PROCEDURE MANUAL
FOR THE BERMUDA TURTLE PROJECT

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SEA TURTLE CONSERVANCY

BERMUDA TURTLE PROJECT

BERMUDA ZOOLOGICAL SOCIETY

BERMUDA AQUARIUM, MUSEUM & ZOO

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Additional information on the Bermuda Turtle Project is available at : http://www.conserveturtles.org/bermuda/

THE BERMUDA TURTLE PROJECT (BTP)

INTRODUCTION

Bermuda was once the site of an important nesting aggregation of the green turtle (*Chelonia mydas*). As early as 1594, mariners stopped at the island to provision their boats with turtles which they used for meat and oil. By 1620, the government was sufficiently concerned about the wanton exploitation of the turtle resource to pass "AN ACT AGAYNST THE KILLINGE OF OUER YOUNG TORTOYSES." The law failed to halt the extirpation of the breeding colony, however, and by the 1920's, nesting by green turtles had virtually ceased. A single nest in 2015 was the first documented green turtle nest in Bermuda in many decades.

Today, the green turtles that inhabit the island's extensive shallow-water habitats are all immatures in "benthic developmental habitat." Immature hawksbills in the same life history stage are also relatively common on the Bermuda Platform. The green turtles have been the primary focus of a tagging study initiated in 1968 by Dr. H. C. Frick, II, a trustee of what at that time was the Caribbean Conservation Corporation (CCC), now the Sea Turtle Conservancy (STC), with support from Dr. James Burnett-Herkes of the Bermuda Aquarium, Museum and Zoo (BAMZ). One of the first scientific investigations of green turtles on their foraging grounds, this project continues today as the Bermuda Turtle Project (BTP), a joint effort of the Bermuda Zoological Society (BZS), the support charity for BAMZ, and the STC. Drs. Anne and Peter Meylan, research associates of the STC and the Bermuda Aquarium, have served as scientific directors of the project since 1992. In 2019 the technical team was joined by STC scientists Dr. Daniel Evans and Richard Herren. Dr. Ian Walker, Principal Curator of BAMZ, leads the Aquarium's participation in the project. Jennifer Gray serves as the Bermuda Co-director for the project. Dr. Gaëlle Roth serves as the Bermuda Co-director and is the project's veterinarian. 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, and volunteers.

The goal of the Bermuda Turtle Project is "to promote the conservation of marine turtles through research and education." By the end of 2021, about 4260 green turtles and 138 hawksbills have been captured by the project and tagged and released so that information can be obtained on size structure of the population, genetic identity, sex ratios, growth rates, site fidelity, and migratory patterns. More than 1500 recaptures have been made of tagged green turtles by the project in Bermudian waters, providing one of the largest data sets in the world on growth rates and movements of free-ranging, immature green turtles. Green turtles tagged in Bermuda have been captured as far away as Nicaragua and Venezuela; the long-distance tag returns are particularly important because they shed light on the migrations of the green turtles that grow up in Bermuda waters. Hawksbill turtles (*Eretmochelys imbricata*) are captured in the net or by diving, and important information is also being gained about this Critically Endangered species. Loggerheads (*Caretta caretta*) and leatherbacks (*Dermochelys coriacea*), are observed in low numbers, primarily as strandings, and are sometimes tagged as part of the project. Kemp's ridleys (*Lepidochelys kempii*), are very rare in Bermuda.

One two-week sampling session is typically held each year. More than 40 sites around the island have been sampled for turtles. Exact positions of the netting sites are recorded with a portable Global Positioning System unit (GPS). 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 1000 feet of 4-inch-mesh net. The net is about 20 feet deep. The capture team sets the net in a circle around turtles on a grass flat. 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 the research vessel or to shore for data collection.

All turtles are tagged, measured and weighed. An external titanium tag is used and an internal PIT tag is inserted. Each tag bears a unique number (Fig. 6D). External tags include a reward message and a return address (Fig. 6E). Blood samples are taken from turtles for several purposes, including sex determination, blood chemistry, stable isotope analysis and genetic analysis.

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SAFETY RULES FOR PEOPLE

1. Before entering the water to help with the BTP, all participants must:
   (1) pass a swim test
   (2) read the Safety Rules For People and the Safety Rules For Turtles
   (3) go through a briefing on safety by a team member and/or catch boat operator
   (4) sign a waiver form
2. The net itself is extremely dangerous. Avoid getting tangled in the net. Swim in buddy pairs, never separating from your buddy and never swim under the net. If the net is at an angle and a turtle is underneath the net, bring the net and the turtle to the surface together from above. Do **not** go under the net to retrieve the turtle. Just as turtles get caught in the net, so can divers. 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. 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. The most dangerous aspect of our turtle catching operations is swimming around boat motors. Snorkelers should always listen for the propeller and stay close to the net. The catch boat will always avoid the net and thus you will be safe next to 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 in 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.
5. 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 likelihood that snorkelers will tire from swimming the net and handling turtles. This second boat may operate on the outside of the net so always be cautious and aware of vessel proximity.
6. No jewelry (including dive watches), cameras or diving knives are worn when swimming around the net because they can lead to entanglement in the net. Scuba fins **without** buckles or clasps are REQUIRED for working around the turtle net.
7. Do not leave the catch boat without specific direction from the catch boat operator. He/she will make sure that the boat is out of gear before sending snorkelers over the side. Remember that the catch boat is towing the net boat and you will have to swim to 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 orient to the net.
8. Hand signals are necessary to communicate with the catch boat. All are done with one hand. The OK signal is one arm arched over to touch the top of the head. If you have caught a turtle, or if see a turtle stuck in the net and need help with it, put one hand up above your head. If you or the turtle or your buddy is in distress, wave your arm and make sure someone in the catch boat sees you. This is an emergency signal and the catch boat team will drop whatever it is doing to assist you.
9. Green turtles rarely bite, but they can, and with painful results! Loggerheads and hawksbills OFTEN bite and can inflict painful injuries. Pay special attention when moving turtles or passing turtles from person to person.
10. The claws on turtles' flippers and the edges of the carapace and the flippers are very sharp. Do not slide your hand along a flipper edge or carapace edge. It can produce a deep cut like a paper cut. Be alert when standing near a turtle or riding with them in the boat.
11. 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 it while you are in the water. Keep an eye out for Portuguese Man-of-War and lionfish, and alert the catch boat immediately if you see any.
12. Never tag a turtle alone. One person is needed to immobilize the flipper while another tags the turtle.
13. Never place objects such as tagging pliers, tags, pencils, etc., on the belly of the turtle, or on or near the flippers. These may become dangerous projectiles.

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 extract 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 him in sight. Summon a catch boat by raising one hand or get another buddy-pair to assist you.
2. 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. Divers need to continue swimming the net until it is completely pulled out of the water.
3. Turtles should not be kept in direct sunlight out of the water for more than 10 minutes. Never set 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. Good shade must be provided (e.g., by tarps, roofs, etc.) and turtles and deck kept wet. There is a hose on the research vessel for this purpose.
   Ensure safety of turtles on the catch boat. Captured turtles placed on the catch boat need to be carefully watched to ensure their safety. They should be placed on their backs and kept at sufficient distance from other turtles so they do not get bitten, scratched or hit by a flipper. 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 sea water frequently, kept in the shade when possible and taken to the research vessel as soon as possible.
4. Turtles should be spaced apart from each other in the boat so that they cannot inflict injury to each other. Nails on the flippers can cause serious damage to eyes. Do not stack turtles on top of each other. They are best stored on a boat with their bellies up. Swimming noodles that have been tied in a loop are available on the research vessel to hold turtles on their backs comfortably.
5. Set turtles down gently after weighing them or when loading them onto the boat. **Never drop a turtle.** Do not rest heavy turtles on their pygal scales during transfer as this may result in breakage. To prevent accidents, make sure you have sufficient assistance when handling turtles.
6. Do not tie ropes on the turtle except for weighing. Use large-diameter, soft ropes to prevent damage to skin and/or cutting off circulation.
7. Use care when returning a turtle to the water from the boat or a dock. Set the turtle in **tail first,** and then pause for a moment with the head out of the water until the turtle realizes it is in the water and pulls away. This will prevent accidental aspiration of water.
8. Fibropapillomas are wart-like tumors that occur on the head and soft tissues (shoulders, neck, tail area, etc.) of sea turtles in many parts of the world. Green turtles are the species most commonly 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 them and keep any affected turtles away from healthy turtles. Avoid direct contact with the tumors. The use of gloves is recommended when handling a turtle with tumors.
9. Turtles should be worked up in a timely fashion. The sooner turtles can be returned to the water and to their normal routine, the better. This means avoiding catching very large numbers of turtles in a single set where this is avoidable and working up turtles as efficiently as possible.
10. 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.

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 ones.**
- **Be prepared to remove your gear if it becomes entangled in the net.**
- **No jewelry may be worn because it can become entangled in the net.**
- **Fins without buckles are required or buckles must be completely taped with duct tape.**
- **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.**
- **One buddy dives for the turtle while the other stays on the surface to assist.**
- **Swim turtle and net to surface, holding on to the turtle.**
- **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 not confident about getting a particular turtle out of the net, back off and signal boat or other swimmers for assistance.**
- **Periodically look up and check surroundings.**
- **Use extreme caution near areas where net is hitched on reef.**
- **If you hear the boat engine, make eye contact with the driver to see if they have words for you.**

HAND SIGNALS	**MEANING**
Waiving arm	Emergency
One arm Straight up	Turtle in net
Either arm up with fingertips touching top of head	Diver ok or turtle escaped
Hand over hand above the head (climbing rope)	Pull in net, end of set (pull turtles up from surface, abandon set in case of emergency)

SAMPLING PROCEDURES

SETTING THE ENTRAPMENT NET

This section describes the steps taken to catch turtles for the BTP. The safety issues associated with this procedure are discussed above and should also be read. Setting the entrapment net involves: selecting sites to be sampled, setting the net, catching the turtles, and taking a GPS reading and other set data. Normally, the set ends when all turtles swimming within the area enclosed by the net have been captured. Snorkelers must remain in the water with the net until all of the net has been retrieved and stowed in the net boat. On rare occasion, the set may need to be aborted and the net taken out before all turtles have been captured.

Site selection. Sites to be sampled are selected ahead of time by the scientific directors in cooperation with the rest of the project team, and a tentative daily plan is made. The site(s) to be sampled on any given day are reconsidered the day the sets are to be made by the project team members present, and the captain of the research vessel. This allows the latest information on weather, prevailing winds, equipment availability, and crew availability to be taken into consideration. Generally, the placement of the set is determined by the location of the proper bottom type at a depth appropriate for the net. On most grass beds on which we set, we have a series of "sets" that are determined by previous sampling at that site. In some cases, the exact location on the grass flat where the net is to be set may be predetermined by a specific need and therefore will be prescribed by GPS coordinates.

The set is made by using the catch boat to tow a net boat from which 1000 ft of net is paid out. Snorkelers are transported in the catch boat and take with them only the equipment they will need while in the water. Leave sunglasses, sunscreen, hats, etc., on board the research vessel. As the catch boat approaches a potential netting site, all crew are expected to remain as quiet as possible. Avoid clanking objects (anchor chain, diving gear, etc.) against the hull and keep voices very low.

Setting the net. When a prospective location has been identified for the set, there is some discussion of where the anchor is to be set, which part of the set (usually the deepest part) will be blocked off first, and in which direction the set is to be made. At some sites, particular attention must be paid to exact depth in the deepest part of the set, and or tidal flow. An experienced member of the crew will get into the net boat to set the net. All other participants stay on board the catch boat at this time and prepare to enter the water by reconfirming buddy pairs, getting their masks cleared, and getting their masks and fins on. Once the set starts and the net goes into the water, the catch boat operator is in charge of the set. On a signal from the catch boat, the person in the net boat releases 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 travels. The person in the net boat stays to the side of the net, grabbing the net only to help straighten it when large amounts of mesh go out together. This person alerts the catch boat if a problem develops with deployment of the net and when the halfway point (marked on the float line) leaves 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 facilitates communication between the catch boat operator and the person setting the net and between the catch boat operator and snorkelers who are about to enter the water. This person must always maintain eye contact with the net-setter during deployment of the net.

