (insufflation) is necessary for visualization, filtered air or medical grade carbon dioxide is used. Upon completion of the procedure, the gas is expelled using one of multiple techniques to exert pressure on the plastron, and the surgical site is closed using an absorbable suture. The turtle is monitored prior to release to ensure recovery and buoyancy control and full recovery from sedation or anesthesia if used. Laparoscopy is typically performed on turtles greater than 120 grams (Wyneken et al., 2003; Wyneken et al., 2007). See Wyneken et al. (2006) for additional specifics on laparoscopy and Harris et al. (2017) for information on conducting laparoscopy in the field.

As part of the laparoscopic procedure, internal organs and tissues (e.g., gonads, muscle, liver, kidney, spleen, and mesenteric fat) may be sampled using surgical endoscopic instruments for physiological, contaminant, and health-related studies.

See Appendix L for example protocols.

Animal Welfare and Safety:

- Laparoscopic protocols should consider aseptic techniques, analgesia, and a response plan for any unplanned complications. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- A single pre-surgical dose of antibiotic may be administered to reduce the chances of post-surgical infections.

- A nonsteroidal anti-inflammatory drug (NSAID) (e.g., ketoprofen, 2 milligrams/kilogram intramuscular (IM) administration; MacLean et al., 2008) may be administered to reduce post-operative pain with no sedation. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- A short-acting general anesthetic may be administered prior to the procedure. Turtles that receive a general aesthetic should be held out of water for at least 1 hour following the conclusion of the procedure and should not be returned to water until it is established that they are fully responsive.
- Sedation is not required but is recommended if conditions allow. If sedation is used, turtles should be held out of the water for at least 1 hour following the conclusion of the procedure and should not be returned to water until it is established that they are fully responsive.
- All disinfecting/cleansing of equipment must follow the protocols in Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- All overturned turtles must be placed on padded surfaces to avoid injury.

NMFS permits require that laparoscopic procedures are performed following veterinarian-approved protocols. The protocols must also be performed under the supervision of an experienced veterinarian. In addition, researchers may only use sedation or anesthesia following a veterinary-approved protocol and when directly attended by a veterinarian. Laparoscopy is not performed on any compromised animals that are obviously weak, lethargic, positively buoyant, emaciated, or that have severe injuries or other abnormalities resulting in debilitation.

Human Training and Safety:

- Laparoscopy and associated tissue sampling are only performed by or under the supervision of a qualified veterinarian.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

8. INSTRUMENT ATTACHMENTS

8.1 Overview

Researchers use a variety of devices to track turtle movements and collect data on habitat use, dive behavior, physiology, foraging events, and associated environmental parameters. Commonly used types of instruments include acoustic, radio [very high frequency (VHF)], and satellite transmitters including pop-up archival tags (PATs), pop-up satellite tags (PSATs), time-depth recorders (TDRs), solar satellite units, animal-borne video, audio, and environmental data collection systems (AVEDS; e.g., Crittercams) or customized animal tracking solutions. The size, weight, and dimensions vary by instrument type, and various attachment methods have been developed based on research objectives, study design, and target species (hard-shelled vs. leatherbacks). Attachment methods include suction-cups, adhesives (e.g., epoxy, resin, putty), and anchored attachments affixed through the carapace or pygal region, including tether attachments.

Each of these methods is discussed in more detail below. Instrument attachment is expected to have minimal effect on individual turtles when conducted according to established guidelines and best practices (NMFS and USFWS, 2008).

Tag selection and attachment methods are important considerations when deploying telemetry devices. In some instances, tag deployment can have a detrimental effect (e.g., increased drag leads to decreased swimming efficiency) on study animals (Watson and Granger, 1998; Godley et al., 2008; Sherill-Mix and James, 2008). Jones et al. (2011) assessed a variety of telemetry devices and attachment procedures and recommended that all transmitters minimize drag impact and maximize hydrodynamic design. The drag of a tag can be more consequential to a turtle than the mass of the tag (Watson and Granger, 1998; Jones et al., 2011; Jones et al., 2013). Drag can be minimized by reducing the ratio of the frontal area of the tag and attachment to the frontal area of the study animal. The shape of the tag can be more important than the frontal area in determining drag. A larger smoother (more hydrodynamic) attachment can result in a larger surface area (including larger frontal area), but a more hydrodynamic form and can drastically reduce drag (approximately 25 percent) compared to a smaller less smooth attachment (Shorter et al., 2014). In a review of acceptable practices for wildlife tagging, Wilson and McMahon (2006) conclude that the short-term attachment of devices with deleterious effects can be considerably less harmful than long-term attachment with supposedly harmless devices. Jones et al. (2011) address this temporal issue by recommending that special considerations be made for the timescale and question being asked when placing tags that cause considerable drag on turtles. They (Jones et al., 2011; Jones et al., 2013) introduce the concept of evaluating the impact of a tag based on its effect on the annual energy budget.

Depending on the study objectives and available technology, multiple tag components may be housed into one tag package to minimize potential effects on sea turtles. In other cases, multiple tag attachments (up to three maximum) that remain attached for varying durations may be needed. For example, a researcher may place a short-term suction-cup attached AVED and a longer-term satellite transmitter on an animal to collect different types of data. The use of multiple tags can also provide information for assessing tag accuracy, tag failure rate, and turtle post-release mortality rate. In some instances (e.g., TDR tags), researchers recapture the animal to remove the tag and download the information recorded. These animals are intentionally captured twice. When selecting tag type, attachment method, and expected attachment duration, researchers must ensure that all selections are appropriate and consistent with research study objectives, are not unnecessarily duplicative (when attaching more than one tag), and are likely to yield meaningful data.

All NMFS-permitted tracking devices and attachment methods must meet appropriate mitigation measures to reduce drag and minimize the risk of entanglement. Telemetry devices and attachment material selection and protocol must be as good as the best available, current published methods, especially with regard to risk for thermal injury. Products not previously used for animal attachment should be tested (including monitoring of temperature) by mock application prior to use on sea turtles. The following considerations must be incorporated into tag

selection and application:

- The frontal area of the tag must be minimized, and the tag must have a low profile.
- The tag must be streamlined and cover as small an area on the sea turtle as possible.
- Adhesives, use of base plates, and building up of adhesive material must be optimized so that the minimum amount is used to obtain the adhesive and hydrodynamic objectives necessary for tag endurance.
- To the degree possible, placing the tag at the peak height of the carapace must be avoided. Tags that are attached to the top of the carapace must be placed slightly anterior or posterior to the peak where uplinks will be maintained. The saltwater switch must still be exposed to the air during breathing, but the frontal area should be minimized.
- The antenna length and diameter must be minimized to reduce risks of entanglement and drag.

Additional permit requirements for instrument attachment include the following:

- Each attachment must be made so that there is minimal risk of entanglement. The transmitter attachment must contain a weak link (where appropriate) or have no gap between the transmitter and the turtle that could result in entanglement. For tethered instruments, the lanyard length must be less than half of the turtle's carapace length. It must include a corrosive, breakaway link that will release the unit after its battery life.
- Adequate ventilation must be provided around the head of the turtle during the attachment of transmitters if attachment materials produce fumes. Turtles must not be held in water during application to prevent skin or eye contact with harmful chemicals.
- For hard-shelled turtles, procedures for drilling through marginal scutes must follow aseptic techniques with two alternating applications of medical disinfectant (e.g., Betadine, chlorhexidine) followed by 70 percent alcohol. A separate drill bit must be used for each turtle. Bits may be reused if sterilized by autoclave or cold sterilization (e.g., gluteraldehyde) before reuse. Similar aseptic protocols must be used for direct attachment of devices to leatherbacks, with sterilized drill bits used for each turtle.
- AVEDS: Attachments must be made so that turtles are able to move freely without impairment.
- During instrument attachments, NMFS permits require that researchers minimize animal holding times. Turtles may be held out of the water for no more than 3 hours. Permit applicants may request to temporarily transport and hold sea turtles for up to 36 hours from time of capture to release in a USFWS-approved facility.
- For the transport, maintenance, and care of turtles temporarily held in a facility, researchers must follow the “Standard Conditions for Care and Maintenance of Captive Sea Turtles” issued by the USFWS[10] (USWFS, 2019) and, if in the State of Florida, the FFWCC Marine Turtle Conservation Handbook,[11] Section 4, “Holding Turtles in Captivity” (FFWCC, 2016).

[10] USFWS Standard Conditions for the Care and Maintenance of Captive Sea Turtles. [Available at https://www.fws.gov/media/standard-conditions-care-and-maintenance-captive-sea-turtles]

[11] Florida Fish and Wildlife Conservation Commission Sea Turtle Conservation Handbook. [Available at https://myfwc.com/media/3133/fwc-mtconservationhandbook.pdf]

8.2 Attachment Method

8.2.a Suction Cup Attachments

Purpose: Short-term instrument deployments (one to several days).

Description:
Suction cups are used for the short-term, non-invasive attachment of AVEDS, VHF, TDRs, acoustic transmitters, and/or cameras to monitor short-term movements, dive behavior, and foraging ecology. Suction-cup tags are often attached upon capture and work-up of an animal (Figure 31 and Figure 32) or remotely by hand or by a long pole to a free-swimming turtle during a close approach by the vessel. The suction cup and tag are attached to the end of the pole, such that, with a small amount of thrust, the suction cup can be placed on a relatively flat surface (e.g., dorsal carapace of a turtle). The use of a pole allows precise placement of the tag on the moist dorsal surface of the carapace between the longitudinal ridges of the carapace on leatherbacks or on the larger (central or costal/lateral) scutes of hard-shelled turtles. Suction-cup attachments have been shown to last for up to 9 days in studies of leatherbacks, although most typically lasted for 48 hours or less (NMFS, 2019). Small amounts of a temporary adhesive (e.g., denture adhesive) may be used to lengthen the duration of attachment. Suction-cup tags are generally outfitted with VHF to allow tracking in real time and to facilitate retrieval by either locating instruments that have detached from animals or removing instruments following the necessary deployment period. The latter does not necessarily require recapture as devices can be detached from free-swimming animals by hand or by using a pole. See Appendix M for example protocols.

Figure 31. AVED tag attached via suction cup to a hard-shelled turtle. Photo courtesy of NMFS SWFSC, NMFS Permit 18238.

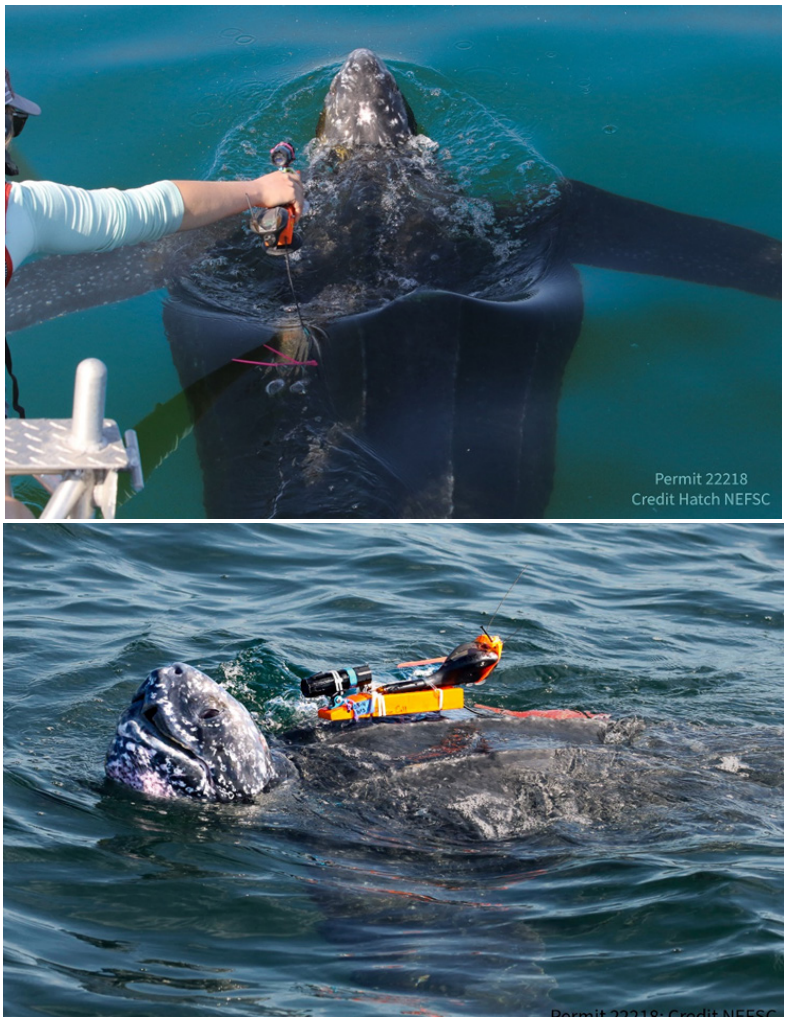

Figure 32. Attaching a transmitter to a leatherback turtle via suction cup. 32a.) Researchers leaning over the side of the vessel to deploy the tag to a surfacing leatherback. 32b.) Leatherback resurfacing with tags successfully deployed. Photos courtesy of NMFS NEFSC (H. Haas), NMFS Permit 22218.

Animal Welfare and Safety:

- Exercise caution when operating boats near free-swimming turtles. Take the engine out of gear if visualization or awareness of the turtle's location is lost near the vessel.
- Ensure that deployment and retrieval take minimal time and are non-invasive.

Refer to the blue box in Section 8.1 for relevant NMFS mitigation measures to reduce drag and minimize entanglements and other requirements.

Human Training and Safety:

- Instrument attachment should be performed or overseen by experienced researchers.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

8.2.b Adhesive Attachments

Purpose: Long-term (months to years) deployment of animal-borne devices to study movement, location, and behavior in hard-shelled sea turtles.

Description:
Devices requiring longer attachment times for hard-shelled sea turtles use an adhesive, such as an epoxy, marine putty, or resin (Figure 33). Instruments attached via adhesive include PATs, PSATs, AVEDS, animal-borne video cameras, acoustic tags, VHF transmitters, and TDRs. These devices are not intended for recovery other than by opportunistic recapture. The carapace is thoroughly cleaned and prepared using a mild abrasive and solvent wash to promote adhesion. Tags are applied to the carapace at a location that minimizes drag while maintaining satellite transmission quality. Often, one larger scute is selected to minimize tag loss due to keratin sloughing. The attachment media may also encompass sections of surrounding scutes but is minimized and shaped to be as hydrodynamic as possible to further reduce drag (Figure 33b). The optimal amount of attachment media will securely and hydrodynamically attach the tag without adding unnecessary media. Neoprene or silicone may be used to accommodate the physical features of smaller, growing sea turtles prior to the application of the adhesive (Mansfield et al., 2012; FIgure 34).

Figure 33. 33a.) Attaching a satellite transmitter using a marine putty base. 33b.) Adding marine putty to the area surrounding the transmitter to create a more hydrodynamic profile. Photos courtesy of NMFS PIFSC, NMFS Permit 21260.

Figure 34. Instrument attachments using neoprene to accommodate physical features of a smaller turtle's carapace. Note that the attachment area is covered with anti-fouling paint to prolong function. Photos courtesy of Ralph Pace and NMFS SWFSC, NMFS Permit 18238.

All surfaces of the transmitter except the saltwater switch and bottom are covered with anti-fouling paint to prolong function (Figure 34 and Figure 35). In some instances, adhesive is used to affix a metal base plate to the carapace on which a tag is attached so that the tag can be released from the turtle after a predetermined time period using a corrosive link. Battery life and duration of attachment depend on the species, life stage, attachment method, habitat type, and individual behavior, as well as programmed features (e.g., duty cycle, transmission frequency) and technical design, but can last a year or more (NMFS, 2019). Tags or base plates detach as the shell grows and the scutes are naturally shed or are scraped off on underwater structures. These factors result in shorter attachment durations for younger juvenile turtles and some other life stages.

See Appendix M for example protocols.

Figure 35. Anti-fouling paint applied to a transmitter and attachment materials and not directly to the carapace. Photo courtesy of NMFS PIFSC, NMFS Permit 21260.

Animal Welfare and Safety:

- During the attachment process, turtles must be held in a well-ventilated area to prevent unnecessary contact with curing compounds used for the attachment.
- All epoxies, resins, and other compounds must be tested prior to use to ensure that they will not cause thermal injury while curing. Temperature safety parameters have not been studied for sea turtle skin. In humans, burns can occur when temperature exceeds 44 °C (111 °F; Ong and Milne, 2016); therefore, this temperature may be applied as a conservative threshold for sea turtles above which extreme caution must be used in the selection and use of products for device attachment. Curing temperatures can be minimized by using the thinnest layer of material required, avoiding building compounds up into thicker formations, and applying cool water to dissipate heat during the curing process.
- When applying anti-fouling paint, researchers must ensure that the paint is only applied over the adhesive material and that it does not come into direct contact with the turtle (Figure 34).
- Researchers must provide shade and adequate ambient temperature during holding.
- Researchers should minimize animal holding time out of the water to the minimum time required for adhesives to cure.

Refer to the blue box in Section 8.1 for relevant NMFS mitigation measures to reduce drag and minimize entanglements and other requirements.

Human Training and Safety:

- Instrument attachment should be overseen or performed by experienced researchers.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

8.2.c. Anchored Attachments

Adhesives are insufficient for some tag attachment purposes due to various factors related to anatomy, growth, and instrument characteristics that lead to detachment of devices. Longer deployments are obtained by direct anchoring of devices with or without the use of adhesives (Renaud et al., 1995; Seminoff et al., 2002b; Epperly et al., 2007b). Small holes are drilled through superficial features of the carapace using sterile, surgical quality drill bits. Various materials and attachment techniques are used to attach devices directly or via tethers. Analgesia is challenging for such procedures involving bone, but it can be provided to some extent by topical spray (e.g., ethylene oxide) or surface application of a local anesthetic (e.g., lidocaine) immediately following drilling. Although not a confident measure of pain perception, researchers typically report minimal to no apparent response from turtles during tag attachments and less than that encountered with other procedures such as flipper tagging.

8.2.c.1 Marginal Scute Acoustic Attachment on Hard-Shelled Turtles

Purpose: Long-term (months to years) deployment of acoustic tags on hard-shelled sea turtles.

Description:
This method is a standard technique used for the attachment of acoustic tags (Seminoff et al., 2002b). Following disinfection of the attachment site, two small holes (e.g., 4 millimeters) are drilled through the outermost edge of the carapace margin along its lateral or caudal aspect using sterile, surgical grade drill bits. Coated wire or zip ties are threaded through the holes to affix the tag. Ties can be secured with small aluminum clamps. Plumber’s putty may also be applied to the scute under the tag to prolong attachments (Figure 36). Deployment time varies but typically can last up to a year (NMFS, 2019). Attachments are often painted with anti-fouling marine paint or silica-based two-part anti-fouling systems (e.g., PropSpeed®). Tags may be painted prior to attachment or while waiting for epoxy/resins to cure the tag and adhesives.

Figure 36. Attaching an acoustic transmitter to the flattest section of the post-marginal scutes, approximately 10–15 millimeters from the edge of the scute. The tag is attached after drilling an 4 to 8-millimeter hole with a sterilized drill bit. 36a.) Initial tag attachment to plumber's putty base. A tie is inserted through the drilled hole and the extra tie length is then removed. 36b.) Tag after extra tie length is removed. 36c.) Building up putty over the tag to create a more hydrodynamic profile. 36d.) Anti-fouling paint can be applied to the tag for extra protection. Photos courtesy of IRG, NMFS Permit 25696/21169, FWC MTP 125/204.

Animal Welfare and Safety:

- When handling the turtle and equipment, researchers should use disposable gloves and change them often to maintain the most sterile environment possible. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Anesthetic must be applied prior to (topical anesthetic) or immediately following (injectable local anesthetic) drilling. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- A sterilized, surgical quality drill bit must be used on each animal and must be disinfected with Betadine and alcohol between uses if multiple holes are drilled in the same individual. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Each drilled hole must not enter the body cavity.
- The skin surface (dorsal and ventral sides) must be thoroughly disinfected prior to drilling. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

- Triple antibiotic ointment may be applied to attachment components.
- Tags are programmed to detach after a designated interval or if certain environmental parameters are met.
- Corrodible links must be used to ensure that the turtle will shed the transmitter package.
- Care should be taken to avoid application of anti-fouling paints to the carapace of the turtle.

Refer to the blue box in Section 8.1 for relevant NMFS mitigation measures to reduce drag and minimize entanglements and other requirements.

Human Training and Safety:

- Instrument attachment should be overseen or performed by experienced researchers.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

8.2.c.2 Tether Attachments

Purpose: Long-term (months) deployment of satellite tags on both hard-shelled species and leatherback turtles.

Description:

Sea turtle researchers have developed methods for instrument attachments that involve wired/tied attachment by drilling through either the posterior marginal scutes or pygal bone (e.g., PSATs/PATs; Morreale et al., 1996). Using this technique, a sterilized drill bit is used to create a hole through one of the pygal bones (caudal-most carapace margin in hard-shelled species) or the pygal (in leatherbacks; Figure 37). In the U.S., the drilled hole typically ranges from 4 to 6.5 millimeters in diameter (NMFS, 2019). Plastic electrician ties, flexible stainless-steel wire, or fishing filament coated in soft tubing (e.g., surgical or vinyl) are passed through the holes to create a tether for the PAT/PSAT (Epperly et al., 2007b). Attachments, such as PATs/PSATs, often need to be painted with anti-fouling marine paint (paints with biocides and fungicides) or silica based two-part anti-fouling systems, such as PropSpeed®. The attachment tethers have a weak link to prevent accidental entanglement and corrodible elements that result in detachment over time. A short tether and breakaway feature of the tether pin in the PATs/PSATs is designed to minimize the potential for entanglement in fishing gear or flotsam. Tags themselves are programmed to detach after a designated interval or if certain environmental parameters are met (typically a year or less). For hard-shelled species, attachment materials (e.g., bolts, wires, line) connected to the scute typically remain attached until the scutes are shed, after the tag unit detaches, depending on the detachment design and corrosive link used (NMFS, 2019). See Appendix N for example protocols.

Figure 37. Leatherback pygal tether attachments 37a.) Leatherback pygal tether tag attachment using flexible stainless steel wire. Photo Courtesy of NMFS NEFSC and SEFSC, NMFS Permit 16733. 37b.) Leatherback pygal tether attachment using fishing filament coated in soft tubing. Photo courtesy of NMFS SWFSC, NMFS Permit 18238.

Animal Welfare and Safety:

- When handling the turtle and equipment, researchers should use disposable gloves and change them often to maintain the most sterile environment possible. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Anesthetic is applied prior to (topical anesthetic) or immediately following (injectable local anesthetic) drilling. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- A sterilized, surgical quality drill bit must be used on each animal and disinfected with Betadine and alcohol between uses if multiple holes are drilled in the same individual. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Each drilled hole must not enter the body cavity.
- The skin surface (dorsal and ventral sides) must be thoroughly disinfected prior to drilling. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Triple antibiotic ointment may be applied to attachment components.
- Tags are programmed to detach after a designated interval or if certain environmental parameters are met.
- Corrodible links must be used to ensure that the turtle will shed the transmitter package.
- Care should be taken to avoid application of anti-fouling paints to the carapace of the turtle.

For specific leatherback monitoring requirements, see Appendix B: Special Considerations for Leatherback Turtles.

Refer to the blue box in Section 8.1 for relevant NMFS mitigation measures to reduce drag and minimize entanglements and other requirements.

Human Training and Safety:

- Instrument attachment should be overseen or performed by individuals trained in these protocols, especially when working with leatherback turtles. See Appendix B: Special Considerations for Leatherback Turtles.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

8.2.c.3 Medial Ridge Attachments for Leatherback Turtles

Purpose: Long-term (months) deployment of satellite tags on leatherback turtles.

Description:
On leatherbacks, transmitters are attached directly to the carapace by drilling through the medial ridge running craniocaudally along the carapace (Figure 38). After cleansing the skin along the medial ridge, two to three small holes are drilled through the ridge using a sterilized drill bit. Monofilament or coated wire tether (swabbed with triple antibiotic ointment) is threaded through an acetal polyoxymethylene resin disk. Once passed through the hole, the monofilament is secured with a second acetal polyoxymethylene resin disk (typically swabbed with triple antibiotic ointment) and a metal crimp so that the tether is tight and secure. Any excess monofilament is cut off, and the process is repeated for the second monofilament tether.

