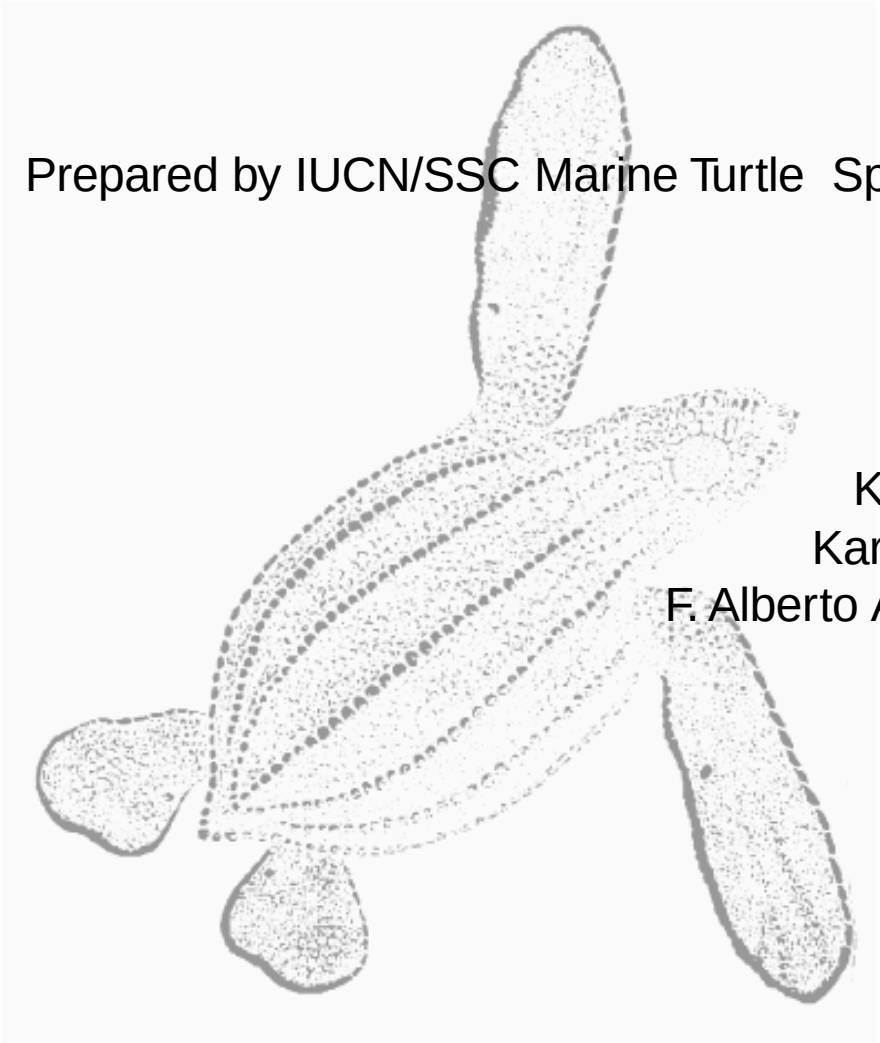
Research and Management Techniques for the Conservation of Sea Turtles

Prepared by IUCN/SSC Marine Turtle Specialist Group



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Preface

In 1995 the IUCN/SSC Marine Turtle Specialist Group (MTSG) published *A Global Strategy for the Conservation of Marine Turtles* to provide a blueprint for efforts to conserve and recover declining and depleted sea turtle populations around the world. As unique components of complex ecosystems, sea turtles serve important roles in coastal and marine habitats by contributing to the health and maintenance of coral reefs, seagrass meadows, estuaries, and sandy beaches. The *Strategy* supports integrated and focused programs to prevent the extinction of these species and promotes the restoration and survival of healthy sea turtle populations that fulfill their ecological roles.

