PROCEDURES MANUAL
FOR THE BERMUDA TURTLE PROJECT

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8th VERSION

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Preface

The primary purpose of this manual is to guide and inform safe and effective practices by Bermuda Turtle Project (BTP) participants by describing the methodology used for the study of marine turtles in the ocean. The well-being and care of the animals we study is a priority in our work. Whilst the content of this manual is specific to the BTP, it is hoped it will be a valuable resource across the vast global network of sea turtle biologists.

The Bermuda Turtle Project conducts its research under a continuous series of Protected Species Licenses granted by the Bermuda Government Department of Environment and Natural Resources.

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History of the Bermuda Turtle Project

Bermuda's connection with sea turtles goes back to the first landings of humans on our shores at a time when turtles were a valuable resource for survival. As early as 1594, mariners stopped at the island to provision their boats with turtles which they used for meat and oil.

In 1610, settlers noted that "on the shores of Bermuda, Hogges, Turtles, Fish and Fowle do abound as dust of the earth." With recorded takes of more than 40 turtles per boat per day, it was not long before the local stocks of sea turtles were noticeably depleted.

William Strachy's narrative of 1610 reads… "the Tortoyses came in again, of which we daily both turned up great store, finding them on Land, as also sculling after them in our Boate stroke them with an Iron goad, and sod, baked, and roasted them. It is such a kind of meat as a man can neither absolutely call Fish nor Flesh, keeping most what in the water, and feeding upon Sea-grass like a Heifer, in the bottom of the Coves and Bays and laying their Eggs (of which we should find five hundred at a time in the opening of a shee Turtle in the Sand by the shore-side."

These historic records tell us not only that sea turtles were nesting abundantly on our shores but also identify the species as green turtles, the only sea turtle species that are vegetarian and graze primarily on sea grass in our inshore shallow waters.

Lefroy (1877) in his Memorials of early settlement of Bermuda, wrote that "the abundance of turtles rapidly came to an end" which led authorities to pass protective laws in 1620. Despite this early legislation, recorded wild nesting of turtles remained low and the adult green turtle population was so reduced that a commercial harvest was no longer profitable. The law failed to halt the destruction of our breeding colony. If our forefathers understood sea turtle biology, effective conservation measures would have been possible.

The juveniles living in Bermuda, and protected by the 1620 law, were from distant populations and would not stay in Bermuda as adults to nest. Sadly, despite the well-intentioned legislation, Bermuda's adult nesting turtles continued to be hunted.

FIRST CONSERVATION LEGISLATION IN THE NEW WORLD!

In 1620, only eleven years after Bermuda's colonization, an Act of the first Bermuda Parliament against the killing of sea turtles was passed. The New World's first conservation legislation written right here on the tiny islands of Bermuda.

In 1870 Bermuda's Attorney General declared that there was no nesting of sea turtles in Bermuda giving us an approximate time reference for the local extinction of our nesting green turtles.

Fast forward 100 years and we see less interest in consumption of sea turtles and more effort to conserve them both in Bermuda and across the globe.

Additional laws protecting sea turtles were passed in 1937, 1947, 1963, 1972 and 1978, which placed various restrictions on weight limits and seasonal fishing activities and imposed a total fishing ban on all sea turtles within Bermuda's territorial waters. In 2003, all sea turtles in Bermuda's territorial waters were listed under the Protected Species Act as Critically Endangered or Endangered, and in 2012, the green turtle, hawksbill turtle, loggerhead turtle and leatherback turtles were listed specifically, forcing attention to management plans for their recovery. If there is one lesson to learn in all of this conservation legislation history, it is the importance of understanding the life history of an animal to properly protect it.

The beginnings of the Bermuda Turtle Project are closely intertwined with Dr. Archie Carr, the man who pioneered the global movement to study and protect sea turtles. Dr. Carr, a stalwart supporter, and Dr. Henry Clay Frick lll, who had a residence in Bermuda, set their eyes on Bermuda to be part of Operation Green Turtle, a Caribbean-wide effort led to restore green turtle populations. The Caribbean Conservation Corporation is world's oldest sea turtle conservation group and is now known as the Sea Turtle Conservancy (STC).

In 1968, in partnership with the Bermuda Government Department of Agriculture and Fisheries (Dr. Jim Burnett Herkes and Dr. David Wingate), Dr. Carr and Dr. Frick flew 25,000 eggs from Costa Rica and Surinam to Bermuda with a hope to reintroduce a nesting colony. The experiment resulted in 16,000 hatchlings emerging on local beaches and naturally finding their way to sea. They also launched the Bermuda Turtle Project (BTP) mark-and-recapture program, making BTP one of the longest, continuous research and conservation program focused on sea turtles in their marine environment.

The failure of the translocation experiment

Given the small number of turtles likely to have reached adulthood and the fact, learnt later, that the sex of sea turtles is determined by temperature during incubation and that the lower temperatures of the Bermuda beaches probably resulted in a preponderance of males, the translocation experiment has generally been considered to have

In 1970, Dr. Frick's daughter, Jane, undertook a study to determine what happened to hatchling green turtles after they left the nesting beach. She would strap on a mask, snorkel and fins and follow the Operation Green Turtles for hours, for miles out to sea.

She found that the hatchlings set a determined course to the open ocean where they would encounter and take up residency in *Sargassum* weed.

In 1978, a Wildlife Rehabilitation Center for marine life was established at the Aquarium by Jennifer Gray with tremendous support from local veterinarians. The center's focus became sea turtles and sea birds. Much was learned about the other sea turtle species that were not part of the mark and recapture program, which took place only in seagrass habitats.

At the end of 1991, Dr. Frick retired from Bermuda and the scientific oversight of the project was turned over to Drs. Anne and Peter Meylan. Both had long associations with Dr. Carr and the STC; in fact, Anne was one of Dr. Carr's last Ph.D. students. With Jennifer Gray as the local coordinator, BTP took on an expanded research effort with new goals and a more rigorous sampling schedule. The ensuing decades would see extensive data collection and analysis leading to multiple scientific publications and a better understanding of a sea turtle's life history.

The outstanding success of the Bermuda Turtle Project is the result of a longstanding partnership with the Sea Turtle Conservancy and Drs. Anne and Peter Meylan. The Meylan's have dedicated their lives to the study and protection of sea turtles and for nearly three decades served as the Scientific Directors of the BTP. In 2020, the scientific oversight of the project was turned over to Dr. Daniel Evans and Richard Herren of the Sea Turtle Conservancy, and they continue to maintain the BTP as an internationally renowned project.

INTRODUCTION

Today, Bermuda is one of several locations in the Atlantic where post-pelagic, immature turtles occur in the complete absence of adults making it an important area where green turtles of this age can be studied in their natural habitat. Where most studies of sea turtles take place on nesting beaches, Bermuda provides scientists and resource managers with a unique opportunity to study the poorly understood juvenile life stage.

Five of the world's seven sea turtle species are known to occur in Bermuda waters with the green turtle being the most abundant. Green turtles in Bermuda consist of a foraging aggregation made up of juveniles in a developmental habitat. A green turtle nest observed in Bermuda in 2015 was the first in almost a century. Immature hawksbill turtles can be found on the coral reefs across the Bermuda Platform. Leatherback turtles are occasionally observed or recorded as strandings while passing by Bermuda on their migrations. Kemp's ridley turtles are a rare occurrence in Bermuda, with only four stranding records. Loggerhead hatchlings spend their first months well camouflaged in floating rafts of *Sargassum* weed. It is not uncommon for juvenile loggerheads to strand on Bermuda shores, often associated with clumps of *Sargassum*. In 1990 and again in 2005, a single loggerhead nest was discovered on a beach in Bermuda. These two nesting events are the first record of loggerheads nesting in Bermuda and the first records of any sea turtle nesting since the early 1900s.

The goal of the Bermuda Turtle Project is to promote the conservation of marine turtles through research and education.

The Bermuda Turtle Project is carried out as a joint program of the Sea Turtle Conservancy (STC) and the Bermuda Zoological Society (BZS), with important roles also filled by government agencies here in Bermuda. While STC scientists Dr. Daniel Evans and Richard Herren are now the Scientific Directors of the project, Drs. Anne and Peter Meylan remain involved as scientific advisors. Dr. Ian Walker, Principal Curator of the Bermuda Aquarium, Museum, and Zoo (BAMZ), leads the Aquarium's participation in the project. Jennifer Gray continues to serve as local Director with Co-Director Dr. Gaëlle Roth who also serves as the project's veterinarian. Barbara Outerbridge is the registrar at BAMZ and is responsible for archiving the original BTP data. Dr. Sarah Manuel, Senior Marine Conservation Officer, serves as the project's liaison to Bermuda's Department of Environment and Natural Resources. The team is assisted by other members of the BAMZ and BZS staff, volunteers, and the students who participate in the annual Sea Turtle Biology and Conservation course.

By the end of 2023, 5312 green turtles and 143 hawksbills have been captured, tagged and released as part of the BTP. The information collected includes the size structure of the aggregation, genetic identity, sex ratios, growth rates, site fidelity, migratory patterns, and health status. More than *1500* recaptures have been recorded in Bermuda waters by the project, providing one of the largest data sets in the world on growth rates and movements of free-ranging, immature green turtles.
More valuable information is gathered from strandings. Necropsies are performed to determine the cause of death so that we can monitor threats, diseases and toxins that can impact marine wildlife.

The green turtles living in Bermuda waters come from nesting beaches elsewhere, grow up here and then depart to mature elsewhere. They will mature on feeding grounds in the US and Caribbean and then return to their natal beaches to nest. Genetic analysis of Bermuda green turtle samples suggests contributions from nesting populations from Florida, Mexico, Cayman, Costa Rica, Cuba, Aves Island, Guadeloupe, French Guiana, Guinea Bissau and a few other small rookeries.

Green turtles tagged in Bermuda have been captured as far away as Nicaragua and Venezuela. These long-distance tag returns are particularly important because they shed light on the developmental migrations of the green turtles that grow up in Bermuda waters and highlight the need for international conservation efforts across all the geographic boundaries travelled by sea turtles.

Green turtles captured and tagged as juveniles in Bermuda waters by the BTP have been found (years later) nesting on beaches in Florida, Mexico, and Costa Rica.

More than 40 sites around the island have been sampled for turtles. The project uses a modification of the turtle fishing method that was historically employed in the Bermuda turtle fishery. A catch boat is used to tow a net boat that contains a long, 4-inch-mesh net. The capture team sets the net in a circle in areas where green turtles are known to feed. Snorkelers swim the perimeter of the net to catch turtles as they become entangled. The turtles are loaded into the catch boat and then transferred to a larger vessel for data collection. All turtles are tagged, measured, and weighed. Turtles captured for the first time are tagged with one to two external flipper tags and an internal PIT tag is inserted. Each tag bears a unique number. External tags include a reward message and a return address for the Archie Carr Center in Gainesville, FL. Blood samples are collected for use in sex determination, blood gas and chemistry, stable isotope analysis and genetic analysis.

Immature hawksbills (*Eretmochelys imbricata*) are occasionally captured in the net or by free-diving, and important information is also being gained about this species.

SAMPLING OBJECTIVES

Given that capture in the entrapment net and subsequent transfer to the catch boat and the research vessel are a significant disturbance to a sea turtle's behavior, BTP is committed to pursue two main sampling objectives efficiently and safely:

- *the safety and well-being of all turtles in all aspects of capture and data collection.*
- *a complete and accurate biological examination of all captured turtles*

Although protected by law, sea turtles face multiple threats while residing in Bermuda waters. Boat, jet-ski, and propeller strikes are common threats because turtles cannot always react quickly enough to get out of harm's way. Entanglement in fishing gear, especially monofilament line left behind by fishers, accounts for the injury and death of many turtles every year. BTP works closely with the BAMZ Wildlife Rehabilitation Centre providing support, equipment, tags, stranding response, medical care and necropsies.

Sea Turtle Stranding Hotline
441 293 2727 #999

Collaborations with other researchers enhance our understanding of sea turtle biology. Jeff Schwenter and Dr. David Owens worked with BTP on determining the changing sex ratios of Bermuda green turtles over time. Larisa Avens has been studying the age and growth of green turtles, including age at recruitment, using skeletochronological analysis of humeri collected during our necropsies. Karen Bjorndal and Alex Gulick have been examining stomach contents of green turtles to provide insights on green turtle diet in Bermuda. Simona Ceriani has analyzed skin biopsies from Bermuda green turtles to study changes in diet using stable isotope analyses. In partnership with Eckerd College, BTP sequences genetics samples which identifies where Bermuda's sea turtles are coming from and allows examination of changes in the make-up of the green turtle and hawksbill aggregations over time.

Beyond active research and advocating for better conservation, BTP is building capacity and enhancing relationships with overseas territories, particularly, those with responsibilities to help manage the source populations for Bermuda's turtles. In pursuit of our mission to protect sea turtles through research and education, BTP has conducted an In-Water Course since 1995. The course brings students to Bermuda to learn about sea turtle biology and conservation. Over the last 28 years, we have shared hands-on experience, knowledge and expertise with 234 students from 41 countries, including many Bermudians. When participants return to their communities, they have shared what they have learned, established new research initiatives, influenced policy and inspired new generations of conservationists.

LONG-TERM MONITORING IS ESSENTIAL

Never has the relationship between sea turtles and seagrass beds been more important to study. Over the past 20 years, Bermuda has experienced a near total collapse of seagrass beds, which peaked in 2020 and 2021 leading to a mass exodus and demise of the juvenile green sea turtle aggregation. Continued monitoring is essential to record further loss, implement effective conservation practice, and hopefully monitor the recovery of species and habitats.

1. SAFETY RULES

Priority of the BTP team leaders is to ensure minimal risk of injury to turtles and participants and to set the standards for carrying out in-water field work. The BTP In-Water research course requires that all participants be 18 years of age and strong swimmers comfortable free diving to 20 feet to retrieve turtles from the capture net. Participants must be healthy and have suitable medical insurance.