This technique has proven to be a successful replacement to the shoulder harness for long-term attachment of devices to leatherbacks with reduced hydrodynamic impacts (Fossette et al., 2008; Byrne et al., 2009; Casey et al., 2010; Jones et al., 2011; Jones et al., 2013). Use of corrodible links is necessary to ensure that the turtle will shed the transmitter package at a predetermined time frame. Currently, transmitters are significantly smaller and lighter than the models used in the past, conform to the shape of the leatherback carapace, and are specifically designed for direct attachment. Deployment time varies, but the attachment can be designed to last from 30 days to up to a year (NMFS, 2019). Attachments are often painted with anti-fouling marine paint or silica based two-part anti-fouling systems (e.g., PropSpeed®).

See Appendix N for example protocols.

Figure 38. 38a.) Coated wire tethers inserted into holes drilled in the central ridge area of a leatherback turtle. Note that holes only penetrated a few millimeters through the carapace ridge. 38b.) Securing the tag with the tethers. 38c.) Tag after tethers are crimped and secured. 38d.) Leatherback with tag securely attached to the medial ridge. Photos courtesy of NMFS SWFSC, NMFS Permit 18238.

Animal Welfare and Safety:

- When handling the turtle and equipment, researchers should use disposable gloves and change them often to maintain the most sterile environment possible. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Anesthetic must be applied prior to (topical anesthetic) or immediately following (injectable local anesthetic) drilling. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

- A sterilized, surgical quality drill bit must be used on each animal and disinfected with Betadine and alcohol between uses if multiple holes are drilled in the same individual. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Each drilled hole must not enter the body cavity.
- The skin surface (dorsal and ventral sides) must be thoroughly disinfected prior to drilling. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Triple antibiotic ointment may be applied to attachment components.
- Tags are programmed to detach after a designated interval or if certain environmental parameters are met.
- Corrodible links must be used to ensure that the turtle will shed the transmitter package.
- Care should be taken to avoid application of anti-fouling paints to the carapace of the turtle.

For specific leatherback monitoring requirements, see Appendix B: Special Considerations for Leatherback Turtles.

Refer to the blue box in Section 8.1 for relevant NMFS mitigation measures to reduce drag and minimize entanglements and other requirements.

Human Training and Safety:

- Tags should be applied by researchers experienced with leatherback turtles. See Appendix B: Special Considerations for Leatherback Turtles.
- The research team should consist of a dedicated observer to monitor leatherbacks during transmitter attachments. See Appendix B: Special Consideration for Leatherback Turtles.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

9. NON-INVASIVE IMAGING

9.1 Overview

Diagnostic imaging includes multiple methods that are commonly used non-invasive techniques in veterinary medicine to visualize internal structures. Applications in sea turtle research include clinical assessment as well as studies related to physiology, anatomy, or abnormal states. Most modalities involve the use of ionizing and non-ionizing electromagnetic radiation, and safety procedures are required to minimize exposure and potential negative health effects. Contrast-enhancing pharmaceuticals may be administered to improve visualization of some tissues and anatomical structures. Some imaging capabilities, such as radiography and ultrasound, exist as portable machines amenable to use in the field, whereas others are large and require turtles to be transported to facilities for use.

NMFS permit applicants may request to temporarily transport and hold sea turtles for up to 36 hours from time of capture to release in a USFWS-approved facility to accommodate imaging methods. Exceptions may be granted pending consultation with NMFS veterinarians.

For the transport, maintenance, and care of turtles temporarily held in a facility, researchers must follow the "Standard Conditions for Care and Maintenance of Captive Sea Turtles" issued by the USFWS[12] (USFWS, 2019) and, if in the State of Florida, the FFWCC Marine Turtle Conservation Handbook,[13] Section 4, "Holding Turtles in Captivity" (FFWCC, 2016).

9.2 Radiography

Purpose: Imaging internal anatomy to visualize normal features or abnormalities.

Description:
Radiography has wide-ranging applications for health assessments, including diagnosis after injury or ingestion of foreign materials (e.g., fishing hooks, plastic). Radiography is non-invasive and uses electromagnetic radiation to image internal structures (e.g., digestive tract), which are recognized by their shape and density. Radiography can also assist in identifying the presence of gas emboli and marine debris. Portable units are readily available for potential field use. Radiation exposure during imaging is well within the safety limits set for general medical use. Intravenously or orally administered contrast material might be used to improve the visualization of specific structures, evaluate function (e.g., digestive and urinary systems), or assess blood flow. However, the size of the animal must be considered when assessing the use of radiographic equipment.

Radiography must be performed following a veterinarian-approved protocol. If the procedure is performed at a facility, see the blue box in Section 9.1 for NMFS holding time and facility requirements.

Animal Welfare and Safety:
- Typically, animals are manually restrained; sedation usually is not required.
- Only contrast agents with demonstrated safety in animals are used.

Human Training and Safety:
- Radiography must be performed by experienced personnel.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during the procedure.

[12] USFWS Standard Conditions for Care and Maintenance of Captive Sea Turtles. [Available at https://www.fws.gov/media/standard-conditions-care-and-maintenance-captive-sea-turtles]
[13] Florida Fish and Wildlife Conservation Commission Sea Turtle Conservation Handbook. [Available at https://myfwc.com/media/3133/fwc-mtconservationhandbook.pdf]

9.3 Computed Tomography

Purpose: Imaging of internal anatomy to visualize normal features or abnormalities.

Description:
Computed tomography (CT) also uses electromagnetic radiation but can be used to obtain better distinction of specific tissue densities, as well as cross-sectional and 3D imaging, which allows better visualization than standard radiography (Valente et al., 2007). CT enables researchers to detect discrete changes in organ size, shape, margin contour, and position, and cross-sectional and 3D imaging offer significant advantages for the detection of pathologies and diagnosing skeletal and soft tissue abnormalities. These machines are large and require turtles to be taken to an imaging facility. Contrast agents are used for CT as with radiography. It typically takes less than a minute to image a sea turtle, depending on the size of the turtle and area of interest.

CT must be performed following a veterinarian-approved protocol. If the procedure is performed at a facility, see the blue box in Section 9.1 for NMFS holding time and facility requirements.

Animal Welfare and Safety:

- Animals may be manually restrained, although sedation or anesthesia are typically necessary to limit the animal's movement. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.
- Only contrast agents with demonstrated safety in animals are used.

Human Training and Safety:

- CT must be performed by experienced personnel.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during the procedure.

9.4 Magnetic Resonance Imaging

Purpose: Imaging internal anatomy to visualize normal features or abnormalities.

Description:
Magnetic resonance imaging (MRI) uses strong magnetic fields and radio waves to image structures within the body. As with CT, MRI units are large, non-portable machines. An advantage of MRI is that this modality provides better imaging of soft tissues and some pathological processes as compared to standard radiography or CT. Sea turtles must remain motionless during imaging, thus sedation or anesthesia is often necessary. Imaging takes longer than that for CT, approximately 20 to 45 minutes on average. Duration depends on the size of the turtle and the aspect of anatomy that is being imaged. See Appendix O for example protocols.

MRI must be performed following a veterinarian-approved protocol. If the procedure is performed at a facility, see the blue box in Section 9.1 for NMFS holding time and facility requirements.

Animal Welfare and Safety:

- Sedation or anesthesia is often necessary. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:

- MRI must be performed by experienced personnel.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during the procedure

9.5 Ultrasound

Purpose: Imaging internal anatomy to visualize normal features or abnormalities.

Description:

Ultrasound uses high frequency sound waves to image internal structures. In sea turtle research, ultrasound is a noninvasive technique used to image internal structures most often used with adult turtles to image the gonads, follicles, and eggs for reproductive studies (Rostal et al., 1990; Owens, 1999; Pease et al., 2010; Blanvillain et al., 2011), but other organ systems and tissues (e.g., musculature, subcutaneous fat layer) can be imaged for other purposes (e.g., nutritional status, body condition; Harris et al., 2016). Portable units allow ultrasonography to be performed under field conditions (Figure 39), and no sedation or anesthesia is necessary with this procedure. Various acoustic windows have been identified for imaging specific organs, and a variety of probes exist to provide the necessary depth of field and resolution. Acoustic coupling gel is applied to the skin to maintain contact with the probe and optimize imaging. See Appendix O for example protocols.

Figure 39. Using portable ultrasound units to image reproductive organs while in the field. Figure 39a.) Hard-shelled turtle. Photo courtesy of NMFS PIFSC, NMFS Permit 21260. 39b.) Leatherback turtle. Photo courtesy of NMFS SWFSC, NMFS Permit 18238.

If the procedure is performed at a facility, see the blue box in Section 9.1 for NMFS holding time and facility requirements.

Animal Welfare and Safety:

- Time conducting ultrasounds must be minimized.
- Typically, animals are manually restrained; sedation usually is not required.
- All overturned turtles must be placed on padded surfaces to avoid injury and only for brief periods of time.

Human Training and Safety:

- Imaging must be conducted by experienced personnel.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during the procedure.

10. REMOTE VISUAL AND ACOUSTIC SURVEYS

10.1 Overview

Visual surveys of sea turtles can be conducted by vessel and aircraft to assess habitat and to document seasonal abundance and spatial distribution of sea turtle populations. Visual surveys from a vessel may be conducted from large research vessels and from smaller motorized vessels. Surveys are typically conducted at slow speeds ranging from 5 to 10 knots. Vessel survey activities may include conducting line-transect surveys or temporarily tracking animals from a distance to observe behavior or retrieve short-term transmitter attachments once shed. Uncrewed survey methods and technology are still evolving. Here, we include basic information on remote visual and acoustics surveys as described in NMFS, 2019.

Vessel and aerial surveys only require a NMFS permit when following a sea turtle for more than 5 minutes or longer. Targeting or pursuing an animal is considered "take" under the ESA and requires a NMFS permit. Uncrewed survey methods are still evolving, and NMFS reviews permit applications on a case-by-case basis as needed.

10.2 Aerial Surveys

10.2.a Crewed Aerial Surveys

Purpose: Visually survey large or remote geographic areas to collect data on sea turtle presence/absence, location, behavior, and habitat use and characterize seasonal abundance and spatial distributions. Also used to locate turtles and facilitate capture.

Description:
Crewed aerial surveys predominantly use a twin- or single-engine, fixed high-wing aircraft (such as the DeHavilland Twin Otter, the Partenavia P68) or a single-engine light utility helicopter. Photographs and video may be taken during flights. Aircraft are typically flown at 500 feet or higher but may be flown as low as 300 feet depending on the type of aircraft, research objectives, and other factors (e.g., weather). Aircrafts are flown at speeds ranging from 35 to 110 knots. Observers in lower and slower aircrafts can detect sea turtles better than in higher, faster aircrafts. During capture efforts, researchers may conduct aerial line transects, and aircraft may act as spotters to guide the capture vessel to the vicinity of turtle aggregations. Aerial surveys often involve limited circling (approximately two to three passes) from a distance only to verify sightings, although in some instances they may be used for activities that require longer observation and/or closer proximity to target sea turtles. See Appendix P for example protocols.

NMFS permits require that encounters during aerial surveys (crewed or uncrewed) are not longer than 45 minutes.

Animal Welfare and Safety:

- Not applicable.

Human Training and Safety:

- Personnel must be trained in conducting aerial surveys and distance sampling.

10.2.b Uncrewed Aerial Surveys

Purpose: Remotely collect data on individual turtles and/or populations to characterize location, presence/absence, behavior, habitat use, abundance, distribution, and seasonal variability. Also used to locate animals and facilitate capture.

Description:

Uncrewed aerial surveys (UASs) may also be conducted with either fixed wing aircraft, such as the Puma AE, or rotary aircraft capable of vertical take-off and landing (VTOL), such as the APH-22 hexacopter. The size of UAS units varies from less than 1 meter in diameter for VTOL units to the 10-foot wingspan of the Puma. Uncrewed aerial systems may be launched directly from the capture vessel to search for animals. Once sea turtles are sighted, the capture vessel will move to the vicinity of a sighted animal to permit observation and evaluation of behavior and condition before attempting capture. The aircraft may repeatedly circle, follow, or hover over an animal for an extended period of time to more accurately define and maintain its location. For UASs, the time spent over the animal will be limited by the UAS unit's battery life, typically 15 to 30 minutes for VTOL units and 45 minutes for fixed-wing units based on currently available models. Fixed-wing UAS units are typically operated at 100 feet or higher, but VTOL units may be flown at lower altitudes of approximately 30–50 feet to collect images (e.g., photogrammetry). See Appendix P for example protocols.

NMFS-permitted UASs must be conducted using equipment that:

- Has an auto-return feature to prevent mid-flight failure and the potential for injury to sea turtles.
- Is piloted or directly supervised by a Federal Aviation Administration (FAA)-licensed, qualified crew member.
- Has a ground control station with a trained, dedicated UAS visual monitor working with the pilot.
- Is operated only within the line of sight.

Animal Welfare and Safety:

NMFS permits require that encounters during aerial surveys (crewed or uncrewed) are not longer than 45 minutes.

- Time spent flying over the animal must be minimized.

Human Training and Safety:

- UAS units must be piloted or directly supervised by an FAA-licensed crewmember.
- Personnel must be trained in operating UAS technology.
- The research team should include a visual monitor who works with the pilot during active operations.

10.3 Uncrewed In-Water Surveys

Purpose: Remotely track individual turtles to identify species and/or location and to characterize movements, habitat use, presence/absence, abundance, distribution, behavior, and environmental conditions.

Description:

Remotely Operated Vehicles (ROVs), including Autonomous Underwater Vehicles (AUVs), may be used to closely track sea turtles and observe and record foraging, diving, and other natural behaviors (Smolowitz et al., 2015; Figure 40). Models in current use for turtle tracking are approximately 2 meters in length (e.g., Ocean Server Iver II, Benthos Mini-Rover, REMUS 100; NMFS, 2019). ROVs are typically equipped with a live-feed video camera, time-depth-temperature sensors, and lights. ROVs may be attached to the research vessel by a tether that can be several hundred meters long. ROVs are typically operated at low speeds (e.g., approximately 1 knot with a maximum of 4 knots) to avoid risk of injury or disturbing animals. Turtles may be tracked for up to several hours per day.

Figure 40. AUV at the surface. Photo Courtesy of NMFS SEFSC, NMFS Permit 21233.

Tracking of animals with an ROV may occur upon sighting a target animal during a vessel survey or upon the release of a captured animal outfitted with an acoustic transmitter. The ROV is deployed from the side of the vessel and maneuvered toward the turtle but typically not approaching closer than 3 to 5 meters (NMFS, 2019). In some instances, two AUVs are deployed together when tracking a tagged animal to increase positional resolution of its location. Autonomous underwater vehicles can be outfitted with sensors to detect acoustic tag transmissions and are able to then alter their path in order to follow a moving transmitter. The AUVs process these detections to position the acoustic tag in 3D space with a resolution of less than 10 meters over 30 times a minute. Working in tandem to increase the spatial resolution, the AUVs can communicate through an acoustic transducer to plan movements and exchange information. When using two units, to minimize influence on turtle behavior, the AUVs are programmed to not get within 15 meters of the estimated turtle location. This distance may vary depending on the study objectives and capabilities (e.g., sensors) of the unit. AUVs may be operated underwater or at the surface to allow for visual monitoring of their positions from the research vessel. Tracking may occur for 10 hours or more depending on the AUV's battery life or turtle tag attachment duration. Throughout tracking operations, researchers may deploy a directional hydrophone over the side of the vessel monitoring the AUVs to constantly monitor the approximate location of the tagged turtle in reference to the AUVs. This will ensure that the AUVs do not lose the acoustically tagged turtle and that the AUVs are maintaining the programmed buffer distance. See Appendix Q for example protocols.

NMFS ESA Section 10 permits are only required when the researcher intends to track and pursue sea turtles.

Animal Welfare and Safety:

- Tethers are too rigid to present a risk of sea turtle entanglement.
- Depending on research objectives, ROVs maintain a minimum distance of 3–5 meters from the turtle of interest.
- Depending on research objectives, AUVs remain at least 15 meters from the estimated turtle location.

Human Training and Safety:

- Personnel must be trained in operating ROVs and AUVs.

10.4 Remote Acoustic Detection and Tracking

Purpose: Remotely detect and monitor turtle movements, habitat use, presence/absence, abundance, distribution, behavior, and other aspects of their ecology.

Description:
Other methods of remote detection of sea turtles include multibeam, side-scan, and imaging sonars (e.g., DIDSON). Vessels deploying these devices operate at low speeds (2 to 7 knots). High-frequency sound pulses (120 kilohertz to 1.8 megahertz) are transmitted by the sonar transducers and reflect off the seafloor and objects in the water column, producing real-time acoustic backscatter images of bottom topography and the location of sea turtles in the study area. When testing the effectiveness of a particular type of acoustic gear for detecting and/or imaging sea turtles, turtles may be followed at a distance once they are detected to keep them within the acoustic beam. See Appendix Q for example protocols.

Animal Welfare and Safety:

- Turtles are followed at a distance. The exact distance depends on the equipment used during surveys.
- The duration of a directed acoustic effort typically does not exceed 5–10 minutes.

Human Training and Safety:

- Personnel must be trained in operating remote detection technologies.

11. INFREQUENT AND/OR HISTORICAL METHODS

11.1 Overview

Research methods evolve over time, and some methods are infrequently used as newer methods are introduced or the research focus changes. Some methods are completely replaced by more modern techniques that are more effective or less invasive than earlier techniques. Here, we describe several standard research methods that are still used today, although less frequently compared to those described in previous sections. We also briefly describe two historical tagging and carapace marking methods that are no longer used in the U.S. Because these methods are no longer used in the U.S., we have not provided any information on animal welfare or human safety and training. However, researchers may still encounter turtles that were previously tagged using these methods and should be familiar with identifying these tags and markings to ensure that recapture data are collected and reported.

11.2 Infrequent Methods

11.2.a Oral Cavity Measurements

Purpose: Characterize anatomic features relevant to diet and fishing gear interaction.

Description:
The jaw and oral cavity are measured for specific research interests. Applying constant pressure to the lower jaw will prompt the turtle to open the oral cavity. All measurements are usually taken using spring and/or dial calipers while the mouth is held open with a bite block or canine mouth gag (i.e., a type of oral speculum available from veterinary equipment suppliers; Figure 41). Canine mouth gag tips are often padded to reduce damage to the beak as the turtle bites down on the gag. Oral cavity measurements can include internal gape width, esophagus width, gape height, upper jaw length, and lower jaw length.

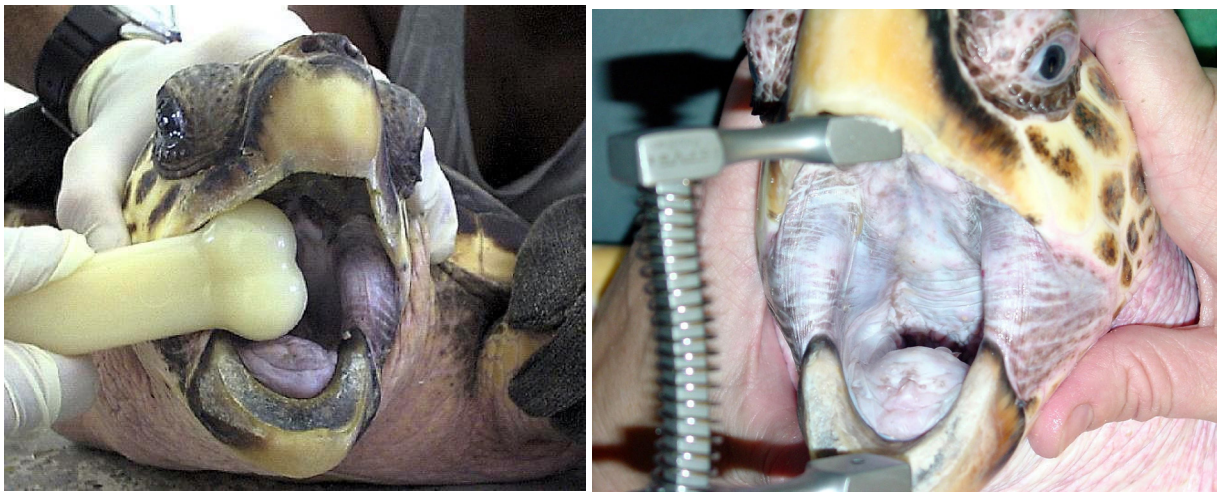

Figure 41. Various instruments (bite block and canine mouth gag) used when collecting oral cavity measurements. Photos courtesy of NMFS SEFSC, USFWS Permit TE676379.

Animal Welfare and Safety:

- To avoid injury to turtles, mouth gag surfaces must have no sharp edges or otherwise be constructed to prevent injury to the mouth or beak (rhamphotheca).
- Equipment that comes in contact with animals must be cleaned and disinfected after each use. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed.

11.2.b Bioelectrical Impedance Analysis

Purpose: Used to measure the relative fat content in relation to nutritional condition.

Description:

Bioelectrical Impedance Analysis (BIA) assesses the relative fat content in turtles by measuring the resistance of body tissues to the flow of a harmless, low-level electrical current. The percent of fat tissue is determined by measuring the speed and strength of the current. In the U.S., researchers typically use a handheld analyzer that runs on a 9-volt battery (NMFS, 2019). The instrument includes an Ohm resistor that allows calibration checks. During testing, small electrodes are placed on the opposite limbs of the body. The test generally takes only 5 to 20 seconds and elicits no response from sea turtles (NMFS, 2019). Other devices require the shallow insertion of electrode needles into the skin of the flippers. Various factors have been reported to influence results, including environmental temperature and post-prandial state (Kophamel et al., 2023).

Animal Welfare and Safety:

- Care must be taken to ensure that turtles do not injure themselves during BIA.
- All equipment must be cleaned between use on each turtle, and the skin should be disinfected if needle electrodes are used. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:

- Researchers should be familiar with the use of these devices and confounding factors that influence results.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during BIA.

11.2.c Tear Collection

Purpose: Physiological studies, hormone analysis, and health assessment.

Description:
Sea turtle lacrimal glands function with the kidneys to maintain electrolyte homeostasis and may be used to study physiological parameters and hormones. Tears are excreted through the lacrimal duct that opens into the caudodorsal conjunctiva of each eye. The tears are very viscous and are not easily amenable to collection by pipettes or similar instruments. Disposal eye spears used in ophthalmic procedures for tissue manipulation and fluid absorption have been used to sample tears from sea turtles (Niemuth et al., 2019)

Animal Welfare and Safety:

- Use adequate animal restraint and careful handling of instruments to avoid accidental injury to the eye.

Human Training and Safety:

- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

11.2.d Oxytetracycline

Purpose: Chemical bone marking for skeletochronology (aging) studies.

Description:
Oxytetracycline (or tetracycline) injections are performed in many species to establish time-specific marks in bone tissue. If an oxytetracycline-injected turtle later strands dead and is recovered, post-mortem analysis to visualize this chemical mark then allows researchers to calculate the frequency of bone growth mark (or ring) formation to validate the use of growth mark counts and spacing for age and growth rate estimation (Frazier, 1985; Coles et al., 2001). This procedure has been performed in various species of sea turtles with minimal effects, and the results indicate that generally a single bone-marking dose of oxytetracycline does not appear

to harm either the animals injected or those in their immediate surroundings (Frazier, 1985; Harms et al., 2004; Gauche et al., 2006; Snover et al., 2011; Innis et al., 2020). However, in some cases involving different dosages and injection frequencies, anorexia, local cutaneous erythema, and muscle necrosis around injection sites have been reported (Innis et al., 2017). Following Goshe et al. (2016), a dose of oxytetracycline [dosage (milliliter) = weight (kilogram) × 25 (milligrams/kilogram)/concentration (milligrams/milliliter)] injected into the shoulder muscle has been demonstrated to create a mark in sea turtle bones at a known point in time (time of injection). In the U.S., administration is according to a veterinary-approved protocol using a single low dose (e.g., 25 milligrams/kilogram; Harms et al., 2004) and intravenous (IV) or IM administration of several smaller volume injections (less than 10 milliliters/site; NMFS, 2019). See Appendix R for example protocols.

Animal Welfare and Safety:

- Administration must be according to a veterinary-approved protocol that defines the dosage, injection site selection and preparation, and protocols (NMFS, 2019).
- All equipment should be sterilized prior to use. See Appendix A: Antiseptic Practices, Pain Management, and Biosecurity.