When about half of the net is set, two pairs of snorkelers usually enter the water, but only on command from the catch boat operator after he or she has taken the motor out of gear. The crew member facilitating communication for the boat operator identifies the first two buddy pairs and has them ready to go overboard. One buddy pair swims along the net towards the anchored end of the net; the other follows the net in the direction of the catch boat. In addition to looking for turtles in the net, these buddy pairs "help the net." The most important problem they fix is the occasional spot where the lead line overlaps the float line, preventing the net from hanging properly. Usually, the lead line can just be lifted off of 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. But they shouldn't spend extended periods 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 and a facilitator, 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.

When the catch boat passes the net float, completing the circle of net, the crew of the catch boat and the person in the net boat (and his/her buddy) work to assure that there is sufficient overlap of the net. The catch boat crew passes a section of float line near the net float into the hands of the person in the net boat and then releases the net boat from the catch boat. The person in the net boat secures the float line to the net boat in such a way as to close the gap between the two ends of the net. He/she then enters the water and with his/her buddy uses the slack in the float line, lead lines, and anchor line to insure complete closure of the net. While the engine is shut off or out of gear during this operation, all remaining snorkelers will be instructed by the catch boat operator to leave the catch boat.

It is extremely important when entering the water to do so only on 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.

Once in the water, swimmers 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, swimmers are likely to see any turtles trapped in the net and are safe from the catch boat(s) which will always keep the running motor away from the net. Do not swim out into the center of the set even if a turtle you are chasing swims out into the center. Do not expend your energy by swimming too fast. It is not a race and swimmers should maintain a steady pace ensuring their bubbies are comfortable with that pace. Sometimes there are extra panels of net near the net boat resulting from the closing off of the net. These need to be checked for turtles, as well. Turtles can be captured in any portion of the net. If there is turbidity, snorkelers will need to take turns diving down to check the deeper parts of the net.

Turtles that are caught in the net must be brought to the surface for air 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 foreflippers. Large turtles are best held by the shell 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 the net up to 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 reverse direction if you find that you are swimming close to other buddy pairs. Personnel in the catch boat(s) may also instruct you to reverse swimming direction in order 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 and pick up the turtle and the net.

While the net is deployed, 4 to 7 buddy pairs swim the net and remove tangled turtles. The catch boat operator and one or (ideally) two assistants now begin to patrol inside the net to receive turtles from buddy pairs swimming the net. For sets that are known to have high density of turtles we will plan to have a second catch boat on hand into which captured turtles can be placed. Snorkelers will need to pay attention to the locations of both catch boats while they are swimming the net, but the presence of two catch boats should reduce the time that snorkelers have to wait to pass captured turtles into a boat. Communication with the catch boat(s) is by means of hand signals. One hand in the air signifies 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. Do not wave your hand. A waving hand is a signal of distress. A waving hand is used only in absolute emergencies in which people 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. It is especially useful if you have raised a hand to indicate a turtle in the net but then the turtle got out. Stay clear of the catch boat(s) if it is near the float line to pick up a turtle. If you need to pass the catch boat at this time 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. 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 (see page X) and inform the catch boat operator if suspicious growths are observed. Evidence of this disease will appear as warts or tumors around the eyes, the neck or at the base of the flippers. Special handling procedures will be needed to prevent transmission of the disease to other turtles.

The catch boat team will mark the time of capture on the plastron of the turtle with red grease marker.

Remember to keep swimming the net until all of the net is removed from the water or you are told to do otherwise. Some turtles, especially recaptures, avoid the net and may not become entangled. We have caught turtles in the last 100 ft. of net that we pulled into the net boat.

Taking the GPS reading and other set data. Once the net has been set and most of the action of taking turtles from snorkelers has passed, the catch boat operator, with assistance from the crew, takes a GPS reading, water depth and temperature data. The GPS reading is taken by moving to the center of the set and holding the GPS unit so that it has a clear view of the sky; that is, boat tops, hands, or masts do not obscure its view of the sky. When the GPS unit gives a new location, record the latitude and longitude on the form in decimal degrees (i.e., 32.66745º N 64.24242º W) in the spaces provided on the Set Data Sheet. There are spaces on the Set Data Sheet for five decimal places. The GPS unit is not waterproof and should always be handled with care and kept in its container inside the GPS bag when not in use.

In addition to latitude and longitude, the locality name, recorder's name, date, time that the set was initiated and time set was completed, and set number are recorded on the Set Data Sheet (page 26). 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 or weighted measuring tape). These physical data are recorded on the appropriate lines on the Set Data Sheet.

When all snorkelers and turtles have returned to the research vessel, 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. The Set Data Sheet is then turned over to the data recorder who will record these physical data on the data sheet for every 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, for some reason, turtles from the set are released elsewhere than at the location where the release GPS was taken, a release GPS position should be acquired at the time of release, preferably with the project's GPS unit.

Ending a set. The catch boat operator ends the set when no more turtles are seen within the net perimeter or, when 15 minutes have elapsed since the last observed capture, or when 20 minutes have passed since closing the net and no turtles are caught. The catch boat operator should record the number of remaining turtles (if any) in the net on the set data sheet. Turtles should not be corralled using the boat to chase or drive them into the net nor should they be rodeo-captured or pursued with segments of the net. This is stressful for the turtles and may endanger both people and turtles.

Aborting a set. The catch boat operator or any BTP team member has authority to terminate a set early (before all turtles are captured) 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 or 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 as if rope climbing. The signal gives snorkelers permission to pull the net up from the surface, freeing any entangled turtles and preventing further captures. Except under extreme circumstances, the set must be monitored by snorkelers in the water until all of the net is back in the boat. If the catch boat has had to leave the set, the catch boat operator assigns one person on the net boat as the headcounter. That person keeps track of all personnel 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.

Retrieving the net. After each set is finished, the net must by 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 that it stays perpendicular to the line of the net. 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 in the bow facing the person pulling the leads who stands in the stern. The person pulling the mesh sits on the gunwale opposite from where the net will be pulled in. These three pull the net simultaneously making an effort 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 off line or is being disoriented by currents or wind. As the net comes in over the side, all foreign objects 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 of 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 will become hung up as it is retrieved and be available to help get the net loose at these sites as needed.

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 (*Endurance)* and the turtles are off-loaded onto the deck. Everyone helps with this job before taking care of their own needs. All turtles should be placed in styrofoam rings (swimming noodles) with their plastron up. The noodles steady the turtles and keep them off the deck. On hot days, one person should use the saltwater hose 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 salt water is going, especially if someone is using a PIT tag reader while you are spraying.

As turtles come onto the deck they are separated into two groups, those with visible external tags are laid in the first rows, starting from the left, and those with no external tags are placed in a new, lower row, starting from the left. Within these categories, turtles are sorted by size from larger to smaller. As turtles come onto the deck, examine them closely again for any evidence of fibropapilloma. Evidence of this disease will appear as warts or tumors around the eyes, the neck or at the base of the flippers. Any such anomaly should be brought to the attention of a team member. As soon as turtles are on the deck, one of the assistant taggers should check turtles without external tags for PIT tags. Any turtles with PIT tags are recaptures and should be marked on their plastrons and moved over with the recaptures that have external tags.

DATA COLLECTION PROTOCOL

Working philosophy. Given that capture in the entrapment net and subsequent transfer to the catch boat and the research vessel are a significant interruption of the turtle's normal behavior, we all need to do everything we can to pursue two objectives:

1) – the safety and well-being of all turtles must be carefully considered in all aspects of capture and data collection.

2) – a complete and accurate workup for each turtle is the best justification for this intrusion on their daily lives.

Overview of turtle work ups. The data collection process is described in detail below. However, the basic steps consist of:

1) Tagging
2) Measuring and weighing
3) Blood sampling
4) [Additional sampling or tracking equipment (i.e. satellite transmitter) attachment]
5) Release of turtles as close to capture site as possible.

The organization of this protocol follows the order of data collection on the project data sheet. See page 15 for sample data sheet.

GENERAL INFORMATION

Only a trained data recorder fills out data sheets. The recorder writes his/her full name on the recorder line on each data sheet (no initials) so that he/she can be contacted if questions arise about the record. The data recorder must have data recording experience, be familiar with the data sheet, be able to deal with rapid delivery of information, and have legible handwriting.

Wherever they appear, dates are always written in the format 4 August 2011, never 8/4/13 or 4/8/13. Month can be spelled out or abbreviated.

All entries are made using #2 pencil only. Use standard block letters and numbers. Be sure to differentiate 1's and 7's and 4's and 9's.

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

No tags are discarded; any tags removed from recaptured turtles, or 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").

Items on the data sheet that are shaded gray are to be left blank temporarily. They are completed later, as appropriate.

Any scar, injury, barnacle or other factor that may make a measurement inaccurate should be noted in the Capture Remarks section of the data sheet. Large barnacles can be carefully removed to facilitate more accurate measurements. All measurements are expressed in metric units (cm, kg).

Before each turtle is released, the left tag number is read to the recorder who quickly reviews the data sheets to be certain that all data have been recorded for that individual and that all tag numbers are listed and correct.

At the end of each day's sampling, the data sheets are reviewed to be certain that all blanks have been filled and all entries are legible. The data sheets are taken back to BAMZ daily for safekeeping.

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 Tag #.

TAGS AND TAGGING

Primary tag number - This space is left blank initially. One of the tags applied to a turtle captured for the first time (typically the left tag) is designated as the primary tag number at the time the data are entered into the data base. 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.

Recording tag numbers. Over 4000 turtles have been tagged in the waters around Bermuda during the course of this project. Also, turtles tagged by other projects have occasionally been found in Bermuda waters. Thus, the probability of catching turtles that already have tags is very good and therefore every turtle is carefully inspected for existing tags. Nearly all turtle projects tag their turtles on the trailing edge of the front flippers. Hind flippers are also checked because some projects tag turtles only on the hind flippers. If no tags are present, the foreflippers are examined for a scar that would be left if a tag had been lost (old tag hole). The universal PIT tag reader should be used to scan the flippers, neck and soft tissue areas of all turtles to find any non-visible, PIT tags. If the turtle has neither tags nor tag scars, the word *none* is recorded on the "at capture" portion of the tag lines on the data sheet. If a tag scar is present, "*none, tag scar present*" is recorded on appropriate left or right tag line. The terms right or left always refer to the turtle's right and left when the plastron (belly shell) is down and the head pointed away from the observer.

If a tag or tags are present, we carefully read both sides of the tag(s) and the complete tag number is recorded on the right or left tag line of the data sheet, as 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 recorded on the data sheet. In this case, it is also noted whether the tag is metal or plastic, and the size of the tag is measured. The color of the tag is also noted. If possible, photograph both sides of any unusual tags.

In every case, be careful to read the entire tag number. Plastic tags applied in Bermuda normally have a two-letter prefix followed by three or four digits. The number on metal tags usually consists of one or two letters followed by three or four digits; some old tags have as many as six digits without a prefix. Make sure that an additional digit or letter is not hidden in the tissue of the flipper. If there is any chance that additional letters or digits are hidden by the tissue of the flipper, the tag should be removed and replaced with a new tag (see below). Any removed tag should be saved in the "recovered tag" container after its number and the number of its replacement have been recorded on the data sheet.

All turtles should be released carrying at least two tags. This does not mean that we put two new tags on every turtle. Some recaptures already have one tag, others two. In many cases these tags are in good enough condition to stay in service. All tags on recaptures are carefully inspected by a team member to determine if they need replacement.

Criteria for replacement 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 very narrow strip of tissue
5- tag is wearing and has become noticeably thin or corroded
6- numbers etched into tag surface are becoming illegible
7- tag has heavy burden of algae, barnacles, etc., that cannot be removed
8- F tags should be replaced on any turtle that has reached approximately 35 cm.
9- MM tags should replace smaller tags on any turtle over 60 cm as these tags have very long retention and higher chance for long-term recovery.

Don't replace tags simply because they are loose or have rotated in the hole. They should meet one of the above criteria.

Choosing the right tag. It is important that when using two metal tags, the tags must be made of the same metal. This is most easily achieved by using two tags with the same prefix. If the tags are of different metals, there is a chance that they will interact chemically which may not be good for the turtle or the tags.