1.1 SAFETY RULES FOR PEOPLE

1. Before entering the water to participate in the BTP, all participants must:
   (1) pass a swim test,
   (2) read the "Safety Rules For People" and the "Safety Rules For Turtles" sections,
   (3) go through a safety briefing given by a team member and/or catch boat operator, AND
   (4) sign a waiver form.

2. The net itself is extremely dangerous.
   Avoid getting tangled in the net. Swim in buddy pairs, never separate from your buddy and only one buddy at a time should dive down. Never swim under the net. If the net is at an angle and a turtle is underneath the net, grab the turtle from above and bring the turtle with the entangled net to the surface. Do **not** go under the net to retrieve the turtle. Just as turtles get caught in the net, so can snorkelers. Be prepared to take your flippers or mask off if they get tangled in the net. If you can't untangle yourself easily, swim to the surface with the net. Your buddy can help you at the surface. The buddy that stays at the surface should always have eyes on the diving snorkeler. Be especially careful if the net is hooked on coral because you and your buddy may not be able to lift the net to get to the surface. A knife is kept on the catch boat to cut the net free of coral, if necessary.

3. Swimming around boat engine motors is also dangerous.
   Snorkelers should always listen for the propeller and stay close to the net. The catch boat will always avoid the net so you will be safe around the net. The catch boat will be operating inside the net, so do not cross the open space within the net. If the boat is approaching the net to retrieve a turtle, give the boat plenty of space to operate. Stay away from the area unless your help is requested. Never swim behind the catch boat.

4. A CPR/first aid-trained person must remain on the catch boat with the operator during a set.
   They assist the operator by constantly counting the snorkelers, watching for signals from snorkelers; recording GPS locations, water temperatures and water depths, pulling captured turtles into the boat, disentangling turtles from the net, and keeping captured turtles wet and out of the sun.

5. NO jewelry (including dive watches), cameras or diving knives, are to be worn when swimming around the net.
   They can get entangled in the net. Only slip-on fins **without** buckles or clasps are allowed for working around the turtle net.

6. Do not leave the catch boat without specific direction from the catch boat operator.
   The operator will make sure that the boat is out of gear before sending snorkelers into the water. Remember that the catch boat is towing the net boat, and you will have to swim away from the boat immediately to avoid being hit. Once clear of the catch and net boat, secure your gear, check on your buddy and swim the net to look for turtles.

7. Hand signals are necessary to communicate with the catch boat.
   All signals are done with one hand. The "I am OK" signal is one arm arched over to touch the top of the head. If you have caught a turtle, or a turtle is stuck in the net and you need help with it, put one hand straight up above your head. If you, the turtle, or your buddy is in distress, wave your arm and make sure someone in the catch boat sees you. This is an <u>emergency signal,</u> and the catch boat team will drop whatever they are doing to assist you.

8. All sea turtles can bite.
   Green turtles rarely bite, and their bite is usually mild. Loggerheads and hawksbills bite often and can inflict painful injuries. Pay special attention to their head when moving turtles or passing turtles from person to person. The claws on turtles' flippers, as well as the edges of the carapace and the flippers can be sharp. Do not slide your hand along a flipper edge or carapace edge, as it can produce a deep cut. Be alert when standing/sitting near a turtle in the boat.

9. Rays are occasionally caught in the net.
   If you see one, stay clear and raise one hand to alert the net boat. Do not try to remove a ray while you are in the water. Keep an eye out for dangerous marine life, such as Portuguese Man-of-Wars and lionfish, and alert the catch boat immediately if you see any.

10. Never place objects such as tagging pliers, tags, pencils, etc. on the plastron of the turtle or on or near the flippers. These may become dangerous projectiles.

1.2 SAFETY RULES FOR TURTLES

1. Turtles can drown in the net.
   The first task of snorkelers patrolling the net is to bring captured turtles to the surface so that they can breathe. If you can free the turtle from the net quickly and take it to the surface, do so; if not, bring the turtle and the net to the surface. If the net is around the turtle's neck, keep the weight of the net from restricting the turtle's breathing. Do not attempt to bring a turtle to the surface (with or without the net) unless you feel confident that you can do it. If you fail, the turtle will struggle in the net, get more tangled up, and use up critical oxygen supplies. If you decide not to attempt to bring the turtle up, keep some distance between you and the turtle but keep it in sight. Get another buddy-pair to assist you or summon the catch boat by raising one hand.

2. Regularly patrol all parts of the net. Snorkelers will begin entering the water shortly after the net starts going in, and they should space themselves out to ensure that all sections of the net are regularly patrolled. Snorkelers need to continue swimming the net until it is completely pulled out of the water.

3. Turtles should not be kept in direct sunlight for more than 10 minutes. Never place turtles on hot pavement, decks, docks, etc. Beware of metal fittings and hatch covers on the deck of the research vessel, which can get especially hot. Shade must be provided (e.g., by tarps, roofs, etc.) and turtles and deck must be kept wet. There is a hose on the research vessel for this purpose.

   Water in the bottom of the catch boat should be kept low enough that turtles' nares are never under water. Turtles should be rinsed/cooled with water frequently, kept in the shade, and taken to the research vessel as soon as possible**.**

4. Turtles placed on the boat need to be carefully monitored to ensure their safety. They should be placed on their backs and spaced apart from each other, so they do not get bitten, scratched or hit by another flipper. Do not stack turtles on top of each other. Swimming noodles that have been tied in a loop are used to hold turtles on their backs comfortably at a slight angle to facilitate breathing.

5. Set turtles down gently after weighing them or when loading them onto the boat. Do not rest turtles on their carapace edges or pygal scales during transfer as they could break. To prevent accidents, make sure you have sufficient assistance when handling turtles.

6. Use care when returning a turtle to the water from the boat or a dock. Set the turtle in tail first**,** pause for a moment with their head out of the water until it takes a breath, then gently release. This will prevent accidental aspiration of water.

7. Fibropapillomas are wart-like tumors that occur around the eyes and other soft tissues (shoulders, neck, tail area, etc.) of sea turtles. Green turtles are the species most affected. The tumors can be as small as a pea, or as large as a grapefruit. They have only been observed once in Bermuda. It is important to check all turtles for tumors and keep any affected turtles away from healthy turtles. Avoid direct contact with the tumors and use gloves when handling a turtle with tumors to avoid contamination.

8. Turtles should be worked up in a timely fashion and returned to the water as soon as possible. This means working up turtles as efficiently as possible and avoiding catching very large numbers of turtles in a single set.

9. When setting at sites known to have very high turtle density, a second catch boat should be available to retrieve turtles from snorkelers in the water. This will reduce the stress on turtles waiting to be removed from the net.

10. Never tag a turtle alone. One person is needed to immobilize the flipper while another person tags the turtle's flipper.

2. TURTLE SAMPLING PROCEDURE

The aim is to safely capture turtles in the most efficient way. The capture method includes:

- a 1000 ft capture net that gets deployed from a small net boat.
- a catch boat that tows the net boat and temporarily holds captured turtles.
- a research boat where turtles are gathered for their work-up and data collection.

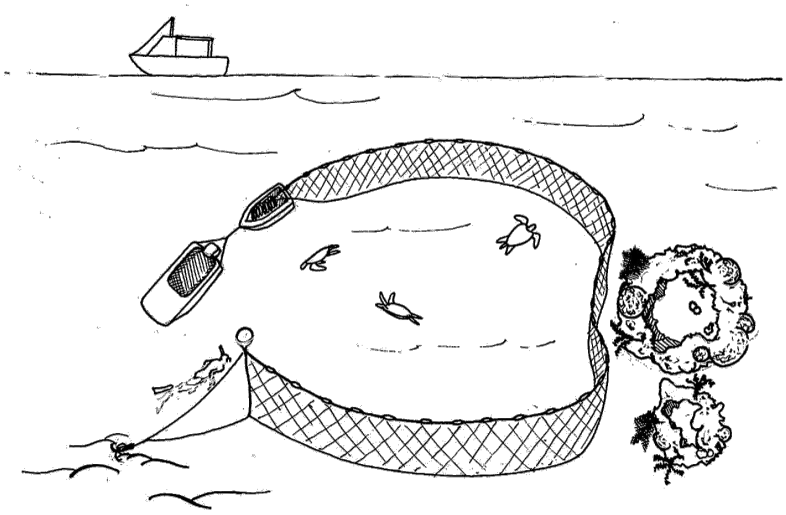


Fig. 1 Research boat, catch boat, net boat, and capture net.

2.1. SAMPLE SITE SELECTION

The placement of the set is determined by the location of the proper bottom type at a depth appropriate for the net. There are historical sets that are determined by previous sampling events. The exact location of these historical sets is identified by GPS coordinates. Certain sets are repeatedly sampled to analyze trends in the capture rate over time.

Sites to be sampled are selected ahead of time by the BTP team leaders, and a tentative daily plan is made. Each day the team leaders and boat captain review the latest information on weather, tides, currents, equipment and crew availability, and boat traffic. Based on this information, site selection may be revised.

2.2. SETTING THE NET

The capture set is made by using the catch boat to tow the net boat from which the net is dragged out (see Fig. 1). Snorkel teams are transported in the catch boat and only take with them the equipment they will need in the water. Sunglasses, sunscreen, hats, etc., are left on board the research vessel. As the catch boat approaches a potential netting site, all crew are expected to remain as still as possible. Avoid clanking objects (anchor chain, diving gear, etc.) against the hull and keep voices very low.

Team leaders will decide where the anchor is to be set, which part of the set will be blocked off first (usually the deepest part), and in which direction the set is to be made. At some sites, particular attention must be paid to the exact depth of the deepest part of the set, and/or tidal flow. An experienced team member will get into the net boat to prepare the anchor with the beginning of the net ready for launch. All other participants stay on board the catch boat and prepare to enter the water with their buddy by getting their masks and fins on. The catch boat operator/captain oversees the set. On a signal from the catch boat operator, the person in the net boat throws out the net anchor, net float and about 20 ft. of float line and lead line from the net boat. Once the anchor catches on the bottom, the net sets itself at the speed at which the catch boat is traveling. The person in the net boat stays to the side of the net at the bow, periodically grabbing the net only to help straighten it when large amounts of mesh go out together. This person also alerts the catch boat if a problem develops with deployment of the net and when the mark points (quarter, half, three-quarters) leave the net boat. The person setting the net must be very careful to keep clear of the rapidly deploying net. Most important is to keep toes pointed to the stern of the net boat so the net can flow over them. A crew member on board the catch boat communicates between the catch boat operator and the person setting the net. This person must always maintain eye contact with the net-setter during deployment of the net. During the setting of the net, the catch boat operator will periodically take the motor out of gear and instruct the first 2 snorkel pairs to enter the water to patrol the net. One buddy pair swims along the net towards the anchored end of the net,

and the other pair follows the net in the direction of the catch boat. In addition to looking for turtles in the net, these buddy pairs "help hang the net." The most important problem they fix is the occasional spot where the lead line overlaps the float line, which prevents the net from hanging properly.

Usually, the lead line can be lifted off the float line and dropped to the bottom and the net corrects itself. If there are areas where large amounts of mesh are wadded up, snorkelers can try to shake these out. However, they should not spend too much time working on the net because turtles get caught as soon as the net is in the water. Under the direction of the catch boat operator, additional buddy pairs are deployed intermittently as the rest of the net is set. A few snorkelers may stay on board until the net is fully deployed to assist with closing the net.

When the catch boat passes the anchored float line, completing the circle of net, the crew on the catch boat and the buddy pair in the net boat work to assure that there is sufficient overlap of both ends of the net. The net boat is then released from the catch boat. The person in the net boat secures the anchored float line to the net boat in a way that closes the gap between the two ends of the net. They then enter the water and with their buddy use the slack in the float line, lead lines, and anchor line to insure complete closure of the net. With the engine shut off /out of gear, any remaining snorkelers will be instructed by the catch boat operator to enter the water.

It is extremely important when entering the water to do so only with instruction from the catch boat operator, to jump clear of the catch boat, and to pay attention to the net boat that is being towed behind the catch boat.

Net description: braided twine (#18 weight of twine), 4" knot to knot, 8" across two sides of each square.

2.3. SWIMMING THE NET AND CAPTURING TURTLES

While the net is deployed, 4 to 7 buddy pairs swim the net and detangle caught turtles. Once all the buddy pairs are in the water, the catch boat operator and one or two assistants will patrol inside the net to receive turtles from buddy pairs swimming the net. For sets that are known to have high density of turtles, a second catch boat is used to receive captured turtles. Snorkelers will need to pay attention to the locations of both catch boats while they are swimming the net, but the use of two catch boats should reduce the time that snorkelers must wait to pass captured turtles into a boat.

Do not expend your energy by swimming fast. It is not a race and swimmers should maintain a steady pace, ensuring their buddies are comfortable with that pace.

Snorkeler pairs remain with their buddies and swim the net until the set is finished, or until they are instructed to do otherwise. By swimming close to the float line and watching the lead line on the bottom, snorkelers are likely to see any turtles trapped in the net and are safe from the catch boat, which will keep the running engine away from the net. Do not swim out into the center of the set even if a turtle swims out into the center. Sometimes there are extra sections of net near the net boat resulting from closing the net. All of these also need to be checked for turtles, as turtles can be captured in any portion of the net. If there is turbidity and you cannot see the bottom of the net from the surface, snorkelers will need to **take turns** diving down to check the deeper parts of the net. Only one buddy should dive down at a time while the other stays at the surface to monitor their buddy.