Human Training and Safety:

- Supervising researchers must be trained and experienced in this method.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during marking.

11.2.e Stomach Pills

Purpose: Investigate stomach temperature fluctuations related to satellite-linked data. Primarily for research involving leatherback turtles but could be used for any species.

Description:

Stomach pills possess a thermistor to detect stomach temperature and a transmitter to relay temperature fluctuation data to a satellite-linked data recorder to provide details on the foraging events and patterns of sea turtles at sea. By design, pills thus far used in sea turtles can only be used in conjunction with an externally attached satellite tag mounted on the turtle's carapace between the first and second vertebral scutes using a marine epoxy or similar substance. This combination allows for transmission of the stomach temperature data along with location data and dive behavior data via an Argos satellite uplink when the turtle surfaces to breathe. The pill is coated with a dissolvable, biocompatible material (e.g., ethylcellulose) to temporarily increase the pill's diameter to increase retention time in the stomach (Casey et al., 2010). Coating diameter is based on pill size (technology is making pills smaller quickly) and the size of the animal. The pill is inserted into the turtle's esophagus using a lubricated flexible rubber tube. Administration of the pill takes only 1 to 2 minutes. Once the coating dissolves, the pill should be small enough to pass through the pyloric sphincter, into the small intestine, and then be passed by the animal, within 2 weeks of ingestion.

NMFS permits require that researchers use "smart" pills or other devices intended to be ingested and passed or otherwise retrieved in accordance with a veterinary-approved protocol. The protocol must include the allowable minimum size of the turtle, a clear explanation of safety relative to body size, monitoring of the transmitter, administration of the pill, and anticipated risk for obstruction and complications.

Animal Welfare and Safety:

- A damp cloth can be placed over the turtle's eyes to keep it calm during the procedure.
- Researchers must ensure that the pill is properly sized for the animal to prevent potential intestinal blockage.

For specific information on performing methods on leatherback turtles, see Appendix B: Special Considerations for Leatherback Turtles.

Refer to the blue box in Section 8.1 for additional NMFS permit requirements.

Human Training and Safety:

- For NMFS personnel requirements for handling leatherbacks, see Appendix B: Special Considerations for Leatherback Turtles.
- All researchers handling turtles need to be aware of the turtle's head/mouth and claws to prevent and avoid injury from being bitten and/or clawed during sampling.

11.3 Historical Tagging and Carapace Marking Methods

11.3.a Coded Wire Tags

Coded wire tags (CWTs) were used to tag turtles starting in the late 1970s (Schwartz, 1981; Higgins et al., 1997). CWTs provided a reliable method for marking hatchling cohorts (not individuals) and were used extensively in captive reared and wild Kemp's ridley hatchlings in the U.S. and in larger yearling Kemp's ridley turtles during NOAA Fisheries' Kemp's ridley headstart project (1978–1992; Higgins et al., 1997). Tag application involves injecting a small section of coded wire using a specialized tag injector into the dorsal surface of a turtle's front flipper near the claw. The tags may be either non-magnetized or magnetized at the time of insertion, but the wire tag must be magnetized for detection with a handheld magnetometer. A non-magnetized tag can be magnetized immediately before detection by passing a magnet over the front flippers where the tags were implanted, before tagging by using a pre-magnetized roll of wire, or by using a magnetized head on the tag injectors (Higgins et al., 1997, Fontaine et al., 1993). Coded wire tags are detected using a wand-type tag detector (magnetometer) or by radiography. Codes on the tags can only be read after the tag is removed via dissection and examined under a microscope. Therefore, CWT can only be read when recovered from deceased animals. See Fontaine et al. (1993), Higgins et al. (1997), and NMFS-SEFSC (2008) for more details on coded wire tag use, application, and detection. Because

tagging and marking methods are no longer used in the U.S., we have not provided information on animal welfare and safety or human training and safety.

11.3.b Living Tags

Living tags were first used on sea turtles in the 1980s (Hendrickson and Hendrickson, 1981). Living tags provided a permanent marking method for sea turtles, and they were particularly useful with post-hatchlings and small juveniles that could not be marked using other tagging methods. A living tissue plug is removed from the plastron and transplanted into the carapace, leaving a permanent, identifiable light spot that grows with the animal on the contrasting dark carapace (Figure 42). See Balazs (1999) and NMFS-SEFSC (2008) for more information on living tags.

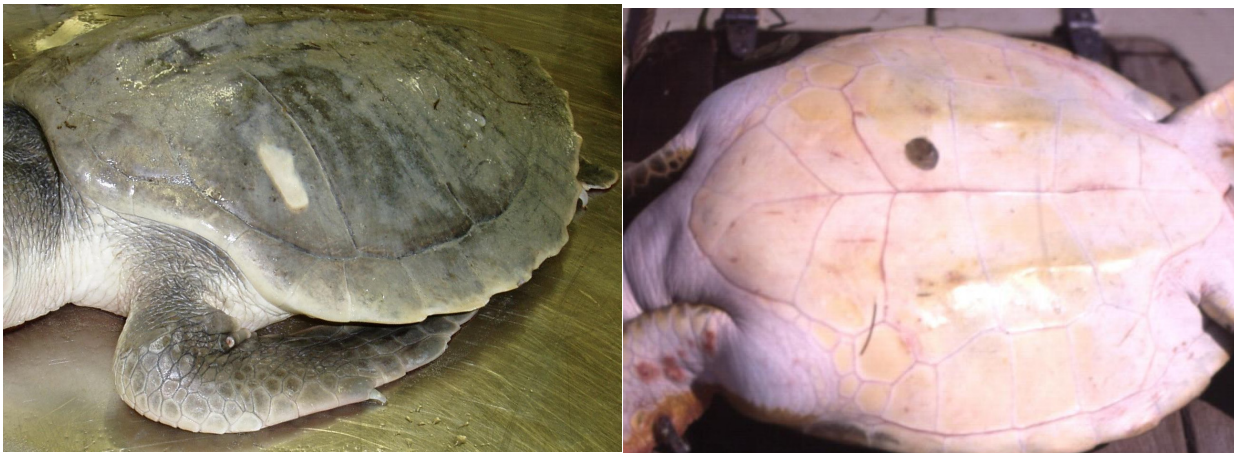

Figure 42. Living tags in juvenile hard-shelled turtles. 42a.) Living tag (rectangular white area) on the carapace of a juvenile Kemp's ridley turtle. 42b.) Living tag (dark circle) on the plastron of a juvenile green turtle. Photos courtesy of FL Coop Unit at UF, NMFS Permit 1299 and FWC MTP 094.

12. CONCLUSIONS

As new technologies are developed and techniques improve, NMFS anticipates that new research procedures or protocols may be proposed by sea turtle researchers. For example, the use of UASs for sea turtle research is expected to expand over time as this technology evolves (i.e., new sensors and payload components that can collect different types of information are likely to be developed). Improvements in sea turtle tag design and attachment methods are also expected as this field of study grows and technology evolves (e.g., the miniaturization of solar cell technology with longer battery life), allowing units to be placed on smaller animals and/or for longer durations. These units may be considered standard methods in the future as their effects meet certain requirements, and the researchers can meet standard mitigation measures.

Since unknown risks may be associated with new or experimental procedures, procedures will only be considered standard methods after reviewing the best available scientific information to determine that (1) the procedure is effective at achieving the desired research objectives, including new research activities, and (2) any adverse effects on sea turtles resulting from the procedure are less than or similar to the adverse effects of any of the procedures previously authorized by NMFS (or equivalent) or described above for the same research objectives. Mitigation measures and best practices related to new standard methods may be revised to minimize impacts to the extent possible.

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Appendix A: Antiseptic Practices, Pain Management, and Biosecurity

Antiseptic and Sterile Techniques

Measures to minimize the risk of infection and cross-contamination between individuals include using clean, aseptic, and sterile techniques (NMFS, 2017; NMFS, 2019). Clean technique usually applies to noninvasive procedures that result in contact with skin or mucous membranes. The aseptic technique is most commonly used for brief, minimally invasive procedures that result in any degree of internal contact (e.g., drawing blood). Sterile techniques apply most often to longer invasive procedures, such as laparoscopy or surgery. Reusable instruments for procedures requiring aseptic or sterile techniques must be sterilized by standard autoclave or cold sterilization procedures. Instruments that do not have internal contact (e.g., tagging pliers and non-disposable PIT tag applicators) must be disinfected using a broadcidal solution and the product-recommended contact time between individuals.

Clean technique includes:

1. Routine hand washing or use of disposable gloves.
2. Cleaning and disinfecting equipment between individuals. To disinfect field equipment, use an appropriate disinfectant such as a freshly mixed 1:10 solution of household bleach (~5–6 percent sodium hypochlorite). To prepare a 1:10 bleach solution, add one volume of household bleach (e.g., 1 cup, liter) to 10 volumes of clean water (e.g., 10 cups, liters). Remove any organic material from equipment before disinfection and spray or soak equipment for at least 10 minutes, using a fresh solution every day.

Aseptic technique includes:

1. Disinfecting hands or using new non-sterile disposable gloves (preferred).
2. Disinfecting the turtle's skin using a surgical scrub (e.g., Betadine® scrub or chlorhexidine gluconate). Alcohol may be used in lieu of a surgical scrub if necessary to avoid interference with research objectives (e.g., stable isotope analysis).
3. Applying 70 percent alcohol (e.g., isopropyl or ethanol) to the turtle's skin (minimum requirement). Multiple applications and scrubbing are used to thoroughly cleanse the procedure site as necessary. A minimum of two alternating applications of surgical scrub and alcohol are used for PIT tag application sites and drilling into the carapace, due to potential increased risk of infection.
3. Cleaning the work area.
4. Using sterile instruments or new disposable items (e.g., needles and punch biopsies) between individuals.

Sterile technique includes:

1. Protocols in accordance with approved veterinary protocols that consider analgesia/anesthesia, use of antimicrobials, anticipated risks and response measures, and exclusionary criteria for animal candidacy.
2. Direct veterinary attendance when possible.
3. Disinfection of hands and use of sterile disposable gloves.
4. Dedicated site (e.g., surgery room) or work area modified to reduce contamination.
5. Surgical preparation of skin.
6. Sterile instruments.

Table 1. Required antiseptic technique for each research procedure.

Research Procedure	**Required Technique**
Handling, gastric lavage, and cloacal lavage	Clean technique
ssue sampling (biopsy punch or comparable)	Aseptic technique
Blood sampling	Aseptic technique
PIT tagging	Aseptic technique; two applications of surgical scrub and alcohol
Flipper tagging	Aseptic technique
Carapace drilling for instrument attachment or bone biopsy	Aseptic technique; two applications of surgical scrub and alcohol
Bone biopsy (other than carapace)	Sterile
Laparoscopy (± biopsy)	Sterile
Large skin, muscle, fat biopsy, other tissue biopsy	Sterile

Handling Turtles with Fibropapillomatosis

If sea turtles with FP may be encountered, a designated separate set of equipment for turtles with FP must be available and used only for turtles with observable FP. Non-disposable equipment that comes into contact with sea turtles with FP (e.g., tag applicators, measuring tape) must not be used on turtles without FP. All measures possible should be exercised to minimize exposure and cross-contamination between affected turtles and those without apparent disease, including the use of disposable gloves and thorough disinfection of non-disposable equipment and surfaces between individual turtles. Appropriate disinfectants can include 70 percent isopropyl alcohol, 10 percent bleach, and other viricidal solutions with proven efficacy against herpes viruses.

Pain Management

Procedures used for sea turtle research include those anticipated to cause short-term pain or distress, such as tagging, as well as more invasive procedures where relatively longer periods of pain or discomfort may result. Detailed information regarding pain management is outlined in the section for each research procedure. The table below provides NMFS (2019)-recommended minimum requirements when considering animal welfare and the relative benefits and risks of different modes of pain management under field and laboratory conditions. Additional measures are encouraged whenever possible, including sedation or anesthesia for invasive procedures (e.g., laparoscopy) when release does not immediately follow the procedure and full recovery can be assessed.

Table 2. Pain management requirements for each research procedure.

Research Procedure	**Minimum Requirement**
Tissue sampling (biopsy punch or comparable)	None
Blood sampling	None
PIT tagging	None*
Flipper tagging	None
Carapace drilling for instrument attachment or bone biopsy	Local anesthetic and/or systemic analgesic**
Bone biopsy (other than carapace)	Local anesthetic and systemic analgesic
Laparoscopy	Local anesthetic and systemic analgesic
Laparoscopy biopsy	Local anesthetic, sedation, and systemic analgesic
Large skin, muscle, fat biopsy, other tissue biopsy	Local anesthetic and systemic analgesic

*Anesthesia is not required for PIT tag injections for turtles when using 10-millimeter tags and a 16-gauge needle for turtles at least 16 centimeters SCL. In general, tags of this size are not injected in turtles <30cm SCL.

**Local anesthetic may be administered by immediate application to the wound following drilling (i.e., “splash block”).

Appendix B: Special Considerations for Leatherback Turtles

Overview

Extra care must be exercised when capturing, boarding, handling, and sampling leatherbacks. NMFS-permitted activities involving directed leatherback research activities incorporate recommendations made by veterinarians with experience capturing leatherbacks (NMFS, 2017; NMFS, 2019). Here, we outline NMFS permit requirements for directed leatherback research as described in NMFS (2019). This information can also be applied to opportunistic leatherback capture in non-selective capture gear.

General Handling Requirements

- Leatherbacks are boarded only if they can be safely brought on board the research vessel.
- Researchers must handle and support leatherback turtles from underneath.
- Researchers must not turn leatherback turtles on their carapace.
- The period of holding must not exceed 1 hour unless deemed medically necessary.

Personnel Requirements

To effectively monitor leatherback turtles during capture and handling, researchers must have a designated medical observer on each capture outing team (NMFS, 2019). Whenever possible, the preference is for an experienced veterinarian (i.e., documented history of working with sea turtles under conditions requiring proficiency in emergency procedures and resuscitation). If a veterinarian is not in attendance, one must be reachable by cellular or satellite phone or radio in case of an emergency. A veterinarian is required to be onboard if invasive procedures are to be performed or if the capture interval will be longer than 1 hour, starting at the time the leatherback is caught in the net. Here, an invasive procedure is considered any non-tagging procedure that pierces tissues deeper than the dermis (e.g., fat biopsy). For any captures, at least one individual must have the dedicated role of monitoring vital rates, behavior, and ensuring temperature control. This individual should not have any other duties that limit their attentiveness to these responsibilities. Moreover, monitoring and delegation of responsibilities should be coordinated such that the period of restraint is as brief as required to accomplish research objectives.

Training Requirements

If no veterinary personnel are on the research team, the chief scientist is responsible for monitoring leatherback welfare during research activities. The chief scientist must be trained by a veterinarian in the following information and procedures:

- Acceptable parameters for heart rate, respiration, temperature, and responsiveness, as defined by baseline data gathered in the field as well as in collaboration with veterinarians and colleagues from NMFS (or equivalent agency).
- Recognition and appropriate response to situations that suggest cessation of animal handling/procedures and initiation of release.
- Safe water reintroduction and monitoring of a turtle in possible distress.
- Appropriate first aid measures for animals in distress. These measures may include intubation, artificial respiration, and administration of pharmaceuticals to stimulate respiration and/or cardiac contraction.

Monitoring in Remote Field Sites

If research is conducted in remote field locations where it is not possible to have a veterinarian or veterinary technician on the capture team, the chief scientist or a senior researcher with significant prior experience with leatherbacks in emergency situations can serve as the medical observer. The medical observer will have the role of monitoring vital rates, behavior, and ensuring temperature control. This individual should not have any other duties that limit their attentiveness to these responsibilities. Moreover, monitoring and delegation of responsibilities are coordinated such that the period of holding is as brief as required to accomplish research objectives.

Monitoring Requirements

Methods for monitoring include:

- Performing a gross examination upon capture, including the assessment of body fat
  - (subjective), activity, alertness, pre-existing injuries, weight, and length.
- Recording the respiratory rate over a 2-minute period, logged every 20 minutes.
- Recording the response to noxious stimuli (either tail pinch or blink response), logged every 20 minutes.
- Recording the heart rate determined by digital or Doppler detection on the femoral artery, ultrasound, rectal/cloacal pulse oximeter, or electrocardiogram, logged every 20 minutes.
- Recording body temperature detected by an anal probe inserted 15 centimeters, logged every 20 minutes.
- Assuring cooling by running ambient seawater over the carapace and forelimbs during the time on deck.
- Relating changes in the animal's condition to the chief scientist so that an ongoing assessment of the animal's condition can be made.

Adverse Reactions

Adverse reactions could be indicated by cardiac arrhythmia, cardiac arrest, respiratory arrest, seizures, or severe blood gas alterations. Veterinarians are still in the process of defining normal and altered blood gas parameters by establishing baselines. The response to adverse reactions would depend on the type of reaction but would likely involve basic supportive therapy including intubation and assisted respirations, IV fluids (for shock and to hasten elimination of drugs through renal excretion), anti-arrhythmic drugs (e.g., IV lidocaine), cardioresuscitory drugs (e.g., IV epinephrine for cardiac arrest), or anti-seizure medication (e.g., IV diazepam).

Monitoring Requirements for Various Restraint/Handling Times

The following monitoring is recommended for short and prolonged periods of restraint and recorded as part of each animal's permanent capture record (NMFS 2019).

Restraint interval <30 minutes

Parameter	**Frequency**
Responsiveness/activity level	Throughout
Respiration rate	Upon capture, every 20 minutes
Heart rate*	Upon capture, every 20 minutes

*Only conducted if boarded/accessible

Restraint interval ≥30 minutes

Parameter	**Frequency**
Responsiveness/activity level	Throughout
Respiration rate	Upon capture, every 20 minutes
Heart rate*	Upon capture, every 20 minutes
Point-of-care analyzer*	Upon capture, every 20 minutes
Body temperature*	Upon capture, every 20 minutes

*Only conducted if boarded/accessible

Below are general guidelines (NMFS, 2019) based on previous studies and medical opinion regarding the alteration of these parameters that trigger immediate assessment by the medical observer and chief scientist:

Parameter	Trigger threshold
Responsiveness	Reduction in response to procedures or noxious stimuli
Respiration rate	Apnea for periods >2 minutes
Heart rate	<20 beats per minute
Blood pH	<7.2 (temperature corrected)
Potassium	>6.8 millimoles per liter
Glucose	<60 milligrams per deciliter
Body temperature	Alteration of initial body temperature by >2 °F or 1 °C (or if temperature exceeds 86 °F/30 °C)

Emergency Field Kits and Emergency Interventions

In addition to animal monitoring equipment, an emergency field kit for intervention will be included in each research excursion (NMFS, 2019). This kit will be available to the medical observer with appropriate training on all items in the kit. The kit includes, but is not limited to, the following:

- Means of ventilatory support (e.g., demand breathing valve, 2-liter Ambu bag, oxygen cylinder)
- Endotracheal tubes (non-cuffed 10, 12, 14, and 16; other sizes as appropriate)
- Oral speculum and appropriately sized blade
- Water-based lubricant
- Disinfectants (e.g., Betadine® scrub, isopropyl alcohol)
- Sterile gauze
- Medical tape
- Needles and syringes (size appropriate)
- Epinephrine (additional medications, e.g., doxapram, lidocaine, and sodium bicarbonate)

The attending veterinarian should be prepared to render aid and resuscitation in the event of an emergency. If a veterinarian is not in attendance, members of the capture team must be trained by a veterinarian in basic resuscitation procedures, which may include endotracheal intubation, ventilatory support, and epinephrine administration. The level of training and expected level of intervention are determined by the designated project veterinarian based on the ability/aptitude of the capture team. Such intervention should follow a previously developed response plan that includes remote consultation with a veterinarian by phone and a written contingency protocol if communication is not possible.

Appendix C: Example Protocols: Selective Capture Methods

Diving from a Vessel

Example 1

Turtles will be captured by hand by a trained group of divers. Hand capture will involve free-diving (approximately 2–20 meters) to capture turtles resting/foraging on bottom substrates. While most captures occur in waters less than 10 meters, occasionally a capture in deeper waters is attempted by trained personnel. All safety procedures are followed, and attempts are only made if the safety of the turtle and personnel are maintained. If turtles take notice of the diver and attempt to flee, they will not be pursued. Capture from slow moving boats will also involve spotting turtles from the boat and easing into the water to hand-capture, or free divers will be towed behind the boat and will signal the captain and then subsequently dive to the resting turtle. Turtles will immediately be brought to the surface and lifted onto the boat, or, for nearshore capture, turtles will be brought to shore and placed in a turtle holding bin.

Dip Nets

Example 1

On occasion, a dip net is used to capture a free-swimming animal. The dip net measures approximately 1.5 meters in diameter and consists of an aluminum hooped net with 4-inch knot-to-knot mesh size hung on the hoop. The pole consists of a telescoping fiberglass or aluminum pole that could expand to 5 meters in length. The boat approaches floating or surfacing turtles from behind, and researchers gently place the net in the water just ahead of the turtle so that it swims into the net. Researchers may also scoop up the turtles from underneath as the boat approaches from behind. Researchers will attempt a maximum of three tries per each individual turtle.

Break Away Hoop Nets: Leatherback Capture

Example 1

Using Crewed-Aircraft for Capture Assistance

During capture efforts, crewed aircraft will survey the study area to encounter leatherbacks and to guide the capture vessel to the vicinity of the focal turtle. Once a turtle is sighted, the capture boat will move to the vicinity of a sighted animal to observe the turtle, evaluate its behavior and condition, and then attempt to capture it if deemed appropriate. As the boat is approaching and during evaluation and capture, the crewed aircraft circles the focal animal in order to more accurately define location. During this period, the natural behavior of leatherbacks is to spend only a small portion of their time at the surface and to resurface in the same general area each time but often too far away for the capture boat to see. Therefore, the use of a spotter plan is essential for the successful capture of leatherbacks. Circling by the aircraft above the focal turtle usually lasts for more than 5 minutes but not more than 30 minutes. During circling, by nature, the aircraft raises to altitudes of 700–1000 feet above sea level and maintains an airspeed of at least 125 knots, which minimizes negative effects of aircraft presence on leatherback behavior.

Animal Capture

Leatherback turtles are captured using a large diameter breakaway hoop net. This method has also been employed routinely by researchers off the U.S. West Coast, Northeast Coast, and off Nova Scotia, Canada, to safely catch leatherback turtles in waters for biological sampling and satellite transmitter attachment (James et al., 2005a; James et al., 2005b; Dodge et al., 2014). The capture method is described in detail by Asper (1975). The breakaway hoop net is custom made so that the hoop is wide enough to fit easily over a leatherback with front flippers loosely held at its side. A crew member is positioned on the bow, ready to guide the hoop net (fitted to a long guiding pole) over the leatherback. The hoop net is fitted with breakaway stays to a cast net, which is then pursed over the turtle. The net is constructed of 4-inch diameter knotless mesh. This design contains the large front flippers and permits greater control of the captured animal by reducing movement of the front flippers through the mesh. The knotless mesh reduces the potential for abrasion. Upon capture of a leatherback, the net is adjusted by hand to provide slack and ensure that the turtle is able to extend its neck to breathe several times alongside the boat prior to being brought aboard the vessel.

Vessels

Vessels used for capture vary based on logistics and distance from shore. Capture and release operations are primarily conducted aboard a 30-foot × 10-foot PackCat aluminum hull landing craft. This vessel has been specially modified for the capture of leatherback turtles and has been used successfully for leatherback capture operations in nearshore waters since 2001. The captured turtle is moved toward the open bow door of the vessel and lifted approximately 1 meter from the waters' surface by the hydraulic bow door. The door and decking are covered with PVC plating that prevents abrasion and permits the sliding of the turtle into the vessel. In areas outside of the coastal study areas that are too distant for access by the small landing craft vessel, the captured turtle will be quickly brought alongside to the stern of the capture boat (approximately 20 meters) and guided

up a short ramp onto a raised platform. This technique has been deployed successfully by researchers sampling leatherbacks off the coast of Nova Scotia (James et al., 2005a) and off the southern U.S. Capture and release operations in coastal and offshore waters are facilitated by the use of a rigid-hulled inflatable boat launched from a larger vessel. Following capture with a breakaway hoop net, as described previously, the turtle is raised to the ship's wet deck or auxiliary platform 1–2 meters above the waterline with the aid of a cargo net. Other similar vessels may be used, as appropriate and required by logistics or other constraints.

