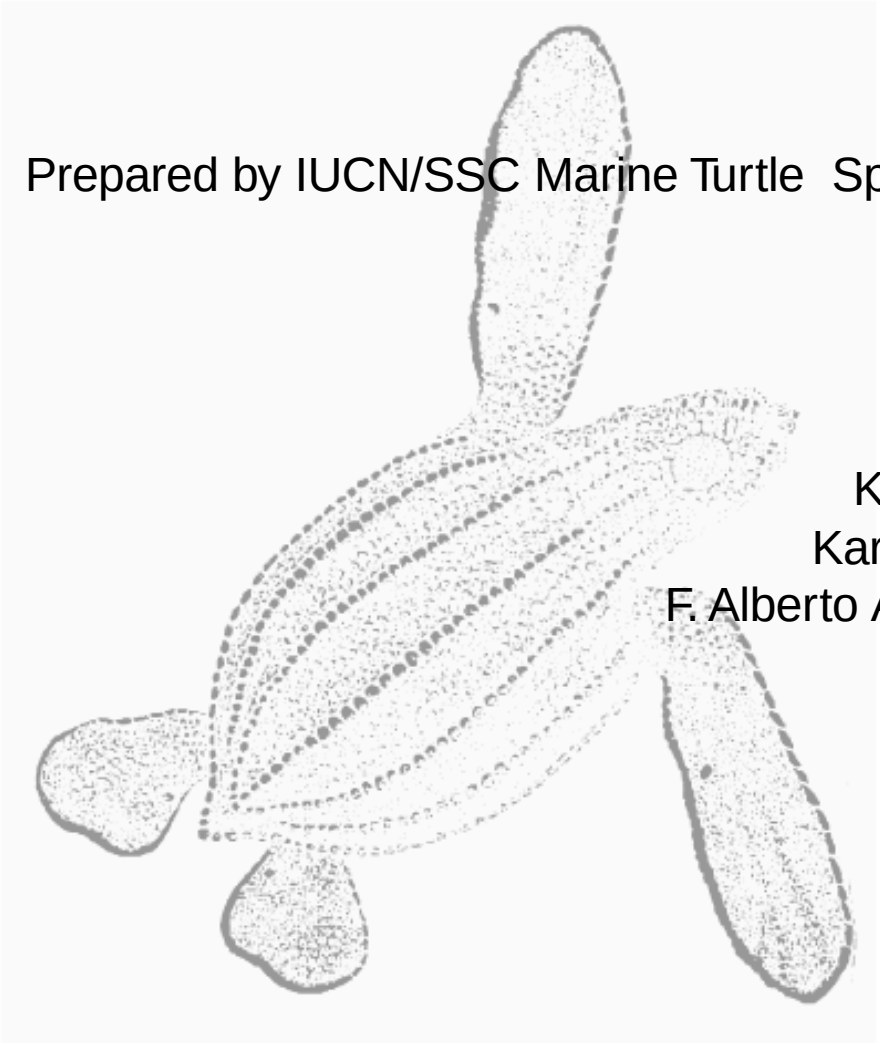
Research and Management Techniques for the Conservation of Sea Turtles

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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Infectious Diseases of Marine Turtles

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A better appreciation of the role of infectious diseases in the ecology of free-ranging marine turtles and as causes of individual and mass morbidity and mortality will require consistent application of appropriate diagnostic methods and careful interpretation of results. This chapter is written primarily for field biologists who may encounter occasional sick, injured, or dead turtles, who may be confronted with mass morbidity/mortality events and want to find out the cause(s), or who may want to incorporate routine health monitoring and surveillance for infectious disease as part of their overall population studies.

The purpose of this chapter is to provide an overview of diagnostic procedures and a guide for the collection and handling of diagnostic samples. It is not possible in the space available to provide an atlas of marine turtle diseases and specific instructions for diagnosing each. Diagnosis and treatment of specific diseases will require the assistance of one or more specialists in clinical pathology, anatomic pathology, microbiology, parasitology, immunology, and reptile medicine. While some diagnostic procedures can be conducted in the field, many will require submission to an experienced laboratory, and proper collection and handling of samples will be critical. Besides, most marine turtle diseases probably have yet to be described, so that understanding general approaches will be more useful. A recent synopsis of the known marine turtle diseases and an introduction to the literature is in Herbst and Jacobson (1995). A more detailed review and description of marine turtle diseases is provided by Lauckner (1985).