Turtles caught in the net must be brought to the surface to breathe as soon as possible. Occasionally, it is possible to pull the turtle out of the net and bring it to the surface, but usually it is necessary to bring up the turtle and the net together. Small and medium-sized turtles are best held at the base of the front flippers with the plastron facing away. Larger turtles are best held by the carapace, with one hand holding the nuchal region just behind the neck and the other holding the pygal region just above the tail. Holding a large turtle this way allows you to guide it to the surface with the turtle providing some of the power. If you come upon a large turtle in the net and you are uncertain that you or your buddy can bring it to the surface, do not snorkel down together to bring it up, wait for another buddy pair to assist you, or raise one hand to summon the catch boat for help. Never lift or pull the net up from the surface to retrieve a turtle. Putting tension on the net will sometimes allow the turtle to get loose from the net and if the lead line is off the sea floor, the turtle will escape from the set. Remain aware of where other buddy pairs are swimming. You may want to change direction if you find that you are swimming too close to other buddy pairs. Personnel in the catch boat may instruct you to change swimming direction to improve coverage of the net. If the mesh of the net is at an angle to the sea floor, do not go underneath the net to retrieve a turtle. Swim down from above the net, and pick up the turtle and the net. There should never be net between you and the surface of the water, so that you have a clear way to the surface. Your buddy at the surface can assist with that by keeping your path to the surface clear of net.

Communication with the catch boat is by use of hand signals (Table 1). One arm up in the air means that you have a turtle in hand, or that a turtle is securely caught in the net at your location, and you need help to retrieve it (Fig. 2). Do not wave your hand. A waving arm is a signal of distress. A waving arm is used only in absolute emergencies in which people or turtles are in immediate danger. The "all clear" or "all OK" sign is one hand arched over and touching the top of your head; use this to let the catch boat know that you are fine and continuing to patrol the net (Fig. 3). It is especially useful if you have raised a hand to indicate a turtle was in the net but is no longer in the net.

HAND SIGNAL	**MEANING**
Waiving arm	EMERGENCY
One arm straight up	Captured turtle or turtle in net (or bycatch in net)
One arm up with fingertips touching top of head	Snorkeler ok or turtle escaped, no assistance needed
Moving hand over hand above the head (like climbing a rope)	Pull in net, end of set (abandon set in case of emergency and release turtles)

Table 1. Hand signals.

Fig. 2 Arm straight up to signal catch boat.

Fig. 3 Arm curved onto top of head – all ok sign.

Stay clear of the catch boat if it is near the float line to pick up a turtle. If you need to pass the catch boat, do so by passing it on the opposite side of the net. NEVER swim behind the catch boat.

When you capture a turtle, bring it to the surface immediately and be sure to hold it with its head completely out of the water so that it does not get water in its mouth or nares. It is best to hold turtles with their head facing away from you. Before passing the turtle to the catch boat, carefully inspect soft parts for the presence of fibropapillomatosis and inform the catch boat operator if you observe any suspicious growths. Evidence of this disease will appear as warts or tumors around the eyes, the neck or at the base of the flippers (see page 13). Special handling procedures will be needed to prevent transmission of the disease to other turtles. Check the turtle for tags while still in the water and be careful tags are not inadvertently left in the net or dropped.

The catch boat team places the turtles on their backs on the deck, keeping a distance between turtles and making sure their heads stay out of water that may have pooled into the catch boat. The time of capture is marked on the plastron of the turtle with a grease marker as this is important information for when the blood samples are taken.

Snorkelers need to keep swimming the net until all the net is removed from the water, or they are asked to do otherwise. Turtles have been caught in the last 100 feet of net that was pulled into the net boat. Some turtles, especially recaptures, avoid the net and may not become entangled.

2.4. TAKING THE GPS READING AND OTHER SET DATA

Once the net has been set and most of the action of receiving turtles from snorkelers has passed, the catch boat operator and assistant take a GPS reading, the water depth and water temperature. The GPS reading is taken by moving to the center of the set and holding the GPS unit with a clear view of the sky (no boat tops, hands, masts to obscure its view of the sky). When the GPS unit gives a new location, record the latitude and longitude in decimal degrees to five decimal places (i.e., 32.66745° N 64.24242° W) in the spaces provided on the Set Data Sheet. The GPS unit is not waterproof and needs to be handled with care and kept in its container inside the GPS bag when not in use.
In addition to latitude and longitude, the location name, the recorder's name, date, time that the set was initiated, time set was completed, set number and net use are recorded on the Set Data sheet (Fig. 4a). Water temperature is taken at, or near, the bottom and the water depth is measured at the center of the set taken with a hand-held sonic depth gauge.

SET DATA:

Date: _____ Set Number: _____

Capture Locality: _____

Amount of net used: _____ Point to Point ☐

Time set started: _____ Time net closed _____

Time net pull started: _____ Time net out: _____

Net Pullers: _____

Turtles remaining inside net at finish _____ Turtles seen outside net set: _____

Depth (ft.) _____ Bottom Temperature: °C _____

CAPTURE GPS (center of set):

Lat. ___ ___.___ ___ ___ ___ ___

Long. ___ ___.___ ___ ___ ___ ___

Release Locality: _____

RELEASE GPS:

Lat. ___ ___.___ ___ ___ ___ ___

Long. ___ ___.___ ___ ___ ___ ___

Other Comments: _____

Recorder: _____

To be completed after all turtles processed

first captures _____ # recaptures _____ Total # turtles _____

Fig. 4a

HABITAT DATA:

Circle seagrasses present: *Thalassia, Halodule, Syringodium*

Circle dominant species: *Thalassia, Halodule, Syringodium*

% sea grass coverage on net circumference _____ % % sea grass coverage of entire set _____ %

Grazing level on net circumference is: (circle one)

Complete moderate little none N/A

General description of grass in whole set:

5-very dense ☐ 4-dense ☐ 3-medium density ☐ 2-sparse ☐ 1-minimal grass ☐ 0-no grass ☐

Algae species present: *Acetabularia Caulerpa Dictyota Halimeda Laurencia Penicillus Udotea,*

_____

Other fauna observed: _____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Bycatch in net: _____

Photos taken: Y/N Photographer: _____ Recorder: _____

Fig. 4b

2.5 ENDING A SET

At the end of a set, a habitat assessment is done. A snorkel pair (often a team member for consistency) is tasked by the catch boat operator to examine and record (via photographic record) the habitat within the capture set. By swimming the circumference of the net once and then across the set (once clear from the catch boat), the snorkeler pair can collect data on habitat characteristics such as the type of substrate, the composition and estimate the percentage of coverage. The snorkelers take pictures to capture the state of vegetation (grazed or ungrazed). Pictures are also taken of unidentifiable or rare specimens. All snorkelers are asked to provide feedback on what fauna and flora they have observed while swimming the net. Once on board the research boat, this information will be added to the Habitat Data sheet (Fig. 4b; on the back of the Set Data sheet).

The catch boat operator ends the set when 20 minutes have passed since closing the net and no turtles are caught, or when no more turtles are seen inside the net perimeter, or when 20 minutes have elapsed since the last observed capture. Additionally, the time from setting the net to pulling the net should not exceed 75 minutes. The catch boat operator should record the number of remaining turtles (if any) in the net on the Set Data Sheet (Fig. 4a).

2.6 ABORTING A SET

The catch boat operator or any BTP team member has authority to terminate a set early for safety reasons, weather, or other extenuating circumstances. These may include but are not limited to the following:

- The set is too deep or murky to allow complete surveillance of the net.
- Thunderstorm and/or waterspout is threatening safety of vessels and/or swimmers.
- Heavy current has laid the net down.
- Currents are threatening to / have moved the net into coral.
- The catch boat is required elsewhere (emergency transport).
- Dangerous species (e.g., Portuguese Man-o-War) are caught in the set.

When the decision is made to abort a set, the net is removed from the water as rapidly as possible. The signal to do this is usually given by the catch boat operator and consists of a hand-over-hand motion, similar to rope climbing. This signal gives snorkelers permission to pull the net up from the surface, releasing any entangled turtles and preventing further captures. Except under extreme circumstances, the set must be monitored by snorkelers in the water until the full net is back in the net boat. If the catch boat must leave the set, the catch boat operator assigns one person on the net boat as the headcounter. That person keeps track of all snorkelers remaining at the set while the net is removed from the water and then has all personnel stay with the anchored net boat until they can be picked up.

2.7 RETRIEVING THE NET

After each set is finished, the net must be pulled back into the net boat and stacked carefully. This is done by having three people in the net boat to haul in the net and one or two swimmers to guide the net boat so it stays perpendicular to the line of the net. Before untying the net and hauling it in, be sure to store snorkel gear safely away from the net and remove any water and/or debris out of the boat. The three people in the net boat determine who will pull the float line, the lead line and the mesh. The person pulling the float line stands on the bow facing the person pulling the lead line who stands on the stern. The person pulling the mesh stands at the gunwale opposite from where the net will be pulled in. These three pull the net simultaneously to keep the net boat perpendicular to the line of the net. It is the job of the swimmers to adjust the direction of the boat if it turns offline or becomes disoriented by currents or wind. As the net comes in over the side, all foreign objects (such as rocks or algae) are removed as quickly as possible.

At some sites, the net can become caught on coral or rocks. Additional buddy pairs are requested to help lift the net off these objects as the net boat approaches. In sets where reef is close to the net, all buddy pairs are asked to be observant of places where the net may become hung up as it is retrieved and be available to help free the net at these sites if needed.

2.8 BRINGING TURTLES ON BOARD THE RESEARCH VESSEL

After the net is retrieved, the catch boat and net boat with all snorkelers return to the research vessel and the turtles are off-loaded onto the deck of the research vessel. All turtles are placed in styrofoam rings (swimming noodles) with their plastron up, to steady the turtles and keep them off the deck. Turtles should be positioned in the noodle at a slight angle (head up) to allow the turtle's neck to be freely extended. Once all turtles are on the research vessel, the data recorder may begin asking snorkelers to provide feedback on the habitat conditions and what fauna and flora they observed while swimming the net. The most common fauna and flora are listed as an addendum to this document.

As turtles are loaded onto the deck, they are separated into two groups, those with visible external tags (recaptures) are laid in the first rows, starting from the left when facing the bow of the boat, and those with no external tags (first captures) are placed in a lower row, starting from the left. Within these categories, turtles are sorted by size from larger to smaller. As turtles are brought on deck, examine them closely for any abnormalities, injuries and/or evidence of fibropapilloma. Any such anomaly should be brought to the attention of a BTP team leader. As soon as turtles are on the deck, turtles without external tags should be scanned for PIT tags. Any turtles without external tags but with PIT tags are still considered recaptures and should have "PIT" marked on their plastrons and be placed with the recaptures that have external tags.

On hot days, the saltwater hose is used to spray the deck to cool it before the turtles are brought onboard and to spray turtles as they come onto the research vessel. Be careful of where the water is spraying, especially if someone is using electronics such as a PIT tag reader.

The catch boat operator takes a GPS reading for the location of the research vessel if this will be the release site. This is added to the same Set Data Sheet on the lines marked "Release GPS" (Fig. 2a). The Set Data Sheet is then given to the data recorder who will record these physical data on the data sheet for each turtle caught in that set. The form is retained with the

completed data sheets by the recorder. If no turtles are captured in a set, the physical data are transferred from the small data form to a blank data sheet and all relevant lines filled out by the recorder who writes in the comment field that no turtles were caught. If turtles from the set are released somewhere other than the capture location, a release GPS position should be acquired at the time of release with the project's GPS unit.

2.9 SEA TURTLE FIBROPAPILLOMATOSIS

Sea turtle fibropapilloma disease (FP) is a debilitating and sometimes fatal disease of sea turtles. It is seen most often in green turtles but has been reported in every species. To date, there has only been a single occurrence of FP in Bermuda. However, because so little is known about the natural routes of transmission of FP, it is best to work on the assumption that it is highly communicable and to take appropriate precautions. The following protocol has been developed to reduce the possibility of FP becoming established in Bermuda.

Recognizing fibropapilloma disease**:** FP disease is most easily recognized by the external tumor-like growths that it produces. The growths vary from smooth to cauliflower-like with small spiky projections. They occur on soft skin tissue, especially around the neck, the eyes, and at the base of the flippers. They can appear as pea-sized to grapefruit-sized growths, and are variable in color (white, pink, red, gray, purple, black). These tumors are well vascularized and will bleed readily when cut or abraded by the net. Tumors can also grow on internal organs and could be observed during necropsies.

Preventing the spread of fibropapilloma disease**:** Healthy turtles with no evidence of external tumor-like growths can still carry the virus that is associated with FP, as well as other pathogenic agents of sea turtles. Extreme caution must be used handling the body fluids of the sea turtles. The tagging punch must be cleaned of tissue, and the punch and tag applicators disinfected with chlorhexidine solution after every turtle. Blood or other body fluids from one turtle should not be allowed to get in contact with another turtle. Do not use instruments that break the skin (e.g., tagging punch) on multiple animals without disinfecting them thoroughly between use. Most instruments (needles, biopsy punch, PIT tag applicator) are single-use only. Frequent hand wiping with sanitizing wipes is recommended.

Capture of a papilloma-bearing turtle in the entrapment net**:** A turtle with obvious FP should be kept isolated from other turtles and should not be placed directly in the catch boat. The turtle should be handled with gloves and be placed into the equipment bucket (removing the GPS and other equipment first). Any equipment that comes into contact with an FP turtle should be seen as contaminated and should be kept separated. The herpesvirus that is associated with the disease, ChHV5, may survive for long periods outside of the host, especially if it is kept wet or moist. Thorough treatment of all possibly infected surfaces with detergents, disinfectants like sodium hypochlorite (Clorox) or chlorhexidine, or prolonged drying is required to make certain that the disease is not transmitted. All possibly infected turtles should be kept away from all areas where turtles are kept, including the decks of the catch boat and the research vessel, and the Aquarium, its tanks, and its water system.

A live turtle with FP should not be tagged, weighed, or measured. It should be photo-documented, and appropriate samples of the tumors should be taken and preserved in 10% buffered formalin without being frozen. FP turtles should be removed from contact with all other sea turtles and kept out of any facility that houses healthy-presenting sea turtles. If the affected turtle has a heavy FP tumor burden and the animal is seriously debilitated, euthanasia should be considered by government veterinarians. If the tumor burden is small or there is suspicion that the tumor is not FP, then the animal should be isolated and have appropriate samples taken for assessment. If FP is confirmed, the turtle could be sent to an appropriate facility (e.g., The Turtle Hospital in the Florida Keys, USA) for treatment and rehabilitation.