Strike Nets

Example 1

The net is deployed from a boat and encircles the turtle. Similar to entanglement nets, the strike net has floats on the top (float line) and weights at the bottom (lead line). Typically, the mesh size is a stretched diagonal mesh of 46 centimeters (23 centimeters^2 mesh) and is large enough to prevent the bycatch of other species. Once deployed, the net is brought back on board promptly (short soak time). If a turtle is encircled by the net, the net will slowly be brought back into the boat so that the circle around the turtle becomes tighter with the aim of the turtle becoming tangled in the net, allowing for easy capture. The turtle will be brought on board the boat for processing either on the boat or onshore.

Appendix D: Example Protocols: Non-Selective Capture Methods

Entanglement Net

Example 1

Turtles are captured using specialized sea turtle entanglement nets (100 meters × 6 meters; 30-centimeter mesh knot-to-knot; equipped with floats approximately every 5 meters). Entanglement nets are used as a set net placed over a mud bottom substrate and/or deployed in the manner of a seine net from the vessel or on shore. An anchor is attached to each end of the net, which holds the net in place while it is deployed and soaked. When using the entanglement net as a seine net, one side of the net is set with an anchor, and the other side is deployed from the vessel in a circular fashion and slowly pulled back onto the vessel or onto the shore within a few minutes of deployment. The deployment of entanglement nets as a seine net is expected to take less than 10 minutes. After deployment of the net as a seine net, the net is checked for a turtle and is immediately retrieved. Seine nets may also be deployed when set nets are in the water; however, the seine would not interfere with a set net. One to four set nets may be set at one time. The configuration of set nets may vary but typically involves setting the nets parallel to each other. If more than one net is set in a given area, the nets are usually set between 200 and 1000 meters from each other. Nets are deployed on average from 1 to 8 hours to maximize capture efforts during each field outing (Eguchi et al., 2010; Eguchi et al., 2012). Actual deployment time depends upon the number of staff present during each outing and the number of captures at any given time. The tangle nets used in this research have a very large mesh size (12–14 inches knot-to-knot) and allow turtles to swim to the surface to breathe after they are entangled. All nets are continuously monitored visually at all times, and to add an additional layer of care regarding animal welfare, nets will be physically checked (lead line pulled to the surface) at least once every 30 minutes. This is

within the reasonable dive limits of green turtles in shallow water areas, like the study site (e.g., Hazel et al., 2009). During all netting efforts, two or more boats are used to monitor the nets.

Example 2

A large mesh gill net is used to capture turtles. The net is 65–150 meters in length with webbing made of 18-gauge twisted nylon twine or #10 monofilament, with a mesh size of 20–51-centimeter stretch (knot-to-knot). The mesh is suspended from a foam core braided polyethylene top line with fixed bullet-shaped polystyrene foam floats at approximately 10-meter intervals. The bottom line consists of a continuous lead core line (e.g., No. 30). Anchors attached to both ends of the net keep it in position and prevent drifting of the lead line. Net heights vary, depending upon the depth in which the net is set (i.e., shorter nets in <5 meters of water and taller nets in >5 meters of water), so that the netting extends throughout the water column. In order to target green turtles, the net may be set among grass beds.

The net is deployed by boat and carefully monitored by hand-over-hand elevation of the top line from the bow of the boat every 30 minutes. When turtles encounter the net and become entangled, they are quickly removed from the net and placed on the deck of the boat. Before deployment of the net, a careful visual inspection of the area is made to ensure that there are no marine mammals present near the study site. In the case where marine mammals are sighted near the netting sight, nets either are not deployed or are pulled in, and netting activity will cease until the area is clear of marine mammals.

Example 3

Large-mesh entanglement nets are constructed of 2-millimeter diameter lines (e.g., monofilament, nylon, braided) with a stretched diagonal mesh of 46 centimeters (23 centimeter^2 mesh). The lengths of the nets range from 20 to 100 meters, and depths range from 1.5 to 8.0 meters (with a maximum water depth of 8 meters). The nets are set at the surface extending vertically through the water column. Floats are embedded in the top line of the net, and the bottom line is weighted. Nets are deployed close to shore (<100 meters from the shoreline) in shallow, sandy, or muddy (estuarine, generally of seagrass or macro-algae) habitats and continuously monitored (hand checked every 0.5 hour) by boat (with a minimum of four crew members). While hand-checking a net, all potential issues are addressed (e.g., algae clumps removed, rays freed, twists in net fixed, or anchor pulled and reset by boat). A net may be set more than once per day at several locations within a study area. Set times vary by location but typically do not exceed 12 hours (nets monitored twice per hour). Typical capture for a 12-hour soak time is 2–6 turtles but can be greater. If many turtles are caught within the first couple of hours of the soak, the nets are pulled to allow adequate time for the researchers to mark, measure, and release the turtles.

Pound Net

Example 1

Pound nets are stationary gear that directs fishes into enclosures or "pounds" by means of leads. A typical pound net consists of a lead, heart, and pound. As fish and other marine life swim along the lead to the heart, they are directed into the pound by way of a mesh tunnel. Leads are 33/4-inch stretched mesh and 137–549 meters (150–600 yards) long, depending on water depth and proximity of the net to shoals. Hearts and pounds are 10–13-centimeter (13/4-inch) stretched mesh. The pound is fitted with escape panels of 14-centimeter (5 1/2-inch) mesh in one of the corners to allow smaller sized fish to escape. This panel is sewn into the back and sides of the pound and against the bottom of the net. The pound ranges from 8 to 8.5 meters (25 feet^2). All netting is constructed from multifilament nylon webbing that is dipped in a copper solution annually to prevent fouling. Nets are set from May to November and are checked daily, including the entire leader, unless conditions prevent safe passage to the nets. The nets are fished by gathering up the bottom of the pound, working from the tunnel wall to the back wall of the pound until the fish are concentrated in the back of the pound. The entire leader is checked every outing. The fish are then rolled into the boat by pulling the gathered netting and fish into the skiff or bailed out using dip nets. Since the majority of the catch remains alive from entry into the pound until capture in the boat, unwanted bycatch is returned to the water alive, resulting in a very efficient method of fishing. Turtles are removed from the net by holding the anterior and posterior sections of the carapace and gently setting the turtle onto the bottom of the boat. The turtle is temporarily restrained within a section of the boat during sampling.

Trawl Net

Example 1

A large mesh net is used primarily for these directed research activities. These trawl nets are the standard paired otter/flat trawl (such that one net is towed on each side) approved by NMFS. The trawl perimeter around the mouth is 137 feet (60-foot head rope + 65-foot foot rope + 2 × 6-foot wing end height); however, the effective fishing "swath" is 12 meters (Dickerson et al., 1995). Trawl design is 4-seam, 4-legged, 2-bridal, and trawl webbing (dipped nylon) is 4-inch bar and 8-inch stretch-mesh (the top's sides of #36 twisted with the bottom of #84 braided nylon line). Net length is 60 feet (cork line to cod end), and the cod end consists of 2-inch bar and 4-inch stretch mesh. The large mesh limits the bycatch collected, and "mud rollers" are included on the foot rope to reduce damage to bottom habitats and sampling gear. Because the purpose of this study is to catch sea turtles, turtle excluder devices will not be utilized. Trawls are conducted for 30 minutes or less (door in, door out).

Turtles may be captured by trawls while foraging or resting on the bottom or in the water column by a midwater trawl or a bottom trawl as it is deployed or retrieved. In some instances, captured turtles may be capable of out swimming a trawl and escaping. More likely, turtles that are overtaken actively seek an escape, moving from side to side in the trawl, making contact with the

trawl webbing. As the turtle tires, it cannot keep pace with the trawl movement and steadily falls toward the cod-end section or bag, where it will remain until the trawl is retrieved. When a turtle is captured, its presence in the trawl may not be noted until after the trawl has been retrieved in its entirety, and the catch is on the deck of the vessel. The turtle may be mixed in with target catch and bycatch, which will be quantified to the extent practicable.

Both randomized and opportunistic sampling methods may be employed depending on time and site-specific needs. Tow times are restricted to 30 minutes or less (door in, door out) in all fishery-independent research captures.

Trawl Types and Specifications

Example 1: Shrimp and Fish Trawls

Shrimp trawls are typically 4-seam or 2-seam in construction with headrope lengths from 12 to 100 feet depending on vessel size and location fished (inshore vs. offshore). Mesh sizes are fairly uniform throughout the Atlantic and southeastern U.S. waters, ranging from 1.25 to 2 inches. The vertical opening of a shrimp trawl is dependent on the target species of shrimp. The vertical opening of a shrimp trawl may range from 3 feet (brown and pink shrimp) to 16 feet (white shrimp). Fish trawls (e.g., flounder, sheepshead, black drum) are similar. Towing speeds vary from 2 to 3 knots depending on the size and horsepower of the towing vessel and the personal preference of the fisher.

Example 2: Channel Nets

Channel nets are typically set to fish for shrimp as well as estuarine fish species such as shad, catfish, and mullet. Channel nets may take different forms depending on the region or area fished. In the U.S., only North and South Carolina authorize the use of channel nets in state waters. In general, channel nets are funnel-shaped, stationary nets that are set in high flow channels, canals, and rivers to catch emigrating fish and shrimp. The mouth of the net is spread by attaching it to poles, stakes, anchors, or buoys across the float line. The net terminates in a cod end, much like an otter trawl, and is emptied by lifting it into a boat or taking the bag to shore if possible. The headrope dimensions of channel nets are variable.

Example 3: Skimmer Trawls

Originally designed to catch white shrimp by fishing the entire water column, today skimmers may also be rigged with low-opening nets and are used to target brown shrimp. The trawl is held open by a metal framework and is fished on the bottom. The size of a skimmer trawl is regulated by U.S. states and can vary from 15 to 30 feet in horizontal opening. The vertical height of the skimmer trawl varies depending on the target shrimp species and may be as much as 12 feet in overall height. Mesh sizes range from 1.25 to 1.6 inches. Skimmer trawls are “pushed” along the side of the vessel, rather than towed as conventional trawl gear. This allows the vessel operator to maneuver the nets in confined areas such as bayous and sloughs or along the edge of channels. Because skimmers are typically rigged to fish higher in the water column, the potential for turtle capture may be greater than a lower opening otter trawl.

Example 4: Flynets and Other High Opening Bottom Trawls

Flynets and other high opening bottom trawls vary in mesh size and headrope length depending on the targeted catch. Flynets are typically two-seam fish trawls constructed of graduated mesh sizes beginning with a large mesh (16, 32, or 64 inches stretched mesh) in the wings of the trawl with a slow 3:1 taper to smaller mesh sizes in the body and extension and mesh sizes as small as 3 inches in the cod end or bag section. The trawls are bottom tending with net sizes ranging from 80 to 100 feet (headrope length). The vertical height of these trawls when fished may be as much as 30 feet. Flynet vessels are single-rigged (towing one trawl) using a net reel for storage. Tow speeds are often between 3 and 4 knots with tow durations ranging from 10 minutes to several hours. High opening bottom trawls, which are used to target scup and black sea bass, may have headrope lengths as long as 150 feet and mesh sizes up to 40 feet. Similar in general design but of much smaller headrope size (40–75 feet) are trawls used to target inshore Loligo squid.

Example 5: Crab and Whelk Trawls

Both crab and whelk trawls are similar to shrimp trawls in general size and design but are rigged to fish hard on the bottom by adding an additional loop chain to the footrope. These gear types are typically fished during the winter months both in the Western Atlantic and southeastern U.S. waters. Trawls fished for whelk and crab are typically low opening, with vertical heights of 2 to 3.5 feet. Mesh sizes (between 1.5 and 4 inches) vary depending on the location fished.

Example 6: Scallop Trawls

Typical scallop trawls are 55- or 65-foot headrope length two-seam nets with body and wings constructed of 5.5-inch, 4- or 5-millimeter braided poly webbing. Wings are 20 to 25 feet long, cut on an 8:1 or 10:1 taper, while the body and belly sections are 20 to 23 feet long, cut on a 10:1 taper. Eight-inch hard plastic floats are used on the headrope with the number varying from boat to boat, while the footrope is lined with a 3/8- to 1/2-inch loop chain either single or double looped along the entire length. Some fishermen also use tickler chains ahead of the trawl to help kick up scallops. No trawl extensions are used, and the tail bag sections are 60 meshes around by 50 meshes deep and are constructed of 5.5-inch, 4- or 5-millimeter braided, double poly webbing. “Whisker” type chaffing gear is used along the underside of the trawl and bag to reduce wear. Boats are normally double rigged and equipped with 8- or 9-foot wooden doors. Fishing usually occurs in less than 40 fathoms, and cable lengths vary from 3:1 to 5:1 ratios of cable to depth depending on water depth. The trawl may have as much as a 5-foot vertical opening when fished.

Appendix E: Example Protocols: Handling, Holding Conditions, and Release

Hard-Shelled Turtles

Example 1

When a turtle is caught, it is immediately brought on board the research vessel and disentangled. Turtles are either promptly transported to the shore, which is approximately 0.5–1 kilometer from capture sites via the research vessel or measured and equipped with transmitters on the research vessel. During transportation, captured turtles are placed on their plastron onto the deck of the boat, which is covered with floor padding and a shade tarp for sun protection. During transportation to the shore, physical immobilization of green sea turtles is not necessary since the animals generally lay quietly on the boat with minimal struggle. Nevertheless, the boat crew will be positioned around any captured turtle to ensure that it does not injure itself in any way. On the rare occasion that two turtles are captured at the same time, animals are kept separate from one another on the vessel. The size of the turtles precludes the use of tubs or buckets; instead, one or two scientists will physically keep the turtles on the opposite side of the padded floor. Once at shore, to prevent injury to turtles and researchers, large turtles will be placed in an adjustable specialized restraint harness designed to carry large animals prior to being unloaded onto the shore. Once on shore, animals are released from the restraint harness and placed on top of a tire or tarp to prevent injury to the plastron. A size-adjustable containment box specially designed to hold turtles is used. All data collection (e.g., measurements, sample collection, and telemetry attachment) takes place either on shore or on the boat. The entire process from the time the turtle is brought on board the boat until its release takes a maximum of 2.5 hours. Animals may be unintentionally captured on more than one occasion during each sampling season. See the SEFSC Techniques Manual for greater detail on handling and measurement techniques (NMFS-SEFSC, 2008).

Example 2

Turtles are lifted over the gunwale at the lowest point or through the “cut-out door” if the vessel is equipped, minimizing the distance that the turtle is lifted, and taken out of the net (where applicable) and quickly examined. If turtles are brought back to shore (for surgical procedures, transmitter attachment, rehabilitation, etc.) or held on the deck of the boat, they are restrained within individual containers (insulated coolers, bins). Each turtle is kept moist and in the shade, maintaining its body temperature above 60 °F, similar to water temperatures at capture. It is safely isolated and immobilized on a cushioned surface, such as a foam pad, automobile tire, or similar

and sheltered from direct sunlight, wind, and rain with a tarp. The turtle is kept moist by misting it with fresh or saltwater or using wet towels. If using towels, particular attention will be paid to the ambient temperature, as evaporative cooling may chill the animal under some conditions. If the turtle is held out of water for an extended period of time (e.g., transport, surgery), petroleum or water-based lubricant jelly is used on the skin. In the event that an injured or ill sea turtle is captured, researchers will follow state and federal sea turtle stranding reporting protocols (pursuant to federal and state protected species permits) and report the turtle to the nearest state sea turtle stranding network rehabilitation or veterinary group.

During transport, the turtle is kept moist and in the shade, maintaining its body temperature above 60 °F, similar to water temperatures at capture. It is safely isolated and immobilized on a cushioned surface, such as a foam pad, automobile tire, or similar. The turtle is kept moist by misting it with fresh or saltwater or using wet towels. If using towels, particular attention is paid to the ambient temperature, as evaporative cooling may chill the animal under some conditions. If the turtle is held out of water for an extended period of time (e.g., transport, surgery), the use of petroleum or water-based lubricant jelly on the skin is preferred.

Example 3

When an animal is captured, it is placed in large inner tubes with a plywood bottom and/or held inside the boat and then processed in the small boat or on shore (Balazs et al., 1987; Blumenthal et al., 2010). On shore, turtles are placed in a purpose-built holding box with protection for the plastron (e.g., yoga mat). The turtle is kept in the shade, and wet towels are used to keep the animal calm and cool. The turtle is monitored to be sure that it is breathing (typically, turtles breathe every 30–50 seconds but can go several minutes between breaths). The turtle is visually examined for overall health, body condition, injuries, the presence of barnacles, or any abnormalities. Turtles that have interacted with fishing gear are examined for injuries and photographed, and the gear is removed. Turtles are released as close to the capture site as possible, given considerations for the turtle's health, at the water's edge or close to the surface of the water, and will be monitored for normal behavior following release. Holding time for each animal will not exceed the amount of time necessary to examine, measure, weigh, tag, and collect tissue samples. Under normal circumstances, an individual will be held for approximately 1–2 hours. When biotelemetry devices are attached, holding time will increase to up to 3 hours.

Leatherback Turtles

Example 1

Once secured aboard or off the stern, the leatherback is taken out of the net, quickly examined, and secured by hand, so that its limbs are held close to its body to prevent injuries to the turtle and personnel, but breathing is unrestricted. The leatherback is covered and shaded with wet toweling and cooled using seawater, which is pumped through a hose onto the turtle continuously. Removal of the net underneath the turtle and weight measurement are conducted during a single procedure. A rope (2-centimeter diameter) is snugly looped around the base of the turtle, creating a "doughnut" at the base of the turtle. Six pieces of wide webbing material (7-centimeter width) are

laced onto the doughnut loop. The six pieces of webbing extend from the rope doughnut and attach to a D-ring assembly at the top of the carapace. Once the lifting apparatus is securely fitted, the turtle is carefully lifted off the deck of the boat, with the aid of a capstan or tripod and pulley system, approximately 1.5 meters, to obtain a weight measurement and allow for the removal of the net beneath the animal. The entire lifting and weighing procedure is accomplished in 15 minutes. Total holding time will be as short as possible, between 30 minutes and 1 hour.

Because of the nature of capture efforts, animals may be unintentionally captured on more than one occasion during each sampling season, as there is no way to visually observe an animal and determine if it is one that has been caught before, prior to capturing it in the net during a current capture session. Once an animal is safely on board the vessel, researchers will look for flipper tags and determine when the animal was last captured. If an animal has recently been captured, it is released as soon as possible. During all research efforts, a veterinarian will be on call to assist if needed. This capture method has been successfully and safely employed to sample leatherback turtles in central California since the early 2000s.

Appendix F: Example Protocols: Examination, Morphometrics, and Monitoring

Photographs

Example 1

All animals are photographed to catalog carapace, body, head, and plastron coloring and any distinguishing marks, old wounds, and/or lesions. In addition, photographs may be taken for media or educational purposes. On certain occasions, videos may be taken to document research procedures and animal condition and/or for media or educational purposes.

Example 2

Upon capture, digital photographs are taken of each turtle's carapace, as well as the left, right, and dorsal sides of the head. After separating digital photographs into species groups, researchers compare the shape and arrangement of facial and carapace scutes among all individual turtles to identify recaptures.

Body Measurements

Example 1

Standard measurements and weight will be collected on all sea turtles captured. If recaptured, weight and measurements are taken to assess growth over time. Turtles are measured using a soft measuring tape to collect curved carapace, plastron, and tail lengths and widths. Forestry calipers are used to measure straight carapace length, width, and body depth.

Example 2

Various measurements (carapace length, width, tail length, etc.) are taken to track growth rates and size distributions and to determine gender. If the turtle can be brought on board or is on land, standard carapace measurements will be taken on every turtle: CCL, SCL (notch-tip) SCL(notch-notch), curved carapace width, and straight carapace width. Measurements are recorded in centimeters rounded to the nearest 0.1 centimeter using a flexible fiberglass tape measure for curved measurements and calipers for straight measurements. For measurements over-the-curve (curved), researchers will follow the curvature of the carapace. If barnacles affect these measurements, researchers will record it on the datasheet. For leatherbacks, usually only curved measurements are taken.

Weighing

Example 1

Turtles are weighed using a tripod designed for lifting the animals weighing up to 600 kilograms, and a digital scale will be used to record body weight. Animals are restrained in a harness designed specifically for lifting turtles and/or ropes during the weighing process. In the harness device, turtles' flippers are kept at their sides so that no flipper damage or other harm can come to them from overly struggling. Animals are restrained for no longer than 15 minutes.

Example 2

The weight of each turtle is collected (1) using an animal carrier placed on a flat electronic scale, which is placed on the ground, or (2) with a tripod (or other piece of equipment that provides lift), cargo net or rope, and a hanging scale.

Heart Rate Monitoring

Example 1 (Hard-Shelled Turtles)

Heart rate is monitored using a Doppler blood flow detector (Pocket-Dop3; Nicolet Vascular, Madison, WI). This is a minimally invasive procedure, where ultrasound gel is applied to the skin, and a handheld instrument (transducer) is passed lightly over the skin above the femoral artery. The transducer sends and receives sound waves that are amplified through a microphone. The sound waves bounce off solid objects, including blood cells. The movement of blood cells causes a change in pitch of the reflected sound waves (called the Doppler effect). If there is no blood flow, the pitch does not change. In other words, this is just a simple ultrasound. The site used to detect blood flow using the Doppler is dorsal to the hip, anterior to the tail base, under the margin of the carapace, with the probe directed dorsomedially toward the kidney.

Example 2 (Leatherback Turtles)

Doppler blood flow monitoring provides the least invasive and most consistent method for heart rate monitoring in leatherback turtles. The most effective doppler models are those designed for human fetal monitoring (e.g., curvilinear probe, 3–5 megahertz). For leatherbacks with deep vessels, a number of probe positions have been explored, but the most consistent site has been under the margin of the carapace at the level of the hip with the probe directed dorsomedially toward the kidney, which may be detecting blood flow in the dorsal aorta, renal artery, or common iliac artery (Innis et al., 2010). Doppler-derived heart rate data from foraging Pacific leatherbacks captured at sea (mean, 25; range, 20–40 beats per minute) are consistent with previous reports in Atlantic leatherbacks (Innis et al., 2010).

Temperature

Example 1 (Hard-Shelled Turtles)

A thermistor with a long flexible probe is used to obtain the core body temperature of green, loggerhead, olive ridley, and hawksbill turtles. Body temperature is recorded as soon as possible after capture. A sterile water-based lubricant gel (e.g., KY® jelly) is applied to the probe prior to insertion. With the turtle under manual restraint, the base of the tail is lifted, and the probe is inserted approximately 15–30 centimeters into the cloaca using gentle steady pressure. The temperature reading on the thermistor continues to rise until it reaches the final core temperature reading, usually in less than 1 minute. The waterproof probe may also be immersed in sea water to obtain sea surface temperature at the capture location for a comparison of animal and environmental temperatures. The probe is disinfected in between use with a Simplegreen™ solution, rinsed with freshwater, and then wiped with alcohol pads.

Example 2 (Leatherback Turtles)

Temperature monitoring in leatherback turtles can be accomplished using a digital thermometer with a flexible thermistor probe. The probe is lubricated with water-soluble lubricant (i.e., KY® jelly) and inserted into the cloaca approximately 30 centimeters to obtain the core body temperature. Normal body temperatures in foraging Pacific leatherbacks (mean, 22.9; range, 18.8–26.7 °C) can be as much as 10 °C higher than sea surface temperature (Harris et al., 2011).

Appendix G: Example Protocols: Identification Tagging

Metal Flipper Tagging

Example 1

All captured turtles are tagged with metal Inconel tags (Style 681, National Band and Tag Company) issued by the NMFS-SWFSC, using the standard technique described in the IUCN Marine Turtle Specialist Group Manual on Research Techniques (Eckert et al., 1999). The minimum sized hard-shelled turtle to receive a flipper tag is 50 centimeters SCL, whereas the minimum sized leatherback turtle to receive a flipper tag is 100 centimeters curved carapace length CCL. The tag is attached to the trailing edge of the left or right front flipper near the carapace. In leatherbacks, Inconel tags are attached to the trailing edge of a fore or hind flipper. One researcher secures the tag to the flipper, while a second person holds the flipper in place to minimize any potential of the animal moving the flipper during the tagging procedure. The tagging procedure is completed within 10 seconds. When an animal is on deck or onshore, every precaution is taken to make the animal comfortable and to minimize injury. The animals are placed on top of padding when on deck or on padding, a tire, or tarp inside a collapsible containment box that is kept in the shade when on shore; their head is covered with a wet cloth to keep them calm. 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. Prior to and after application, the skin at the injection site will be cleaned using the aseptic technique. The tagging equipment and tag are sterilized prior to tagging each turtle. Tag retention for these tags varies; although some tags are retained for 30 years or more, some loss occurs after 2–4 years. Researchers will search for existing tags prior to administering a new tag. If an existing tag is present, the animal will not be re-tagged. However, if an existing tag is present but in unreadable condition, the tag may be removed and the animal re-tagged in a different location.