Principles

To understand infectious disease in populations one must understand the distinction between being infected with a disease causing agent and having disease (overt illness) caused by that agent. As a rule, infection will be relatively common in a population but clinical disease rare. For any disease agent in a population of turtles there will be individuals that have never been infected, individuals that are infected but are not sick, those that are both infected and sick, and individuals that were infected but are now immune. The interactions of factors that influence whether infection is expressed as clinical disease in a population can be very complex.

Different diagnostic tests can be used to detect or monitor prior or current infection or clinical illness. The results of any single diagnostic test must be interpreted in the context of the entire picture, including the history and pattern of disease in the population, clinical signs, results of other tests, and gross and histopathologic lesions. Detection or isolation of an infectious agent or detection of antibodies to that agent provide only partial information in an investigation of a morbidity/mortality problem. In some instances, findings may be completely incidental to the real cause of the disease.

Field Observations: Signalment, History, Clinical Signs

All detective work involves thorough description of the scene and preservation of the physical evidence. Careful and complete description of the health problem by the field biologist is the first and most critical step in arriving at a diagnosis. The species, age, size, and sex of animals affected (signalment), the onset, duration, and course of the problem (history), the observed clinical signs, and lesions will define the problem and guide the selection of diagnostic approaches. For example, mass stranding of turtles in apparently

good body condition following a sharp drop in water temperature might suggest a peracute infection or hypothermic stunning whereas a similar stranding event in summer might result from a peracute infection or a toxin. Although clinical signs such as weight loss or depression may be non-specific, any conclusions about etiology or pathogenesis based on results of diagnostic tests would have to be consistent with these observations.

In most cases, identification of disease processes and causes of morbidity and mortality come from carefully conducted complete necropsies of dead and moribund turtles (see Jacobson, this volume) and physical examination and biopsy of visible lesions in live turtles. When investigating a population morbidity or mortality event, it is often more informative to euthanize and necropsy a sick turtle rather than one that died spontaneously, because one is more likely to find active primary pathologic processes in the former case while chronic inflammatory responses and secondary infections may obscure these findings in the latter.

Fecal Analysis: Parasitology

The entire gastrointestinal tract contents should be screened at necropsy for the presence of intestinal helminths. Fresh fecal samples from live turtles can be examined by direct smear, flotation, and sedimentation techniques for patent protozoal and helminth infections (Sloss *et al.,* 1994).

Clinical Microbiology

A thorough diagnostic workup of suspected viral, bacterial, or fungal diseases would include attempts to isolate and identify the microbial agent in culture. Specimens must be collected and transported in a way that preserves pathogen viability with minimal changes in the floral composition caused by overgrowth of the specimen by faster growing species. Blood, tissue fluids, exudates, or tissue biopsies to be submitted for microbial culture must be collected under aseptic conditions using sterile instruments and technique so that the specimen is representative of the microbes found in the lesion rather than contaminants. These samples can yield spurious and confounding results and are not worth collecting if they cannot be handled properly and transferred to an experienced clinical microbiological laboratory in a timely manner.

Contact should be made with the receiving laboratory well in advance so that they can advise the field worker about the laboratory's capabilities, submission deadlines, proper collection materials, and transport media. Although many species of bacteria and fungi can be cultured using standard media and procedures, many other microorganisms, such as *Mycoplasma* and *Mycobacterium* species, require specialized culture media and conditions. Other organisms, such as *Chlamydia* species and viruses, require a permissive cell culture system for isolation. A diagnostic laboratory must be identified that has access to specialized media and cell lines and is prepared to carry out the culture procedures required by these agents. Even routinely isolated species may require modifications in procedures to optimize recovery. It is important to remember that failure to isolate a certain microorganism does not rule it out as a potential cause of the disease under study. Appropriate culture systems for some potential pathogenic bacteria, fungi, and viruses have not yet been developed.

Blood Work

Blood is a very useful and easily obtained diagnostic material. Blood-borne pathogens and parasites can be identified in blood smears. Blood cultures may help to detect systemic bacterial infection. Complete and differential blood cell counts and plasma biochemistry analysis can detect a problem and help point to the type of injury that has occurred. For example, an elevated plasma uric acid concentration suggests disease in the kidneys, whereas an elevated creatine kinase level suggests that muscle tissue has been damaged. Plasma also can be tested for the presence of antibodies to specific agents (antigens) and for the antigens themselves.