Sea turtles and humans have been linked for as long as people have settled the coasts and plied the oceans. Coastal communities have depended upon sea turtles and their eggs for protein and other products for countless generations and, in many areas, continue to do so today. However, increased commercialization of sea turtle products over the course of the 20th century has decimated many populations. Because sea turtles have complex life cycles during which individuals move among many habitats and travel across ocean basins, conservation requires a cooperative, international approach to management planning that recognizes inter-connections among habitats, sea turtle populations, and human populations, while applying the best available scientific knowledge.

To date our success in achieving both of these tasks has been minimal. Sea turtle species are recognized as "Critically Endangered," "Endangered" or "Vulnerable" by the World Conservation Union (IUCN). Most populations are depleted as a result of unsustainable harvest for meat, shell, oil, skins, and eggs. Tens of thousands of turtles die every year after being accidentally captured in active or abandoned fishing gear. Oil spills, chemical waste, persistent plastic and other debris, high density coastal development, and an increase in ocean-based tourism have damaged or eliminated important nesting beaches and feeding areas.

To ensure the survival of sea turtles, it is important that standard and appropriate guidelines and criteria be employed by field workers in all range states. Standardized conservation and management techniques encourage the collection of comparable data and enable the sharing of results among nations and regions. This manual seeks to address the need for standard guidelines and criteria, while at the same time acknowledging a growing constituency of field workers and policy-makers seeking guidance with regard to when and why to invoke one management option over another, how to effectively implement the chosen option, and how to evaluate success.

The IUCN Marine Turtle Specialist Group believes that proper management cannot occur in the absence of supporting and high quality research, and that scientific research should focus, whenever possible, on critical conservation issues. We intend for this manual to serve a global audience involved in the protection and management of sea turtle resources. Recognizing that the most successful sea turtle protection and management programs combine traditional census techniques with computerized databases, genetic analyses and satellite-based telemetry techniques that practitioners a generation ago could only dream about, we dedicate this manual to the resource managers of the 21st century who will be facing increasingly complex resource management challenges, and for whom we hope this manual will provide both training and counsel.

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Tissue Sampling and Necropsy Techniques

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The antemortem and postmortem sampling of tissues is necessary to fully understand the causes of lesions, disease, and mortality in living and dead marine turtles. In the living animal, sampling of single or multiple tissues is referred to as a biopsy. Biopsies are collected for biological and pathological studies. While the postmortem examination of a human is referred to as an autopsy, the postmortem examination of an animal is referred to as a necropsy. This chapter reviews biopsy (tissue sampling) and necropsy techniques, and discusses why they are important and when they should be done.

Biopsy Techniques

Biopsies are routinely collected to better understand the nature of a lesion and to determine the most appropriate therapy. Biopsies may be collected from various tissues to provide information relative to the life history of the population being studied. Skin biopsies have been collected for genetic studies, and bone biopsies have been collected for aging studies. For specific information regarding the collection of tissue samples for studies of genetic origin, the reader is referred to FitzSimmons *et al.* (this volume).

Blood is a fluid tissue and is the most common biopsy collected by biologists in the field. In juvenile and adult marine turtles, blood is generally collected from the cervical sinus (Owens and Ruiz, 1980); in neonates, blood is often collected from the heart (cardiocentesis), with the needle passed through the overlying plastron (Samour *et al.,* 1984), or from the cervical sinus (Bennett, 1986). At either site, the integument should be cleansed with 70% ethanol prior to sampling. When cardiocentesis is performed, a surgical glue (cyanoacrylate), such as Vetbond® (3M Animal Care Products, St. Paul, Minnesota 55144 USA) or Nexaband® (Veterinary Products Laboratory, Phoenix, Arizona 85013 USA), should be used to cover the hole left in the plastron after the needle is withdrawn. Otherwise, pathogens in water can migrate through the hole into the heart, resulting in infection (pericarditis). See Owens (this volume) for detailed instructions in blood sampling.