Example 2

Animals greater than 30 centimeters SCL are tagged with cleaned and disinfected flipper tags on both rear flippers. The tagging location is disinfected using an aseptic technique with povidone-iodine solution or chlorhexidine gluconate scrub, followed by a 70 percent alcohol scrub, prior to tagging. For rear flipper tagging, turtles are temporarily placed on their carapace, and the tag is

applied to the trailing edge of the rear flipper just proximal to the first scale. Tagging data, including PIT tag data, are archived with the CMTTP, managed by the ACCSTR at the University of Florida.

Example 3

Turtles are 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 Inconel tags are attached to the trailing edge of a fore or 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 up to 20 years, some loss occurs after 2–4 years.

PIT Tagging

Example 1

All turtles (juveniles, sub-adults, and adults) are tagged with PIT tags, a small (14-millimeter length × 2-millimeter diameter) electromagnetically coded glass-encased "microchip." Tags are individually packaged in a disposable pre-sterilized needle applicator to eliminate the possibility of contamination. Prior to and after application, the skin at the injection site is cleaned using an aseptic technique. In hard-shelled turtles, PIT tags are injected intramuscularly into one or both of the triceps muscle complexes in the front flippers. In leatherbacks, PIT tags are injected intramuscularly into the right, left, or both shoulder muscles. PIT tags are injected using a specially designed sterile PIT tag injector (Avid; McDonald and Dutton, 1996; Wyneken et al., 2010). There is some information that PIT tags may move after application (Wyneken et al., 2010). Tag location consistency is an important aspect in mark-recapture studies as it facilitates the ease and accuracy of identifying an individual during recapture events (Wyneken et al., 2010). Inserting two PIT tags increases the probability of detecting one of the tags in the future and allows for the measurement of tag loss. PIT tags injected intramuscularly in the triceps muscle have a reduced risk of migration and infection, as compared with PIT tags placed subcutaneously in the trailing edge of the flipper blade (Wyneken et al., 2010). Preliminary PIT tag retention studies in leatherbacks have shown high retention when injected into the shoulder muscle (McDonald and Dutton, 1996; Dutton et al., 2005).

Example 2

Upon capture, the dorsal surface of both front flippers is scanned with a PIT tag scanner in order to determine whether the turtle already has a PIT tag. If no tag is found, the triceps superficialis muscle of the left front flipper is cleaned using an aseptic technique with povidone-iodine solution or chlorhexidine gluconate scrub, followed by a 70 percent alcohol scrub used twice in preparation for PIT tagging. PIT tags are scanned before insertion to ensure that they are functional. Using a PIT tag injector, the skin of the flipper is pierced, and the needle is inserted parallel to the surface, just under the skin and into the triceps superficialis muscle. The injection site is swabbed with povidone-iodine solution after tagging, and pressure is applied to the tag site to stop bleeding if needed. Lastly, the flipper is scanned to ensure that the tag is functioning. The PIT tag readers used are capable of reading all frequencies currently in use by sea turtle researchers.

Example 3

Turtles are PIT tagged with small electromagnetically coded, glass-encased microchips: Destron Tx 1406L. The tags are individually packaged in a disposable pre-sterilized needle applicator to eliminate the possibility of cross-contamination. PIT tags are injected between the digits of the fore/hind flipper or intramuscularly within the triceps or shoulder region. They are read with a scanner and are designed to last the life of the turtle.

Appendix H: Example Protocols: Carapace Marking

Etching

Example 1

Shell etching is conducted by using a Dremel tool to engrave a shallow (1–2-millimeter deep and approximately 2-centimeter high) groove (i.e., numbers, letters, or symbols) into a carapacial scute; a light-colored non-toxic paint is then applied to the inscription (Balazs, 1995). A simple and durable carapace marking method to individually recognize turtles from a distance constitutes a valuable research tool. The ability to identify turtles in this manner enhances data collection and sharply reduces the level of disturbance during encounters after the initial flipper tagging.

Plastron Delineation

Example 1

Areas of softness on the plastron in males are temporarily marked using a type of permanent marker. The animal is turned for a short period of time with the carapace side down in order to mark the plastron. Photographs of the outlined area will be taken and compared based on testosterone levels to determine if the animal is close to reproductive status. The animal is placed on either padding or a tire to prevent any potential injuries to the animal.

Example 2

For studies related to reproductive activities of adult male turtles, plastron marking for softness may be conducted. Using a minimally invasive protocol, turtles are placed in a recumbent position (carapace down) with a protective barrier underneath (e.g., yoga mat, innertube, tire, etc.) (Blanvillain et al., 2008; Blanvillain et al., 2011). A marker is used to delineate the whole plastron and the area of softness on the plastron. These markings are used for reproductive comparisons with hormone/ultrasound analysis. A photograph is obtained and analyzed using the software Plastron, which was specifically developed to calculate the area of softness relative to the whole plastron.

Appendix I: Example Protocols: Biological Sampling: Swabs, Epibiont Collection, Keratin/Scute Sampling, and Fecal and Urine Collection

Swabs

Example 1: Cloacal and Buccal Swabs

Periodically, cloacal and buccal swabs are collected to assess if they represent a viable way to collect DNA samples in the field, as well as to determine the reproductive status of female turtles through cloacal cytology (changes during estrus cycle). DNA quantity and quality are compared with the other tissue collection techniques described above. A stainless-steel pry bar is used to carefully open the turtle's mouth, followed by a canine mouth gag to hold the mouth open (Forbes and Limpus, 1993). The pry bar is used very gently, paying close attention not to damage beak keratin. Efforts to open the mouth are discontinued if the turtle shows sustained resistance. The soft tissue portion of the buccal area is swabbed with a Whatman® Omni Swab for approximately 6 seconds. Cloacal samples are collected by gentle insertion of another Whatman® Omni Swab approximately 5 millimeters into the cloaca for about 6 seconds. Both swabbing methods involve gently scraping the epithelium with the swab. Immediately after collection, each swab is placed inside a 15-milliliter conical tube and placed on ice for field storage until transfer to a −20 °C laboratory freezer. No liquid preservative is used in the tube to prevent the absorption of liquid into the sample swab, which might dilute the cells (Miller, 2006; Lanci et al., 2012). For reproductive status assessment, the cloaca swab will be moistened with saline and the same procedure followed as described above. The cells obtained on the swab are then rolled onto slides to examine cytology.

Example 2: Cloacal and Lesion Swabs

To conduct histologic evaluation and/or antimicrobial susceptibility testing, cloacal and lesion samples are collected and examined. The turtles are overturned temporarily onto the carapace and restrained. The external opening of the cloaca is scrubbed with 10 percent povidone-iodine to

disinfect the area. After securely gripping the tip of the tail, a sterile culturette tip (e.g., BBL CultureSwab™, Becton Dickinson and Company, Sparks, MD) is inserted approximately 1 inch into the cloaca. For the culture of external lesions, the culturette tip is gently inserted into the deepest area of the wound. In both cases, the culturette tip is rotated approximately 360 degrees and removed from the cloaca or lesion. The culture is placed immediately into a sterile transport medium (e.g., BBL Port-A- Cul™ Tubes, Becton Dickinson and Company, Sparks, MD) for overnight shipment to a laboratory for bacterial culture and antimicrobial susceptibility testing. Some media tubes also may be stored at −80 °C in liquid nitrogen prior to testing.

Example 3: Nasal Swabs

Using a clean technique, a sterile cotton-tipped or Dacron-tipped applicator of suitably small diameter to fit through the nasal opening is inserted into a naris (e.g., BBL CultureSwab™, Becton Dickinson and Company, Sparks, MD) while restraining the head to ensure that there is no movement that could cause the applicator stick to break. As soon as minimal resistance is encountered, the applicator is twirled and extracted from the naris and is used to prepare cytology slides, placed in sterile transport media for bacterial or viral culture, or placed in a dry cryovial for PCR diagnostics. Applicator sticks that are pre-scored to allow easy breakage into the transport tube are not used to minimize the chance of breakage while the swab is in the nasal passage.

Epibiont Collection

Example 1

Using a hoof scraper or other blunt instrument, barnacles or other epibiota are carefully pried from the turtle's carapace, taking care not to remove the underlying scute. For epibiota present in areas other than the carapace, the corner of a plastic putty knife or forceps tips are used to gently pry up the edge of the specimen in question and then pull the entire organism away from the epithelium. If bleeding occurs, pressure is applied to the affected area using a Betadine® swab. The sample is sealed in a freezer-style, zip-topped bag, and labeled using a permanent marker, with the date, the turtle's identification number, master tag number, and species code. The epibiota may also be placed in sample jars filled with ethanol.

Keratin/Scute Sampling

Example 1

Scute samples are taken from one or more of the eight posterior marginal scutes of the carapace and will be used to determine contaminant levels in the tissue. Collection locations are thoroughly cleaned with a plastic scrubbing pad, clean room wipes (lint-free, non-abrasive, free of particle generation and extractable chemicals), high purity water, and 2-propanol. Keratin is scraped from the radial edge, where the dorsal and ventral surfaces form a thin edge and the keratin and underlying tissue can be discriminated. A disposable stainless-steel biopsy tool is used to obtain up to four 0.2–0.5-gram cores of scute material from each turtle (Reich et al., 2007). The total amount of scute taken does not exceed 1.0 gram per animal, per sampling event. Keratin layers of approximately 1 millimeter in thickness representing the entire depth of scute deposition will be

analyzed to provide a time-series of serially deposited keratin tissue; this tissue will be analyzed for contaminants and stable isotopes (Day et al., 2005). Scute sampling does not penetrate living tissue or draw blood. Samples are stored in acid washed containers and kept in a −20 °C freezer until analysis. Contaminant analysis is conducted at San Diego State University or University of California, Davis by acid digestion and an inductively coupled plasma metal spectrometer. Hg, Cu, Zn, Cd, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and chlordane levels will be assessed in each sample because they have been found to have the highest concentrations of all the contaminants in nearshore marine ecosystems of southern California (Fairey et al., 1998). Researchers have reviewed the National Institute of Standards and Technology Pacific sampling protocol for sea turtle necropsies, which describes taking scute samples using the blade of a knife. This technique works well for a majority of green turtles; however, the use of a knife blade is not advised for Eastern Pacific green turtles due to the skin-like covering of the carapace for this species.

Example 2

Keratin may be collected from the outermost edge of the eight most posterior marginal scutes of the carapace for mercury analysis (Day, 2003). Scutes free of fouling organisms/epibiota and those that appear to have keratin of sufficient thickness and texture to provide a sufficient sample mass while minimizing the risk of penetration through the keratin layer are targeted for sampling. A relatively thin edge of keratin, where the keratin and underlying bone can be discriminated, is usually present where the dorsal and ventral surfaces of a scute meet. Thus, it is possible to avoid scraping too deeply, causing injury to the turtle and contaminating the sample with untargeted tissues. To obtain a keratin sample, the turtle is placed on its plastron on a slightly elevated platform with approximately 15–20 centimeters of the posterior edge of the carapace overhanging the edge of the platform. While one researcher is restraining the turtle's rear flippers, two other researchers will prepare to collect the sample. The carapace, 2 centimeters dorsal and ventral to the edge of the scutes, is scrubbed vigorously with a plastic scrubbing pad to remove sloughing keratin. If there are no areas free of epibiota, the plastic scraper is used to clear the target area as thoroughly as possible prior to scrubbing. Afterward, the scrubbed area is rinsed with high purity distilled water and isopropanol, and the remaining foreign matter and debris are removed using cellulose-based cleanroom wipes or cotton gauze, distilled water, and isopropanol or high purity 95 percent ethanol. Finally, the lateral edges of the prepared marginal scutes are removed by shaving off the edges of the scutes parallel to the edge being sampled using a disposable, sterile scalpel blade. Keratin splinters may also be collected by carefully sliding a sterile biopsy punch along the outer edge of the scute, parallel to the body axis, allowing the shavings to fall directly into a polyethylene sample bag held by a second researcher wearing Kevlar gloves to prevent injury. Typically, the posterior lateral corner of each scute will yield the thickest sample without penetrating the keratin and contaminating the sample with untargeted tissue. This should yield small shavings or splinters of keratin approximately 1 millimeter in thickness, totaling 10 centimeters of 1-millimeter thick shavings.

Fecal and Urine Collection

Example 1

Biotoxin analysis is a valuable technique to determine the potential influence of red tides and other dinoflagellate blooms on the health of sea turtles. Freely voided urine and/or feces are opportunistically collected from green, loggerhead, olive ridley, and hawksbill turtles. These fluids are collected via sterile syringe from the floor of the containment box or boat deck. Urine and feces are frozen and subsequently analyzed for exposure to biotoxins (domoic acid and saxitoxin). This is a component of a larger study to understand the impacts of biotoxins on the health of west coast sea turtles.

Example 2

Fecal samples are collected either after turtles have defecated during biological sampling or by digital extraction of feces from the cloaca. Those turtles that do not defecate during the sampling period are temporarily overturned onto the carapace and restrained. Using a clean technique and while wearing lubricated latex gloves, researchers will insert one finger into the cloaca of the turtle to feel for the presence of a fecal mass. If one is detected, researchers will remove it, placing it into either a polyethylene bag or a conical centrifuge tube.

Example 3

Urine is collected opportunistically (researchers will not attempt to express urine; it will only be collected if the turtle urinates) from male turtles to examine it for the presence of spermatozoa to assist in determining reproductive status. Urine is refrigerated to avoid damaging any spermatozoa that may be present.

Appendix J: Example Protocols: Biological Sampling: Biopsies and Tissue Sampling

Skin Biopsy

Example 1

Skin samples are collected from each captured turtle using a sterile 6-millimeter biopsy punch or forceps and razor. The samples are taken from the epidermis layer of the neck tissue (approximately 1 millimeter deep), and the area is cleansed with alcohol prior to tissue sampling. Alcohol in lieu of an aseptic technique is necessary to avoid interference with research objectives (e.g., stable isotopes). Samples are stored on ice and frozen and/or stored in a saturated salt solution for genetic and/or isotope analysis. Skin samples are preserved and archived at the NMFS SWFSC for future genetic analysis and foraging ecology studies using stable isotope analysis. Analysis of samples is performed primarily at NMFS SWFSC; however, samples may be shipped to collaborators for processing. The stable carbon, nitrogen, and sulfur isotope signatures of these samples and potential prey species are analyzed to determine the trophic status of each turtle and (potentially) identify recently arriving turtles (Suryan et al., 2009). Mitochondrial and nuclear genetic analyses are performed in order to determine the stock origin of the animal.

Example 2

After turning the turtle over on its carapace (when possible), the ventral surface of the rear flipper is wiped 5–10 centimeters from the posterior edge using an aseptic technique with 10 percent povidone-iodine or Betadine® 7.5 percent surgical scrub (Purdue Frederick, Stanford, CT), followed by an application of 70 percent alcohol. Wearing a glove to protect the hand that is holding the flipper, the researcher cleans the vial cap or plastic dive slate with alcohol underneath the treated rear flipper. A new biopsy punch is pressed firmly into the flesh as close to the posterior edge as possible and rotated one complete turn, cutting all the way through the flipper to the plastic cap. A new biopsy tool is used when collecting from each different animal to prevent cross-contamination. The tissue punch process is repeated with the same punch to end up with two plugs from one animal. The punched area is wiped with 10 percent povidone-iodine. If necessary, cyanoacrylate tissue glue such as Nexaban® (Veterinary Products Lab, Phoenix, AZ) or an over-the-counter equivalent such as Super-Glue® or Krazy-Glue® can be used for hemostasis.

Example 3

Skin sampling is conducted for genetic analysis to determine nesting beach origin (genetic stock), disease related studies, and stable isotope analysis for foraging ecology research. Two skin samples (approximately 6 millimeters in diameter) are collected (e.g., neck or hind flipper) from each turtle using a new and/or sterilized (with 10 percent bleach and 70 percent isopropyl alcohol) biopsy punch, scissor, or razor blade. Skin samples are preserved (e.g., saturated salt, ethanol, etc.) and archived at a repository for future analyses.

Example 4: In-Water (Leatherback Turtles)

In the event that a turtle is not brought on board, skin may be obtained from the carapace of free-swimming leatherbacks for DNA and isotope analysis using a biopsy pole. This sampling does not require the capture of the animal. The sampling gear consists of a 1-centimeter diameter sterile stainless-steel corer attached to a 2–4-meter anodized steel sectional aluminum pole. During collection, the researcher will come alongside the leatherback in the water and scrape the carapace at an oblique angle to collect a superficial skin sample. The 1-centimeter depth of the corer will prevent injury to the turtle. The head and neck will be avoided. This method is routinely used by NMFS observers sampling leatherbacks in the water that have been caught on longlines (NMFS-SEFSC 2008).

Samples are stored on ice and frozen and/or stored in a saturated salt solution for isotope analysis. In the laboratory, approximately 0.60 milligram of diet and tissue samples are loaded into sterilized tin capsules and analyzed by a continuous-flow isotope-ratio mass spectrometer in the Stable Isotope Laboratory at the University of Florida (carbon and nitrogen analysis) and Washington State University (sulfur analysis).

Tissue Sampling

Example 1: Muscle Biopsy

Muscle tissue is obtained from either the iliotibialis muscle of the rear flipper or the deltoidus muscle, which protracts and abducts the front flippers during swimming. Only a veterinarian or other highly trained individuals using sterile surgical instruments will conduct this procedure. This procedure is not performed on any compromised animals (e.g., those that are emaciated, with heavy parasite loads or bacterial infection). The incision area is thoroughly cleaned using a sterile technique with 95 percent ethyl alcohol and 10 percent povidone-iodine solution, and then, up to 2 milligrams/kilogram 2 percent lidocaine (e.g., Vetoquinol Inc., Lavaltrie, Canada) is injected intramuscularly, intradermally, and subcutaneously into the incision area 10 minutes before the sample is taken. A 1.5-centimeter incision is made in the skin using a disposable scalpel blade. Scissors are then used for blunt dissection to expose the muscle. The muscle tissue is grasped with tissue forceps, and the surgical scissors are used to excise approximately 200–300 milligrams of muscle tissue. The excised tissue is wrapped in aluminum foil or placed in a suitable storage vial and immediately frozen in liquid nitrogen. The veterinarian uses a monofilament absorbable suture (e.g., polygloconate, Maxon™, US Surgical, Norwalk, CT; polydioxanone, Ethicon PDS II™, Piscataway, NJ; or poliglecaprone, Ethicon Monocryl®) to close the incision area, and a simple

interrupted pattern with 3-0 suture is used to pull muscle tissue together. A horizontal mattress using a 2-0 suture is used to close the skin incision. The incision area is treated with topical antibiotic cream (e.g., povidone-iodine ointment or triple antibiotic ointment), and the turtle can be given a single dose of antibiotic at a site other than the incision site to reduce the risk of infection.

Example 2: Fat Biopsy (Leatherback Turtles)

Subcutaneous fat biopsies may be collected from adult or large immature leatherback sea turtles during in-water captures. These biopsies are performed by a veterinarian or other highly trained individual skilled in sterile techniques. Fat biopsies are collected from the fat pads in the dorsal shoulder region. Fat biopsies will be collected using the sterile technique as described in NMFS permit "Requirements for handling and sampling sea turtles" and the fat biopsy method previously validated in this species (Harris et al., 2016). The person collecting the biopsy sample wears sterile gloves to avoid contamination of the site. For surgical preparation, the skin is scrubbed in a circular motion from the center to the periphery with three alternating washes of either 10 percent povidone-iodine (Betadine®) surgical scrub or 4 percent chlorhexidine diacetate (Nolvasan) surgical scrub, followed by 70 percent isopropyl alcohol. The biopsy site is infused with lidocaine hydrochloride 2.0 percent injectable solution (up to 2 milligrams/kilogram), subcutaneously and intradermally at least 10 minutes prior to the biopsy procedure to minimize pain and discomfort to the animal. The efficacy of the local anesthetic is assessed by observing the turtle's response to a needle prick to the biopsy site prior to skin incision; additional lidocaine is administered as necessary. The flipper adjacent to the biopsy site is manually restrained during the procedure. A superficial skin incision (1–2 centimeters) is made with a sterile disposable scalpel blade. The fat sample (0.4–4.0 grams) may be collected either using sterile scissors (excisional biopsy) or using an 8-millimeter sterile punch biopsy inserted into the incision site. In the event of excessive bleeding, constant pressure will be applied with sterile gauze, and epinephrine may be applied to the biopsy site to facilitate vasoconstriction and hemostasis. The incision site is closed using absorbable sutures in a simple continuous cruciate pattern, taking care to close the dead space to reduce the risk of seroma or hematoma formation, followed by application of cyanoacrylate veterinary tissue glue. A digital photo of the biopsy site is taken prior to release, which is included as part of the turtle's medical record. Fat samples will be analyzed for lipid composition and persistent organic pollutants.

Appendix K: Example Protocols: Biological Sampling: Blood Collection and Esophageal/Gastric Lavage

Blood Collection

Example 1

Trained personnel will perform all blood and tissue sampling on each captured turtle. New sterile disposable needles are used on each animal. Care is taken to ensure that no injury results from the sampling. If a turtle cannot be adequately immobilized for blood sampling, efforts to collect blood are discontinued. Venous blood is collected from the dorsal cervical sinus using 20–21-gauge needles (Owens and Ruiz, 1980). Sample collection sites are scrubbed with povidone-iodine topical antiseptic solution and/or alcohol prior to sampling. Attempts to extract blood (needle insertions) from the dorsal sinus are limited to a total of four; two on either side of the neck. The maximum volume of blood collected from each turtle will represent <5 milliliters/kilogram. Blood samples are analyzed at the NMFS SWFSC. Analyses will determine the mitochondrial DNA and nuclear DNA profile of each turtle, which will help determine its origin and address questions about local stock structure. Samples are also used in stable isotope studies to address foraging ecology. In addition, testosterone concentrations from blood plasma samples are used to determine the sex of individual turtles. A health assessment may also be conducted by completing hematology and toxicology studies to determine exposure to marine contaminants and biotoxins.

Example 2

The turtle is restrained, and its head is gently pulled forward and downward until it is fully outstretched to facilitate the filling of the bilateral cervical sinus. The neck region is rinsed and cleansed with water, and then, an aseptic technique is used with povidone-iodine solution or chlorhexidine gluconate scrub followed by a 70 percent alcohol scrub prior to sampling. A syringe and needle or a vacuum tube, needle, and holder system are used to collect the sample. To collect a sample from turtles less than 0.5 kilogram, a 23-gauge 0.5-inch needle is used; from turtles 0.5 to 5 kilograms, a 21-gauge 1-inch needle is used; for turtles larger than 5 kilograms, a 21-gauge 1.5-inch needle is used. The needle is inserted on either side of the midline of the neck (depending on the size of the turtle, from 0.53 centimeter lateral to the midline) about 1/3 to 1/2 way toward the back of the head from the anterior edge of the carapace. A maximum of two attempts is made on each

side of the neck. If the turtle begins to move during the procedure, the needle is immediately removed. The needle is inserted at approximately 90 degrees to the plane of the neck and is not moved laterally to locate the sinus to avoid causing tissue damage. Once the needle is inserted, suction is applied, and the needle is moved slowly up and down until the sinus is located. The needle will not be removed from the neck while still applying suction, as this can damage the sample. A maximum of 5 milliliters/kilogram of body weight is extracted.