Any time that a suspected FP turtle is handled, all equipment used during handling and necropsy should be disinfected with 10% Clorox before being used again or returned to the Aquarium. Gloves must always be worn. Do not transport any carcasses using Aquarium vehicles and do not transport it to the Aquarium for necropsy or freezing.

Emergency Facilities and Procedures**:** A site at which FP-suspected animals could be safely kept for short periods must be identified. The fisheries compound at Coney Island might be considered. There are appropriate-sized tanks and water available and space where a single turtle could be isolated. The logistics of complete cleanup and sterilization of equipment could be handled at this site too.

2.10 In-water SAFETY SUMMARY

- Without exception, all swimmers must have a buddy and must stay with their buddy while swimming the net.
- People with experience retrieving turtles from the net should initially be paired with inexperienced participants.
- Be prepared to remove your gear if it becomes entangled in the net.
- No jewelry may be worn, as it can become entangled in the net.
- Slip-on fins without buckles are required.
- When swimming the net, always stay close to the float line.
- Never swim under the net.
- Signal the boat if you develop a cramp or for some other reason must stop swimming.
- Only one buddy dives for the turtle, while the other stays at the surface to assist.
- Holding on to the turtle, swim both turtle and net to surface.
- Untangle buddy, then turtle.
- If turtle can't be untangled, relieve pressure from net on the turtle, especially around the neck and head.
- Hold turtle high enough that its face is out of the water, so no water enters mouth when turtle breathes.
- If you are not confident about getting a turtle out of the net, back off and signal the boat or other swimmers for assistance.
- While swimming the net, periodically look up and check surroundings.
- Use extreme caution near areas where net is hitched on the reef.
- If you hear the boat engine, make eye contact with the driver to see if they have anything to say.

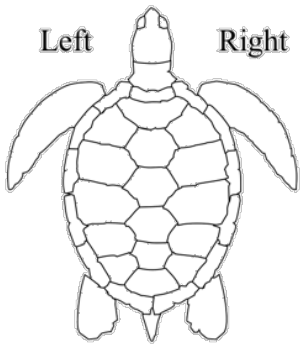
Buddy team of 3 snorkelers swimming the net.

3. TURTLE WORK UP AND DATA COLLECTION PROCEDURES

3.1 OVERVIEW AND IMPORTANT CONSIDERATIONS

Turtles should be worked up sequentially in an organized manner, recaps first, then new captures. This creates less confusion, takes less time, and allows for the most accurate data collection. Working up turtles is best done with a team or small group where each person or group has a specific task. It is also important to have an experienced person recording the data who has a complete understanding of each data field, who can deal with rapid delivery of information, and has legible handwriting. The data recorder should write his or her full name on the data sheet so that he or she can be contacted if questions arise. Dates should be written out (e.g., 4 August 2023 and not 8/4/23 or 4/8/23, which creates confusion). The month can be spelled out or abbreviated. All entries should be made using #2 pencil and written in standard block letters and numbers. Be sure to differentiate 1's and 7's, 3's and 8's, and 4's and 9's. In addition to numbers, letters need to be in CAPITALS and clearly written. Be sure to differentiate H's and M's. Lastly, standard practice in field work is to never erase mistakes but cross them out and add the correction next to it.

Important Note: The terms left and right always refer to the turtle's left and right side when the plastron (belly shell) is down, and the head is pointed away from the observer.



3.2 PRIOR TO TAGGING A TURTLE

The first thing to do is to determine if the turtle has been previously tagged (e.g. recapture). This requires a careful check for PIT tags for all turtles with no external tags.

Primary tag number – For first time captures, the first flipper tag applied to a turtle captured for the first time is designated as the primary tag number. This is typically the left flipper tag and the smallest number. All subsequent captures for a turtle are compiled under this number. For recaptured animals, the primary tag number will be recorded on the data sheet after consulting the capture database.

Locating Existing Tags – Carefully inspect flippers for existing tags. The probability of recaptures in Bermuda is high due to the thousands of turtles that have been tagged through this project and the relatively small area of the Bermuda Platform. Nearly all turtle projects place flipper tags on the trailing edge of the front or rear flippers, proximal to the joint. If no tags are present, the flippers should be closely examined for scars or tag tear-outs, indicating a tag was there, but it has been lost. If a tag scar is present, "*none, tag scar present*" is recorded on the appropriate "at capture" left or right space on the data sheet. If the turtle has neither flipper tags, nor tag scars, the word "*none"* is recorded on the "at capture" portion of the data sheet.

Many projects, including BTP, insert passive integrated transponder (PIT) tags into the soft tissue. The universal PIT tag reader should be used to carefully scan all flippers, neck and soft tissue areas of all turtles to find any non-visible, PIT tags. You should scan slowly to avoid the risk of missing a previously PIT-tagged turtle. Protect the reader by keeping it in a clear and durable plastic bag. Turn the reader on, place the reader close to the skin of the turtle, and then press and hold the READ button. Continue to hold the READ button while moving over the area to be scanned. Be sure to use the entire reading surface of the scanner when trying to detect the tag. By tilting the reading surface at different angles during a scan, you improve your chances of detecting a tag. Turtles that have not been previously tagged are called "new recruits."

Recording Existing Tags – Carefully read both sides of flipper tag(s). The complete tag number is recorded on the right or left tag space on the data sheet, where appropriate. If the tag does not have a University of Florida return address on it, the complete message from the back of the tag is also recorded on the data sheet. In this case, the material (metal/plastic) and color of the tag is noted, and the size of the tag is measured. If possible, photograph both sides of any unusual tags.

FLIPPER TAG HISTORY

More than 25 years ago, projects in the U.S., Bermuda, and the Caribbean, often used two-part plastic roto-tags commonly used on livestock. In Bermuda, plastic tags were applied to the trailing edge of the front flippers and normally had a two-letter prefix followed by three or four digits. It is unknown how many projects still use these or how many turtles might still have these tags on them. Because they are rarely used on sea turtles today, plastic tags have likely fallen off over the years and would be rare to encounter.

It is important to carefully read the entire tag number. The number on metal flipper tags usually consists of one to three letters followed by three or four digits; some old tags may have as many as six digits without a prefix. Make sure that no digits or letters are hidden by flipper tissue or epibionts. If any letters or digits are hidden by the tissue, the tag should be removed and replaced with a new tag (see below). Any removed tags should be saved in the "recovered tag" container with its number and the number of its replacement tag recorded on the data sheet.

All turtles should be released carrying at least one tag type. Most turtles will have two metal flipper tags and a PIT tag. Some recaptures will already have one or two flipper tags. Other turtles will have less that two flipper tags due to injuries, other abnormalities, or size. However, all turtles will carry a PIT tag.

All tags on recaptures are carefully inspected by a team member to determine if they should be replaced. Criteria for tag replacements are:

1. Tag cannot be read without removal.
2. Tag is causing disfigurement or swelling of flipper.
3. Clasp on tag has unlocked.
4. Tag is held in place by a narrow strip of tissue.
5. Tag is wearing and has become noticeably thin or corroded.
6. Numbers etched into tag surface are becoming illegible. Tag has heavy burden of algae, barnacles, etc., that cannot be removed.
7. F tags should be replaced on any turtle that has reached 35 cm.
8. MM tags should replace smaller tags on any turtle over 50 cm as these tags have very long retention and higher chance for long-term recovery.

3.3 FLIPPER TAGGING TURTLES

Flipper tags should be the same material and size. The type of flipper tag used is based on turtle size, using the following guidelines:

- Turtles < 30 cm SCL: One F tag (Monel) on <u>left</u> front flipper (Fig. 9A).
- Turtles 30 – 35 cm SCL: F tag (Monel) on both left and right front flipper.
- Turtles 35 – 50 cm SCL: MB tags (Inconel) on both left and right front flippers (Fig. 9B).
- Turtles > 50 cm SCL: MM titanium tag (large titanium) on both left and right flippers (Fig. 9C).

<u>Use tags in numerical order</u> - Tags are used in numerical order to facilitate recording, efficient processing of turtles, and later reconstruction of the sampling effort. The lower tag number always goes on the left flipper. Misapplied tags are recorded as "wasted" and saved in the "wasted tag" container so that if a question arises about the number of an applied tag or a label on a blood sample, it can be easily resolved.

<u>Tag and tag site preparation</u> - To minimize the risk of infection, tagging pliers, tags and the tag site are treated with disinfectants. Prior to pre-punching or tag application, the punch and/or tagging pliers with the tag are dunked first in chlorhexidine. then freshwater and dried with paper towels. Lastly, the area where the flipper tags will be placed is thoroughly wiped down on both sides with a povidone/betadine-soaked cotton ball. The same is done for the PIT tag site.

<u>Tag site preparation, pre-punching, and flipper tag application -</u> Tag application requires two people to do the job well and safely. These steps are done with the turtle on its back. One person wipes the external tag target areas and the PIT tag target areas with a povidone/betadine-soaked cotton ball and stands in front of the turtle's head to immobilizes the flipper to be tagged. The second person punches tag holes, if necessary, and applies the tags. The front flippers are best immobilized by holding the limb at the shoulder (i.e., close to the body). You should avoid holding sharp objects near the flippers or putting objects down near the flippers, as they may be swatted by the turtle.

Pre-punching - All the metal tags used by the project are self-piercing. They cut through the tissue and the lock closes when correctly applied. However, for F and MM tags, a hole can be pre-punched to improve the application success. These holes are punched with a leather punch (Fig. 9D) at the same location where self-piercing tags are applied, which is on the trailing edge of the fore flipper, just on the inside of the large round scale on the under surface of the flipper. This corresponds to the 2nd proximal dorsal scale on the trailing edge of the flipper. The distance from the edge of the flipper varies depending on the size and thickness of where the leather punch must pierce the flipper, but the location should ensure that approximately one-third to one-half the length of the tag extends beyond the edge of the flipper once the tag is closed. When the correct position is located, the hole is punched with a single smooth motion, closing the punch until a click is heard. The hole is then checked to ensure that the punched-out tissue has been removed. The punch is cleared of tissue and immersed in 10% chlorhexidine and rinsed in clean water before being used on the next turtle.

Flipper tag application - Each tag is applied with its specially designed applicator. There are different-sized applicators for different-sized metal tags (Fig. 9A, B, C). The tag is firmly and squarely seated in the applicator with the sharp point aimed for the opening in the other end of the tag. Flipper tags are applied to the trailing edge of the front flipper, to the inside of the large round scale on the ventral side. Dorsally, the tag will end up on either the 2nd or 3rd proximal scale (usually the 2nd). The tag should be placed so that approximately one-third to one-half of its length extends beyond the edge of the flipper. The exact distance from the edge varies depending on the size and thickness of the flipper. When the correct position is located and swabbed with betadine/povidone, the tag is applied with a deliberate smooth motion, closing the applicator as far as it will go, but not applying excessive pressure. You should always check the underside of the tag to ensure that the tip has passed through the hole, is completely bent over, and is securely in place. If the application malfunctioned, the tag must be removed by hand or with needle-nosed pliers. Inconel tags can often be reshaped, and another application attempted. Otherwise, try again with a new tag.

No tags are discarded; any tags removed from recaptured turtles, or ones that are bent or broken during application, are saved. There are separate containers for tags removed from recaptured turtles “recovered tags” and those misapplied “wasted tags”.

Tag numbers of existing tags are recorded on the appropriate line on the data sheet as is their final disposition (i.e., tag removed). Misapplied tags are listed in the margin of the data sheet of the turtle to which application was attempted (i.e., “Tag X4355 wasted”).

3.4 PASSIVE INTEGRATED TRANSPONDER (PIT) TAGS

PIT tags are inserted into the right front flipper between the radius and ulna. Applying PIT tags is more invasive than applying flipper tags and should only be done by experienced individuals. When properly applied, they provide a permanent ID available to anyone with a universal PIT tag reader.

As mentioned in the above section, all captured turtles should be checked for pre-existing PIT tags using the universal reader. Scan the front flippers, rear flippers and other soft parts, particularly the shoulder. If a PIT tag is detected, the number is recorded on the data sheet. If no PIT tag is found, “*None*” is entered on the “AT CAPTURE” line on the data sheet. If the turtle is a recapture without a PIT tag, one will be inserted.

In alignment with U.S. Federal permit regulations, BTP has started using 10 mm PIT tags because the smaller size and needle is less impactful. The tags themselves are small inert cylinders, a little smaller than a grain of rice, that are injected under the skin using an applicator. The 10 mm tags we are using are produced by Biomark and use a syringe applicator. They are sterilized and packaged for single-use. Always save the needle cover and cover the needle on the applicator immediately after the PIT tag is injected. Needles should be disposed of in the SHARPS container.

Before applying a PIT tag, locate the appropriate site between the radius and ulna of the right front flipper. The major joint in the flipper is between the humerus and the radius and ulna. You should be able to feel a depression between the radius and ulna. This is where the PIT tag should be inserted. Before application, the area where the tag will be injected should be cleaned with a fresh povidone-iodine saturated cotton ball.

The tag is injected from proximal to distal (i.e., towards the flipper tip) subcutaneously between the radius and ulna (Fig. 9G). To do this, insert the needle just under the scales so that about half of the needle length is visible. Then use the plunger to push the tag out of the applicator and into the tissue. Gently remove the needle at the same angle you inserted it, while checking to make sure the tag does not come out. If bleeding occurs at the site, apply pressure with a betadine/povidone-soaked cotton swab until the bleeding stops. If necessary, apply a small amount of surgical glue to close the wound.

After PIT tagging a turtle, remove the adhesive labels containing the tag number and bar code from the tag package, and give them to the data recorder. These labels will be placed on the capture sheet. Then use the PIT tag reader to scan the flipper and confirm the tag number with the data recorder.