The most common solid tissue biopsied is the skin. In most situations, a local anesthetic agent such as 2% lidocaine hydrochloride (Lidocaine HCl, Phoenix Pharmaceuticals, St. Joseph, Missouri 64506 USA) can be used around the site. The biopsy site and surrounding tissue should be treated to a surgical scrub; that is, the site should be cleansed with three alternating applications of 70% ethanol and a surgical iodine soap *(e.g.,* Betadine Surgical Scrub®: The Purdue Frederick Co., Norwalk, Connecticut 06856 USA) before the sample is obtained. Sterile surgical gloves should be used. The sample can be obtained using a scalpel blade (#10 or #15) or a biopsy punch *(e.g.,* Disposable Biopsy Punch: Premier Medical, Norristown, Pennsylvania 19404 USA). Following removal of the sample, the defect can either be sutured or left to heal by granulation.

Depending upon the type of lesion being biopsied, single or multiple samples are collected. Subsequent preservation of the sample will depend upon the various diagnostic tests to be used. For histologic evaluation, a portion of each sample should be fixed in neutral buffered 10% formalin (NBF), with a tissue to fixative volume ratio of 1:10. NBF can only penetrate 6 mm in 24 hr, so the tissue should be thin enough to allow adequate fixation. If tissues are to be stored beyond 48 hr in a fixative, they should be transferred from NBF to 70% ethanol at this time. If samples are to be submitted for microbial isolation attempts, they

should be cleansed with sterile saline to remove the overlying alcohol and Betadine scrub prior to being placed in an appropriate transport media or sterile container for shipment to a diagnostic laboratory. Since freezing results in crystallization artifact, tissues for histologic examination should never be allowed to freeze. For specifics on shipment of samples, the individual collecting the samples should contact a diagnostic laboratory in advance to receive specific information on transport of samples.

Biopsies also can be obtained from visceral structures. While potentially achievable in the field, in most situations this will be performed in a veterinary hospital, under general anesthesia. A gas anesthetic such as isoflurane *(e.g.,* Aerrane®, Fort Dodge Animal Health, P. O. Box 25945, Overland Park, Kansas 66225-5945 USA) is most commonly used. Biopsies can be obtained from the gastrointestinal tract using a flexible fiberopticscope and biopsy device. Biopsies from visceral structures such as the kidney or liver can be obtained either through a laparotomy incision or using an ultrasound guided technique and various automated biopsy devices. Again, consult a veterinary hospital for the various options available.

Necropsy Techniques

To determine cause(s) of death, a thorough postmortem evaluation should be performed. The quality of the necropsy will depend upon the background and training of the person doing the examination. Ideally, the person should have good experience and knowledge of sea turtle anatomy. Information on sea turtle visceral anatomy can be found elsewhere *(e.g.,* Rainey, 1981). Whether the necropsy is conducted in the field or in a veterinary diagnostic facility will determine the depth of the examination. Be prepared to collect the following samples: (1) tissues for histopathology; (2) tissues for electron microscopy; (3) samples for microbiology; (4) tissues for toxicology; (5) stomach content samples; and (6) parasites.

Ideally the necropsy should be performed as soon after death as possible. If the necropsy is delayed, the carcass should be either placed in a refrigerated room or placed on crushed ice. Avoid freezing the carcass since this will cause artifactual changes in tissues. To be most informative, necropsies should be done within 24 hr of death.

Marine turtle necropsy procedures have been described (Campbell, 1996), and a marine turtle necropsy guide has been published (Wolke and George, 1981) and should be consulted for detailed information. Equipment needed for a necropsy are listed in Table 1. It is important to wear appropriate clothing that can be washed following completion of the necropsy. This includes rubber boots or protective covering of shoes and rubber gloves. To reduce the chance of inhaling foreign material and potential pathogens, a face mask should be used at all times. Necropsy report sheets vary among institutions (an example can be found in Wolke and George, 1981) and have not been standardized. Pertinent information should be recorded including species of turtle, weight, carapace and plastron length and width, sex (verified by internal examination), weather conditions, and times at start and finish of the necropsy. Ideally one person should do the postmortem examination and another the recording of the information. Alternatively, a tape recorder can be used and the information transcribed later. For captive animals, a summary of the clinical course of the turtle should be recorded. For wild turtles found dead in the field, the stranding data sheet should be attached to the necropsy report. Photographs should be taken of the entire carcass, both dorsally and ventrally, and of any lesions.