Example 3

Blood sampling is conducted for genetic, health, stable isotope, and/or endocrine analysis to obtain genetics for nesting beaches, assess the health status (e.g., contaminant load, nutritive status, etc.), foraging location and trophic level, and the sex of individuals. Following a previously published technique, a blood sample is collected (≤3 milliliters/kilogram) by inserting an individually packaged pre-sterilized needle, attached to a "vacutainer" blood collection tube (a sterile tube with a closure that is evacuated, which creates a vacuum inside the tube allowing for the collection of the volume of blood specified on the tube; range, 2–10 milliliters) or 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). No more than two attempts are made per dorsal sinus (four attempts total per turtle); and a new needle will be used between blood sample attempts. Potential future uses of the samples may include persistent organic pollutant measurements, heavy metal measurements, plasma chemistry health panels, endocrinology, proteomics, metabolomics, genomics, immunology, virology, and many others. Blood samples may be used in conjunction with skin samples to perform bulk and compound-specific stable isotope analysis. Different tissues [i.e., blood (plasma, whole, red blood cell), skin, and scute] have different isotope turnover rates and therefore give data on acute versus long-term foraging ecology (Seminoff et al., 2006a; Seminoff et al., 2009). Blood samples are also used for health status [percent cell volume, amino acid levels (in relation to forage), and contaminants].

Example 4 (Leatherbacks)

Blood samples are analyzed at the NMFS SWFSC. Analyses will determine the mitochondrial DNA and nuclear DNA profile of each turtle, which will help determine its origin and address questions about local stock structure. Samples are also used in stable isotope studies to address foraging ecology. Health assessment may be conducted by completing hematology and toxicology studies in collaboration with the contract veterinarian with NOAA SWFSC.

Esophageal Lavage

Example 1

Esophageal lavage is conducted in some animals immediately after capture to collect stomach samples for analysis (Forbes and Limpus, 1993; Seminoff et al., 2002a). The minimum size of the turtle for conducting lavage is ≥50 centimeters SCL. This procedure involves inserting two soft plastic tubes (3/4 inch outside diameter) down the esophagus to the pre-stomach area (i.e., crop in Atlantic green turtles) where recently ingested food is located prior to entering the stomach. The tube does not enter the stomach. The tips of both tubes are smoothed to remove any ridges and minimize abrasion of esophageal mucosa. Clean seawater is inserted through the inflow tube to

flush food particles from the pre-stomach area. Contents flow out of the stomach via the outflow tube and are caught in a 5-gallon bucket placed below the turtle's head. The procedure takes 5–10 minutes. Fecal and regurgitated food samples also may be collected opportunistically to identify prey items and to perform toxicological and parasitological analyses. Only researchers with training and long-term experience with this technique will conduct lavage. Samples will be fixed in 10 percent formalin in seawater for 48 hours then switched to 40 percent alcohol seawater solution.

Example 2

The feeding habits of wild turtles can be determined by a variety of methods, but the preferred technique is gastric lavage or stomach flushing. This comparatively simple and reliable technique has been used to successfully sample the gut contents of various vertebrate animal groups without harm to the animals (Forbes, 1999). This technique has been successfully used on green, hawksbill, olive ridley, and loggerhead turtles ranging in size from 25 to 115 centimeters CCL. Turtles are restrained by placing them upside down on the carapace on an automobile tire with the rim removed. The tire is placed on a platform raised several feet above the ground to allow easier access to the turtle during the lavage process. Prior to lavage, researchers will adjust the turtle's position on the tire so that the anterior part of the body is lower than the posterior to allow gravity to assist with collection of esophageal contents. Depending on the size and activity level of the turtle being lavaged, one to two individuals will restrain the flippers and hold the head so that the neck and, therefore, the esophagus remains straight and in line with the longitudinal axis of the body. The turtle is prompted to open its mouth by either gently tugging on the skin of the throat or working two lengths of soft, large-diameter rope in between the jaws to hold the jaws apart from one another. Once the mouth is open, a standard veterinary canine oral speculum (small, medium, or large, depending on the size of the turtle) is inserted just posterior to the anterior tip of the rhamphotheca to keep the jaws from closing. The powerful jaws of larger loggerheads may necessitate the use of a short length of 5-centimeter diameter PVC to keep the mouth open. Both the bars of the oral speculum and any pipe used for this purpose are wrapped with soft, rubber tape to prevent damage to the rhamphotheca. Once the mouth is securely open and the turtle's position has been stabilized, two lengths of clear, flexible vinyl tubing are lubricated with vegetable oil or lubricating gel, such as KY® jelly, and inserted into the esophagus, passing to either side of the oral speculum. The first tube, used to retrieve food items from the esophagus, is approximately 1 meter in length with a wall thickness of 2 millimeters and an inner diameter of 3–5 centimeters, depending upon the size of the turtle. The second tube, used to introduce water into the esophagus to flush out food particles, is 3 meters in length with a wall thickness of 2 millimeters and an inner diameter of 5 millimeters. The ends of both lengths of tubing are rounded by melting them using a flame and allowing them to cool prior to use to ensure that the tubing will not damage the walls of the esophagus during insertion. The tube's exterior is aligned with the turtle to premeasure the distance to the caudal margin of the pectoral scute of the plastron, roughly corresponding to the level of the stomach, and the distance on the tube for that particular turtle is marked with either tape or erasable marker. The tubes are passed no further than this mark or no further than they will pass without resistance. Although the lengths of tubing will obstruct the glottis during the lavage process, care will be taken not to accidentally introduce the ends of the tubing into the glottis opening. An alternative method is to lubricate a soft plastic veterinarian's stomach tube with

vegetable oil and cautiously insert it into the mouth and throat area. Then, seawater can be pumped through the tube using a veterinarian's double action pump, gently moving the tube back and forth along the length of the esophagus. To initiate lavage, fresh water is pumped into the esophagus using a double-action, veterinary stomach pump while the introduction tube is gently moved up and down the length of the esophagus. If water does not begin to flow from the retrieval tube within a few seconds after introducing water into the esophagus or if return water flow is low, the position of the retrieval tube is adjusted to remove any obstruction. If the situation does not resolve, water flow into the esophagus will be stopped. Barring any such difficulties, the retrieval tube is gently moved up and down the esophagus for 30–45 seconds while water flowing through the tube is collected in a bucket. The contents of the bucket are then strained through a fine-mesh sieve, and any food particles are preserved in 10 percent buffered formalin for future analysis. After the completion of lavage, the water flow is stopped, and the posterior of the turtle is elevated slightly to allow the introduction and retrieval tubes to drain. Once drained, the first tube (introduction tube) is removed first, followed by the retrieval tube and the mouth gag or PVC pipe. At this point, the anterior part of the turtle's body is elevated slightly relative to the posterior to allow any remaining water to drain into the esophagus, away from the glottis, so that the turtle can take a breath.

Example 3

Esophageal lavage will be performed on a subset (generally less than 20/year) of captured animals (not including nesting females) immediately after an encounter in order to collect food samples for diet analysis (Legler, 1977; Seminoff et al., 2002a). This procedure involves inserting 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 when the technique is performed by a trained individual, it poses very little risk to the turtle.

Turtles are placed on their backs (carapace down) on a padded table or in an innertube with their posterior ends slightly elevated so that the head is lower than the body. This allows for maximum drainage from the mouth. The front flippers are restrained by field staff to prevent the animal from moving during the procedure. The mouth is opened by holding the head securely and gently prying the beak open with an avian veterinary speculum. A veterinary canine mouth gag is inserted at the anterior end of the mouth and expanded to keep the mouth open. A plastic tube (with no sharp edges, rounding the ends of both lengths of tubing by melting them with a flame and allowing them to cool prior to use), lubricated with vegetable oil or spray, is gently inserted into the esophagus, and seawater is introduced using a manual, self-priming diaphragm hand pump at low pressure to flush out food particles from the esophagus and anterior crop. For turtles 35–50 centimeters SCL, a tube with an outside diameter of 8 millimeters is used. For turtles over 55 centimeters SCL, a tube with an outside diameter of 12 millimeters is used. Total flushing time is limited to 90 seconds. Food items are collected in a mesh bag that is placed over the turtle's head. The flushing process takes less than 1 minute, and the entire procedure takes approximately 5 minutes.

Appendix L: Example Protocols: Laparoscopy and Associated Internal Tissue Sampling

Laparoscopy

Example 1

This procedure is only to be performed by trained individuals or under the supervision of trained individuals in a facility. To prevent infection, aseptic techniques are followed. Prior to the procedure, a general anesthetic (short-acting injectable) and/or an antibiotic (to minimize any potential infection) may be administered. The dosage will be based on the size of the turtle.

Examples of potential antibiotics and dosages are summarized in Table 3. Turtle temperature is maintained at capture temperature. Aseptic techniques (e.g., sterile gloves, sanitized instruments) are used to the greatest extent possible while in the field. The animal is restrained briefly on its carapace in an inverted or lateral position for surgery, the entry site (prefemoral fossa) is scrubbed, a 1–2-centimeter incision with a sterile scalpel blade is made, and entry into the peritoneal cavity is achieved by using a trocar. Insufflation (approximately 0.5 liter of air) of the body cavity may be necessary for visualization of internal organs, and air may be expelled after gonadal examination (administration of sterile saline fluids into the coelomic cavity may also displace air and provide postoperative care). The wound is sutured using a mattress-pattern closure after the removal of all air. The sutures will be nominally absorbable sutures (e.g., Monocryl suture with a reverse cutting needle); the suture size is dependent upon turtle size but will be 2-0, 3-0, or 4-0. To reduce post-procedure pain with no sedation, an anti-inflammatory (non-steroidal) drug may be administered. All turtles are monitored after the completion of laparoscopy for normal activity prior to release.

Table 3. Examples of general anesthetic or antibiotic recommended dosages.

Drug	Dosage	Source
ketamine/dexmedetomidine; atipamezole (reversal drug)	4–5 mg/kg ketamine mixed with 20–25 mcg/kg dexmedetomidine IV; 0.2–0.25 mg/kg IM	Chittick et al., 2002
alfaxalone	5 mg/kg IV	Phillips et al., 2017
ketoprofen	2 mg/kg IV or IM	Thompson et al., 2018
ceftazidime	20 mg/kg IM	Stamper et al., 1999
oxytetracycline	25 mg/kg IM	Harms et al., 2004
enrofloxacin	20 mg/kg oral	Jacobson et al., 2005
ticarcillin	50 or 100 mg/kg IM	Manire et al., 2005
amikacin	5 mg/kg IM	Carpenter, 2005
lidocaine	≤2 mg/kg intradermal and subcutaneous, 2 mL topical	Thompson et al., 2018; Shertzer et al., 2018

Example 2

Gonad and liver biopsies are conducted in a facility, and only individuals thoroughly trained in the laparoscopy of marine turtles or those directly supervised by individuals so trained will conduct this procedure. Aseptic techniques are used at all times to prevent infection. This procedure is not performed on any compromised animals (e.g., those that are emaciated or have heavy parasite loads or bacterial infections).

Turtles are maintained at temperatures similar to capture temperatures and are restrained briefly on the carapace in an inverted or lateral position for surgery. Following a surgical scrub (either three alternating applications of 70 percent ethanol and surgical iodine scrub, 70 percent isopropanol, povidone-iodine scrub, and chlorohexidine wipe), a local anesthetic (lidocaine, maximum of 2 milligrams/kilogram) is injected into the muscle and dermis of the peritoneal wall of the prefemoral fossa. At operating temperatures of above 78 °F, a minimum of 10 minutes and a maximum of 45 minutes after the lidocaine injection are allowed prior to surgery. For lower temperatures, greater drug effect onset times (e.g., a minimum of 15–20 minutes when operating between 72 °F and 78 °F) are allowed. A single pre-surgical dose of antibiotic may be administered to reduce the chances of post-surgical infections. A short-acting general anesthetic (see anesthesia protocols below for details) is administered prior to the procedure.

Following sedation, a 1- to 2-centimeter incision is made just through the skin; the trocar and sleeve are used to push through the muscles and peritoneal wall into the body cavity. Care is taken to avoid entry that is too far posterior (where the trocar might strike the kidney) or entry that goes too deep (where the trocar might strike the lung or intestine). After achieving entry into the peritoneal cavity, the location of the trocar is verified with the laparoscope prior to inflating the body cavity with filtered air. Inflation (known as insufflation) is sometimes necessary to visualize the internal organs.

After completing the examination, all air is removed from the body cavity prior to suturing the wound. Introcoelomic fluids (sterile 0.9 percent saline or other IV fluid solution at up to 3 percent body weight or 30 milliliters/kilogram) may be administered as supportive perioperative care to maintain fluid and electrolyte balance and to help displace air in the body cavity during evacuation.

A single deep suture and two superficial sutures are used to seal the wound using a nominally absorbable suture. The suture size depends on the size of the turtle but will be 2-0, 3-0, or 4-0. The deep suture is a horizontal mattress pattern to eliminate dead space, and the superficial sutures may be either a buried, subcuticular horizontal mattress, external simple interrupted, horizontal mattress, or cross-mattress, depending on the surgeon's preference. All wild turtles are held in tanks temporarily to ensure that they are fully responsive following sedation and that they are able to perform normal swimming and diving activity prior to release.

Associated Tissue Sampling

Example 1: Gonad Biopsy

This procedure can be performed in the course of laparoscopy for sex determination by a veterinarian or other highly trained individual. Propofol may be administered (5 milligrams/kilogram IV; Maclean et al., 2008) as a short-acting (depending on ambient temperature considerations) general anesthetic prior to the procedure. An NSAID (e.g., ketoprofen, 2 milligrams/kilogram IM; Maclean et al., 2008) may be administered to reduce post-operative pain with no sedation, but special care should be taken with green turtles. A single pre-surgical dose of antibiotic may be administered to reduce the chances of post-surgical infections. After manually restraining the turtle, the inguinal area is scrubbed with 10 percent povidone-iodine solution. Lidocaine hydrochloride (e.g., Phoenix Pharmaceuticals, Inc., St. Joseph, MO), up to 2 milligrams/kilogram, is infused intradermally and subcutaneously around the proposed incision sites in the inguinal areas 10 minutes prior to the procedure to block any pain and discomfort to the turtle. The rear flipper on the side of the incision is then pulled back and toward the opposite side, causing the skin to remain taut. A 2-centimeter incision in the inguinal fossa will be made using a disposable scalpel blade; blunt dissection of the connective tissue is accomplished using surgical scissors. Once the gonad is identified, the incision is extended about 3–4 millimeters, and the biopsy guide is attached over the scope or a biopsy port is opened if the trocar is so equipped, and the biopsy tool is fed into its port. Using endoscopic cup biopsy forceps, a 1–2-millimeter piece of the side of the cranial 1/3 of the gonad (about 1/3 the way down) is sampled, avoiding vascular areas (the gonad sits on top of some of the renal blood vessels). Also, care is taken to make sure that the paramesonephric duct (i.e., the oviduct in females) is not lying on the sampling site. Sampling 1/3 of the way down from the cranial pole of the gonad will avoid accessory ducts (epididymus, vas deferens, Wolfian ducts, etc.), thus allowing access to the greater concentrations of follicles in the caudal ends of the ovaries. In addition, if researchers were to sample all the way cranially, this may disrupt the epididymus/vas deferens of males. Using a clean hypodermic needle, samples are retrieved from the forceps cup, placed into microcentrifuge tubes (e.g., Eppendorf®) filled with 10 percent buffered formalin, and stored at room temperature. If any bleeding occurs (it is exceedingly rare for it to bleed beyond the surface sampling site), 1 cc of intracoelomic fluids (e.g., Lactated Ringer's solution, 0.9 percent saline solution) will be administered. The incision is closed using

simple interrupted sutures using a monofilament nominally absorbable suture, such as one of the following three options (Govett et al., 2004): polyglyconate (e.g., Maxon™, US Surgical, Norwalk, CT), poliglecaprone 25 (e.g., Monocryl®, Ethicon, Somerville, NJ), or polydioxanone (e.g., PDS II™, Ethicon), followed by cyanoacrylate tissue glue on the surface.

Example 2: Liver Biopsy

Liver biopsy samples for toxicology analysis may be collected in the course of laparoscopy for sex determination. This procedure will not be performed on any compromised animals (e.g., those that are emaciated or have heavy parasite loads or bacterial infections). Propofol may be administered (5 milligrams/kilogram IV; Maclean et al, 2008) as a short-acting (depending on ambient temperature considerations) general anesthetic prior to the procedure. An NSAID (e.g., ketoprofen, 2 milligrams/kilogram IM; Maclean et al., 2008) may be administered to reduce post-operative pain with no sedation, but special care should be taken with green turtles. A single pre-surgical dose of antibiotic may be administered to reduce the chances of post-surgical infections. After laparoscopic examination of the gonads, the laparoscope and sleeve will be left in place and a second 1-centimeter skin incision made in the same inguinal space as the laparoscope. A second trocar is then advanced into the body cavity at a location that can be verified by the laparoscope as safe from any contact with internal organs. Once the trocar is in the body cavity, a 4-milimeter cup biopsy instrument is advanced into the field of view and guided to the liver. The biopsy is then taken at a location at the margin of the liver with minimal observable vascularity, avoiding the vascular areas (the gonad sits on top of some of the renal blood vessels). Care is taken to ensure that the paramesonephric duct (that will be the oviduct in females) is not lying on the sampling site. Using endoscopic cup biopsy forceps, a 1–2-millimeter piece of the liver will be sampled by firmly clamping the desired tissue with the cutting cup biopsy tip and retracting until the tissue comes away. Two biopsies of approximately 0.1 gram (1–2 millimeters) are obtained each from each turtle. A hypodermic needle is used to get the samples out of the forceps cup and into microcentrifuge tubes (e.g., Eppendorf®) filled with 10 percent buffered formalin. The biopsy site is observed directly for hemorrhage; if clotting fails to occur rapidly, a small piece of absorbable gelatin sponge hemostatic device (e.g., Gelfoam®, Pharmacia & Upjohn, Kalamazoo, MI) is inserted via the instrument port and applied to the biopsy site to promote clotting. A single deep suture and two superficial sutures are used to seal the wound using a monofilament nominally absorbable suture, such as one of the following three options (Govett et al., 2004): polyglyconate (e.g., Maxon™, US Surgical, Norwalk, CT), poliglecaprone 25 (e.g., Monocryl®, Ethicon, Somerville, NJ), or polydioxanone (e.g., PDS II™, Ethicon), followed by cyanoacrylate tissue glue on the surface. The suture size depends on the size of the turtle but will be 2-0, 3-0, or 4-0. The deep suture is a horizontal mattress pattern to eliminate dead space, and the superficial sutures may be either a buried, subcuticular horizontal mattress or an external simple interrupted, horizontal mattress, or cross-mattress, depending on the surgeon's preference. Turtles receiving propofol are held out of water for 1 hour following the conclusion of the procedure and are not returned to the water until fully responsive. All animals are monitored temporarily in tanks to ensure that normal swimming and diving activities have returned prior to release.

Administration of Drugs: Sedation/Anesthesia

Example 1

Following Chittick et al. (2002) and Harms et al. (2007; 2009), ketamine 5 miligrams/kilogram and dexmedetomidine 25 micrograms/kilogram (or medetomidine 50 micrograms/kilogram) are administered by IV in the dorsal cervical sinus for the duration of up to 1 hour. Reversal is achieved using atipamezole 0.5 milligram/kilogram half IV (dorsal cervical sinus), half IM (deltoid muscles). Other drugs that may be used during surgical procedures at the discretion of the veterinarian include lidocaine 2 milligrams/kilogram local anesthetic at the surgical site prior to surgery, ketoprofen nonsteroidal analgesic 2 milligrams/kilogram IM (deltoid muscles; Tuttle et al., 2006; Maclean et al., 2008), oxytetracycline presurgical prophylactic antibiotic, and bone marker (Harms et al., 2004).

Example 2

Following Maclean et al. (2008), propofol 5 milligrams/kilogram is administered by IV in the dorsal cervical sinus for the duration of up to 30 minutes. No reversal is required. Other drugs that may be used during surgical procedures at the discretion of the veterinarian include lidocaine 2 milligrams/kilogram local anesthetic at the surgical site prior to surgery, ketoprofen nonsteroidal analgesic 2 milligrams/kilogram IM (deltoid muscles) (Tuttle et al., 2006; Maclean et al., 2008), oxytetracycline presurgical prophylactic antibiotic, and bone marker (Harms et al., 2004).

Example 3

Following Phillips et al. (2017), alfaxalone 5 milligrams/kilogram is administered by IV in the dorsal cervical sinus following povidone-iodine solution. No reversal is required. Other drugs that may be used during surgical procedures at the discretion of the veterinarian include lidocaine 2 milligrams/kilogram local anesthetic at the surgical site prior to surgery, ketoprofen nonsteroidal analgesic 2 milligrams/kilogram IM (deltoid muscles) (Tuttle et al. 2006; Maclean et al. 2008), oxytetracycline presurgical prophylactic antibiotic, and bone marker (Harms et al., 2004).

When general anesthetics have been used, either propofol or alfaxalone alone or ketamine/dexmedetomidine with dexmedetomidine reversed with atipamezole, turtles could be released within 3 hours, unless judged that they are not ready for release. The turtles could be held overnight to ensure that they are swimming and diving normally before release.

When local anesthetics (lidocaine) or nonsteroidal analgesics (ketoprofen) have been used, no recovery period is required because there is no central nervous system depression.

Appendix M: Example Protocols: Suction and Adhesive Instrument Attachments

Suction Cup Attachment

Example 1 (Leatherback Turtles)

Small suction cups are used to attach telemetry packages that include one or more of the following: VHF transmitter, GPS transmitter, TDR, accelerometer, and/or video camera. These suction-cup tags are attached to free-swimming leatherbacks (without capture and with minimal disturbance to the individual) for the purpose of monitoring short-term turtle movements and collecting data on diving behavior and foraging ecology, including prey identification and consumption rates. Suction cup attachment occurs while the turtle is in a free-swimming state, virtually unaffected by the research team. Suction cups have previously been permitted and used successfully by NMFS SWFSC to attach transmitters to leatherback turtles. The tag provides a means of "marking" a turtle for subsequent capture, while obtaining data on diving and foraging behavior. The tag can remain attached for hours or days and allows the research team to efficiently relocate the turtle by monitoring VHF transmissions for subsequent capture/tagging operations as described above. The suction-cup-tag derived data allow for measuring foraging rates and identifying prey species. Movement data are used to address questions regarding the use of prey patches, variability of dive profiles during daytime and nighttime hours, and how environmental factors affect prey resources and turtle behavior.

The suction-cup tag is placed on free-swimming leatherback turtles via a small boat and a 6-meter-long pole. The suction-cup tag is attached to the end of the pole, such that with a small amount of thrust the suction cup can be precisely placed between the longitudinal ridges of the dorsal carapace of the turtle. Turtles are slowly approached, and the pole is used to apply the tag as the animal comes to the surface for a breath, within 1–2 meters of the vessel. The approach and tagging require about 5–10 seconds, and the vessel immediately retreats from the position of the turtle.

There are two types of instrument housings for suction-cup tags. The smaller housing is a short tube made of PVC material (2-centimeter diameter) that can accommodate two VHF transmitters and a TDR and is attached with a single 8-centimeter diameter suction cup. One VHF transmitter and TDR are surrounded by syntactic foam, providing buoyancy such that when the suction cup detaches, the tag floats like a spare buoy with the antenna oriented vertically out of the water. The second VHF tag is oriented 90 degrees from the first and is designed to transmit each time the animal surfaces to breathe. The larger instrument housing that can also include a small video camera is attached with two 8-centimeter suction cups. This so-called "SeeTag" has the same components as the TDR/VHF tag previously described but also includes a GoPro video camera housed within a water-tight container, similar to the designs developed by Marshall (1998), Harvey et al. (2001), and Davis et al. (2004).

Retrieval of the tag is facilitated by 1) recovering the tag after it falls off the carapace or 2) removing the tag from the free-swimming turtle with the aid of a pole and blunt hook. The tag is usually retrieved within 1–8 hours of deployment, but if it is not retrieved manually, the suction-cup tag is expected to release on its own within 1–5 days. The VHF transmitters can be tracked in real-time at distances of 1–6 kilometers with the aid of a handheld antenna and at greater distances with the aid of aircraft. Video and dive data require retrieval of the tag. The maximum number of telemetry tags applied to a single animal is three, although no more than two at the same time, including a suction cup attachment on a free-swimming turtle prior to capture.