3.5 MORPHOMETRIC DATA

To minimize the variation in the way measurements are taken, only BTP team members will take the measurements that get recorded. Turtles with severe anomalies or injuries that interfere with an accurate measurement or compromise the turtle's health will not be measured. Instead, note the injury or anomaly in the "Capture Remarks" section of the data sheet. Scars, minor injuries, barnacles or other factors that could make a measurement inaccurate should also be noted in the "Capture Remarks" section of the data sheet. Large barnacles can be carefully removed to facilitate more accurate measurements. When using the calipers, care must be taken that the tips of the calipers are in contact with the proper endpoints for the measurement (see Fig. 5B). All measurements are expressed in metric units (cm, kg).

PLASTRON LENGTH - The plastron is measured on the midline from the anterior edge of the intergular scutes to the posterior edge of the anal scutes using calipers. There are sometimes extra scales at the posterior end of the plastron, and these should be ignored. The plastron is measured from bone to bone where it is covered by the scales (Fig. 5D). To improve accuracy, this measurement is taken three separate times, changing the position of the calipers in between, to ensure accuracy.

STRAIGHT CARAPACE WIDTH - Straight carapace width is measured with the calipers at the widest point across the carapace (Fig. 5D). It should be measured whilst the person is standing squarely over the turtle, perpendicular to the turtle's midline. Again, care must be taken that the very tips of the calipers are in contact with the edge of the carapace. The measurement bar of the calipers must be parallel to the surface of the plastron and at right angles to the midline of the turtle. It is helpful to use the marginal scales on the edge of the carapace as a guide to be certain that you are measuring straight across the shell. However, this will not work for turtles with irregularly arranged marginal scales.

TAIL MEASUREMENTS - Two tail measurements are taken with the turtle on its carapace with a soft measuring tape (Fig. 5E). Accurate measurements require two people. One person places the start of the measuring tape at the end of the anal scutes and stretches it to the tip of the tail, in line with the midline of the plastron. The second person holds the tail down so that both the cloaca and the tip are visible. With the tail down and fully extended, the tape is stretched out to the tip. The distance from the plastron to the center of the cloaca (Plastron-To-Cloaca) and the distance from the plastron to the tip of the tail (Plastron-To-Tail Tip) is recorded.

STRAIGHT CARAPACE LENGTH - Straight carapace length is measured with calipers. This is the straight-line distance in centimeters (to the nearest tenth) between the center (midline) of the nuchal scute along its anterior edge to the posterior edge of the carapace at the point where the two posterior marginal scutes meet on the midline (The posterior marginals are called supracaudal scutes or pygals; Fig. 5A and 5C). This measurement is also called the minimum straight carapace length or notch-to-notch. Care must be taken that the very tips of the calipers are at the points between what is being measured (Fig. 5B). This way one can be certain that the measurement bar of the calipers is exactly parallel to the line being measured. To improve accuracy, this measurement is taken three separate times, changing the position of the calipers in between, to ensure accuracy.

BODY DEPTH - The body depth is the maximum depth in cm of the turtle's body and is measured with calipers. It is measured by 1 person holding the turtle up horizontally, and the second person holding the calipers vertically where the carapace and plastron surface are the most spread apart (Fig. 5F).

WEIGHT - Turtles are weighed using a net and electronic scale. The scale should be zeroed or tared with the net and hooks before weighing. The turtle is then placed in the net, which is wrapped over or around the front and rear flippers and then hooked together with a carabiner. The turtle is then lifted, with the carabiner set on the hook of the scale. The people lifting the turtle stand by to steady the turtle and make sure the weighing net does not slip. The reader takes the reading (to the nearest 0.1 kg.) and gives that information to the data recorder, along with the left tag number of that turtle. The net and turtle are then lifted off the hook and the turtle is removed from the net and gently placed down on its noodle. The scale should be re-tared with the net and hooks for each turtle.

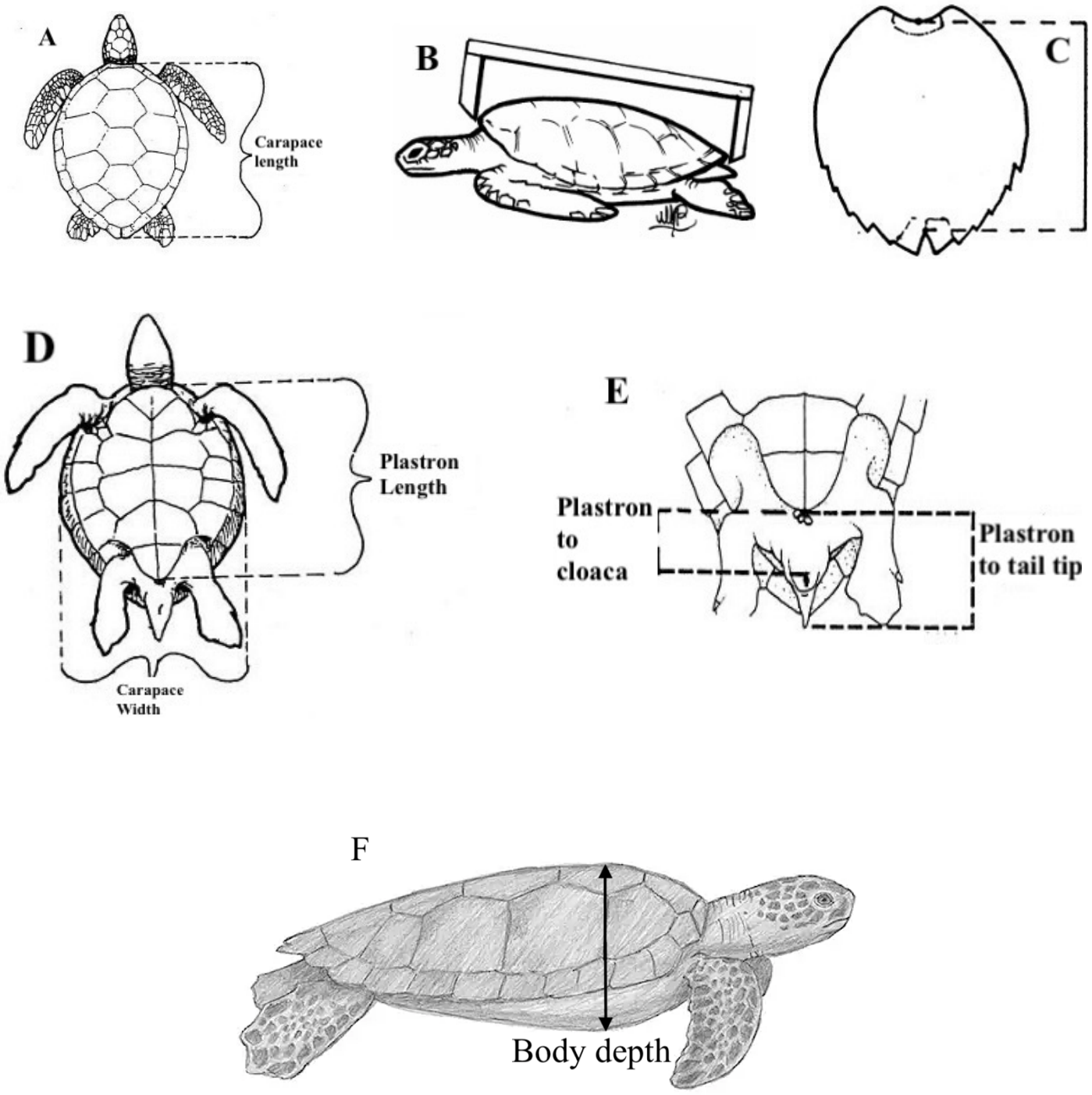


Fig. 5 Measurements taken from each turtle.

A, B, C Straight Carapace Length notch-to-notch (SCLnn or SCL min).
D Plastron Length (PL) and Straight Carapace Width (SCW).
E Tail lengths: Plastron to Cloaca (PL-CLO) and Plastron to Tail tip (PL-TT).
F Body Depth.

3.6 BLOOD COLLECTION PROTOCOL

The type of blood sample taken from a turtle depends on whether it is a first capture or a recapture. A turtle captured for the first time will have two different types of blood samples taken. A BTP recapture will have its sampling history checked in the look-up list that is kept on board. It is possible that all data has been previously collected, or that one of the samples is still needed. The sampling history of recaptures must be confirmed prior to taking any samples. The plastron of recaptured turtles is marked with the type of samples that are needed (if any), using a waterproof grease marker:

- P = Plasma, for testosterone levels (green top vacutainer – lithium heparin tube).
- L = Lysis, sample for genetics (red top vacutainer – for whole blood to add to Lysis buffer).
- H = Health assessment (part of the whole blood is used for blood gas analysis, plasma is used to run a chemistry panel).
- O = No samples needed

No marks are written on the plastron of first captures as both samples (P and L) will be collected.

When a turtle is in poor condition a veterinary assessment may dictate that no sample is to be taken to reduce the risk of further compromising the turtle's health.

Blood samples are collected by BTP staff that are appointed for this activity on the CITES permit.

Blood samples are used to determine the sex, the genetic origin, and (for some individuals) stable isotope concentrations. Two different blood tubes need to be filled to collect the samples that will provide this information. Additionally, a subsample of turtles will be selected randomly to be part of a health assessment. This means that a part of a blood sample is used to analyze blood chemistry values and percentage of red blood cells.

Vacutainers are the tubes used for collecting the blood. A **green top vacutainer is coated with lithium heparin to stop the blood from clotting**. After centrifuging a green top vacutainer, the plasma (the supernatants on top of the cells) can be used to measure testosterone levels (for sexing) and chemistry analytes (if part of the health assessment). A **red top vacutainer does not have any coating so the blood will clot very quickly**. The whole blood collected in a red top vacutainer is immediately transferred into a lysis buffer and gently mixed so that genetic material from the red blood cells can be analyzed.

<u>Preparation for blood collection:</u>
All required supplies and equipment are checked before blood sampling begins. These include sanitizing hand wipes, laser thermometer, Q-tips or cotton balls, povidone-iodine solution, needle holders, double-ended 20 and 22 gauge needles, red and green top vacutainer tubes, centrifuge, forceps, pipettes, cryovials (white top and red top), 10 ml blue top tubes (filled to 2 ml mark with lysis buffer), fine tip permanent markers, and a test tube rack on ice inside a small cooler.

The size of the needle is selected based on the size of the turtle: a smaller size needle (22 gauge) is used for turtles 35 cm carapace length or smaller and a larger needle (20 gauge) is used for turtles over 35 cm carapace length.

Two additional people are needed to assist the person collecting the blood samples: one to hold the turtle and another to pass supplies.

<u>Venipuncture/ Taking the sample:</u>
The selected turtle is checked for marks on its plastron, confirming what samples need to be collected.

If a turtle is part of the health assessment, the turtle's temperature is taken with a laser thermometer pointed at the axillary region and the time of venipuncture is noted. The temperature and the unit (Celsius or Fahrenheit) are shared with the data recorder. The turtle is restrained by hand. The person that holds the turtle sits in a comfortable spot and is handed the turtle with its head down, plastron facing them, and front flippers held against the carapace. The holder clamps the flippers against the back of the carapace (Fig. 9H). The holder then rests the turtle's shoulders on their knees, with the turtle is sitting at a 45' angle. The downward angle of the turtle helps the blood to pool in the dorsal cervical sinus. The dorsal neck area is cleaned with povidone-iodine, removing any barnacles, algae, or other debris. The bleeder (the person taking the sample) cleans his/her hands with a sanitizing wipe. The turtle's head is held by the back edge of the skull and gently pulled down. The neck is kept straight and extended to have a clear view of the neck. The turtle's eyes, throat and ears are avoided as these are sensitive and fragile areas. The hyoid area is kept free for the turtle to breathe. The bleeder sits directly in front of the turtle to visualize the four quadrants of the neck, with the supraoccipital bone showing the midline.

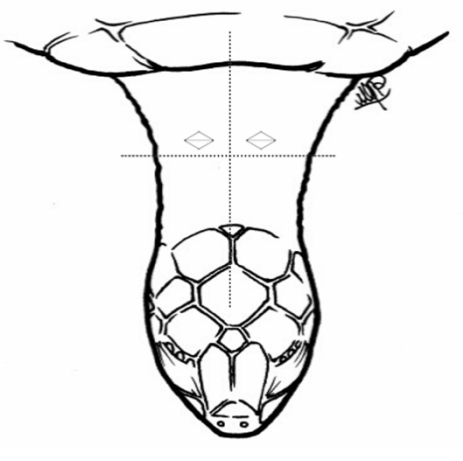
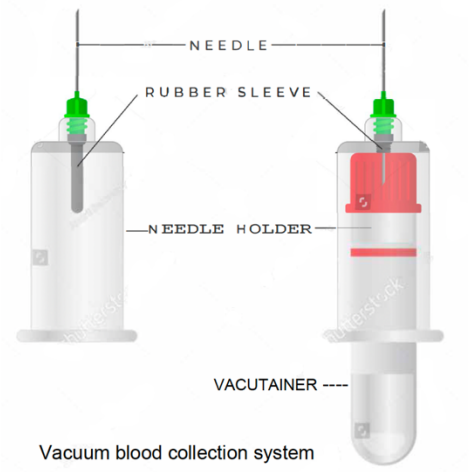


Fig. 6 Quadrants of the neck.



A double-ended needle is locked into a plastic needle holder, and a green vacutainer is placed in the tube, but not yet loaded onto the needle. The cover is taken off the needle and the needle is inserted approximately midway between the midline and the lateral edge of the neck, at a point 2-3 cm above the midpoint between the skull and shell (Fig. 6). The midline of the neck is avoided because this is the location of the spinal cord. When the needle is inserted just under the skin, the green top vacutainer is pushed onto the needle, creating a vacuum. The needle is then slowly pushed deeper in the tissues along a single trajectory. The needle is not twisted or moved side to side as this can cause cutting of the tissue. If blood does not enter the vacutainer along the first trajectory, the needle is very slowly withdrawn until about 10% remains under the skin, and then a new trajectory/angle is attempted. Up to 2 separate needle sticks can be tested per side. If both fail, the other side of the neck is sampled. The vacutainer often begins to fill as the needle is being slowly withdrawn. If the needle is pulled out of the skin, the vacuum is lost and a new needle and vacutainer are needed. A new vacutainer is used when switching sides because occasionally vacutainers lose their vacuum and fail to secure a sample.