If no tags are present, turtles are double-tagged (see below for application procedures) using appropriate-sized tags as follows:
- turtles under 35 cm SCL: F tags (monel) on both sides;
- turtles approximately 35-60 cm SCL: M, K or X tags (inconel) on both sides;
- turtles over approximately 60 cm SCL: MM titanium tag (large titanium) on both sides.

If tags are present. R and F tags are replaced with larger tags as soon as the turtle reaches 35 cm because they appear to have a fairly high rate of loss. All large turtles (over 60 cm) should be tagged with two large titanium (MM) tags. Bermuda turtles carrying these tags have been observed many years later on nesting beaches in Costa Rica and Mexico.

Tags are always used in numerical order. Tags are used in numerical order to facilitate recording, processing of turtles in a timely fashion, and later reconstruction of the sampling effort. The lower tag number always goes on the left side. Misapplied tags are recorded as wasted (see above) 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 is more easily resolved.

Tag and tag site preparation. To minimize the risk of transmission of pathogens, tagging pliers, tags and the tag site are treated with disinfectants. Tags are soaked in a 10% chlorhexidine solution for 15 seconds and rinsed in fresh water between turtles. Also, just prior to prepunching or tag application, the punch and or tagging pliers with tag are dunked in chlorhexidine then freshwater. Furthermore, the tagging site on the flipper where the external tags will be placed is thoroughly wiped down on both sides with a povidone/betadine-soaked Q-tip as is the site for the PIT tag.

Tag site preparation, prepunching, and tag application Tag application requires two people to do the job well and safely. These steps are done with the turtle on its back. One person stands in front of the turtle's head and immobilizes the flipper that is to be tagged; the second wipes the external tag target areas and the PIT tag target area with a povidone/betadine-soaked Q-tip; punches tag holes as necessary; and applies the tags. The tip of the flipper should be well extended behind the turtle's head so that the hole can be punched in, or the tag applied to, a smooth, flat surface. Remember to keep your face well back from the flipper's possible trajectory. Avoid putting objects such as pliers on the turtle's belly during tagging as they may become dangerous projectiles.

Prepunching. All of the metal tags used by the project are designed to be self-piercing. They cut through the tissue and lock closed when correctly applied. However, a hole is prepunched for most tags to improve the application success. These holes are punched with a leather punch at the same location where self-piercing tags are applied This site is on the trailing edge of the foreflipper, just on the inside of the large round scale on the under surface of the flipper. This site corresponds to the 2nd or 3rd large scale on the trailing edge of the dorsal surface of the flipper. The distance from the edge varies depending on the thickness of the flipper in the area where the leather punch must pierce the flipper. When the correct position is located, the hole is punched with a single smooth motion, closing the punch until a sharp click is heard. Do not bear down on the punch and release your grip as soon as you hear the click. The hole is then checked to be sure 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.

Tag application. Each tag is applied with a specially designed applicator (Fig. 6F). There are different-sized applicators for different-sized metal tags so care must always be taken to use the correct applicator. 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. Self-piercing tags are applied to the trailing edges of the foreflipper just to the inside of the large round scale on the underside. We place the tag in either the 2nd or 3rd ventral scales (usually the 2nd). The tag is placed so that about one-half of its length extends beyond the edge of the flipper. The distance from the edge varies depending on the thickness of the flipper in the area where the tag will pierce the fin. When the correct position is located and swabbed with betadine/povidone, the tag is applied with a single smooth motion, closing the applicators as far as they will go but not applying excessive pressure. The tag is always checked to be certain that the sharp tip has passed through the hole and has bent over to secure the tag in place. If this has not happened, we attempt to secure the tag with the tagging pliers or other pliers. If it does not secure, the tag is removed. Inconel tags are sometimes reshaped and another application attempted. Otherwise, we try again with a new tag. We do not discard misapplied tags. They are noted as being destroyed and are saved in a container for misapplied "wasted" tags.

After each turtle is tagged, the leather punch is cleared of tissue with a blunt tool kept in the tagging kit for that purpose and all the punches and the tagging pliers are immersed in a Chlorhexidine solution to disinfect them. This immersion is followed by a fresh water rinse.

PASSIVE INDUCED TRANSPONDER (PIT) TAGS

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

All captured turtles should be checked for PIT tags using the universal reader. Turn the reader on and continuously press the READ button while moving the reader along first the left, then the right front flipper. Scan the rear flippers and other soft parts, particularly the shoulder areas. If a PIT tag number is found by the reader, the number is entered on the data sheet in the space allocated for this type of tag. 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, we add one. We PIT tag all turtles before release.

The PIT tag itself consists of a 10 or 12 mm long cylindrical tag that is injected under the skin using a PIT tag gun. The PIT tags are pre-sterilized and packaged for single use only. Always save the needle cover and cover the needle 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 left 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 located. Before application, the area where the tag will be injected should be cleaned with a fresh betadine or povidone-saturated Q-tip.

The tag is injected from medial to lateral (PIT tag gun pointed towards flipper tip) subcutaneously in the forearm between the radius and ulna (Fig. 6G). To do this, insert the tip of the needle under the skin between the radius and ulna; then push the plunger to move the tag out of the applicator and into the connective tissue. Gently remove the needle in the same angle you inserted it, monitoring the tag does not come out. Watch for bleeding after injection. If blood flows from the wound, elevate it and apply pressure with a betadine/povidone-soaked swab until the flow stops. If necessary, apply a small amount of surgical glue to close the opening.

As soon as the PIT tag is successfully applied, the adhesive strip with the tag number and bar code that comes with each applicator package should be transferred to the data sheet. The person applying the pit tag should then read the injected tag with the reader and confirm the number with the data recorder.

PIT TAG LOCATION. RF (right front flipper) **LF** (left front flipper). The left is preferred.

CAPTURE INFORMATION

SPECIES. Any turtle whose identification is in doubt should be identified 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. Three species regularly occur in Bermuda waters: the green turtle **(CM)**, hawksbill **(EI)** and loggerhead **(CC).** Leatherback **(DC)** and Kemp's ridleys occur extremely rarely. Hybrids between sea turtle species are not common but are known from Bermuda. Possible hybrids should be carefully photographed.

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. Fibropapillomas are small (pea-sized) to large (softball sized) growths on the soft parts of turtles. They appear most commonly on the neck and head (especially around the eyes) and at the base of the flippers. As turtles are unloaded from the catch boat, we look at the face and neck of each one for any wart-like growths. When they are set down on their carapace on board the research vessel, ventral soft parts are checked for these growths. **If any suspicious growths are found, a team member is immediately notified**. Fibropapillomatosis is contagious among turtles but is not known to affect people, but we take precautions anyway. **See WHAT TO DO WITH A PAPILLOMA TURTLE (page 26).**

CAPTURE DATE. The complete date is written out in the form: day month year with the month spelled out (e.g., 16 August 2011). It is always the date on which the animal was captured that is entered on this line even if the animal is tagged or the data is completed at a later date.

OBSERVATION TYPE. Four observation types are coded on the data sheet: **First (observation)**, **Recapture**, **Stranding** and **Tag return**. Turtles captured during the normal operations of the BTP are recorded as a **First observation** if they have no 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 either dead or alive. A turtle with no tags but with tag scars visible 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 in turtles with 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 listed in the reward message on the tags. This office has, in turn, received the tag and information from whomever caught the turtle, usually a fisherman somewhere in the Caribbean. 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. In cases where turtle tags or dead turtles with tags are turned over to the project in Bermuda by third parties, these should be listed as tag returns 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 applied to the same grass flats, the BTP has established a set of standardized names for the most frequently fished sites (page 13) or the map (page 14). 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 is always used for samples taken at that location.

GPS (capture) READING. 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 be entered below the capture location on the data sheet is given to the recorder on a "Set Data" form by the catch boat operator, along with data for temperature, depth, set time, and the release location and release GPS reading. See the section on "Taking the GPS reading and other set data" on page 5 for more details.

DATA RECORDED BY. The full name of the person recording the data is entered here. The data recorder is also responsible for ensuring that all of 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 of 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.

MEASUREMENTS

In order to minimize the variation in the way measurements are taken, only BTP team members will take the measurements. Do not take the measurement if the turtle has some anomaly or injury that prevents a normal measurement. Instead, note shell or tail damage or other obstructions that interfere with accurate measurement in the Capture Remarks section of the data sheet. All measurements are expressed in metric units (cm, kg). When using the calipers, care must be taken that the very tips of the calipers are in contact with the endpoints for the measurement (see Fig. 1E).

PLASTRON LENGTH. The plastron is measured <u>on the midline</u> from the anterior edge of the intergular scutes to the posterior edge of the anal scutes using the tree calipers (Fig. 1B). Usually there are no extra scales at the anterior end of the plastron but there are often one to three small round scales at the junction between the anal scutes and the skin. These small scales are ignored and the plastron is measured to the end of the bone where it is covered by the anal scales (Fig. 1B, C).

STRAIGHT CARAPACE WIDTH. Straight carapace width is measured with the tree calipers at the widest point across the carapace (Figure 1B, page 14). It should be measured with the turtle on its back and the person standing squarely behind 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 shell. 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 scales on either edge of the shell as a guide to be certain that you are measuring straight across the shell. This will not work for turtles with irregularly arranged marginal scales.

TAIL MEASUREMENTS. Two tail measurements are taken with the turtle on its carapace; a third is calculated later using these two measurements (Figure 1C on page 14). These two measurements are made at the same time with a soft measuring tape. Taking these measurements accurately requires two persons working together. One person places the origin of the tape at the end of the anal scutes and stretches it towards the tip of the tail, in line with the midline of the plastron. The second person holds the tail in such a way that both the cloaca and the tail tip are visible. When the tail is relaxed and fully extended posteriorly from the plastron, in the same plane as the plastron, the tape is stretched out for the length of the tail. Then both the distance from the plastron to the anterior edge of the cloaca **(Plastron-To-Cloaca)** and from the plastron to the distal tip of the tail **(Plastron-To-Tail Tip)** can be measured and reported to the data recorder.

STRAIGHT CARAPACE LENGTH. Straight carapace length is measured with the tree calipers. It is the straight-line distance in centimeters (to the nearest tenth) between the center (midline) of the nuchal scale along its anterior edge to the posterior edge of the shell at the point where the two 12th marginal scales meet on the midline (Figures 1A and 1D, page 14). Care must be taken that the very tips of the calipers are at the points between what is being measured (Figure 1E, page 14) for this and all measurements made with the calipers. This way one can be certain that the measurement bar of the calipers is exactly parallel to the line being measured.

WEIGHT. Turtles are weighed using a net and a hanging, electronic scale. The scale is zeroed. The turtle is placed in the net and lifted into place under the scale. The net or caribiner is set on the hook of the scale. The persons lifting the turtle stand by to steady the turtle, watch that the weighing net does not slip, and advise the scale reader when the turtle has stopped moving. The reader takes the reading (to the nearest 0.1 kg.) and gives that information to the data recorder, along with the tag number of that turtle. The net and turtle are then lifted off the hook and, the turtle is placed on its noodle. The scale is regularly tared by weighing the net and hooks (without turtle).