The procedure for attaching telemetry devices is as follows:
A free-swimming leatherback is approached, and a suction cup mounted video camera is attached with a pole (no capture). This device is tracked with a small VHF transmitter contained within the housing of the video camera apparatus and remains attached to the carapace for hours/days. If the apparatus does not fall off the carapace unassisted, it will be removed manually with a pole.

No more than two transmitters are attached at one time (one satellite-linked transmitter and one acoustic transmitter).

Adhesive Attachments

Example 1: Fiberglass Resin and Two-Part Marine Epoxy (General Biotelemetry Transmitters)

Biotelemetry tags are attached to turtles to determine movements and habitat use. Prior to attachment, the transmitters are treated with an environmentally safe anti-fouling paint (e.g., PropSpeed®). The attachment area on the carapace is lightly sanded (hard-shelled turtles only) to remove algae and cleaned with alcohol or acetone. Care is taken to avoid fumes collecting in the holding enclosure, and the use of solvents or solvent rags close to the turtle's head is avoided. Biotelemetry devices (e.g., satellite, sonic, and archival) are attached to the carapace in two ways depending on species and habitat: 1) The first is with thin coats of fiberglass resin as described in Balazs et al. (1996). A non-toxic elastomer compound is 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 is widely used and is an accepted, safe, and effective method for transmitter attachment on hard-shelled turtles (Balazs et al., 1996). The turtles are held for no longer than 3 hours until the resin has cured and then released. 2) The second method is with a thin coat of two-part marine epoxy as described in Hart and Fujisaki (2010). A 0.75-centimeter layer of epoxy is applied to the footprint area of the tag on the carapace. The tag is placed on an epoxy footprint with enough pressure to cause the epoxy to ooze out on the sides. The epoxy is then pulled up along the sides of the tag with a spatula until it hardens. Generally, an epoxy putty (e.g., JB WaterWeld) is applied around the tag, which is then painted with marine anti-fouling paint. Variations of the resin or epoxy attachment techniques are used as deemed appropriate (e.g., turtle size or tag size).

Example 2: Fiberglass Resin or Quick-Set Epoxy (Acoustic Transmitters)

Sonotronics™ or Vemco acoustic transmitters (Sonotronics-CHP-87-L; dimensions = 90 millimeters long × 18 millimeters in diameter; weight = 11.5 grams; Vemco-V16-4x; dimensions = 68 millimeters long × 16 millimeters in diameter; weight = 24 grams) are used to track turtles. Tag dimensions and capabilities may change as technologies evolve. Tags are programmed to transmit signals in 35.0 to 69.0 kilohertz, a frequency range that is outside the hearing capacity of green turtles (30 hertz–1 kilohertz; Ridgeway et al., 1969). Each "ping" from the transmitter lasts between 528 and 942 milliseconds, depending on the transmitter configuration. Transmissions are 145 dB (reference 1 micropascal) and have a transmission range of up to 1 kilometer, although transmissions typically attenuate to 0 percent within 250 meters of the tagged animal, due to the extreme shallow depths within the study area (maximum depth = 6 meters) coupled with the presence of seagrass and other benthic features. The decibel level is extremely low out of water with pings actually indistinguishable to the human ear.

Acoustic transmitters are attached to the carapace with thin coats of fiberglass resin as described in Balazs et al. (1996) or quick-set epoxy. The attachment area on the carapace will be cleaned and lightly sanded to remove algae. A non-toxic compound such as plumber's putty or elastomer is used to "cushion" the transmitter and hold it in place during the attachment procedure. A thin coat of laminating resin or quick-set epoxy is then applied to the carapace and transmitter. For the fiberglass technique, 4–5 strips of fiberglass cloth are pasted over the transmitter to attach it (three to four 5-inch × 1.5-inch pieces overlaid on top of the transmitter perpendicularly and one to two 3-inch × 1.5-inch pieces placed parallelly on top of the transmitter; Balazs et al., 1996). Based on the recaptures of sonic transmitter-equipped turtles, these transmitters generally fall off the turtle within 6 months, although two turtles that had transmitters that remained attached for up to 1 year have been encountered. The capture of these turtles was opportunistic as it is virtually impossible to target specific turtles to remove sonic transmitters.

In the event a turtle bearing a non-functional acoustic tag is captured, all fiberglass resin and the tag are carefully removed from the animal using a scraper tool and fine grit sandpaper. To date, no adverse effects from acoustic tags have been observed from recaptured turtles.

Acoustic transmitter-equipped turtles are tracked with one of three techniques. First, ship-based (active) tracking is carried out with an acoustic receiver with a directional hydrophone (Sonotronics, Tucson, AZ, Vemco VR100 and VH110). To minimize the disturbance to turtles, each resighting position is determined by maneuvering the tracking vessel to within 10–20 meters of the turtle and recording the location of the tracking vessel with a GPS (Garmin, England; error range = ±3 meters to ± 12meters). Distances from telemetered turtles are determined from direct observation of surfacing turtles or estimated from the strength of the sonic signal at one-tenth gain with a directional hydrophone. For active tracking, a small vessel platform such as a 17-foot Boston Whaler (with a Honda 60- or 75-horsepower outboard motor) is used. Two types of Vemco acoustic active tracking transmitters are used for active tracking of turtles. These transmitters are the same size but convey different sensor information. V16-AP coded acoustic transmitters contain 3D accelerometer sensors and provide detailed information on summed vector acceleration and also contain a depth sensor. In addition, regular continuous pulse acoustic transmitters (V16-2L) are used for active tracking. For more information see Smith et al. (2019).

Example 3: Corrosive Link (TDRs)

TDRs (MK-10, Wildlife Computers, Redmond, WA; dimensions = 67 × 17 × 17 millimeters; 30 grams or newer models as they become available) are seated in a tubular-shaped syntactic foam drogue (20-centimeter length, 7-centimeter diameter) that has a hydrodynamically optimized dome and conical tail portion composed of incompressible syntactic foam. For tracking and retrieval, each TDR drogue has an internally mounted VHF radio transmitter (MOD 050, Telonics, Inc., Mesa, AZ; dimensions = 55 millimeters long × 17 millimeters in diameter; transmission range = 148.0–140.0 megahertz) and an acoustic tag (CHP-87-L, Sonotronics, Tucson, AZ). TDRs log time-of-day, depth (resolution = 0.5 meter), temperature, and light levels (lumens). Data collection intervals are set at 10 seconds (depth) and 1 minute (temperature and light levels) and initiated by a saltwater switch.

To ensure the prompt recovery of the TDRs, an automatic release mechanism consisting of two interlocking plates is used: one fixed to the turtle's carapace with a nylon mesh apron and a 5-minute quickset epoxy and the second attached to the TDR drogue with hose clamps. To offset the slight positive buoyancy of TDR drogues, the bottom plate is counterweighted to achieve neutral buoyancy. A screw-and-groove assembly links the anterior portion of these plates; the rear portion is connected with a galvanic mercury link that, upon immersion in seawater, dissolves at a constant rate. Upon dissolving, a spring mechanism will force the rear of the top plate upward, thereby disengaging the front portion. Detachment occurs as soon as the spring mechanism is engaged and usually takes place within 5–15 days of deployment. The slight buoyancy causes the units to float to the surface. Captive trials and one recapture of a wild turtle demonstrated that base plates are on average shed from the carapace within 10 days of TDR detachment. The attachment location does not interfere with flipper or head movements. Units weigh 0.8 kilogram out of water but will be neutrally buoyant in water due to the counterweighing system described above.

These data are used to examine fine-scale movements and diving of green turtles within foraging areas. This sampling technique allows for the determination of the depths of dives as turtles forage on the seafloor. The movement data are used to address questions regarding the use of our study

sites and how environmental factors, especially temperature, affect the distribution of green turtles in the study site. Short-term tracking, prey sampling, and long-term monitoring of green turtles in the area provide important information regarding the foraging ecology and habitat use of this endangered species.

Example 4: Fiberglass Resin or Two-Part Quick-Set Epoxy (Satellite Transmitters)

One of the following (or a newer version as models may change over time) is deployed on turtles: 1) Wildlife Computers "Splash" tag, variable dimensions based on configuration; 2) Wildlife Computers "Spot 5" tag, variable dimensions based on configuration; or 3) Wildlife Computers MK-11 GPS tag. The A-1010 is a location-only tag. The Spot-5 tags record location and depth data. Note that a maximum of one satellite tag will be deployed on any single turtle.

Transmitters are attached to the carapace with thin coats of fiberglass resin as described in Balazs et al. (1996) or 2-part quick-set epoxy. The attachment area on the carapace is lightly sanded to remove algae. A non-toxic compound such as plumber's putty or an elastomer is used to "cushion" the transmitter and hold it in place during the attachment procedure. For fiberglass resin, a thin coat of laminating resin is applied to the carapace and transmitter, and 6–8 strips of fiberglass cloth are 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). For epoxy resin, a smooth mound of epoxy resin around a tag is created to hold the tag in place. It can take anywhere between 15 minutes to 2 hours for the resin to completely cure. Curing is dependent upon the type of resin used and the outside ambient temperatures. Once cured, the turtles are then released back into the study area at the point of capture. A subset of satellite transmitter-equipped turtles may also be fitted with an ultrasonic transmitter to track short range movements. The number of animals outfitted with a transmitter or combination of transmitters is dependent upon the study question(s) and the amount of funding that is available to allocate to the purchase of transmitters or number of transmitters supplied via research partners. If the number of transmitters on hand in a particular season is low, valuable data are still gained by tagging only a few turtles. Transmitters may stay on for approximately 1 year. No adverse effects have been observed in recaptured animals that have previously had a satellite transmitter attached.

Example 5: Two-Part Quick-Set Epoxy (Satellite Transmitters)

Satellite tags are applied directly to the carapace at a location that minimizes drag while maintaining satellite transmission quality by the use of quick-set, two-part low heat epoxy (Godley et al., 2002). This section of the carapace allows the base antenna on the transmitter to break the plane of the water's surface. Attachment media may also encompass sections of the surrounding scutes but will be minimized and applied to be as hydrodynamic as possible. Prior to attachment, all epibionts are removed from the attachment site using a hoof scraper if necessary. The attachment site is then scrubbed with a scrub brush, rinsed with fresh water, dried with a towel, and lightly sanded with sandpaper. Once smooth, the entire area is wiped with a small amount of acetone or alcohol. The epoxy components are discharged from the cartridge in equal amounts via a caulk gun and are incorporated in a specialized mixing nozzle so that no modification of amounts is required. A small amount (<50 grams) of two-part cool setting epoxy is then used to create an even base for the transmitter to rest and to secure it to the carapace. Once the base has hardened, epoxy is used

to further secure the edges of the transmitter to the carapace. The size and weight of the satellite transmitter used depends on the size of the turtle. The tag and attachment materials do not exceed 5 percent of the turtle's body weight. Drying time varies between 20–60 minutes depending on ambient temperature and humidity. The turtle is released at or near the point of capture. Ideally, turtles are tagged on the boat and held no longer than 1.5 hours, barring unforeseen weather or logistical events.

Example 6: Neoprene Mounts for Small Juveniles

Transmitter types for small juveniles (<50 centimeters SCL) include Microwave Telemetry's PTT-100 9.5-gram solar-paneled satellite bird tags and Wildlife Computers SPOT 311 or equivalent tags. The total weight and dimension of each tag are approximately 11 to 13 grams and 38-millimeter length × 17-millimeter width × 12-millimeter height. Tag dimensions and capabilities may change as technologies evolve but are not expected to increase in size or weight. Tags are attached as follows:

1. The carapace is prepared by sanding the attachment site and transmitter with 60–100 grit sandpaper to improve adhesion of the epoxy. Between 1.5-millimeter and 3.0-millimeter neoprene with nylon backing is cut into pieces approximately 3–4 centimeters larger than the transmitters, resulting in a piece approximately 10 × 14 centimeters with rounded edges. An outline of the neoprene is then traced onto the attachment site of the carapace using a washable marker. Room temperature vulcanizing silicone is then used to outline the scutes at the attachment site, which will act as a barrier to epoxy along these areas of shell growth. Once the silicone sets, quick-set epoxy is applied to the attachment site, taking care to avoid the silicone and allow growth along the scutes' suture lines. The neoprene is then placed on top of the epoxy, nylon side up. Once secure, the transmitter is attached to the neoprene with a quick-set epoxy.

2. The carapace is prepared by sanding the attachment site and transmitter with 60–100 grit sandpaper to improve adhesion of the epoxy and acrylic base. Two strips (approximately 40-millimeter length × 5-millimeter width) of 5-millimeter neoprene wetsuit material are glued on either side of the turtle's vertebral ridge using surgical or cosmetic cyanoacrylate adhesives (e.g., 3M VetBond™ and OnRite™ Perma Rite #9 Plus Hard Bond). Approximately 15 to 22 milliliters of clear All Glass™ aquarium silicone is used to affix the tag to the neoprene and shell and to shape a streamlined surface around the attachment site. This epoxy is placed over scutes that are first treated with a base coat of manicure acrylic (Kiss® Acrylic Fill Kit, Kiss Products), applied per the manufacturer's instructions. The turtle's shell is lightly sanded and cleaned using 70 percent isopropanol and allowed to air dry. The carapace is then treated with a 2 percent chlorhexidine diacetate disinfectant solution and air dried again prior to tag attachment. The antenna is positioned cranially. The acrylic mixture is "painted" on the turtle's shell, thinly covering vertebral scutes I to IV and part of the adjacent costal scutes. When dry, the tag is attached directly on top of the acrylic, using PowerFast™ epoxy. See Mansfield et al. (2012) for more details.

Example 7: Neoprene Mounts for Small Juveniles

Small (5–9.5 grams) satellite tags are attached using a reliable silicone-neoprene attachment with an acrylic base coat that maximizes tag retention (Mansfield et al., 2012). Turtles' carapaces are cleaned, lightly sanded, and treated with a manicure acrylic base coat. Thin strips of neoprene are glued on either side of the turtles' vertebral ridge near the second vertebral scute. Aquarium silicone is used to attach the tag to the neoprene and to create a smooth, flexible, hydrodynamic surface around the attachment site to allow for normal shell flexion while diving and subsequent turtle growth. Tag retention has been up to 219 days (average approximately 72 days; Mansfield et al., 2012). Turtles are released in proximity to their capture site per permit specifications. Tags are expected to remain attached for a period of 30–90 days before the turtles shed the tags from their carapace (rapid growth of the turtles at this size result in regular shedding of keratin from the turtles' shells). Thus, the endpoint for this study is when each tag ceases to transmit. These methods were developed for loggerheads but may require slight modifications for other species. However, no additional procedures are required for other species (e.g., the acrylic base coat might not be used on green turtles). Turtles as small as 5 centimeters SCL could be tagged, depending on emergent tag technologies, but most will be larger (up to approximately 30 centimeters SCL).

Example 8: Integrated Tags with Multiple Attachment Methods

In order to determine vertical and horizontal movement, activity level, empirical temperature, and surrounding environment, a bio-logging approach using Fast-loc GPS, depth, temperature, acceleration, and video data loggers is used. Three types of tag components are housed in one hydrodynamically optimized unit measuring 15 centimeters × 12 centimeters × 5 centimeters and weighing 1.8 pounds. The dimensions and weight of the tag are expected to decrease as technology evolves. The Gyro data logger (Logical Product, Japan) measures 3-axis acceleration, 3-axis geomagnetism, 3-axis gyroscope, depth, and temperature and is housed in a specialized acrylic housing. The video camera consists of a GoPro camera (2.6 ounces) also encased in acrylic housing, and the GPS tag consists of an Mk10-AFB (or newer model, Wildlife Computers).

In addition, SeeTags (Watson et al., 2010) may be used, which have the same components as the TDR/VHF tag but additionally have a camera located at the end of a gooseneck that attaches to a water-tight container with the video recorder, similar to those used in the work conducted by Marshall (1998), Harvey et al. (2001), and Davis et al. (2004). Only turtles (green, loggerhead, olive ridley) ≥50 centimeters SCL and where the integrated tag weighs no more than 5 percent of the body weight are equipped with integrated tags.

Each integrated tag is attached to a float made of copolymer foam (Nichiyu Giken Kogyo, Saitama, Japan) in which a VHF transmitter (130 BB, Advanced Telemetry Systems, Isanti, MN) and a time-scheduled release mechanism (Little Leonardo Co.) are embedded. Integrated tags are attached to the crown of each turtle's carapace using epoxy putty (in total 120 grams; Konishi Co., Ltd., Osaka, Japan), two-component epoxy resin (in total 28 grams; ITW Industry Co., Ltd., Osaka, Japan), and a plastic cable tie connected to the time-scheduled release mechanism (Okuyama et al., 2011). The tags are designed to have a slight positive buoyancy (+100 grams) to pop-up at the scheduled time. Although the integrated tags will increase drag, as occurs with satellite

transmitters (Watson and Granger, 1998; Jones et al., 2011), and will add a slight negative buoyancy to the turtles, no behavioral changes were noted during visual observations in preliminary attachment tests in holding tanks.

In addition, integrative tags may be attached with a two-plate mechanism: the top plate is linked to the cameras with two 10-centimeter diameter hose clamps; the bottom plate is attached to the carapace with a nylon mesh apron and a 5-minute quick-set epoxy. The front of the plates is connected by an interlocking assembly, and the back is connected with the burn-wire connector and backup corrosive mercury link. To offset the slight positive buoyancy of the tag, the bottom plate is counterweighted to achieve neutral buoyancy. Integrated tags are programmed to detach within 14 days after deployment, at which time a charge from a 9 volt battery housed internally within the tag is sent to the burn-wire, causing the wire to corrode and break, thereby disengaging the plates. Once detached from the base plate, the slight positive buoyancy of the camera causes the unit to float to the surface. Captive trials and one recapture of a wild turtle demonstrated that base plates are shed from the carapace within 10 days of camera detachment (Seminoff et al., 2006b). The attachment location does not interfere with flipper or head movements. See Seminoff et al. (2006b) for additional details on attachment techniques and research value. From the camera images, species are determined, as are relative size, number, and proportion of food items consumed during each dive, along with time and depth information, allowing assessment of foraging success.

A VHF receiver (TR-4, Telonics Inc., Mesa, AZ) with a 3-element Yagi antenna and an acoustic receiver (VR100 VEMCO Ltd., Nova Scotia, Canada) with a directional hydrophone are used to recover floating integrated tags.

Appendix N: Example Protocols: Anchored Instrument Attachments

Marginal Scute for Acoustic Attachments for Hard-Shelled Turtles

Example 1

For long-term stable isotope sampling, two biopsy punches of carapace scutes are collected using sterile, disposable 6-millimeter AcuPunch or Sklar tools on turtles >30 centimeters CCL. Collection will follow procedures in Vander Zanden et al. (2010). Two samples from the third lateral scute are collected preferably from the right side, but if there are abnormalities or epibionts, the left side is used. The area is cleaned with alcohol swabs prior to sample collection. One sterile 6-millimeter biopsy is placed at the sampling location on the posterior medial region of the third right lateral scute, applying a little pressure. The first application of pressure makes an outline in the scute, followed by applying more pressure with a twist, which allows the punch to remain at that sample site without moving. The punch is pressed in about ¼ of its depth to get all the layers of the scute. A small cracking sound indicates the biopsy punch has reached the bottom of the scute. At that point, the punch is rocked from right to left to sever the sample completely. Clean forceps are used to remove the sample from the biopsy punch or from the carapace if it remains on the turtle. The sample is placed into a 2-milliliter cryovial. A new biopsy punch is used to take a second scute sample adjacent to the first, following the previous steps. The samples are placed in the same cryovial and labeled. Forceps are thoroughly cleaned between turtles with alcohol swabs, and the cryovials are placed in an air-conditioned room for at least 48 hours with the lid loose, but not completely off, to allow the sample to dry. After 48 hours, the lid is tightened and the vial stored. This procedure is conducted using a new, sterile biopsy punch (which takes out one 6-millimeter plug of the top section of the carapace for each turtle) and by thoroughly disinfecting the sampling area prior to and after the procedure with 91 percent isopropyl alcohol. Samples are transported back to land in a cooler and stored in a regular freezer in the laboratory until the time of analysis.

Marginal Scute Tether Attachments for Hard-Shelled Turtles

Example 1

PATs are attached to the carapace by tether. The dorsal and ventral surfaces of postcentral scutes are first scrubbed with a scrub brush, and a hoof scraper is used to remove epibionts if needed. Tethering hardware and drill bits (3/16-inch titanium) are soaked in 10 percent povidone-iodine for at least 15 minutes. The dorsal and ventral surfaces of the postcentral scutes are thoroughly scrubbed using an aseptic technique using two applications of a surgical scrub (e.g., povidone-iodine) and 70 percent alcohol. The eyestrap is positioned as far forward (toward head) as possible on the postcentral scutes to capture the underlying bone but without intercepting the integument on the ventral surface. The eyestrap (pad eye) is aligned on the postcentral scutes and, using the holes as a guide, drilled once quickly through the scute using a disinfected titanium drill bit. A blood clotting gel is used to stop bleeding if needed. Next, the hole area is flooded thoroughly with 10 percent povidone-iodine, and a bolt of appropriate length is inserted through the eyestrap, with nylon washers against the carapace and the plastron and a stainless washer between the eyestrap and the nut or head of the bolt. The thimble of the PAT tether is then threaded over the eyestrap, and the second bolt is inserted following the same steps. The bolts are secured with washers and lock nuts using a screwdriver and wrench, and any excess length is trimmed from the bolts if necessary.

Battery life and duration of attachment are estimated to be one year. Tags and attachment material do not weigh more than 5 percent of the turtles' weight. Argos transmitting PATs operate within frequencies 401.618 megahertz to 401.680 megahertz. The largest PATs measure approximately 40 millimeters in diameter at their widest point; overall length of the tag, not including the antenna, is 175 millimeters. The total weight is 75 grams. Other models (e.g., mini-PATs) may be smaller. Sensors may include depth and temperature.

Pygal Tether Attachments for Leatherback Turtles

Example 1

Disposable gloves are worn by the researcher. The drill bit is sterilized via autoclave before use in the field or by using 10 percent povidone-iodine and soaking for at least 15 minutes. The dorsal and ventral areas of the turtle near the pygal region are thoroughly scrubbed using an aseptic technique using two applications of a surgical scrub (e.g., sterile gauze sponges saturated with 10 percent povidone-iodine) followed by 70 percent alcohol. A single hole is drilled through the center of the pygal region with a 1/4-inch brad point drill bit. Next, the hole is flooded thoroughly with lidocaine. Clotisol can be used to stop bleeding, if necessary by squirting drops into the hole. Surgical tubing is swabbed with triple antibiotic ointment and passed through the hole with enough excess to be looped around to create a surgical tubing-only loop with the tag tether inside the tubing. The monofilament tether is then secured using two crimps on the tether above the tubing on the dorsal side of the turtle to secure the loop. Only surgical tubing is in contact with the turtle. Excess monofilament is trimmed if necessary.

Example 2

The pygal region of the carapace (the overhanging posterior projection of the carapace) is an ideal location for the attachment of tethered tags (e.g., PSATs) or archival tags and have been used successfully in previous studies (Morreale et al., 1996). The tethered PSAT or archival tag is anchored by drilling a hole (approximately 4 millimeters) through the pygal using an orthopedic drill bit (cleaned with antiseptic). Prior to drilling, the attachment site is sterilized with three separate applications of antiseptic (e.g., povidone-iodine) and isopropyl alcohol and then desensitized with a topical anesthetic (e.g., ethyl chloride). Lidocaine 2 percent gel or solution is applied topically to each drill tract (2 milliliters per site). In addition, an intramuscular injection of an NSAID (e.g., ketoprofen) is administered into the pectoral muscle at the time of the procedure. After drilling, flexible stainless-steel wire or fishing filament coated in soft tubing (surgical or vinyl) soaked in antiseptic (e.g., Betadine®) is threaded into the hole. The monofilament line or stainless-steel wire is anchored to the pygal by passing the line through a 5-centimeter diameter button or fishing bead soaked in antiseptic (e.g., Betadine®) beneath the pygal. The line/wire is then threaded back through the pygal and through the single piece of tubing and a 5-centimeter diameter button or fishing bead soaked in antiseptic (e.g., Betadine®) located at the upper end of the pygal. The line continues above a dorsal crimp and attaches to the PSAT or archival tag. The length of the tether does not exceed 15–20 centimeters. This method offers several modifications from the pygal attachment method used in leatherback telemetry studies by Morreale et al. (1996). The short tether and breakaway feature of the tether pin in PSATs are designed to minimize the potential for entanglement in fishing gear or flotsam.