If during the blood sampling the turtle starts to move or struggle, or the boat begins to rock excessively, the needle is withdrawn completely, and the process is paused until the turtle or boat settles.

The green top vacutainers are coated with an appropriate amount of lithium heparin for the tubes to be filled with blood until the vacuum stops. Once the vacuum stops, the green top tube is switched out for a red top (without removing the needle) to take a 0.5 ml sample. If the green, heparinized tube is filled to less than 50% of capacity, that sample is set aside and a new attempt is made on the other side of the neck. The person assisting the bleeder helps with switching out the vacutainers whilst the bleeder holds the needle steady. To avoid aspiration of tissue, the vacutainer is taken off before the needle is removed from the neck.

Immediately after obtaining the samples, the needle is withdrawn and covered with the plastic cover and discarded into the SHARPS container. The turtle is placed in normal horizontal position and any bleeding is controlled with manual pressure.

The bleeding assistant begins to process the samples immediately. The uncoated, red top sample is the priority since it will clot quickly. The 0.5 ml blood sample is poured into a 10 ml blue-top plastic vial prefilled with 2 ml of lysis buffer, and is then gently inverted several times to mix the blood with the lysis buffer.

<u>Labeling the blood samples</u>

Labeling the samples accurately and legibly is as important as collecting the samples. The labels on the samples will be read by technicians at various labs who are not familiar with the project, the tag letters, or number series. Samples with labeling errors or without labels cannot be used and must be discarded.

- Be sure all numbers and letters are unambiguous: 1 and 7 (you can put a strike through 7); 4 and 9 (open 4); M and H.
- Print, using standard printed block letters and numbers.
- Use the specified marker for each vial or tube as some markers will not last in the ultrafreezer.
- Be careful to accurately record prefixes. We use tags with the following prefixes: BP, MB, MM, M, F, and K. (ex. MB708 and MM708 are entirely different turtles).

Always write out the month or use an abbreviation for it (e.g., 4 Aug 2023). Do not use all numbers (8/4/16) because the order for day and month can be unclear. All samples should at least have the left tag number of the turtle, the abbreviated species ID (Cm = green; Ei = hawksbill; Cc = loggerhead) and the date written on the etched portion of the side of the tube. For example:

MB141
Cm
22 Aug 2022
Set 1

Tube	Label	Contents	Test	Notes
Green top tube	Left tag # Date Species Set # Body temperature for health assessment turtles only	Filled with whole blood; centrifuge will separate sample into plasma and blood cells.	On whole blood: blood gas, manual packed cell volume, blood smear.	After centrifugation: plasma and blood cells to be transferred to appropriate cryovials.
Red top tube	*None (collecting vial will be discarded)*	0.5 ml Whole blood	Genetic analysis	Transferred immediately into blue top lysis tube.
Blue top tube	Left tag # Date Species Set #	0.5 ml Whole blood in 2 mls of buffer lysis	Genetic analysis	Buffer will break down blood cells and stabilize DNA.
White or clear top cryovial	Left tag # Date Species	Plasma only, max 1.8 mls (collected from green top tube)	Total protein, chemistry, testosterone levels	Plasma is the supernatant after centrifuging. Cryovials cannot be relabeled once frozen.
Red top cryovial	Left tag # Date Species	Blood cells only (collected from green top tube)	Stable isotopes	This is the sediment after centrifuging.

Table 2. How to label blood tubes.

Preparing the samples for analysis:
After labeling the samples, the tubes are either placed in the blood cooler or are immediately processed if the turtle was selected to be part of the health assessment.

The green top vacutainer for health assessment is passed to the lab team that will run a few tests on board:

- Blood gas measurements are collected by running a CG4 cartridge with whole blood through the i-STAT machine. The time of analysis and the results are noted on the health data sheet for that turtle.
- 1 (or 2) microhematocrit capillary tube(s) are filled with whole blood from the green top vacutainer. One end of the capillary tube is sealed with clay. Place microhematocrit capillary tubes in individual, labeled slide holders (label with left tag# and date). These will be taken to the BAMZ hospital lab for manual hematocrit reading.
- A drop of whole blood is smeared on a microscope slide with cover slip. Label the slide with left tag # and date. Place the labeled microscope slide into the slide holder, together with the capillary tube of that turtle. Take to BAMZ hospital lab for slide staining with Wright Giemsa.

After all turtles from a set have been worked up for the day, the processing of all the blood samples is completed.

The green top vacutainers are centrifuged to separate plasma from the blood cells. If the sample is also being used for a Health Assessment, do not spin the sample until all necessary health tests have been completed. Samples are centrifuged for 10 minutes at 3400 rpm, taking care to balance the load in the centrifuge using blanks before spinning. The start time of the centrifuge is noted. Centrifuged samples are carefully removed from the centrifuge, taking care not to disturb the layers, and are placed into a rack for pipetting.

Each sample is pipetted by carefully removing the top of the vacutainer and collecting the clear plasma with a Pasteur pipette. A new pipette is used for every turtle. Each plasma sample is deposited into two 2 ml cryovials with a white top, taking care not to fill the vial above the 1.8 ml mark, as the samples will expand when frozen. If the layers are accidentally disturbed and the blood cells and plasma mix, the sample is centrifuged again.

The red blood cells (sediment) from the centrifuged green top vacutainers are pipetted into a separate red-top cryovial. All cryovials are neatly labeled with the left tag number of the turtle, the initials of the genus and species and the date of collection. This is always done using a permanent marker to be certain that the writing will persist during long periods in the ultrafreezer. The filled cryovials are returned to the blood cooler in a plastic bag with other blood samples from the same set. The plastic bag is

labeled by date, capture location and set number. The empty green vacutainer is discarded into the vacutainer box. The pipette, but not the pipette bulb, is deposited into the SHARPS container.

After all plasma samples have been spun and cryovials labeled, the tag numbers on all blood samples are checked against the data sheets to be sure that all samples are completely labeled, that numbers are legible, and that numbers on labels correspond to numbers on data sheets for the day's turtles. Lysis samples are separately bagged per set and the Ziploc bag is also labelled by date, capture locality and set #.

The blood cooler goes to the aquarium at the end of the day to analyze the chemistry of the plasma samples, before they go into the freezer. The cooler and test tube rack are cleaned and brought back on board for the next day.

In the lab of the aquarium, the blood analysis for selected turtles for health assessment is continued. The microhematocrit tubes are centrifuged with a specific centrifuge and the manual hematocrit is read on the chart.

A drop of plasma is used to read the Total Protein on a refractometer.

0.1 ml of plasma is taken from the white top cryovial to load the Reptile rotor in the VetScan Chemistry Analyzer. The time of analysis is noted and results are added to health data sheet. All leftover plasma (1.8 ml) is kept for hormone analysis and as a back-up sample in a designated freezer, along with the RBC in red top cryovial for stable isotopes, which is all done in a lab overseas.

The microscope slide with the blood smear is stained with Wright Giemsa stain and buffer. These will be kept for complete blood count, which examines the different sizes and structures of blood cells.

For each health assessment turtle both a BTP data sheet and a health assessment data sheet are completed.

Plasma and red blood cell samples are stored together in a freezer; blue-top lysis samples are stored in an air-conditioned lab.

3.7 TISSUE BIOPSY PROCEDURE

When attempts to get a blood sample from a turtle fail, a tissue biopsy is taken as an alternative sample for genetic and stable isotope analyses.

The critical precaution in taking any DNA samples is to avoid contamination with non-target DNA. This requires careful technique and is best done with two people: one to hold the turtle and flipper steady, the other to collect the sample with a sterile biopsy punch. The following items are required:

- a 4-mm single use biopsy punch (pre-sterilized in package)
- a plastic, white-capped sample vial pre-filled with 5 ml saturated salt (SS) buffer. The vial should be pre-labeled "SS"
- a small plastic dive slate or piece of plexiglass to serve as a clean surface on which the biopsy punch can be used
- fine-tipped forceps to remove samples that get lodged in the biopsy punch
- ultra-fine tipped permanent marker
- alcohol wipes

One person holds the turtle on its back and holds the hind flipper firmly. The second person cleans the skin on the trailing edge of the flipper on both sides with an alcohol wipe. The dive slate or plexiglass plate is also disinfected with an alcohol wipe and is placed behind the cleaned portion of the flipper. The biopsy punch is used to cut a round piece of connective tissue from between two scales on the turtle's flipper by pushing the punch through the flipper firmly against the dive slate (Fig. 9I) (do not take a sample of scale). The skin biopsy is dropped into the 10 ml saturated salt buffer-filled tube with white top by tapping the punch against the side of the sample tube or by using the sterile forceps to extract the biopsy from the punch.

Once the biopsy is immersed in the sample tube, the tube is tightly closed and the etched side of the tube is labeled with the turtle's left tag number, the genus and species abbreviation, and the date of collection. For example:

MB l 4 l
CM
22 AUG 2023

The biopsy punch should never be used on another animal and should be discarded in the SHARPS container after use. The dive slate and forceps are thoroughly cleaned with alcohol after each biopsy and are stored in a Ziploc bag.

The white-capped tubes with tissue samples are placed in a Ziploc bag labelled with the date, capture locality and set number, and are stored in an airconditioned lab. This can be the same bag that has the lysis samples.

3.8 ESOPHAGUS LAVAGE PROCEDURE

Lavage is the collection of a diet sample from the mouth and esophagus (Balazs, 1980). We do this on a randomly selected subsample of turtles, usually the same turtles we use for the health assessment. This procedure is done by trained and experienced people only.

Equipment needed:

- Two 5-gallon buckets
- Sea water
- Clean, veterinary lavage tubes
- Veterinary double action stomach pump (check that lavage tubes connect to pump)
- Vegetable oil spray (optional)
- Mouthpiece
- Soft measuring tape
- 4% formalin solution (Toxic)
- Sieve (to filter out food particles)
- Spoon or Forceps
- White or colored tape
- Sample jars with air-tight lids
- A minimum of 4 people (one person to hold the turtle, one to pump the water, one to open the turtle's mouth and one to feed the tube into the esophagus)

Procedure:

1. A clean 5-gallon bucket is filled with seawater collected from the same area where the turtle was captured.
2. The flipper tag is confirmed with the recorder that this green turtle was selected for the health assessment.
   * Fibropapilloma turtles will not be used for lavage samples *
3. The size of tube is selected based on the size of the turtle. The diameter of the tube will be 0.63 cm for small juveniles (>25cm SCL but < 40 cm SCL) or 0.94 cm for larger juveniles, subadults and adults (> 40 cm SCL).
   The maximum length of the tube that will be inserted orally is set by measuring the distance from the tip of the mouth to the intersection of the inter-gular and gular scute on the plastron. The portion of the tube that will be inserted should be marked by tape and lubricated with vegetable oil. The tube is connected to the water pump.
4. The turtle is held upside down on its carapace at a 45° angle (with its head lower than the tail). This angle helps minimize the risk of aspiration as gravity will pull the lavage fluids downwards, out of the turtle's esophagus and mouth. The front flippers are secured by the person holding the turtle.
5. The mouth of the turtle is gently opened with a bite block or similar tool and the oral cavity is checked for any abnormality, injury or disease.
6. The lubricated tube is inserted into the mouth, carefully passing the trachea opening and advanced into the esophagus until the tape mark. The turtle will slowly swallow the tube, and this is when we will begin slowly but steadily pumping seawater from the bucket. The length of the inserted tube is checked regularly so it does not go past the maximum length (tape mark).
7. While seawater is pumped through the tube, the tube will be moved gently back and forth in the esophagus. Food items will be collected from the backwash into a second clean, five-gallon bucket that is placed directly below the turtle's head. The pumping of the water takes about 20 seconds. The water pumping stops and the tube is gently pulled out.
8. The contents of the receiving bucket are poured into a sieve.
9. Food items are collected from the sieve using a spoon or forceps and are stored in a 4% formalin solution for later analysis. The sample jar is securely closed and wrapping wax is used for extra protection against leakage. Formalin is a toxic chemical and should not be inhaled, ingested or come into contact with your skin and/or eyes. Gloves should be used when opening these sample jars.
10. The turtle is held at the 45° angle until no more water is coming out. The turtle is then gently placed back onto its plastron.
11. The data sheet for the turtle is completed, making note of the lavage procedure and contents that have been collected.
12. Sample jar is labeled with date (month is written out), tag ID (left front flipper tag), species abbreviation (CM), capture location and lavage sample with permanent marker.
13. All used materials are cleaned with soap and seawater, not with chemicals.