Necropsies start on the outside and move internally in a methodical manner. The exterior of the turtle should be thoroughly examined, and all gross abnormalities described. Drawings of marine turtles, both dorsally and ventrally, should be used to indicate location of lesions (this is best accomplished if the data sheet includes a standard silhouette). Wounds to the shell and soft tissues are noted. Any other changes such as swellings to join spaces of long bones and cutaneous or subcutaneous masses are recorded. Samples of all significant lesions should be collected for histopathology. Samples are placed in neutral buffered 10% formalin (NBF), with a tissue to fixative volume ratio of 1:10. If hard tissue such as long bone is collected, it should be fixed in a container separate from the soft tissues to allow adequate penetration and fixation.

The overall appearance of the turtle will dictate whether to continue with a full necropsy. If the turtle is in an advanced state of postmortem change, such as bloated with gas, skin discolored, or scutes falling from the shell, collection of tissues for histopathologic evaluation will be unrewarding.

The necropsy progresses with the turtle in dorsal recumbency (plastron up). The plastron is removed intact by separating it from the carapace along the marginal bridge, on both sides, and from the skin at areas of attachment. The gular area of the lower jaw

Table 1. Necropsy equipment list.

1. Coveralls or other appropriate clothing
2. Rubber boots or shoe covers
3. Rubber gloves
4. Mask
5. Camera
6. String, labels, assorted bottles, water proof pen
7. Forceps (several sizes)
8. Tissue cutting board
9. Necropsy knives and sharpener
10. Scalpel blades (#20 and #10) and handle
11. Postmortem shears
12. Alcohol lamp or butane burner
13. Matches or lighter (for flame)
14. 70% alcohol
15. Containers with neutral buffered formalin
16. Fixative for electron microscopy such as Trumps solution (should be kept chilled)
17. Sterile whirl-pack bags (i.e., sterile plastic bags that can be sealed)
18. Cryotubes
19. Microbial culturette swabs
20. Microbial transport media
21. Dry ice and ice chest or cooler
22. Balance (up to 250 g)
23. Stryker saw
24. Calipers
25. Microscope slides
26. Necropsy sheet and notebook

is incised just medial to, and along the edges of the mandible. The incision is extended into the oropharynx, and once completed, the tongue, glottis, and proximal trachea can be lifted and exteriorized. This allows visualization of the oral cavity, with sampling of tissues as needed. Portions of tongue and glottis are collected for histology. As tissues are sampled for histology, the transition area between healthy and abnormal tissue should be collected. This is often an important area to look for pathogens. The trachea and esophagus are severed just cranial to the base of the forelimbs and removed from the carcass as a unit. Next, the forelimbs and hindlimbs and their associated girdles are removed. When this is done, the entire coelomic cavity can be visualized.

Before any further samples are collected, this is a good time to scan the coelomic cavity for any obvious lesions. All lesions noted should be described in terms of size, color, shape and consistency. If excess or discolored fluid is seen in the coelomic cavity, a sample should be obtained for culture. A small amount of fluid can be placed on a microscopic slide and a smear made for future cytologic examination. Visually scanning the coelomic cavity for changes and collecting samples at this stage of the necropsy is important to ensure that minimally contaminated samples are collected for microbiology. As the necropsy progresses, contamination of tissues is inevitable. Samples of lesions may be swabbed with appropriate culturettes or portions collected aseptically (using either sterile or flamed instruments), placed in a sterile container, and transported to a diagnostic laboratory for culture. The manner in which the sample is transported will depend upon the cultures attempted. For the most part, samples should be transported either on crushed or dry ice. If the animal is recently dead (within 1 hr), heart blood can be collected for culture of aerobic organisms. Again, consult a veterinarian or diagnostic laboratory for selection of appropriate transport media.