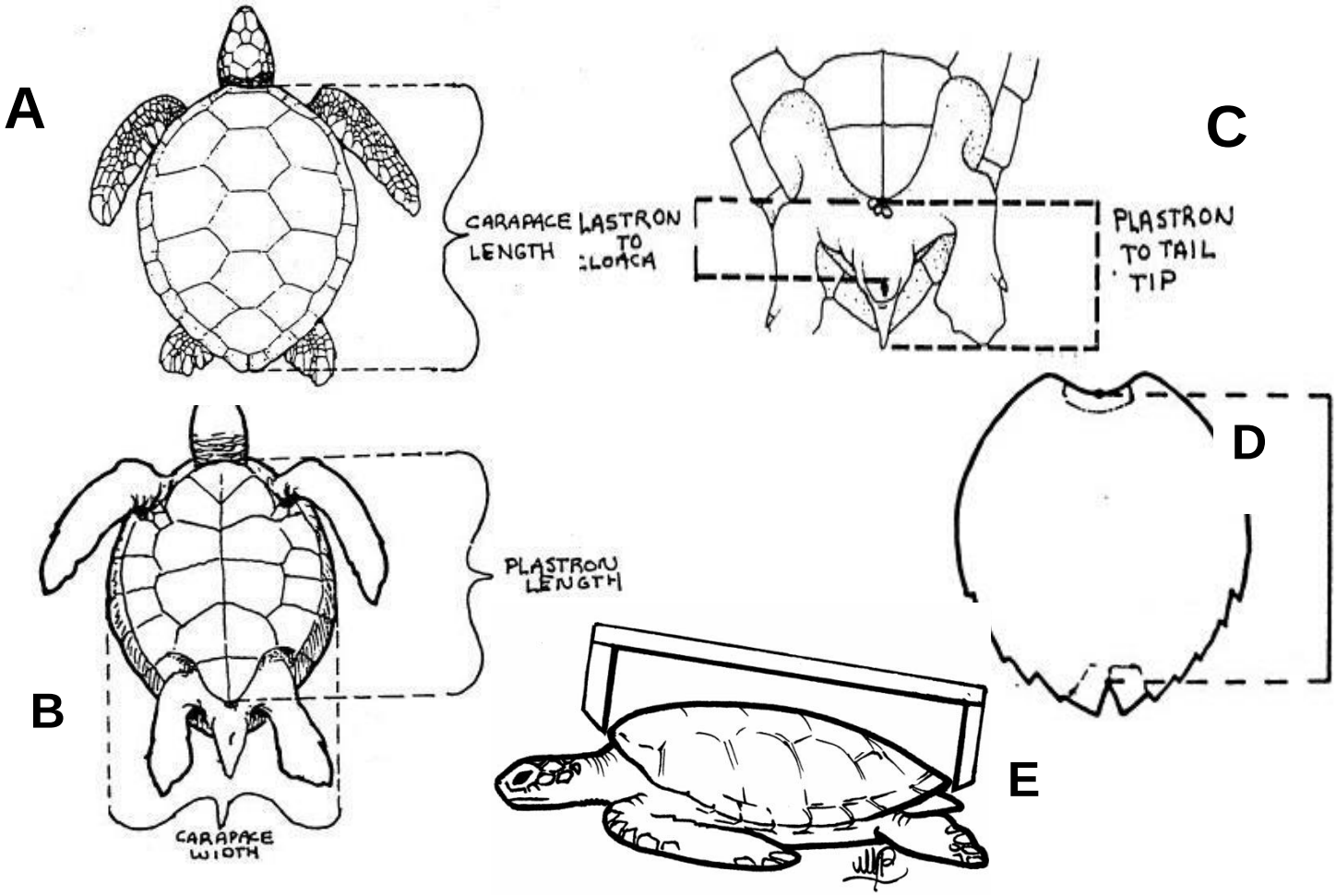

Figure 1. Measurements of the shell of sea turtles in Bermuda. A, D, E, straight carapace length notch-to-notch (SCLnn or SCL min), B, plastron length (PL) and carapace width (CW), C. Tail lengths: plastron to cloaca (PL-CLO) and plastron to tail tip (PL-TT).

BLOOD/TISSUE SAMPLES COLLECTED: P L R B O N. Blood samples are typically collected from every turtle. For first captures these include plasma, stable isotope, and genetic samples. Only plasma and stable isotope samples are taken from recaptures. The existence of a previous genetic sample should be checked on the Comprehensive Tag List on board the research vessel by the assistant recorder before blood samples are taken from recaptures. A record 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 in taken instead (see skin biopsy protocol on p.20) and this is indicated by circling the **B**. **O** (other) is circled if some other kind of sample is taken. If no samples are taken, the **N** is circled. A complete blood sampling protocol is found on page 16. If only one sample is taken, the single appropriate letter is circled. If multiple samples are taken, they are all indicated. This line is never left blank.

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 was released even if the animal was tagged or the data were recorded on a previous date.

RELEASE LOCATION. The name of the release location, like the name of the capture location, should come from the standardized list of site names. If we add any new release locations, they should be added to the list of site names.

GPS (RELEASE) READING. The "Set Data Sheet" to be completed by the catch boat driver will have both a capture GPS location and a release GPS location. In many cases, the release GPS is taken from the deck of the research vessel while it is still at the position where turtles are turned loose. If, for some reason, turtles are released elsewhere, a new GPS reading should be taken, preferably with the project's GPS unit, and recorded on the Set Data Shee**t**

CAPTURE REMARKS. Miscellaneous observations are recorded here. Any notable injuries (such as shark bites or missing flippers) can be noted here. 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.).

Sample #:	BERMUDA TURTLE PROJECT BERMUDA AQUARIUM MUSEUM AND ZOO	
PRIMARY TAG NUMBER:		
TAG(S) ON LEFT at capture: X5289	at release: X5289	
TAG(S) ON RIGHT at capture: X5290	at release: X5290	
PIT TAG # at capture: 039065095 at release: (attach ID here)		Pit Tag Location: RF (LF)
SPECIES (circle) (CM) EI DC CC	FIBROPAPILLOMA Y (N)	
CAPTURE DATE: (write out month) 27 February 2003		
OBSERVATION TYPE: First (Recapture) Stranding Tag-Return		
CAPTURE LOCATION: Cow Grounds		
GPS (Capture): 32.31912 N	64.87295 W	
DATA RECORDED BY: Jane Tucker	SET #: 2	SET TIME: 13:15
PLASTRON LENGTH (cm)		37.5
STRAIGHT CARAPACE WIDTH (cm)		36.6
PLASTRON-TO-CLOACA (cm)		5.5
PLASTRON-TO-TAIL-TIP (cm)		8.2
STRAIGHT CARAPACE LENGTH (notch-to-notch in cm)		44.7
WEIGHT (kg)		12.4
BLOOD/TISSUE SAMPLE: (circle) (S) (L) N O B S = SERUM L = LYSIS N = NO SAMPLE O = OTHER B = SKIN BIOPSY		
LAPPED? Y N SEX: M F MATURITY STATUS: _____ COMMENTS: _____		
RELEASE DATE: (write out month) 27 February 2003		
RELEASE LOCATION: Cow Ground		
GPS (Release): 32.31850 N	64.87360 W	
CAPTURE REMARKS: missing tip of right front flipper	WATER DEPTH: 8.3 m / ft. (circle one)	WATER TEMP: 19 °C SURFACE or (BOTTOM) (circle one)

Figure 2. Sample completed Bermuda Turtle Project data sheet. Note that latitude and longitude are recorded in decimal degrees.

Locality	Total Sets	Locality	Total Sets
Annie's Bay	19	**Outside Daniels Head**	73
Bailey's Bay	53	**Cowground Flat**	68
Blue Hole	34	**Vixen**	67
Chub Head	1	**Tudor Hill**	62
Cocoa Bay	6	**Bailey's Bay**	53
Coney Island	1	**Fort St. Catherine**	51
Cowground Flat	68	**Ely's Flat**	35
Crescent (No Lat/Long)	2	**Blue Hole**	34
Crescent East	30	**Crescent East**	30
Crescent West	17	**Ferry Reach**	21
Devils Flat	1	**Annie's Bay**	19
Dockyard Camber (Inside)	1	**Crescent West**	17
Dockyard Camber (Outside)	1	Nonsuch Island	16
Ely's Flat	35	**Walsingham Bay**	16
Ely's Harbour	10	Somerset Long Bay	15
Emilys Bay	1	North Rock	13
Ferry Reach	21	Ely's Harbour	10
Ferry Reach East	1	Grotto Bay	10
Five Star Island	6	Long Bay	10
Fort St. Catherine	51	Rockfish Shoal	10
Franks Bay	2	Cocoa Bay	6
Goat Bay	1	Five Star Island	6
Green Bay	1	Paradise Lakes	6
Grotto Bay	10	Wreck Hill	6
Hawkins East Bay	3	Smith's Island Bay	5
Long Bay	10	White Flats	5
Mullet Bay	3	Hawkins East Bay	3
Nonsuch Island	16	Mullet Bay	3
North Rock	13	Tucker's Town	3
Outside Daniels Head	73	Crescent (No Lat/Long)	2
Paradise Lakes	6	Franks Bay	2
Rockfish Shoal	10	Stocks Harbour	2
Smith's Island Bay	5	Chub Head	1
Somerset Long Bay	15	Coney Island	1
Stocks Harbour	2	Devils Flat	1
Tucker's Town	3	Dockyard Camber (Inside)	1
Tudor Hill	62	Dockyard Camber (Outside)	1
Vixen	67	Emilys Bay	1
Walsingham Bay	16	Ferry Reach East	1
Well Bay	1	Goat Bay	1
White Flats	5	Green Bay	1
Wreck Hill	6	Well Bay	1
Total	689	Total	689

Table 1. Distribution of samples among netting sites for 1992 – 2015. Standard locality names employed by the Bermuda Turtle Project are used in this table.

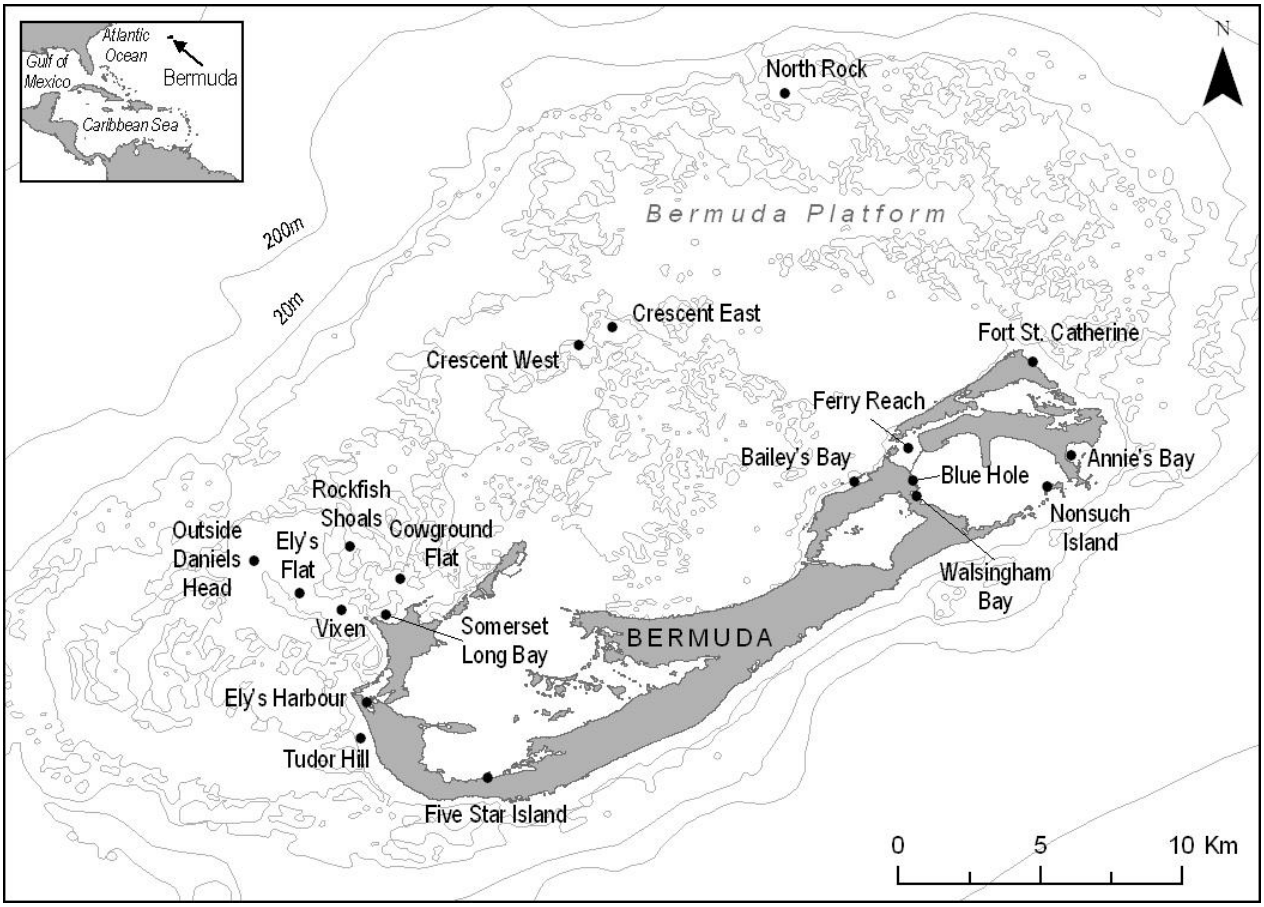

Figure 3. Locations that are regularly sampled for sea turtles by the Bermuda Turtle Project.