3.9 CAPTURE DATA AND REMARKS

BERMUDA TURTLE PROJECT
BERMUDA AQUARIUM MUSEUM AND ZOO

Sample #:

Please do not write in shaded fields. Primary Tag Number: FL0791

Tag(s) on Left at capture: X at release: MB 3377

Tag(s) on Right at capture: X at release: MB 3378

Pit Tag # at capture: 018 308 052 at release: (attach ID here) **Pit Tag Location:** RF (LF)

Species (circle) (CM) EI DC CC **Fibropapilloma** Y (N)

Capture Date: (write out month) 08 AUG 2022

Observation Type: First (Recapture) Stranding Tag-Return

Capture Location: Stocks Harbour Central

GPS (Capture): 32.37035 N 64.68953 W

Data Recorded By: Barb Outerbridge **Set #:** 2 **Set Time:** 1:28

				Average Mst's
Plastron Length (cm)	36.4	36.4	36.3	36.4
Straight Carapace Width (cm)				34.3
Plastron-to-Cloaca (cm)				5.5
Plastron-to-Tail-Tip (cm)				8.7
Straight Carapace Length (notch-to-notch in cm)	44.6	44.7	44.7	44.7
Carapace Depth (cm)				17.2
Weight (kg)				12.80

Blood/Tissue Sample: (circle) S L R O (B) N

S = Serum L = Lysis R=Red Blood Cells O = Other B = Skin Biopsy N = No Sample

Photos? (Y) N **Diatoms?** Y (N) **Health check?** Y (N) **Lavaged?** Y (N)

Comments: _____

Release Date: (write out month) 08 AUGUST 2022

Release Location: Stocks Harbour Central

GPS (Release): 32.37040 N 64.68905 W 458

Capture Remarks: **Old Tag Hole?** LF RF

Flipper Damage? LF RF LR RR **Flipper Missing?** LF RF LR RR

Damage affecting measurements: **SHELL** YES **TAIL** YES

Large Notch(es) in shell? YES

Other/Comments:

Water Depth: 3 m / (ft.) (circle one)

Water Temp: 30 °C Surface or Bottom (circle one)

Revised:03 Aug 2022 12 AUG 2008 Mullet Bay

Fig. 7 A completed data sheet for a recapture that had a PIT tag but no flipper tags.

For each turtle, a data sheet is completed by the recorder, in pencil. An example is shown in Figure 7.

Primary tag number – For first time captures, one of the applied tags (typically the left tag) is designated as the primary tag number in the database. All subsequent sightings for this turtle are compiled under this number. For recaptured animals, the primary tag will be entered after consulting existing tagging records.

Tag(s) on Left/Right – These spaces are completed with the newly applied (first time captures) or pre-existing (recaptures) flipper tag numbers.

PIT tag # / PIT tag location– This is where the PIT tag number is copied. PIT tag location is noted as Right Front (RF) or Left Front (LF).

SPECIES - Any turtle whose species ID is questionable should be determined by a team member. Any turtle that is not a green turtle should be photographed to show dorsal and ventral views, plus a close-up of the side of the head. Two species regularly occur in Bermuda waters: the green turtle (CM) and hawksbill (EI). Loggerhead (CC) are occasional, Leatherback (DC) and Kemp's ridley (LK) occurrences are extremely rarely. Hybrids between sea turtle species have been documented in Bermuda. Possible hybrids should be thoroughly photographed with views from every aspect, including head shots from the side.

FIBROPAPILLOMATOSIS - Y (yes) N (no). When turtles are taken from the catch boat and placed on board the research vessel for tagging and data collection, they are sorted into two groups, those with tags and those without. When checking for tags, one should also check for fibropapillomas. If any suspicious growths are found, a team member should be immediately notified. FP is contagious among turtles but is not known to affect people, but we take precautions anyway.

CAPTURE DATE - The complete date is written out on the form: day – month – year, with the month spelled out (e.g., 16 August 2022). It is always the date on which the animal was captured that is entered, even if the animal is tagged or the data is completed at a later date.

OBSERVATION TYPE - First, Recapture, Stranding or Tag Return. Turtles captured during the normal operations of the BTP are recorded as a "First observation" if they have no external or PIT tags and as a "Recapture" if they have one or more tags previously applied by the project (including a PIT tag). The "Tag return" category is usually reserved for foreign recaptures but would also be used for any healthy BTP turtle captured in Bermuda waters by non-project efforts. The "Stranding" category is for any untagged turtle that is obviously in bad health (i.e., not able to swim or dive well enough to avoid capture by hand) or is found washed up on shore (dead or alive). A turtle with no tags but with visible tag scars is marked as a "First observation" because there is no way to connect it with its previous tag history. The presence of tag scars is always noted on the data sheet. Special care should be taken to look for PIT tags, even in turtles without tag scars.

"Tag returns" are observations of turtles made by people not associated with the project. In most cases, tag returns will be reported to us via the Gainesville, FL office, which is listed in the reward message on the tags. This office has, in turn, received the tag and information from whomever caught the turtle. It is important that data (species I.D., size, weight, etc.) collected by project team members be distinguished from data reported by third parties. Details concerning third parties (including name, address, and phone) should be included under capture remarks. Turtle tags or dead turtles with tags that are turned over to the BTP project by third parties should be listed as domestic tag recaptures or strandings, depending on whether the turtle is healthy vs. sick/injured/dead. If the turtle is returned alive and healthy by a third party, and can be released by a team member, the observation should be considered a tag return. In nearly all cases, a foreign tag return will mark the end of a turtle's record.

CAPTURE LOCATION - Since multiple names are sometimes referred to the same grass flats, the BTP has established a set of standardized names for the most frequently fished sites on the map. The standard name should be entered on the data sheet. If new sampling sites are added, a standard name for each is added to the list and will always be used for samples taken at that location.

GPS (capture) - The GPS unit is taken along in the catch boat on all sampling sessions and a reading is taken from the center of the set by the catch boat operator and crew. The GPS reading (in decimal degrees to 5 decimal places) is entered on the Set Data sheet (Fig. 2A) along with the temperature, depth, set time, and the release location and release GPS reading. The Set Data sheet is given to the recorder by the catch boat operator when returning to the research vessel. See the section on "Taking the GPS reading and other set data" on page 11 for more details.

DATA RECORDED BY - The full name of the person(s) recording the data is entered here. The data recorder makes sure that all the data sheets are accounted for and turned over to a BTP team member at the end of each day.

SET # AND SET TIME - In addition to the GPS location, the data sheet includes spaces for four physical descriptors of the set. Set number and set time are recorded in the middle of the data sheet. Water depth (in feet) and bottom water temperature (in ^{o}C) are recorded in the bottom right-hand corner. All these data are made available to the data recorder by the catch boat operator who records them on a Set Data form during the set.

RELEASE DATE - The complete date is written out in the form: day - month - year with the month spelled out (e.g., 16 August 2016). It is always the date on which the animal is released even if the animal was tagged and measured on a previous date.

RELEASE LOCATION - The name of the release location should come from the standardized list of site names. If we add a new release location, it should be added to the list of site names.

GPS (RELEASE) READING - The Set Data sheet is completed by the catch boat driver, which has both a capture GPS and a release GPS location. Often the release GPS is taken from the deck of the research vessel while it is still at the position where turtles are released. If turtles are released elsewhere, a new GPS reading should be taken with the project's GPS unit and recorded on the Set Data sheet.

CAPTURE REMARKS - Miscellaneous observations are recorded here, including any notable injuries (shark bites or missing flippers). If the observation is a tag return, all details are entered here about the person reporting the information (name, address, phone number), date of report, habitat type, circumstances of capture, final disposition of turtle (i.e., dead /released alive, etc.).

BLOOD SAMPLES COLLECTED: P (plasma) L (lysis) R (red blood cells) B (biopsy) O (other) N (no sample)
Confirmation of the blood samples collected is made by circling the appropriate letter on the data sheet: **P** for plasma sample, **L** for genetics sample in lysis buffer, **R** for red blood cells saved for stable isotope analysis. If a genetic sample is needed but no blood is obtained, a skin biopsy is taken (see skin biopsy protocol) and is indicated by circling the **B** (biopsy). **O** (other) is circled if some other kind of sample is taken. If no samples are taken, the **N** is circled. If only one sample is taken, the single appropriate letter is circled. If multiple samples are taken, all applicable letters are circled. This line on the data sheet is never left blank. The number of plasma samples taken is usually noted on the data sheet.

3.10 PICTURES

For each turtle, pictures are taken to be used as additional ID and to capture their body condition. The left flipper tag number, the date and the set number are written on a white erase board. The board is placed behind the turtle so that each picture includes an identifier for the turtle. A picture is taken from the left lateral side of the head, the plastron, the carapace, as well as the body depth as seen from its left side (see pictures in addendum).

3.11 RELEASE OF A TURTLE

Before a turtle is released, the left tag number is read to the recorder who reviews the data sheet to confirm that all data has been recorded and that tag numbers are correctly listed. The recorder must confirm that the turtle is ready for release.

Turtles are released as close to their capture location as possible, either from the research boat, or by taking the turtles back to the capture location with the catch boat. A turtle is released by holding it at the base of the front flippers, with the carapace facing the holder, and lowering the hind flippers in the water first, keeping the turtle vertically in the water with its neck and head out of the water (Fig. 8). Once the turtle takes a breath, the turtle is gently released.

At the end of each day's sampling, the data sheets are reviewed to ensure that all blanks have been filled, samples in hand match the data sheets, and all entries are legible. The data sheets are taken back to BAMZ daily for safekeeping and checking. The data sheets for any given date are organized in the data notebook first by Set #, then by Observation Type (recaptures, then first captures), then in alphanumeric order by Primetag #. The Primetag number may need to be looked up before the page order is finalized if the turtle has had multiple tags. In the meantime, the left-flipper tag number at capture can be used (for recaptures).

Fig. 8
Release of a turtle in the water.

4. ADDITIONAL SAMPLING

4.1 SATELLITE TAGGING PROCEDURE

Record the ID of the TAG's Platform Terminal Transmitter number on the turtle's data sheet and switch the TAG on. Ensure that the TAG is functioning by checking to see that the TAG is showing up in the Argos web portal system. Pay careful attention to the mechanism for turning your TAG on and off, as it varies by manufacturer.

Ahead of time:

- Cut the fiberglass cloth into five pieces.
    - Fiberglass Base - A rectangular or oval base approximately 50% larger than the base of the TAG.
    - Side Strips - Two 1" wide (25mm) strips that are approximately 50% longer than the length of the TAG.
    - End Strips - Two 1" wide (25mm) strips that are approximately 50% wider than the width of the TAG.
- Cover any wet/dry, and depth (if applicable) sensors on the Tag with painter's tape to protect them from resin or paint.
- Lightly sand the top and edges of the TAG if it will be painted. If you are unable to paint the tag before releasing the turtle, do not sand the TAG.

For the attachment: you MUST remember to remove the tape from the sensors before releasing the turtle.

Place the turtle in a large bin or have one or two people help restrain the turtle. The turtle does not need to be immobilized the entire time, but there are instances when its movements should be restricted as much as possible. It may be helpful to place a dark, damp cloth over the animal's eyes but be careful not to cover the nares as the animal will need to breathe. This may help calm the turtle and will protect its head from cleaners, resin, and paint.

1. Select the best location for the TAG. It should be dorsally centered on the carapace for best hydrodynamics and near the head of the turtle. For smaller TAGs, consider a single-scute attachment on the second central dorsal scute from the head. Most sea turtle satellite TAGs are designed to have the antenna near the turtle's head.
2. Clean the attachment area (at and around the attachment site) with fresh water (salt water is ok to use, but fresh is preferred) and the scouring pad. This may need to be done several times with different scouring pads to remove all of the algae and epibiota growth. Once clean, dry the attachment site completely using a cloth damped with Isopropyl alcohol.
3. Once the carapace is dry, lightly sand the attachment area with 80 grit (medium) sandpaper. (Avoid excessive sanding or sanding over the suture lines between scutes, as the carapace can be oily and may leach body fluid). Vigorously dust the sanded area.
4. Clean the attachment area with acetone/Isopropyl alcohol and a clean cloth to maximize the adhesion of the resin to the carapace. Do not touch after cleaning.
5. Repeat this sanding/cleaning process once more with 80 grit sandpaper, or until the cloth comes away clean.
6. Use 60 grit (course) sandpaper to scratch lines into the scutes of the attachment area, again avoiding the suture lines. While the previous sanding was to remove biota and oils, the process may have made the carapace very smooth. This step creates grooves in the carapace to help the resin adhere to the carapace.
7. Dispense about 1/3 (4 squeezes for a standard sized satellite tag) of the epoxy resin into a mixing cup. Mix thoroughly for 60 seconds and spread a thin layer (no more than half of the mixed resin) of resin onto the attachment area with a tongue depressor where you will place the fiberglass base. Do not stir the resin too vigorously; use a steady motion to completely mix the resin.
8. Immediately lay the base fiberglass cloth on top of the wet resin. Quickly apply more resin with the tongue depressor and a brush to wet the fiberglass completely. It will dry in approximately 5 minutes.
9. Use epoxy putty to cover the entire bottom of the TAG which will bond to the fiberglass/resin base. Cut off 3/4 of the epoxy putty stick and remove the end sticker and plastic cover. Wearing gloves, kneed/mix until a uniform color. This should take about two to three minutes. Work quickly because once the putty is mixed, it only has a 5-minute working time.
10. Divide the epoxy in half and make two "logs" that are the length of the tag. Remove the Peel Ply backing, if present, from the TAG and position these logs along the bottom of the TAG. Press and smooth the "logs" downward and towards the middle of the TAG base so it covers the entire base. Make sure there are no air pockets in the putty. If the turtle has a pronounced dorsal ridge, you may need to build up putty along the TAG's side edges to straddle the ridge. Position the TAG centrally on the turtle and press it down firmly, gently shifting it from side-to-side to set it in and squeeze some of the epoxy putty out from under the TAG base. If necessary, add additional putty to any gaps between the TAG and carapace until it is fully filled in. Use a gloved finger to press the epoxy putty up along the sides of the TAG and smooth the surface. Allow it to set for about 10 minutes or until you cannot indent the putty with your fingernail.

11. Apply resin and fiberglass strips over the epoxy putty. These will often overlap the edge of the TAG and onto the carapace. Cut the strips as needed to stay on a single scute if possible. The strips will go along the back, sides, and front edge of the TAG. Mix more of the resin to attach the fiberglass strips. Generally, it takes 3 squeezes of the applicator for each side and 2 squeezes for each end. For each side/end, first spread resin on the area, place the fiberglass, and then apply more resin (like when applying the based layer of fiberglass. Repeat this step along each side of the TAG starting with the back. The side strips should cover the ends of the back strip. The front piece is the final strip of fiberglass to be placed, and it should cover the ends of the two side strips.
12. Be careful not to cover any sensors. There should be no epoxy or fiberglass over the top of the TAG.
13. Once the epoxy is dry, paint the resin/fiberglass footprint, the TAG and the Argos antenna with a coat of Marine Anti-Fouling paint. Do not paint any sensors. If you have several days before the attachment, you can apply two coats of Marine Anti-Fouling paint to the TAG, allowing 24 hrs of drying time between coats. Make sure to place tape over any sensors before painting.
14. While the paint is drying, REMOVE THE TAPE from all sensors. Allow the antifouling paint to dry as long as you can before releasing the turtle.