In continuing the necropsy, all major organs are identified (Rainey, 1981) and samples collected including the following: tongue, skeletal muscle, glottis, trachea, lungs, thymus, thyroid, adrenal gland, pancreas, heart, liver, gall bladder, esophagus, stomach, small intestine, large intestine, bladder, reproductive organs and tract, and brain.

For electron microscopy, small portions (1 mm^3) of relevant tissue should be fixed in Trumps solution (McDowell and Trump, 1976). If a change suggestive of a viral infection, such as the presence of inclusions, is found by light microscopic examination of NBF fixed tissue, a small portion of tissue can be processed for electron microscopy. It is even possible to use paraffin embedded tissue in identifying the presence of viruses. Most viruses are preserved fairly well in paraffin.

For heavy metal analysis, samples of kidney, liver, brain, skeletal muscle, pancreas, skin, stomach contents, feces, and urine can be collected, placed in separate Teflon® FEP (fluorinated ethylene propylene) bags (plastic may be used if necessary), and frozen on dry ice or in an ultrafreezer until submitted. The use of titanium knives and Teflon sheets is recommended. If these are not available, an alternative is to tease apart tissues using bare fingers rinsed in alcohol, and then to place the samples in Teflon bags (Becker *et al.,* 1994). Instruments must be cleaned between collection of different tissues/samples to avoid contamination from sample to sample.

For analysis of organic compounds, fat, liver, kidney, and skeletal muscle should be collected. Specimens can be collected individually in acetone-rinsed glass jars, covered with acetone rinsed aluminum foil (rinse the shiny side and put it toward the inside of the glass jar) before replacing the lid (Beasley, pers. comm.). This will avoid contact between the specimen and the rubber seal. Jars may be filled as full as possible and refrigerated until extracted for organic contaminants. This will lessen the loss of volatile compounds into the air at the top of the jar. Samples should be submitted to an appropriate laboratory as soon after collection as possible. If samples cannot be submitted quickly, jars can be filled to about 3/4 of the jar's capacity and frozen (at least to -20 F) until analyzed. Breakage may be less likely if jars are tilted when freezing. Plastic jars and bags also can be used; however, there may be transfer of interfering substances to the tissues from the plastics. Chlorinated plastics (polyvinylchloride) and plastics with phthalate esters in them, may present problems (Beasley, pers. comm.). If used, be sure to give your analyst some of the same type of empty jars or bags. In this way the bags can be tested for interfering/contaminating substances.

When collecting helminths for identification, trematodes should be placed in a dish containing tap water, which is placed in a refrigerator overnight to allow parasites to relax. They should then be placed in an AFA (alcohol-formalin-acetic acid) solution consisting of 8.5 parts 85% ethanol, 1 part commercial formalin, and 0.5 part glacial acetic acid. Nematodes should be dipped in concentrated glacial acetic acid or hot 70% ethanol for fixation and then transferred to a mixture of 9 parts 70% ethanol and 1 part glycerin. All material presented to a parasitologist should have complete data including host species, host organ or tissue, collection locality, date of collection, and collector.

At the end of the necropsy, the carcass should be disposed of in accordance with local regulations.

Postmortem examinations are the best way to try to establish causes of mortality in marine turtles. However, determining the specific cause(s) of death is not possible in all cases. Even the best necropsy may turn out to be a diagnostic conundrum. Many pesticides and contaminants may not result in light microscopic changes in tissues and trying to establish a causal relationship is difficult, especially since lethal doses for these compounds have not been determined. Still, necropsies provide invaluable information about causes of morbidity and mortality which cannot be derived through any other means. Unfortunately, there are relatively few reports on causes of mortality in free-ranging marine turtles (Glazebrook and Campbell, 1990).

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