BLOOD/TISSUE SAMPLING PROTOCOLS

BLOOD SAMPLING PROTOCOL

The availability of all required supplies and equipment is checked before blood sampling begins. These include: lap rack (with a pair of ropes, for large turtles), a scrub brush, sanitizing hand wipes, Q-tips, iodine (Betadine or Povidone), needle holders, 20 and 22 gauge needles, centrifuge, forceps, cryovials, 10 ml blue-top tubes filled to 9 ml mark with lysis buffer, cryomarkers, red and green top vacutainer tubes, and a test tube rack on ice inside of a small cooler. At least two assistants are needed in addition to the person collecting the blood samples, one to pass supplies, the other to label vacutainers and communicate with the data recorder. 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. For all larger turtles, a 20-gauge needle is used.

If under 35 cm carapace length, the turtle to be bled is restrained by hand by a third assistant. The holder sits in a comfortable spot with knees at a right angle and slightly spread. The holder is handed the turtle with its head down, plastron facing the holder and forelimbs held against the top of the carapace. The holder uses four fingers of each hand to clamp the flippers against the back of the shell with thumbs on the plastron (Figure 6H). The holder then rests the turtle's shoulders on their knees. Turtles over 50 cm should be restrained with ropes on a lap rack with the plastron down as shown in Figure 4B, D. Once the turtle is secure, assistants lean the rack against the gunwale with the turtle's head down in front of the person taking the blood sample. The neck area is cleaned with water and betadine, removing any barnacles, algae, or other debris.

When the neck is clean and the turtle securely set in the holder's hands, it is allowed to rest for about a minute. This allows blood to pool in the dorsal cervical sinuses. During this time, bleeder and assistant clean their hands with a sanitizing wipe. The turtle's head is held by the back edge of the skull and gently pulled down to extend the neck. It is extended downward toward the deck and held in this position until all samples are drawn. Be careful to avoid the turtle's throat and ears as these are sensitive areas and the hyoid area must be free for the turtle to breathe. The top of the neck on either side of the midline is cleaned with Betadine or Povidone. The person who will draw the sample sits directly in front of the turtle in order to visualize the four quadrants of the neck, as illustrated in the Figure 5H, feeling for the supraoccipital bone to verify the midline.

A needle is locked into a plastic needle holder and the cover removed but kept handy. When the turtle is calm, the needle is inserted just barely through the skin at the target point which is approximately midway between the midline and the lateral edge of the neck, at a point 2-3 cm above (posterior to) the midpoint between the skull and shell. When the needle is under the skin, a green top vacutainer is inserted into the needle holder. The needle is then very slowly pushed straight into the neck along a single trajectory. We make certain not to move the needle

from side to side since this will result in a cutting action. We are also careful not to "twist" the needle as this can result in tearing of tissue. If blood does not enter the tube along the first trajectory, the needle is very slowly withdrawn until about 10% remains under the skin, and then a new trajectory is attempted. We systematically test up to three different angles of entry. If all three fail, we move to the other side of the neck. We frequently find that the vacutainer begins to fill as the needle is being slowly withdrawn. If the needle is pulled out of the skin, the vacuum will be lost and a new vacutainer will be needed. We use a new vacutainer when switching sides because occasionally vacutainers lose their vacuum and are the cause of failure to secure a sample. If a small but inadequate sample is obtained on the first side, we usually change the needle to avoid problems of clotted blood in the needle. We make no more than three attempts on each side of the neck.

It is critical to avoid the midline because this is the location of the spinal cord. We also make sure to keep the animal's neck straight and extended so that the bleeder has a clear view of the head and neck for orientation purposes. If the turtle starts to flap or struggle, or the boat begins to rock excessively, the needle is withdrawn completely and we wait for the turtle to settle or waves to subside.

Depending on the size of the animal, we fill one or two 4 cc heparinized vacutainer tubes (green top) and then (without removing the needle) take a 0.5 ml sample in an untreated tube (red-top). We fill the green top, heparinized tubes with blood until the vacuum stops since it contains an appropriate amount of lithium heparin for a full tube. If we fail to fill the heparinized tube to less than 50% of capacity we set that sample aside and try for a sample on the other side.

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

Before it is released, both tag numbers are read to the recorder and the types of blood samples collected are reported. At this point, the data recorder checks to be certain that all data have been collected from the turtle; she/he then informs the assistants if the turtle is ready for release.

As the turtle is being set down and prepared for release, a bleeding assistant begins to process the samples right away. The 0.5 cc untreated, red top sample is the first priority since it will clot very quickly. It is poured into a 10 cc blue-top plastic vial prefilled with 9cc of lysis buffer and the vial is gently inverted several times. The left tag number of the bled turtles and abbreviated species ID and the date are written on the etched portion of the side of the blue-top vial in the format:

MB141
Cm
22 Aug 2011

The tag number and species abbreviation are also written on the top of the vial. The same tag number and the set number are also written on the green top vacutainer(s), which is (are) then placed in the rack in the blood cooler. Heparinized samples are held on ice until centrifuged. Lysis samples also go into the blood cooler but are kept out of direct contact with the ice.

As soon as possible after all turtles from a set have been worked up for the day, we complete the processing of the blood samples. The heparinized (green top) samples are centrifuged to separate plasma from cells. Samples are centrifuged in the vacutainers for 6 minutes at 5000 rpm, taking care to balance the load in the centrifuge using blanks before spinning. Spun samples are 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. Use a new pipette for every turtle. Each plasma sample is dripped into two or three, 2 ml cryovials, taking care not to fill the vial above the 1.8 ml full line because the samples will expand when frozen. The red blood cells are pipetted into a separate 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, as shown for blue-top vials above.

This is always done using a cryomarker 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 plasma and red blood cell samples from the same set. The empty green vacutainer is placed 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 correspond to numbers on data sheets for the day's turtles. Lysis samples are bagged and labeled separately from plasma and red blood cell samples. All are labelled by date and set # in ziploc bags. Plasma and red blood cell samples are stored in the freezer at the Aquarium; lysis samples are stored in an air-conditioned office or lab.

IMPORTANT PRECAUTIONS:

-We don't bleed turtles in a rush, or on a pitching vessel. The turtle, the assistant and the blood sampler all need to be relaxed and patient.

-We move the needle gently and slowly in and out in a straight line, never side to side or twisting, when it is inside the turtle.

-The person doing the bleeding always discards the needle immediately into the SHARPS container. It is NEVER set on the deck or on a lap.

-We never use the same needle on more than one turtle.

-We avoid letting blood or any other body fluid from one turtle get on another turtle.

-We avoid getting any blood or other body fluids from turtles into open cuts of humans.

-We wash hands thoroughly with sanitizing wipes before and after drawing blood from every turtle and after processing blood samples.

-Gloves are available if desired.

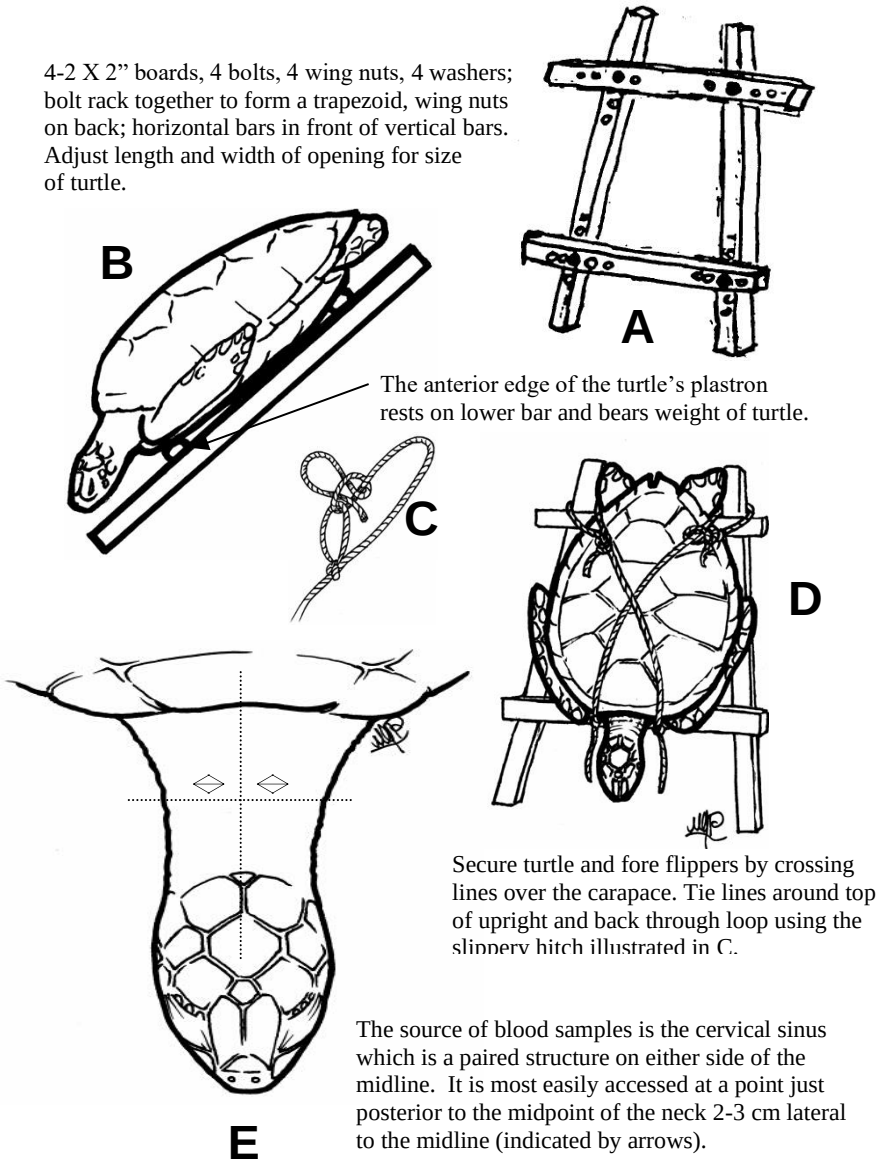

Figure 4. Illustrations for blood collection from large turtles. A, bleeding rack; B, lateral view of turtle on rack; C, slippery hitch for tying turtle; D, frontal view of turtle on rack; E, detail of neck with site of dorsal cervical sinus.

SKIN BIOPSY PROTOCOL

Because all of the cells of each turtle contain the same DNA, a skin biopsy is an excellent alternative to a blood sample for genetic analysis. The critical precaution in taking this or any DNA sample is to be certain to keep any non-target DNA from being preserved with the sample. This requires careful technique.

Taking a biopsy sample is best done with two people--one to hold the flipper and turtle steady, the other to collect the sample with a sterile biopsy punch.

To take the sample and preserve it, you will need the following items which are kept together in a biopsy kit:

- a 4-mm single use biopsy punch (pre-sterilized in package)
- a (sterile) plastic sample vial pre-filled with 5 ml saturated salt buffer (SS; use white-capped vials for tissue samples in saturated salt buffer to distinguish them from blood samples in blue-capped vials containing lysis 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
- permanent marker
- alcohol wipes

To take the sample, two people should work together. Gently put the turtle on its back. Hold the hind flipper firmly and clean the skin on the trailing edge of the flipper, on both sides with an alcohol wipe. Use an alcohol wipe to clean the dive slate or plexiglass plate and place it behind the cleaned portion of the flipper. Use the biopsy punch to punch a round piece of skin from the turtle's flipper by pushing the punch through the skin, firmly against the dive slate (Fig. 6I). Place the round skin biopsy into the saturated salt buffer-filled tube. The biopsy can be dropped into the buffer by tapping the punch against the side of the sample tube. If this fails to dislodge the sample, use the sterile vacutainer needle or forceps to extract the biopsy from the punch. Be sure the sample becomes immersed in the buffer.