The different types of assays that can be performed on whole blood or plasma have different collection, handling, and storage requirements that may limit their practicality in certain field conditions. All blood samples should be collected from a vascular space, such as the dorsal cervical sinus, following adequate training and recommended procedures (see Owens, this volume). Typically, 3-5 ml of whole blood should be adequate for most analyses. Turtles readily tolerate having up to 1 ml blood per 100 g body weight removed if necessary. Thin film blood smears, for performing differential white cell counts, should be made by spreading a drop of whole blood on a microscope slide, immediately following collection. This minimizes clumping and changes in blood cell morphology that can occur with standing. For complete blood cell counts, a sample of unclotted whole blood

must be sent to the laboratory as soon as possible, usually within 24 hr. Whole blood can stored for short periods in a refrigerator and shipped on wet ice.

Plasma for biochemical assays must be removed from whole blood rapidly. A clinical centrifuge for use in the field is essential. Delays in separating the plasma from whole blood will cause changes in many biochemical parameters. For example, plasma glucose will decrease as it is consumed by the still living cells and potassium will increase as it gradually leaks from cells. Sample hemolysis as well as prolonged storage at -20°C will cause drastic changes in the activity of certain enzymes. The plasma should be submitted immediately for biochemical analysis or stored in liquid nitrogen or an ultra cold freezer (<-70°C).

Results of plasma or serum biochemistry analyses may also vary with the type of analyzer used and the quality control program of the laboratory (Bolten *et al.*, 1992). As with clinical microbiological samples, arrangements should be made before field work begins so that blood samples can be submitted to a single clinical pathology laboratory that is set up to analyze turtle material. The laboratory should have established reference ranges for the species being studied. Variation in data, due to different collection, handling, and analysis methods among studies and among samples within a study, make data interpretation difficult and should be minimized.

Plasma (1-2 ml) should also be archived in several aliquots for serodiagnostic testing. Plasma samples for antibody detection can be stored in a conventional freezer (-20°C), but care should be taken to avoid repeated thawing and re-freezing of samples as this affects test sensitivity. Packed cell volume (PCV) (or hematocrit, Hct), which is the percent of blood volume consisting of cells, can be measured at the time of plasma separation. Low PCV (<30%) is not only a useful gauge of blood loss following trauma, but can also indicate a chronic disease problem such as parasitism, infection, anorexia/starvation. Usually, a microcentrifuge and capillary tubes are used when measuring PCV, but a standard clinical centrifuge and flat-bottomed tubes can be used instead.

Serodiagnostic Tests: Serology

Serodiagnostic tests are performed on serum or plasma to detect either the presence of antibodies to a particular disease causing agent or the presence of circulating antigens from the disease causing agent itself. The former type of test is used to determine whether individuals in a population have ever been exposed to a particular disease causing agent, by the fact that they have mounted a humoral immune (antibody) response against it. The latter type of test is used to determine whether the individual has an ongoing exposure *(e.g.*, active infection), by the fact that they presently have foreign substances (antigens) derived from the disease causing agent circulating in their blood. The high sensitivity and specificity of these types of tests make them extremely valuable in population health monitoring (disease surveillance), in which most infections are subclinical, and in testing specific hypotheses (differential diagnosis) about the causes of specific disease outbreaks.

The fact that antibodies and some antigens are stable in frozen plasma for many years makes it possible to perform retrospective epizootiologic studies that can yield valuable information on the long-term health history of turtle populations and help pinpoint the time, perhaps long before clinical disease became recognized, when a new infectious agent entered a population.

Molecular Diagnostic Tests

The science of detection and characterization of pathogenic organisms has made tremendous advances with the development of nucleic acid hybridization (Southern and Northern blotting, *in situ* hybridization) and amplification techniques (polymerase chain reaction) and the ever increasing availability of specific nucleic acid probes and primers (Persing *et al.*, 1993). While molecular diagnostic tests exist for many bacteria and fungi shared between turtles and other vertebrates, those for pathogenic organisms unique to marine turtles are still under development. Nevertheless, turtle biologists should anticipate the eventual availability of these tests and collect the appropriate specimens. Fortunately, either formalin fixed or deep frozen tissues (< -70°C) can be used for many applications. For research requiring non-degraded DNA and RNA, fresh tissue samples must be frozen immediately and stored in liquid nitrogen.

Specific Diseases

The primary role of the turtle biologist who is not also a disease specialist, in diagnosing specific infectious diseases, is to recognize and describe potential disease problems in the population and to collect and preserve the appropriate samples. The following sections briefly describe the types of samples that would be needed for the major infectious disease agents.