Medial Ridge Attachments for Leatherback Turtles

Example 1

Leatherbacks are handled using disposable gloves to maintain the most sterile environment possible. The hardware used for attachment is soaked in 10 percent povidone-iodine prior to use. Sterile gauze sponges saturated in 10 percent povidone-iodine or presoaked 10 percent povidone-iodine swabs are used to cleanse the central ridge area of the leatherback several times. A portable drill with a sterilized drill bit is used to drill two small holes through the ridge. If necessary, a blood clotting gel such as Clotisol® is used to stop bleeding by applying drops into the holes after first cleaning the dropper tip with an alcohol swab. The hole only penetrates a few millimeters horizontally through the carapace ridge and does not enter the body cavity. The holes are thoroughly flooded with 10 percent povidone-iodine. One monofilament or coated wire tether (swabbed with triple antibiotic ointment) is then threaded through an acetal polyoxymethylene resin disk that has been swabbed with triple antibiotic ointment (e.g., Neosporin®) on the bottom. Once passed through the hole, the monofilament is secured with a second acetal polyoxymethylene resin disk (swabbed with triple antibiotic ointment) and a crimp so that the tether is tight and secure. Any excess monofilament is cut off, and the process is repeated for the second monofilament tether.

Example 2

A satellite transmitter is attached to the medial ridge of the turtle's carapace, posterior to the midpoint of the carapace, where the medial ridge is most prominent (usually posterior to the widest area of the carapace). This location provides the greatest bight for attachment and lessens drag effect in comparison with attachment near the leading edge of the carapace (Jones et al., 2011). The attachment site is sterilized with three separate applications of Betadine® antiseptic and isopropyl alcohol. Following attachment site preparation, two small (4.5-millimeter diameter) holes are drilled into the medial ridge at a horizontal angle with an orthopedic drill bit. Each hole only penetrates a few millimeters into the carapace ridge and will not enter the body cavity. The drill bit is dipped in Betadine® prior to the creation of each hole and is cleaned with soap and soaked in chlorhexidine between animals. To minimize the pain associated with the tag attachment process, lidocaine 2 percent gel or solution is applied topically to each drill tract (2 milliliters per site). In addition, an intramuscular injection of an NSAID (i.e., ketoprofen) is administered into the pectoral muscle at the time of the procedure. Surgical tubing, treated with Betadine®, is inserted into the holes. The surgical tubing acts as a sheath for the tether attachment. Flexible braided stainless-steel wire (1.8-millimeter diameter) is slid into the surgical tubing inside the holes. The stainless-steel wire functions as a tether to attach the transmitter. One end of the tether has a loop (secured with a corrodible stainless-steel crimp) prior to insertion, and a loop will be crimped to the other end after insertion. A tag base is then formed over the ridge using fast-setting, non-adhesive, cold curing silicone putty. The putty does not compress at depth and conforms to the shape of the ridge. The transmitter (Wildlife Computers, model Mk10 "SPLASH ridgemount") is designed specifically for direct attachment to the medial ridge of a leatherback turtle. Current model numbers include F-294A (single tether, 85 × 87 × 41 millimeters; weight, 215 grams) and F-295A (two tethers, 129 × 78 × 40 millimeters; weight, 233 grams).

Example 3

On leatherback sea turtles, transmitters are attached directly to the carapace at the medial dorsal ridge (Hamelin and James, 2018). The attachment site is sterilized with three separate applications of antiseptic (e.g., povidone-iodine) and isopropyl alcohol and then desensitized with a topical anesthetic (e.g., ethyl chloride) prior to drilling. Lidocaine 2 percent gel or solution is applied topically to each drill tract (2 milliliter per site). In addition, an intramuscular injection of an NSAID (e.g., ketoprofen) is administered into the pectoral muscle at the time of the procedure. Tags are attached at the medial dorsal ridge at the most prominent portion of the ridge, generally posterior to the greatest width of the carapace, in order to reduce drag (Jones et al., 2011; Jones et al., 2013). An orthopedic drill bit (cleaned with antiseptic) is passed horizontally through the medial ridge to create two approximately 4.5-millimeter diameter holes by a few millimeter depth (not entering the body cavity). Then, a flexible polymer tubing (e.g., Tygon®), cut to size and soaked for 1 hour in antiseptic (e.g., Betadine®) in individual plastic bags, is passed through the drill tracks. A base for the tag is created from silicone putty. A 1.8-millimeter diameter flexible stainless-steel wire coated in plastic is passed through the transmitter via the flexible polymer tubing to secure the transmitter to the dorsal ridge using a stainless-steel crimp. The crimp will corrode, allowing the tag to detach (approximately 1 year after attachment).

Appendix O: Example Protocols: Non-Invasive Imaging

Magnetic Resonance Imaging

Example 1

MRI, in comparison to radiography and CT, allows for a more detailed evaluation of soft tissue structures. Imaging facilities may be up to 3 hours away. Turtles are transported on foam padding and kept moist and within their preferred temperature range. Prior to conducting MRI, turtles are sedated with midazolam (1–3 milligrams/kilogram IM or IV), propofol (2–5 milligrams/kilogram IV), or ketamine/dexmedetomidine (2 milligrams/kilogram and 25 micrograms/kilogram, IM or IV, respectively) and then positioned in ventral recumbency. MRI is performed using a commercial unit, and images are made in multiple planes using weighting selected to highlight the tissue or pathology of interest (e.g., T1, T2, T*2, spin-echo, FLAIR). Scans are completed in 20 to 30 minutes.

Ultrasound

Example 1 (Leatherback Turtles)

Ultrasonography is used to non-invasively measure the depth of the subcutaneous fat layer to quantify the nutritional condition of captured turtles. A portable veterinary ultrasound machine (Sonosite Vet 180 Plus, C60 5-2 megahertz transducer) is used to obtain images of the subcutaneous fat and underlying musculature at five anatomical sites: right shoulder, central neck, left shoulder, right hip, and left hip. Ultrasound gel is applied to the skin, and the probe is held against the skin for several seconds until an image is obtained. The resulting image is frozen on the screen, measurements of the subcutaneous fat are recorded, and the image is saved and later downloaded to a laptop computer. In addition, ultrasonography is used to visually determine the presence of gonads.

Example 2

Ultrasound technology is used to determine the sex and reproductive status of turtles. Turtles are placed in a recumbent position (carapace down) with a protective barrier underneath them (e.g., yoga mat, inner tube, tire, etc.), ultrasound gel is applied to the skin, and a field-portable ultrasound machine is used to visualize the gonads (testes/epididymides or follicles/eggs) of adult turtles to determine sex and reproductive status via the left/right hip region (cranial to the femurs but lateral to the plastron). This is a minimally invasive procedure that has been used in other sea turtle studies (Pease et al., 2010; Blanvillain et al., 2011).

Appendix P: Example Protocols: Aerial Surveys

Crewed Aerial Surveys

Example 1

Crewed airplane-based aerial surveys occur using either the NOAA de Havilland Twin Otter Aircraft or a contracted Partenavia P.68 platform (both are twin engine, fixed-wing aircrafts) flying between 500 and 800 feet. The altitude flown depends upon various factors, such as the weather (i.e., cloud ceiling), locations of nesting sea bird colonies, or target species. The most conservative altitude is used depending upon conditions. Photographs and video may be taken during flights. During capture efforts, aircrafts are used to conduct line transect surveys extending from nearshore to offshore waters and to act as spotters to guide the capture vessel to the vicinity of turtle aggregations. Once turtles are sighted, the capture vessel moves to the vicinity of a sighted animal to permit observation and evaluation of behavior and condition before attempting capture. The aircraft may circle an animal to more accurately define location for over 5 minutes.

Example 2

Aerial surveys may be used for collecting information on turtle presence, species, identity, behavior, habitat, proximity to fishing gear, and body size and condition. The aerial survey craft is most likely a fixed wing such as a Piper Supercab PA-18 or a Helio Courier, although other options include a twin- or single-engine, fixed high-wing aircraft, such as the DeHavilland Twin Otter, or a helicopter, such as the two-bladed, single-engine light utility helicopter Robinson R22 or R44. Photographs and videos may be taken during flights, and VHF receivers may be used from the aircraft. Typical altitudes for both fixed and rotary platforms are 500–700 feet as long as marine mammals are not spotted, but they may be flown as low as 300 feet on the rare instances when higher altitudes are not adequate. Each encounter does not exceed 45 minutes. Crewed aircrafts are flown at speeds ranging from 35 to 110 knots. If a marine mammal is spotted, the pilot will move to 1000 feet or higher depending on the species (1500 feet for right whales).

Once a target animal is spotted, the aircraft starts a circling pattern to maintain visual contact. There are different types of circles that the pilots could make, depending on what is needed. For example, if the plane is working in tandem with a small boat, and the small boat is already working one turtle when the spotter plane sees another turtle, the spotter plane may make some very high and very wide circles to keep an eye on the general area where the second turtle is. A spotter plane may also make the same type of circle if a new turtle is spotted and to keep a general eye on the

location while the tagging boat is enroute. This type of circling is expected to have little or no effect on the turtle. If a boat is in very close proximity to the turtle and is ready to directly interact with the turtle, the spotter plane may make closer or tighter circles so that it can see the turtle coming to the surface a few seconds before the boat operator can see the turtle. In these situations, to help direct a boat, the spotter pilot may say something like "at your 3:00, 1.5 boat lengths away." This helps the small boat operator orient the boat in the correct direction and be ready to interact with the turtle when it surfaces. In previous surveys, this type of circling did not affect the turtle's behavior, though researchers acknowledge that it has more potential to affect behavior than a higher and larger circle. If the plane's presence affects the turtle's behavior and makes it dive, it reduces the researchers' chances of successfully capturing, tagging, or filming the animal. Therefore as a matter of course the plane keeps enough distance at all times so as to not affect the turtle's behavior. Either of these circles (large, high or smaller, lower) could also be used if a plane is working independently of a boat to determine turtle presence in certain times and areas or for photogrammetry. If a turtle is seen on an initial pass but species identification was not possible, the pilot may circle to collect the species identification information, or a pilot may circle in order to get another attempt at photographing the turtle.

Uncrewed Aerial Surveys

Example 1

Because crewed aircraft support is often unavailable when weather conditions are appropriate and turtles are available and can be prohibitively expensive, the use of UASs is a useful technique to overcome some of these hurdles. Researchers anticipate using the APH-28 ranger, a long-endurance hexacopter from Aerial Imaging Solutions, LLC or a comparable unit as technology evolves. This unit is relatively quiet with sound levels close to ambient (e.g., wave noise) at 30-meter (100-foot) altitudes. The aircraft weighs about 1.3 kilograms (2.0 kilograms with a battery and E-PM2 camera) and has a 0.81-meter span across the rotors. Depending on payload and environmental conditions, the aircraft can achieve flight durations of up to 35 minutes. The aircraft's attitude, altitude, and heading are stabilized by an electronic control system that incorporates three gyros, three accelerometers, a magnetic compass, a barometric pressure sensor, a GPS receiver, and 8 microprocessors.

This UAS platform has a long track record (>2000 flights) of safe and successful use by scientists from NMFS SWFSC, and all have airworthiness certificates from the NOAA/Office of Marine and Aviation Operations Aircraft Operations Center. The use of the APH-28 ranger platform allows researchers to take advantage of existing equipment owned by collaborators (i.e., RC units and ground stations) and eliminates the requirement to train pilots on an entirely new aircraft system. This aircraft is a slightly modified version of the APH-28 that has already proven to be a very successful platform used in operations from the Antarctic to the Aleutian Islands. To increase endurance for this current research, the developer of the aircraft will use more efficient 14-inch propellers, reduce the weight of some structural components, and upgrade to newer high voltage lithium polymer (LiHV) batteries.

Researchers will launch the UAS unit from a small vessel, large research vessel, or land-based station. The UAS unit is flown by a team of at least two individuals on all flights; a pilot in command (PIC) and a ground station operator (GSO). Both will be FAA Part 107 Certified Remote Pilot licensed to operate the UAS unit.

The PIC is in command of each flight operation. The PIC operates the remote-control unit and maintains visual contact with the aircraft. The GSO operates the ground station and monitors video feed and aircraft systems data (altitude, attitude, distance, heading, and battery status). The PIC maintains situational awareness of the surrounding area, making certain that no other aircrafts encroach on the operational airspace. The UAS unit will remain at least 30 feet above a turtle. This will allow researchers to identify distinguishing marks or external injuries of individual turtles. The UAS unit is operated in daylight within visible line of sight, in uncontrolled air space, or with specific FAA authorization if operating in controlled air space. The UAS unit has an auto-return feature in case of failure. The ground control station (single station) is on the vessel or at the ground-based station, located 50–500 meters from the turtle(s) being filmed.

Initially, researchers will use test locations to search for green sea turtles (*Chelonia mydas*), which are resident to both study areas (Eguchi et al., 2020). These locations provide calm sea states and shallow waters, albeit with turbid waters at times. The UAS unit can be launched from land or the 17-foot Boston Whaler research vessel at these locations. Researchers will test various sampling designs (e.g., speed, altitude, transect lines, and boat/shore based) to optimize sampling at each location.

Example 2

UASs may also be used as part of the survey program. UAS units can be fixed wing, such as the Puma AE, or rotary units capable of VTOL, such as the APH-22 hexacopter. The size of UAS units varies for the different models with fixed wings typically larger than VTOLs. Potential models include Puma, Altavian Nova, eBee, eBee Plus, albris, APH-22, DJI Phantom, 3DR Iris+, 3DR Solo, Freefly Cinestar 6, Mikrokopter, or other similar low impact models. The maximum wingspan of the largest UAS (Puma) is 9 feet 2 inches. All models have an auto-return feature, are piloted by a trained, qualified crew member, have a ground control station with a trained UAS visual monitor working with the pilot, and are operated within line of sight. In the past, researchers have had FAA authorization to fly fixed wing and rotary drones for turtle research. Researchers will continue to work with the Federal Aviation Administration and NOAA Aircraft Operations Center on appropriate and updated Operational Risk Management Assessment authorizations for future years. Two NOAA Principal Investigators are pilots trained and certified to fly APH-22s. If researchers undertake research with other UAS units, additional CIs will be added (as pilots) or pilots will take additional training to obtain additional certifications. UAS units may be launched from the shore or directly from the capture vessel to search for animals. If a UAS unit is used for photogrammetry, the vessel will be further away from the animal, at a distance far enough that it is unlikely to disturb the animal but close enough to be able to use the vessel as a ground control platform for the UAS unit. The aircraft may repeatedly circle, follow, or hover over an animal for an extended period of time to more accurately define and maintain its location. For UASs, the time spent over the animal is limited by

the UAS unit's battery life, typically 15 to 30 minutes for VTOL units and 45 minutes for fixed-wing units based on currently available models, though advances in battery life are expected during the course of this permit life. Fixed-wing UAS units are typically operated at 100 feet or higher, but VTOL units may be flown at lower altitudes no lower than 40 feet to collect images (e.g., photogrammetry).

Appendix Q: Example Protocols: Uncrewed In-Water Surveys and Remote Acoustic Detection

Uncrewed In-Water Surveys (Using ROVs and/or AUVs)

Example 1

Turtles will be tracked using AUVs. This method can increase the positional resolution of the location of tagged turtles, provide more position estimates, and reduce potential influences on turtle behavior from research activities as the tracking system and researchers can remain further away from turtles during tracking. The AUVs are Ocean Server Iver II models (Fall River, MA) that are approximately 2 meters in length, 15 centimeters in diameter, and are propeller driven. These vehicles are fitted with MAP 600 acoustic telemetry systems (Lotek Inc., Ontario, Canada), which enable the AUVs to detect acoustic transmissions from Lotek tags. These hydrophones sit 25 centimeters below the body of the AUVs and are separated by 2.4 meters on an external PVC frame. The AUVs are able to process these detections in order to position the acoustic tag in 3D space with a resolution of <10 meters over 30 times a minute. The AUVs are able to then alter their path to follow a moving transmitter. Working in tandem to increase the spatial resolution, the AUVs can communicate through an acoustic transducer to plan movements and exchange information.

For tracking using AUVs, turtles are tagged with an acoustic and radio transmitter (MM-M-16-50, Lotek Inc.; dimensions = 68 millimeters long × 16 millimeters in diameter; weight = 26 grams; acoustic tag = 47 millimeters long × 16 millimeters in diameter). On occasion, these are coupled with an accelerometer data logger (accelerometer = 3 centimeters × 5 centimeters long, 2 centimeters deep). This device allows for high frequency sampling (30 hertz) of 3D acceleration and gyro orientation. The transmitters operate at 76 kilohertz and pulse every 2–5 seconds. After the turtle is released, the AUVs are tendered to the last known location of the turtle, and they are placed in the water. To minimize influence on turtle behavior, the AUVs are programmed to not get within 15 meters of the estimated turtle location. Although the AUVs are capable of operating underwater, AUV operations are limited to the surface during active tracking. This will allow constant monitoring of the status of the AUVs through Wi-Fi communications in addition to visually observing the positions of the AUVs from the research vessel. Occasionally, the AUVs are programmed to do underwater missions for which they will be equipped with cameras (Hero 2, GoPro Inc.) to film the benthic substrate. These AUVs are battery operated, and battery life during these operations is expected to be between 6–8 hours. AUVs are deployed in tandem and operate at the same time; however, in order to extend the length of continuous tracks, AUVs may be deployed

one at a time. This will enable the AUVs to be duty cycled, allowing one to charge while the other is tracking. Duty cycling the AUVs can allow researchers to have continuous tracks lasting >10 hours. All AUV operations, including deployment, monitoring, and recovery, are aboard a 17-foot Boston Whaler, equipped with a 60–75-horsepower motor. Additionally, for monitoring the AUVs, operations may be conducted aboard a 14-foot Tracker tin boat equipped with a 6-horsepower motor. Throughout all tracking operations, the vessel monitoring the AUVs has a VH110 directional hydrophone deployed over the side of the vessel, which is connected to a VR100 acoustic receiver onboard (Vemco, Nova Scotia, Canada). This is the same method that is used for current active tracking of tagged turtles. This will enable researchers to constantly monitor the approximate location of the tagged turtle in reference to the AUVs. This will ensure that the AUVs do not lose the acoustically tagged turtle and that the AUVs are maintaining a 15-meter buffer.

As many as two AUVs are deployed at the same time, which allows for more accurate positions. However, most of the time only a single AUV will be used for tracking. The AUV typically travels at a rate of about 2 knots; however, it is programmed to periodically stop. The average speed during tracking is about 1 knot. It is unlikely that the AUV would collide with an untagged turtle, and if it did, it would cause little damage due to the typically low operation speed.

Example 2

ROVs, including AUVs, may also be used to closely track sea turtles and observe and record foraging and diving behaviors. Possible models include the REMUS 100, the Benthos mini-Rover, and the Deep Trekker. All ROVs used are small, portable ROVs and none exceeds a volume of 30 cubic feet. ROVs are attached to the research vessel by a tether that can be several hundred meters long. Tethers are too rigid to present a risk of sea turtle entanglement. ROVs are outfitted with additional sensors for environmental sampling and/or for additional video recording capabilities. Tethered ROVs may be either battery operated or have a live cable that supplies electricity; in either case, there will be a tether to the ROV.

Tracking of animals with a ROV (which includes autonomously operated vehicles) may occur upon sighting a target animal during an aerial or vessel survey or upon release of a captured or in situ tagged animal. The animal may be outfitted with an acoustic transmitter that can be used as a target by which an autonomously operated vehicle can track a turtle. The ROV is deployed from the side of a vessel and maneuvered toward the turtle, not intentionally approaching closer than 2 meters. In some cases, a turtle may float or swim closer to the ROV; the turtle may even come in contact with the ROV (e.g., mouthing the ROV). Remotely operated vehicles are operated at the surface or submerged. Tracking occurs for up to 12 hours, depending on multiple factors. For example, a longer duration track could build a more robust ethogram of behavioral states (Patel et al., 2016) and could also better document behavioral changes associated with environmental conditions.

Remote Acoustic Detection

Example 1

Turtles are tracked remotely with an array of stationary acoustic receiving stations (passive tracking; SUR-1, Sonotronics, Tucson, AZ; VR2W, Vemco, Nova Scotia, Canada) placed strategically throughout the study areas. NOAA Fisheries has partnered with nearby institutions to develop these remote arrays so as to learn about the movements and residency patterns of turtles in areas of special management within the study area.

Example 2

Multibeam, side-scan, and imaging sonars (e.g., DIDSON) are used from mobile (fixed to the hull or over-board mounted on the side of vessel) platforms to detect sea turtles. Vessels operate at low speeds (2–7 knots), and therefore, the potential for injury to turtles in survey areas from vessel strike is minimal. High-frequency sound pulses (120 kilohertz to 1.8 megahertz) are transmitted by the sonar transducers and reflect off the seafloor and objects in the water column, producing real-time acoustic backscatter images of bottom topography and the location of sea turtles (as well as other marine organisms) in the study area. Acoustic survey methods for sea turtles hold the potential for abundance estimation through quantification of turtle encounters relative to the volume of water sampled. This approach would be beneficial in several respects: 1) providing abundance data essential for improving the status of sea turtle stock assessments; 2) collecting presence/absence data in a manner less stressful to the turtles than capture; and 3) generating information on sea turtle size, distribution, and behavior in addition to abundance (depending on the type of acoustic gear used).

Appendix R: Example Protocols: Infrequent Methods

Oxytetracycline

Example 1

A subset of juvenile turtles is injected intramuscularly in the dorsal shoulder musculature with the antibiotic tetracycline. The purpose of the injection is to mark the bones of the sea turtle at the time of the injection so that they can be used in the calibration of bone growth if the turtle strands dead. Because researchers are interested in understanding the resorption of rings at the center of the humeri, they are most interested in marking young animals (<65 centimeters SCL). This information is also necessary for the validation of skeletochronological studies (Frazier, 1985). The quantity of tetracycline, injected into the shoulder muscle, depends on the weight of the animal. Animals are weighed to calculate the proper dosage (dosage = weight (kilogram) × 25 (milligram/kilogram)/concentration (milligram/milliliter) (NMFS-SEFSC, 2008). Oxytetracycline is administered via multiple intramuscular injections (1.5-inch, 18-gauge needle) in the dorsal shoulder musculature, not to exceed 10 milliliters per site (NMFS-SEFSC, 2008). The skin is scrubbed with Betadine® to disinfect the site prior to injection. Prior to injection, the product's expiration date and concentration are verified. Injections are given with an 18-gauge needle and a 3-cc, 5-cc, or 10-cc syringe, depending upon the total dosage. Animals with an SCL > 70 centimeters will have the dosage split into two or more equal volumes to administer in each shoulder as is recommended in NMFS-SEFSC-579 (NMFS-SEFSC, 2008). All procedures to prevent contamination are followed. Following injection, the site is wiped again with Betadine® to minimize the chance of infection. No adverse effects were noted in 20 captive juvenile loggerhead sea turtles that received intravenous and intramuscular injections of the same antibiotic at a higher dose of 25 milligram/kilogram (Harms et al., 2004).

Example 2

In certain circumstances, sea turtles may be injected with the antibiotic oxytetracycline. One dose administered prior to hook removal, skin biopsy, and tagging could offer the same beneficial fecal prophylactic effects as presurgical antibiotics in preventing post-surgical infections. In addition, tetracycline marks the bones of the sea turtle at the time of injection so that they can be used in future aging studies if the turtle later strands dead. The quantity of tetracycline to be administered depends on the weight of the animal, which may be estimated from its straight carapace length (SCLN-T) if the actual weight is unknown. If the actual weight of the turtle is known or if using a different concentration of the drug, the dosage is calculated using the formula: dosage (milliliter) = weight (kilogram) × 25 (milligrams/kilogram)/concentration (milligrams/millimeter). The product's expiration date and concentration are verified prior to administration. While wearing disposable gloves, the researcher draws the necessary dosage with a disposable syringe from the bottle using a 3-cc syringe for antibiotic quantities 0.6–2.9 millimeters and a 5-cc syringe for larger

quantities. Animals with a SCL > 70 centimeters will have their dosage split into two equal volumes and administered in each shoulder. Bones salvaged from tetracycline marked sea turtles are stored dry at the National Sea Turtle Aging Laboratory in Beaufort, NC.