Once the biopsy is in the sample tube, label the etched side of the tube with the turtle's tag number, the genus and species abbreviation, and the date of collection as follows:

MB141
Cm
22 Aug 2011

Record the tag number on the lid, as well. Be certain that the sample tube is tightly closed. The biopsy punch and the needle (if one is used) should be placed in the SHARPS container after use. It should never be used on another animal. The dive slate and forceps need to be thoroughly cleaned with alcohol (alcohol wipe) after each biopsy and stored in a Ziploc bag.

INSTRUCTIONS FOR LABELING BIOLOGICAL SAMPLES

General

--Labeling the sample accurately and legibly is as important as collecting the samples. Samples with labeling errors or without labels may have to be discarded.
--The labels on the samples in many cases will be read by technicians at various labs who are not familiar with the project, the tag letters or the tag number series. Please write the tag numbers as clearly as possible, so that anyone could read them. Check your work.
--Be sure your 1's and 7's are clear and different (you can put a strike through 7's); be sure your 4's and 9's are unambiguous.
--Print, using standard printed block letters and numbers (nothing fancy or stylized, not script).
--Use the specified marker for each vial or tube. 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. MB708 and MM708 are entirely different turtles.
--Always write out the month, or at least a part of it, e.g., ***4 Aug 2016***. Do not use all numbers, e.g., 8/4/16. Different countries treat the order for day and month differently.

Green-top Vacutainers

Record on label: *Tag number* (tag on turtle's left flipper)
Set #
Contents: Whole blood to be used for hormone analysis (blood gas analysis, manual hematocrit, blood smear for selected turtles)
Comments: These tubes will be centrifuged within a few hours and plasma transferred to permanently labeled 2-ml cryovials. The green vacutainers can be temporarily labeled with any waterproof pencil, sharpie or cryomarker.

Red-top Vacutainers

Record on label: Nothing
Contents: Whole blood to be used for genetic analysis
Comments: 0.5 ml of whole blood collected in red-top vacutainer is transferred immediately to blue-top 10-ml plastic vial that contains 9 ml of lysis buffer. The red-top will be discarded and the blue-top vial labeled immediately.

Blue-top Plastic tubes

Record on label: *Tag number* (tag on turtle's left flipper)
Date (write out month, e.g., 4 August 2016, or 4 Aug 2016)
Species (Cm = green, Ei = hawksbill, Cc = loggerhead)

Contents: Whole blood (0.5 ml) and lysis buffer (9 ml) for genetic samples

Comments: Invert several times to mix whole blood and lysis buffer. Write in label area (etched, cloudy area). Use fine-point permanent Sharpie. Tighten lid as securely as possible.

White-top Plastic tubes

Record on label: *Tag number* (tag on turtle's left flipper)
Date (write out month, e.g., 4 August 2016, or 4 Aug 2016)
Species (Cm = green, Ei = hawksbill, Cc = loggerhead)

Contents: Skin biopsy in 5 ml saturated salt buffer for genetic or stable isotope analysis

Comments: Write in label area (etched, cloudy area). Use fine-point permanent Sharpie. Tighten lid as securely as possible.

2-ml Cryovials

Record on label: *Tag number* (turtle's left flipper)
Date (write out month, e.g., 4 August 2016, or 4 Aug 2016)
Species (Cm = green, Ei = hawksbill, Cc = loggerhead)

Contents: plasma or red blood cells; full line is at 1.8 ml; do not fill above this line to allow room for expansion during freezing.

Comments: Write in label area (white area) with ultra-fine-point sharpie or cryomarker. These vials cannot be relabeled once frozen so it's essential to label them well the first time.

Instructions for Pipetting Plasma Samples

--Balance the tubes in the centrifuge using blanks if necessary.
--Spin at 3400 rpm for 10 minutes, or follow centrifuge guidelines.
--Remove tubes carefully with the forceps. Put in rack. Avoid jarring the samples.
--Organize the spun vacutainers in the rack and label every cryovial with appropriate tag number, species abbreviation and date. Keep the vials for one turtle grouped together to avoid confusion. Double check tag numbers on the vacutainer and the cryovials to be sure they are the same. If there are any questions about tag numbers, consult the data sheets.
--Using **ONE** Pasteur pipette per turtle, fill 2-ml cryovials to fill line (1.8 ml). If samples are too full, the tops will pop off in the freezer. We usually fill 2 cryovials per turtle. Fill each vial to full line before starting the next one. The lab needs a certain minimum amount for an analysis and we typically send them only one vial.
--Don't allow the plasma to enter the bulb. If it does, the bulb will have to be thoroughly cleaned.
--If you accidentally disturb the layer below the plasma, you can re-centrifuge the sample. Some green tubes have a gel layer to keep the plasma separate from the cells.
--Close the lids of the cryovials completely and tightly.
--Discard all used pipettes in the sharp's container and vacutainers in the Biomedical Waste bucket after you process each sample. Do not put a used pipette down on the table where it might accidentally be used for a different turtle.
--Store all the filled cryovials for a particular set in one zip-lock bag labeled with:

Date
Capture Locality
Set #

--Labeled bags with samples go in Blood Cooler with ice. Add ice from food cooler as needed.
--The Blood Cooler goes to the aquarium promptly at the end of the day to analyze the plasma samples before they go in a designated spot in freezer.
--Cooler and test tube rack get rinsed and put where they will make it back on board the next day.

AT THE DAY'S END, CHECK ALL SAMPLES AGAINST THE DATA SHEETS TO BE SURE TAG NUMBERS HAVE BEEN CORRECTLY READ AND ALL SAMPLES TAKEN ARE ACCOUNTED FOR. NUMBERS SHOULD MATCH UP.

Handling of Lysis and Tissue Samples

Store the lysis and tissue samples in one zip-lock bag for each set labeled with:

Date
Capture Locality
Set #

These samples can also be stored in the Blood Cooler with the hormone samples. They go in a designated unrefrigerated spot at the aquarium. They should never get warm.

Handling of Red Blood Cells

Store the red blood cell samples in the zip-lock bag with the plasma samples for each set labeled with:

Date
Capture Locality
Set #

These samples can also be stored in the Blood Cooler with the hormone samples. They go in the freezer at the aquarium at the end of the day. They should never get warm.

PROJECT LOGISTICS

TASK LIST FOR VOLUNTEERS AND STUDENTS

Volunteers may be asked to help with any of the following tasks. To be sure that all tasks are completed as safely and quickly as possible, each volunteer may be assigned a particular role as listed in the second part of this section.

1. Snorkeling the net to locate captured turtles.
2. Recovering turtles from the net.
3. Receiving captured turtles into the catch boat, freeing them from the net and keeping them wet.
4. Helping to transfer captured turtles from catch boat into the main research vessel.
5. Assisting catch boat operator with recording GPS locations, water temperatures and water depths at net sites.
6. Pulling the net into the net boat and stacking it for the next set.
7. Keeping the turtles on board the research vessel wet and, as much as possible, in shade.
8. Assisting with tagging, including preparing tags and sterilizing equipment.
9. Looking up capture histories to determine if a genetic sample exists for recaptures.
10. Assisting with weighing.
11. Assisting with tying and untying turtles from the rack for blood collection.
12. Assisting with blood collection and processing.
13. Assisting with collection of skin biopsies.
14. Assisting with release of processed turtles after checking with recorder that tag numbers are correctly recorded and that all data have been entered on data sheet.
15. Cleaning up equipment and the research vessel during return to the dock.
16. Off-loading gear and assisting captain of the research vessel with final clean up.

ROLES FOR VOLUNTEERS AND STUDENTS

Certain tasks like measuring, recording and taking blood samples will normally be done by project personnel. Other tasks will be done by students/volunteers. Not all volunteers/course participants will be expected to do all tasks every day. Instead, you will be asked to assume one of the following roles one day and then move on to a new role the following day:

1-ASSISTANT RECORDER.
Upon arrival on board in the morning, make certain that a sufficient number of unused data sheets (50) are in the data clipboard along with sharpened pencils.
When the catch boat returns to the research vessel with captured turtles: help unload turtles making sure that recaptures go to the front row, new turtles to the right side and listen for any remarks that eventually should be recorded on the data sheets. Also check that Assistant tagger 2 is checking for PIT tags in turtles without external tags. Look up the histories of tagged turtles to determine whether we already have a genetic sample for them. Mark the plastron of recaptured turtles with the types of samples that are needed using a waterproof marker.

During tagging of turtles: keep regular contact with tagger to make sure that any tag numbers reported are heard and recorded properly by the data recorder. Also, keep track of any removed tags – be sure you know which turtle they have been removed from.
When measuring starts: make sure that the data recorder is receiving the data as it is being reported.
When weighing starts: listen for weighmaster's report of weights and help recorder get them on the correct data sheet.
When bleeding starts:. Listen for reports of blood collection and help to organize tissue sampling for new turtles for which no genetic (blood lysis) sample could be obtained.
After all turtles from the last set are bled: sit in cabin with assistant bleeders and blood keeper with all of the day's data sheets and verify tag numbers and quantities of plasma and genetic samples.
At end of day: Make sure that there are enough blank data sheets for the next sample session.

TAGGER. The tagger will normally be a permanent project member. This individual makes decisions about what size tags to use on different-size turtles and which tags need to be replaced. Assistant tagger 1 and 2 work with the tagger. Assistant tagger 1 supplies tags, Assistant tagger 2 helps to put them on.

2-ASSISTANT TAGGER 1.
Upon arrival on board in the morning: make certain that tags are available in sufficient quantity for the day's tagging, make certain that the tagging pliers for each kind of tag are available, set up tags and pliers on tagging table.

When we return to the research vessel with captured turtles: help unload turtles and help captain of the research vessel set up swimming noodles to make cradles for turtles.
During tagging of turtles, provide Tagger and Assistant Tagger 2 with correct-sized tags and tagging pliers as requested by Tagger and make certain that the tagging pliers and punch are rinsed in Chlorhexidine solution, rinsed with fresh water and wiped between turtles. Supply tagger and assistant tagger 1 with small pliers as needed to remove tags that need to be replaced or to reshape new tags that don't go on during the first attempt.
When all tagging is complete: assistant taggers should help with blood sampling by helping to tie weighed turtles on racks for bleeding, setting tied turtles against gunwale for bleeding (or holding small turtles for bleeding), removing turtles from bleeding area, taking turtles off rack after bleeding, checking with recorder that data are complete, and upon OK from recorders, releasing turtles.
During bleeding, Assistant Tagger 1 and 2 are responsible for taking tissue samples from any new turtle for which no other genetic sample is obtained.
After all turtles are worked up: rinse, dry and store tagging pliers for use on the following day; restock tags, and oil tagging gear especially, if it is the end of the week or end of the sampling session. Check supplies of each tag type to be sure that there are enough tags on board for the next day's sampling.

3-ASSISTANT TAGGER 2.
Upon arrival on board in the morning: make certain that betadine and Q-tips are available in tagging gear, that PIT tag box contains a sufficient number of PIT tags and that both readers are working. Also, find biopsy kit and make sure sufficient numbers of biopsy punches are present along with white-top vials prefilled with saturated salt buffer and a marking pen.
When we return to the research vessel with captured turtles: check all turtles without external tags for PIT tags. Move any turtles with PIT tag to recapture side of vessel and mark them as a recapture by writing the PIT tag number on the plastron with a red waterproof marker.
During tagging: assist Tagger by wiping tagging sites with betadine-soaked Q-tips, receiving tags from Assistant Tagger 1, and holding fins of turtles for tagging. Help remove tags that Tagger determines require replacement, report tag numbers to recorder and assistant recorder. Make sure that wasted and recovered tags are reported and placed in the proper containers.
When all tagging is complete: assistant taggers help with blood sampling by helping to tie weighed turtles on rack for bleeding, setting tied turtles against gunwale for bleeding (or holding small turtles for bleeding), removing turtles from bleeding area, taking turtles off rack after bleeding, checking with recorder that data are complete, and upon OK from recorders, releasing turtles.
During bleeding: Assistant Tagger 1 and 2 are responsible for taking tissue samples from any new turtle for which no other genetic sample (whole blood in lysis) was obtained.
When all turtles are worked up: repack PIT tag box, add PIT tags to box as needed. Check on supply of PIT tags for next day. Check biopsy kit to be sure that supplies of biopsy punches, alcohol wipes and saturated salt buffer-filled, white-top vials are sufficient for the next day.