4.2 FECAL COLLECTION PROCEDURE

If a turtle produces stools while on board, a fresh fecal sample can be collected. A fecal sample analysis can provide information about the turtle's diet and the presence of internal parasites.

The fecal matter is collected with a disposable fecalyzer cylinder cap and strainer. The cap is lifted and the green strainer is taken out. The narrow end of the green strainer is inserted into the fecal matter to take a small sample and then is placed back into the holder. The cap is closed and the cylinder is labeled with the left flipper tag, species abbreviation and date. It is best to store the sample in a cool place but not to freeze it. A small Ziploc bag or sample jar can be used as an alternative to a fecalyzer.

In the lab two different screening methods are used: a direct smear and a flotation technique.

For the direct smear, the green strainer is removed from the cylinder and the narrow end is dabbed onto a microscope slide. A cover slip is placed on top of the sample and the slide is ready to be viewed under the microscope (10x or 40x objective).

The flotation technique uses solutions which have a higher specific gravity than the organisms to be floated, so that the organisms rise to the top and the debris sinks to the bottom. This technique produces cleaner materials to view under the microscope, but the disadvantage is that not all parasite eggs float (trematode eggs), or that the walls of some eggs and cysts can collapse in the solution. The most used solution is Zinc Sulfate. The solution is added into the cylinder until it is half full. The green strainer is twisted a few times to dissolve the fecal matter into the solution. More solution is added until a meniscus forms. A cover slip is placed on top and left in place for 15-20 minutes. Afterwards, the cover slip is placed onto a microscope slide and is ready to be read under the microscope.

4.3 HABITAT SAMPLING

If, during the habitat assessment or during a turtle work up, an unidentifiable or rare specimen is found, pictures should be taken to document it. One of the benefit of pictures is that they do not cause habitat or species disruption. The pictures can be used for identification and for educational purposes. In certain cases, it may be beneficial to take a sample of a specimen, such as algae, barnacles, or leeches. Each sample should be stored in a labeled sample jar or sample bag that is securely sealed. To preserve a sample, a fixative solution is added, such as isopropyl or ethanol > 70%. Formaldehyde can also be used, but exposure to this toxic chemical needs to be avoided by using gloves, by handling it in a ventilated area and by making sure the solution won't leak. The collection of a sample is recorded on the appropriate data sheet.

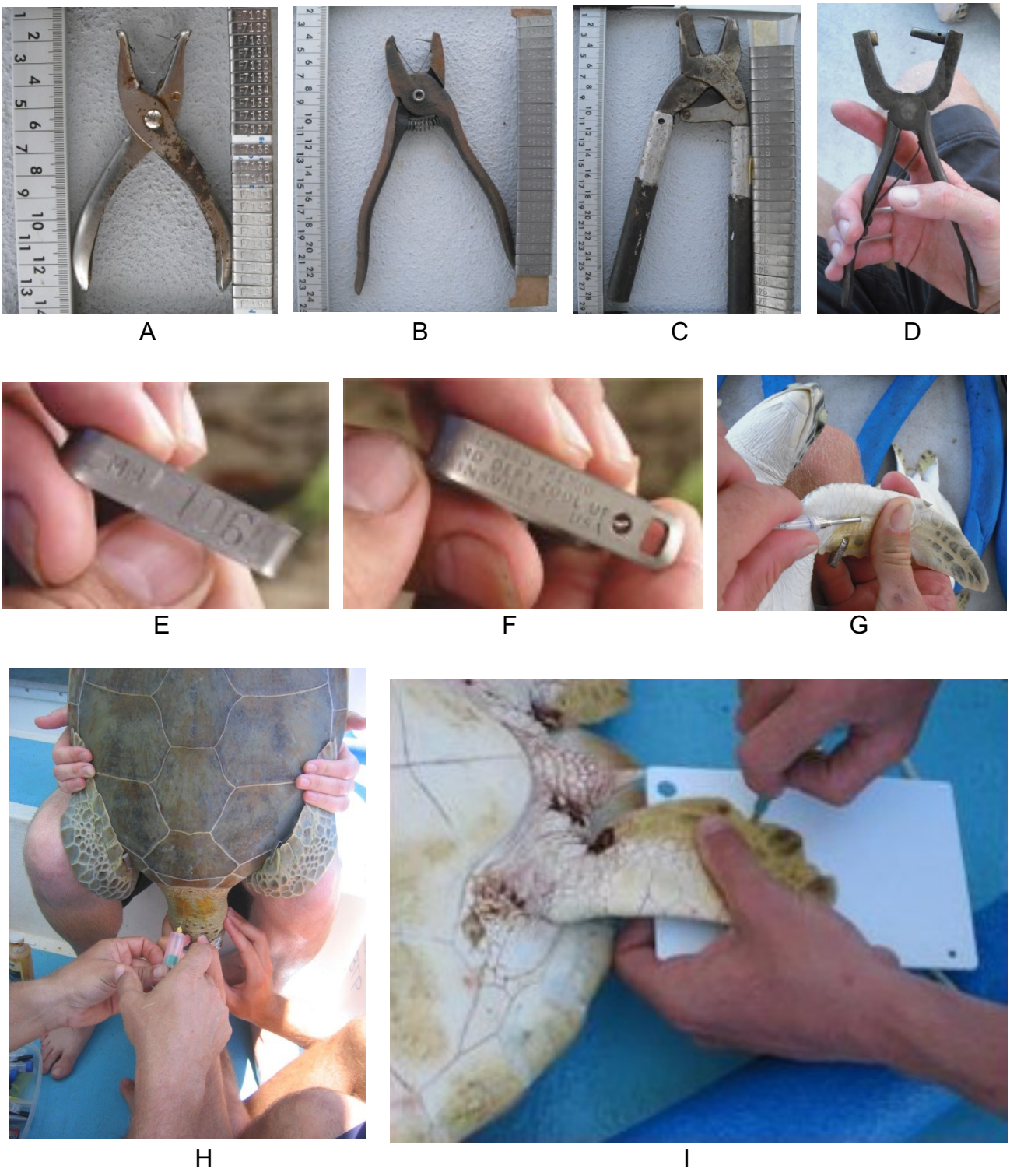
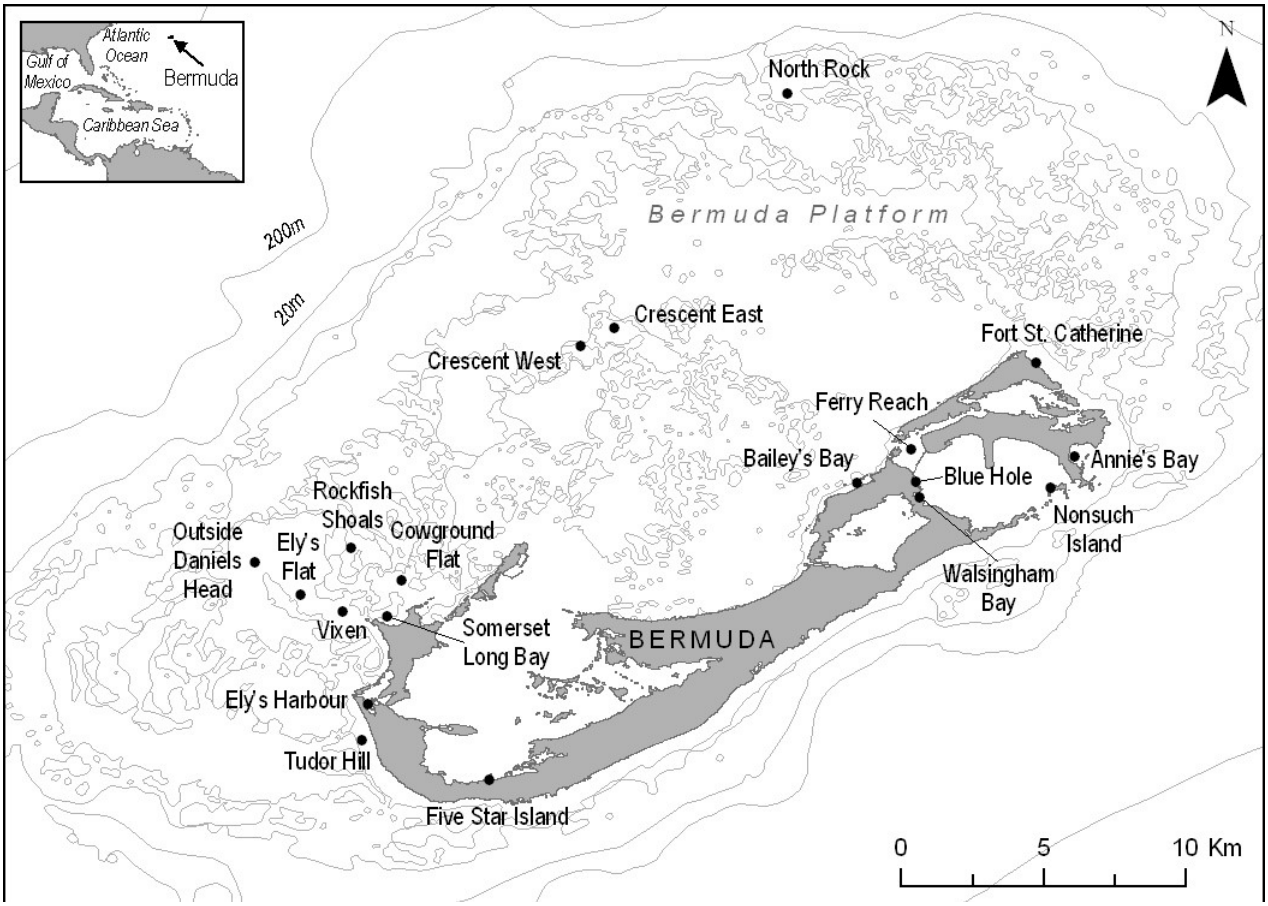
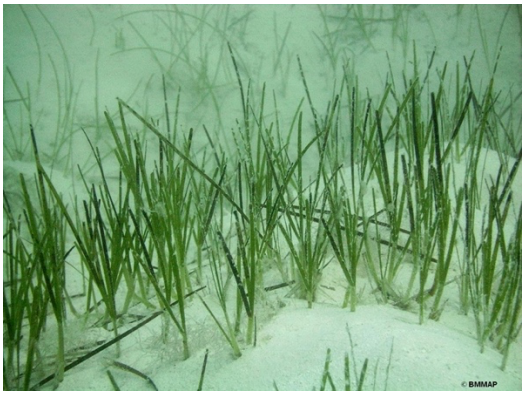


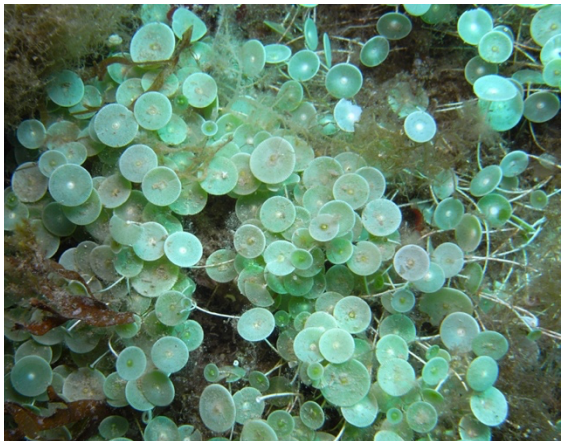
Fig. 9 Illustrations of tagging and sample collection. ***A****) F tags (Monel) and applicator (for turtles smaller than 35 cm SCL).* ***B****) MB tags (Inconel) and applicator (for turtles 35 – 50 cm SCL).* ***C****) MM tags (Titanium) and applicator (for turtles 50 cm and above SCL).* ***D****) Leather punch used to prepunch before application of tags.* ***E****) Numbered side of MM titanium tag.* ***F****) Reward message side of MM titanium tag.* ***G****) Implanting PIT tag using syringe applicator.* ***H****) Holding position for taking blood sample from cervical sinus, with fingers holding flippers against carapace and weight of turtle held on holder's knees.* ***I****) Taking a tissue biopsy sample from the tip of the hind flipper using a biopsy punch and dive slate.*

Appendices



MAP. Locations that are regularly sampled for sea turtles by the Bermuda Turtle Project.

Seagrass species found in Bermuda	
	
Thalassia testudinum (Turtle Grass) Wide and flat blades, the largest of all 4 species.	*Halodule* sp. (Shoal grass) Slender/narrow flat blades.
	
Syringodium filiforme (Manatee grass) Cylindrical blades.	*Halophila decipiens* (Paddle grass) Paired, oval blades.

Calcareous green algae species found in Bermuda	
	
Halimeda	*Udotea*
	
Penicillus (Merman's shaving brush)	*Acetabularia* (Mermaid's wine glass)

Pictures to be taken of each individual turtle:

1. Dorsal view of carapace – full body

2. View of the plastron – full body

3. Lateral view showing body depth - full body.

4. Lateral view of head (left side).

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Online presence

http://www.bermudaturtleproject.org/

https://www.facebook.com/Bermudaseaturtles

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Lefroy J.H. (1877) Memorials of the discovery and early settlement of the Bermudas or Somers Islands, 1515-1685.

This is the 8th version of the BTP Procedures Manual first produced in 1992 by Peter Meylan, Anne Meylan and Jennifer Gray.

Previous updates were done in 1994, 1999, 2003, 2008, 2013, and 2016.