MEASURER. In order to minimize measurement error, it will always be a principal project member who uses the calipers and reads the measuring tape.

4-ASSISTANT MEASURER.
Upon arrival on board in the morning: make certain that large and small calipers are on board and in working order. Locate soft tapes in tag bag.
When we return to the research vessel with captured turtles: help to unload turtles and get out swimming noodles to make cradles.
Before measuring starts: verify with taggers the order in which turtles have been tagged and are arranged on the deck.
During measuring: position and hold turtles for measuring, assist with all measurements, help to report measurements to recorder and assistant recorder, and turn turtles over to weighing crew.
After all turtles are measured: assistant measurer assists with bleeding and release of turtles. This includes helping to tie weighed turtles on rack for bleeding, setting tied turtles against gunwale for bleeding (or holding small turtles for bleeding), removing turtles from bleeding area, taking turtles off rack after bleeding, checking with recorder that data are complete, and upon OK from recorders, releases turtles.
After all turtles are worked up: roll up and store measuring tapes and make sure calipers are in a safe location.
When the research vessel docks at days end: Assistant measurer stays on board to help stow all turtle gear in the cabin and rinse the deck as instructed by the captain or project members.

5-WEIGHMASTER.
Upon arrival on board in the morning: make certain that electronic scale is on board and is charged (or has a working battery).
When we return to the research vessel with captured turtles: help unload turtles and get out swimming noodles to make cradles, then makes sure weighing hook is in position for weighing and that scale can be zeroed.
During work up of turtles: is responsible for zeroing scale, preparing scale hook, reading scale and making sure that all readings are in kg. Reports all readings to recorders.
After all turtles are weighed: help with bleeding by removing turtles from bleeding area, check with recorder that data are complete, release turtles.
Stay on board research vessel at days end: Make sure scale is taken off for recharging and identify person responsible for recharging. Help captain and project members with clean up of vessel as needed.

6-WEIGH AND RACK #1.
7-WEIGH AND RACK #2.

Upon arrival on board in the morning: both make sure bleeding racks for larger turtles are on the deck and lines are cleared.
When we return to the research vessel with captured turtles: help to unload turtles and keep turtles cool using saltwater hose until turtles are ready for weighing.
When turtles are being measured: turn and hold turtles for measuring team; weigh turtles; prepare turtles for blood sampling

When all turtles have been weighed, help with bleeding by removing bled turtles from bleeding area, and check with recorder that data are complete, release turtles.
After all bleeding is finished, clean used equipment.

BLOOD SAMPLING.
Blood samples must be collected by BTP staff designated for this activity on the CITES permit.

8 and 9 ASSISTANT BLOOD SAMPLERS.
Upon arrival on board in the morning: make certain that both bleeding kits are stocked with green and red top tubes, 20 and 22 gauge needles, needle holder, Q-tips and marker. Also, reread instructions for labeling samples on page 19.
During bleeding: assist bleeder with passing vacutainers. Helps to keep track of blood samples, keeps vacutainers ready for bleeder. Helps weighing assistant with bringing turtles for bleeding and removing turtles after bleeding.
When all sampling is finished for the day: works with blood keeper to spin and pipette plasma samples. Green top tubes are centrifuged and the blood plasma is pipetted into two 2ml plastic cryovials. The tubes are labeled with the tag number that is on the green top vacutainer, the species abbreviation and the date. Work with assistant recorder to verify that all numbers on cryovials and blue top lysis samples match those in the day's data sheets. Before leaving vessel at day's end, check that sufficient supplies of vacutainers, cryovials, needles, pipettes and other supplies are available for the next day's sampling.

10-BLOOD KEEPER.
Upon arrival on board in the morning: make certain that small cooler is present and has a layer of ice and test tube rack inside; make sure that both bleeding kits are stocked with blue-topped tubes with 9 cc of lysis buffer and check with assistant tagger that biopsy kit is stocked with white- topped tubes with 7 cc saturated salt buffer. Also, reread instructions for labeling samples (see page 19).
During bleeding: keep full, blue-top lysis vials handy, put ½ cc from red top sample into lysis buffer, mix gently, and label tube; label green top vacutainers and put them on ice; report blood sampling success to recorder and assistant recorder.
When all sampling is finished for the day: work with blood keeper to spin and pipette plasma samples. Green top tubes are centrifuged and the blood plasma is pipetted into two or three 2ml plastic cryovials. The cryovials are labeled with the tag number that is on the green top vacutainer, the species abbreviation, and the date. Work with assistant recorder to verify that all numbers on cryovials and blue top lysis samples match those in the day's data sheets.

ALL STUDENTS AND VOLUNTEERS: Upon return to the dock at the end of the day, place all personal gear on the dock. Assist crew in identifying which equipment will stay on board and where. **Assistant measurer and weigh master will stay on board to help the captain and team members clean and secure the research vessel.** They will help Captain in emptying trash, stowing gear (especially swimming noodles), rinsing decks, closing windows and emptying food and drink coolers. They may also transport scale and GPS unit to a location where they can be plugged in for recharge.

EQUIPMENT CHECKLIST

large and small calipers
camera gear
first aid kit
blank data sheets (about 50; check to see that they are current version)
health assessment sheets
GPS unit with 8 spare AA batteries
soft plastic measuring tape
charged weighing scale
weighing net/ ropes
clipboard
Procedures Manual
sharpened #2 pencils
hole punch for MM tag application
hole punch for F tag application
pliers for F tag application
pliers for M tag application
pliers for MM (titanium) tag application
F, M, and MM tags
plug cleaner (used to remove tissue plug from punch)
needle nose pliers for removing bad or worn out tags
file for filing points of plastic tags.
Betadine / Povidone
10% Clorox solution
rack with two quart jars, one for 10% Chlorhexidine solution for disinfecting pliers and other tools, and one for fresh water for rinsing
paper towels, Q-tips
hand sanitizing wipes
WD 40 for oiling equipment at the end of each session
hoses for wetting and cooling turtles
PIT tags, PIT tag gun, reader, and spare batteries

SKIN BIOPSY SUPPLY CHECKLIST

10 ml white top plastic vials prefilled with 5 ml saturated salt buffer
permanent marker
forceps
dive slate
4-mm biopsy punches
Betadine/Povidone in Tupperware box with cotton swabs
Alcohol swabs

BLOOD SUPPLY CHECKLIST

one adjustable rack
two ropes for rack
scrub brush
Q-tips
Betadine/Povidone in Tupperware box with cotton swabs
needle holders
20 and 22 gauge needles (with Hemaguard)
red top (untreated) 4 ml vacutainers
green top (lithium heparinized) 4 ml vacutainers
10 ml blue top plastic vials
lysis buffer (9 ml per sample needed)
test tube racks (one of which fits in blood cooler)
Pasteur pipettes and bulbs
cooler with ice
cryomarkers
centrifuge
biological waste/SHARPS containers
vacutainer waste box
I-stat blood gas machine
Blood gas cartridges
Microhematocrit tubes and cardboard holders

SUPPLY LIST FOR CATCH BOAT

GPS unit with spare batteries
Set Data Sheets
Ziploc bags
Mechanical pencils
Grease pencil
thermometer on a string
sonic depth gauge
latex gloves to handle FP turtles
Dive gear

SEA TURTLE FIBROPAPILLOMA DISEASE: WHAT TO DO WITH A FIBROPAPILLOMA-BEARING TURTLE

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

Recognizing fibropapilloma disease: Fibropapilloma disease is most easily recognized by the external tumor-like growths that it produces. These can occur on any of the soft tissues of the turtle but are most commonly seen on the softest areas of the head and neck, especially around the eyes, and at the base of the fore and hind flippers. They will appear as pea-sized to grapefruit-sized growths, variable in color but usually pink to red, or gray to black. They often have a floral appearance, with a surface texture like a head of cauliflower, but may also be smooth. These tumors are well vascularized and will bleed readily when cut or abraded by the net. Tumors can also grow internally and we should watch for them when doing necropsies.

Preventing the spread of fibropapilloma disease: Healthy turtles with no evidence of the external tumor-like growths can carry the virus that apparently causes FP, as well as other pathogenic agents of sea turtles. Thus, we must continue to use extreme caution with the body fluids of the sea turtles we handle. The tagging punch must be cleared 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 on another turtle during sampling or at any other time. Do not use syringe needles or other instruments that break the skin (e.g., PIT tag applicators, tagging punch) on multiple animals without disinfecting them thoroughly between animals. Frequent hand wiping with sanitizing hand wipes is recommended.

Capture of a papilloma-bearing turtle in the entrapment net: A turtle with obvious fibropapilloma should not be placed directly in the catch boat, especially with other turtles. It seems likely that if we see fibropapilloma again it will appear in newly arrived, smaller turtles. We should handle the turtle with gloves and put the turtle (and used gloves) into the equipment bucket (removing the GPS and other equipment first) in order to isolate the turtle. The bucket should be scrubbed thoroughly with Chlorhexidine solution before being used again.

Turtles with obvious fibropapilloma should not be taken on board the research vessel or to the Aquarium. The virus that is associated with the disease may survive for long periods outside of the host, especially if it is kept wet or moist. Thus, thorough treatment of all possibly infected surfaces with detergents, disinfectants, or prolonged drying would be required to make certain that the disease would not be transmitted. Thus, 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 fibropapilloma should not be tagged, weighed or measured. It should be photo-documented, appropriate samples of the tumors should be taken and preserved directly in 10% buffered formalin without being frozen, and the animal should be removed from contact with all other sea turtles and kept out of any facility that houses sea turtles. If the affected turtle has a heavy tumor burden that seems clearly to be fibropapilloma and the animal is seriously debilitated, euthanasia should be considered by government veterinarians. Samples of several tumors should be preserved in 10% buffered formalin. If the tumor burden is small or there is suspicion that the tumor is not FP, then the animal should be isolated and appropriate samples taken for assessment. If found to have FP, the diseased animal could be sent to an appropriate facility (e.g., The Turtle Hospital in the Florida Keys) for further observation and possible rehabilitation.

It is very important to confirm any possible cases of fibropapilloma. This can best be done by collecting biopsies for complete pathological evaluation. Thus, a biopsy kit with gloves, 10% buffered formalin, appropriate-sized vials, scalpels, a small plastic ruler, and chlorhexidine solution for clean up, should be assembled. This could be used for taking samples from a badly infected individual after it was euthanized, a mildly affected individual that would remain in isolation until the samples could be examined, or a dead stranded animal with suspicious tumors.

Stranding of a papilloma-bearing turtle: If a papilloma-bearing turtle is dead when it strands, it should be photo-documented at the stranding site. Photographs should be made of all surfaces, and a description recorded of the tumors, including measurements. If the turtle is fresh enough, a necropsy should be performed provided that the necropsy can be done under isolation conditions to avoid contaminating facilities where turtles are kept. If a complete necropsy cannot be performed, then a sample of the suspect tumor should be preserved in formalin for pathologic evaluation and the carcass disposed of (incinerated or buried on land). Even if the carcass is too poor to necropsy, get a sample of suspect tissue and dispose of the rest.

Any time that a suspect turtle is handled, all equipment used during handling and necropsy should be disinfected with 10% Clorox before being returned to the Aquarium. Gloves must be worn at all times. Do not transport the carcass using Aquarium vehicles and do not transport to the Aquarium for necropsy or freezing.

If a papilloma-bearing turtle strands alive, isolate it in a suitable-sized container at an appropriate location and take biopsies of suspect tissue for evaluation. The turtle should remain in isolation until the evaluation of the biopsy is complete. Based on the biopsies and the extent of any infection, a decision will be made as to whether the turtle should be euthanized or sent to an outside facility for rehabilitation.

Do not take papilloma-bearing turtles to the Aquarium. The possible rehabilitation of a single individual is not worth the risk of introducing papilloma to the entire Bermuda population.

Emergency Facilities and Procedures: A site at which FP suspect 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 kept on its own. The logistics of complete cleanup and sterilization of equipment could be handled at this site.

SET DATA:

Date: _____

Capture Locality: _____

Set Number: _____

Time set started: _____

Time set finished: _____

Turtles remaining inside net at finish: _____

Depth: _____

Bottom Temperature: _____

CAPTURE GPS (center of set):

Lat. ___ ___.___ ___ ___ ___ ___

Long. ___ ___.___ ___ ___ ___ ___

Release Locality: _____

RELEASE GPS:

Lat. ___ ___.___ ___ ___ ___ ___

Long. ___ ___.___ ___ ___ ___ ___

Recorder: _____

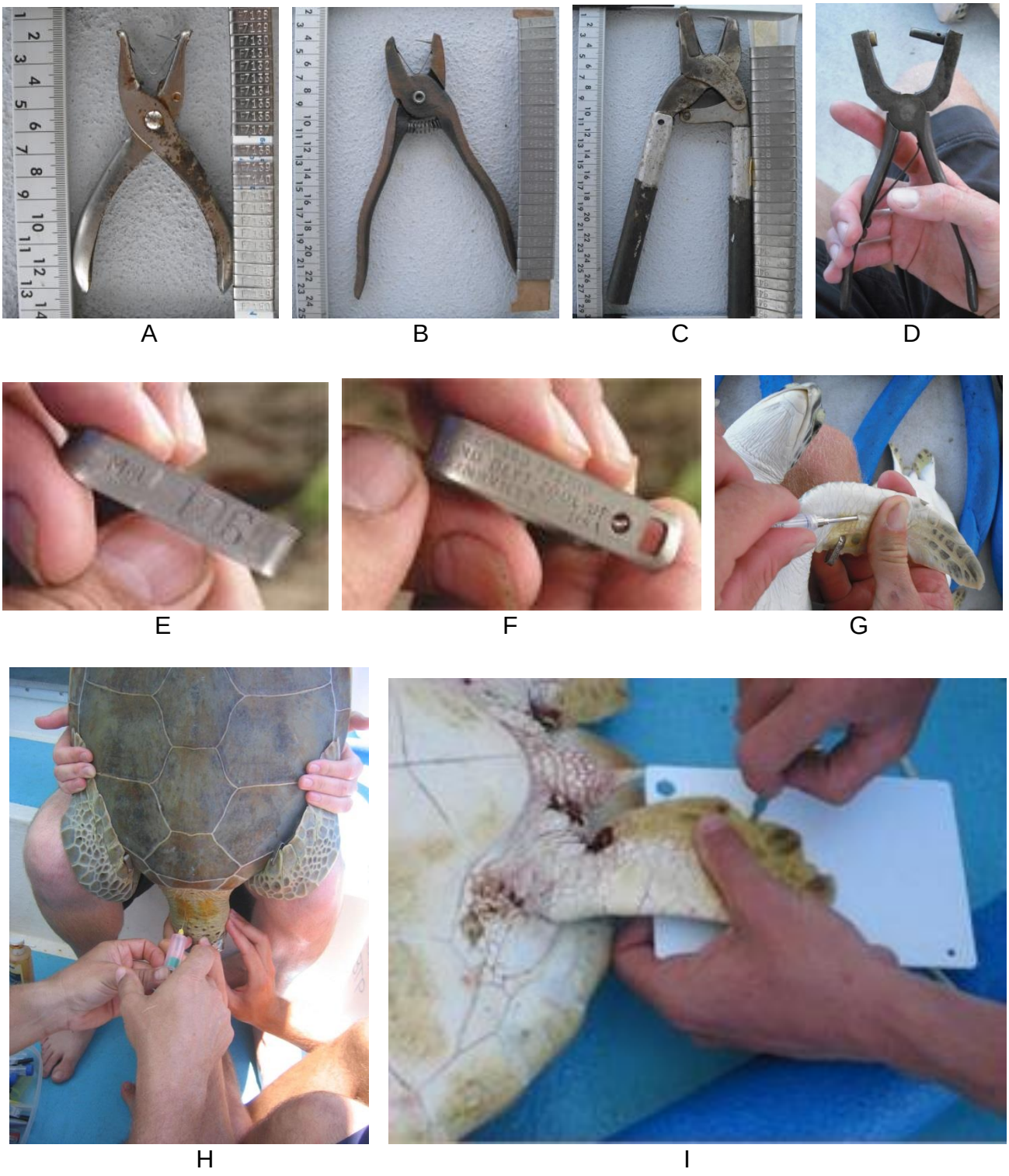

Figure 6. Illustrations of tagging and sample collection. A) F tags (monel) and applicator (for turtles smaller than 35 cm). B) M tags (inconel) and applicator (for turtles 35 – 60 cm). C) MM tags (titanium) and applicator (for turtles 60 cm and above). D) leather punch used to prepunch before application of MM tags. E) Numbered side of MM titanium tag. F) Reward message side of MM titanium tag. G) Implanting PIT tag using syringe type applicator. Tag is injected towards the tip of the flipper with the applicator needle well under the skin. H) Correct holding position for taking blood sample from cervical sinus with fingers holding flippers against shell and weight of turtle held on holder's knees. Note that the turtle is held vertical and the head holder is keeping the neck extended and vertical. This turtle is close to the maximum size that we would hold by hand; larger turtles would be tied onto a rack (Figure 5). I) Taking a tissue biopsy sample from the tip of the hind flipper using biopsy punch and dive slate.

ADDENDUMS:

Esophagus Lavage Protocol for Green turtle (Chelonia mydas)

Green turtles eat mostly seagrass and algae in various parts of the world. Bites taken from these underwater pastures will slowly move through the esophagus whilst the ingested salt water is expelled through the mouth. We will sample food items recently caught in the esophagus of a subsample of the captured green turtles, using the lavage technique (Balazs, 1980).

Preparation:

- Two 5-gallon bucket filled with clear seawater
- Clean, veterinary lavage tubes
- Veterinary double action stomach pump (check that lavage tubes connect to pump)
- Mineral oil/ vegetable oil
- Mouthpiece (to keep mouth open)
- Measuring tape
- 4% formalin solution
- Sieve (to filter out food particles)
- Spoon or forceps
- White or colored tape
- Sample jars with air-tight lids
- Team of minimum 3 people (1 person to hold the turtle, 1 to pump the water and 1 to open the turtle's mouth and feed the tube into the esophagus)

Procedure:

1. A clean 5-gallon bucket is filled with clear seawater collected from the same area where the turtle was captured. Collect 12 liters (2.5 gallons) of clean seawater.
2. A green turtle is randomly selected from the set.
   The flipper tags are confirmed on the data sheet for the selected turtle.
   *Fibropapilloma turtles will not be used for lavage samples *
3. The correct size of tube is selected based on the size of the turtle:
   The diameter of the tube will be ¼" for small juveniles (< 40 Cm SCL; minimum size 25 cm SCL) or 3/8" for larger juveniles, subadults and adults (> 40 Cm SCL).
   The maximum length of the tube that will be inserted 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 maximum length of the tube should be marked by tape.
   The portion of the tube that will be inserted is lubricated with vegetable oil.
4. The turtle will be held upside down on its carapace at a 45° angle (with its head lower than the tail). This angle helps to 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 opened with a bite block or similar tool and the oral cavity will be checked for any abnormality, injury or disease.
6. The lubricated tube is inserted into the mouth, carefully passing the opening for the trachea and inserted into the esophagus. 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 is not inserted past the maximum length.
7. While clear seawater is pumped into the tube using a veterinary stomach pump, 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 turtles head. This step should take less than 1 minute.
8. The contents of the receiving bucket will then be poured into a sieve.
9. Food items collected from the sieve using a spoon or forceps will be stored in a four percent formalin-seawater solution for later analysis, Secure the jar lid tightly.
10. The turtle is held at the 45-degree angle until no more water is coming out. The turtle is then gently placed back onto its plastron.
11. Data sheet for the turtle is completed: make 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, lavage sample with permanent marker.
13. Clean used materials.

Addition of Health Assessment of Green Turtles (Chelonia mydas) in Bermuda

The goal is to establish a baseline of health assessments from free-ranging green turtles in the developmental habitat and to document health status trends or changes over time in the Bermuda green turtle aggregation.

Procedures for capture and handling will follow the BTP Procedures Manual. The goal is to sample 5, randomly selected turtles per set to achieve representation from all sets. For each animal selected, a BTP data sheet AND a health assessment data sheet will be completed. No blood will be taken from turtles with a Body Condition Score of 1/5, to not further compromise the health of the turtle. A careful, full clinical exam will determine the plan required for such a fragile turtle.

Short Step by step outline from BTP Procedure Manual:

1. Capture turtle; catch boat to mark capture time on plastron with grease pencil
2. Check for FP
3. Check for ID Tags (external and PIT tag) - Start data sheet for turtle
4. Health check, BCS
5. Tag turtle if not already tagged (front flipper tags and PIT tags)
6. Take measurements and weight
7. Take pictures of turtle (left lateral head, carapace, plastron, depth; include tag# in pictures)
8. Take body temperature and collect blood samples – start Health assessment data sheet for turtle
9. Label and prepare blood samples for analysis
10. Take skin biopsy if needed
11. When both data sheets are completed and confirmed, turtle can be released

Sampling procedure:

The same procedures as described in the BTP Procedures Manual are followed.
After the application of ID tags and taking of standard measurements and photographs, the procedure for blood sampling is followed. For Health Assessment turtles a part of their blood sample is used to analyze blood gasses, chemistry, manual hematocrit and total protein. A blood smear is made. The analysis and the time of processing are completed on the Health Assessment Data Sheet for each selected turtle.

1. Take body temperature of turtle (infrared body temperature in pectoral soft tissue area) and note temperature on health data sheet.
2. Note the time of venipuncture (withdrawal of blood) for each turtle on health data sheet.
3. Venipuncture is taken from dorsal cervical sinus, after appropriate disinfection of the site.
4. Fill 1 green top lithium heparin vacutainer till vacuum is gone – gently rock 10x (green top vacutainer holds 3.5mls of whole blood). Label this green top vacutainer with the tag # (left front flipper) and the body temperature.
5. Fill red top vacutainer with few drops of blood (0.1 ml ideal, max 0.5mls) – immediately transfer approx. 2 drops into a 10-ml blue top vial filled with lysis buffer (used for genetics).
6. Label the blue top tube with tag #, species (CM) and date (written out).
7. Immediately run a CG8+ or CG4 cartridge from whole blood (green top) on i-Stat machine for the blood gasses and make a note of the time of analysis. Note the results on health data sheet.
8. Fill 1 (or 2) microhematocrit capillary tube(s) and place 1 drop onto a microscopy slide, then seal one end of the capillary tube with clay. Place microhematocrit capillary tubes in individual, labeled slide holders (label with tag#). To be taken to the BAMZ hospital lab for manual hematocrit.
9. Smear the drop of blood on microscope slide with cover slip. Label the slide with tag # and date. Place labeled microscope slide into the slide holder, together with the capillary tube of that turtle. Take to BAMZ hospital lab for slide staining.
10. Centrifuge the green top vacutainer for 3400 rpm for 10 minutes or follow centrifuge guidelines. Make note of centrifuge start time.
11. Pipette only the plasma layer and transfer it in a white top cryovial. Label the white top cryovial (tag #, full date, species) and note time of separation. Place in Set bag in cooler and take to BAMZ hospital lab for further analysis.
12. The centrifuged RBC layer will be transferred to a red-top 2ml-cryovial. Add to Set bag in cooler (to be frozen once at BAMZ).
13. Once in the lab, centrifuge the microhematocrit tubes and read manual hematocrit on chart.
14. Use plasma from microhematocrit tube to read Total Protein on refractometer. Note on Health data sheet.
15. 0.1ml plasma from white top cryovial is used for the Reptile rotor in the VetScan Chemistry Analyzer. Note time of analysis and add results to Health data sheet.
16. All leftover plasma (1.8 ml) is kept for hormone analysis and back-up sample in freezer, together with the RBC in red top cryovial for stable isotopes.
17. Stain the microscopic slide with the blood smear with Gram stain. These will be kept for blood count.
18. Keep the blue top vial with lysis buffer in room temperature, to be analyzed for genetics overseas.