Viruses

Preliminary diagnosis of viral disease usually comes from histopathologic examination of fixed tissues obtained by biopsy or at necropsy. Coupled with history and clinical signs, the occurrence of characteristic cytopathology such as cell degeneration (swelling and lysis), syncytia formation (fusion of adjacent cells), and intranuclear or intracytoplasmic inclusion bodies, provides the first clue that a viral agent may be involved. Electron microscopic examination of these fixed tissues may confirm the presence of virus-like particles and provide a preliminary identification of the agent. Complete diagnosis is achieved by virus isolation from fresh or frozen (< -70°C) samples in an appropriate tissue culture system, followed by immunological and molecular characterization of the isolate. In cases where an appropriate cell culture system has not been developed for the agent, further identification may be achieved by agent specific immunohistochemical techniques using agent specific antibodies or by agent specific molecular biochemical techniques, if these are available.

Minimally, a field worker should collect lesion tissues in neutral buffered 10% formalin. Electron microscopic (EM) examination can be performed on formalin-fixed and even paraffin-embedded tissue specimens. However, special fixatives for EM should be used when description of ultrastructural pathology will be important (see Jacobson, this volume). It is also important to save frozen tissue specimens (held at or below -70°C, preferably in liquid nitrogen) for virus isolation. Although some viruses may remain intact and infectious for very long times at ambient temperature, the most environmentally sensitive viruses rapidly lose infectivity unless they are rapidly frozen and stored below -70°C (Fenner *et al.*, 1974). Fresh tissue samples placed in virus transport media (serum-free cell culture media containing antibiotics and antifungals) can be shipped on ice to a laboratory that has a variety of cell lines (including sea turtle cell lines) for virus isolation. However, frozen tissue provides a resource for additional isolation attempts.

Bacteria / Fungi

Gross and histologic examination of lesions usually provides first evidence of bacterial or fungal disease. In addition to routine hematoxylin and eosin, special tissue stains, such as tissue Gram stains (Brown and Brimm), silver impregnation stains (Warthin-Starry, Gomori Methamine Silver), and acid fast stains (Zeihl-Nielson), can help narrow the range of possible agents. Smear preparations of lesion exudates or impression smears of affected tissues can be made, stained, and examined in the field. Submission of specimens for bacterial and fungal culture should follow the guidelines discussed above (clinical microbiology). Immunodiagnostic and molecular diagnostic techniques can also be applied to fixed or frozen tissues or to culture isolates.

Protozoa

The protozoal diseases that have been described in marine turtles so far are primarily pathogens of the gastrointestinal tract. While fecal analysis (direct smears, floatation) can be an aid in diagnosis, many gastrointestinal protozoans may be commensals and finding the organisms within characteristic histologic lesions is the best way to identify pathogenic species. Protozoal infections of other organs will also require histologic diagnosis.

Metazoan Parasites

Specimens of ectoparasites and epibionts should be saved in formalin for identification. Helminths (trematodes and nematodes) can be discovered by careful examination of the gastrointestinal tract and other hollow organs and their contents at necropsy. Adult cardiovascular trematodes (Spirorchidae) are found by careful examination of heart, lungs, and major blood vessels, and sieving of blood. Collection of worms should be as thorough (quantitative) as possible so that the diversity of fauna can be examined later. Fecal sedimentation and flotation will help identify helminth ova, including those of cardiovascular flukes, which must reach the gastrointestinal tract lumen for access to the environment. Eggs of cardiovascular flukes can also be recovered by sedimentation from tissues that have been digested with enzymes (Dailey and Morris, 1995; Herbst *et al.*, 1998).

The association of parasites with their host often has a long coevolutionary history and evidence of parasitism is a common incidental finding. Demonstration of significant pathology is necessary to directly implicate particular parasites as a cause of morbidity and mortality.

Special Precautions

The phylogenetic distance and physiological differences separating reptiles from humans lowers the risk of disease transmission from marine turtles to man. However, marine turtles may harbor a number of bacterial species that are known human pathogens

or are opportunistic pathogens in a wide range of vertebrate species. These include *Mycobacterium, Salmonella, Vibrio,* and *Chlamydia* species (Acha and Szyfres, 1987). In addition, there is insufficient information about other infectious agents of turtles to be certain of the risks. Field workers should realize that these risks exist and have appropriate materials available to immediately clean and disinfect wounds received while handling these animals. Workers should immediately seek medical attention if even minor wounds become infected or if they become systemically ill after working with turtles. Gloves should always be worn when performing necropsies.

Another concern is the possible accidental spread of infection among turtles by biologists who fail to take sufficient preventative precautions. Instruments such as needles, tags and tag applicators, laparoscopes, endoscopes, stomach tubes can transfer infectious agents very efficiently. Inexpensive disposable materials such as scalpel blades and needles should not be used on more than one animal. Instruments that are used repeatedly must be sanitized or disinfected between animals. Adapting decontamination techniques to the field, although difficult, should be attempted seriously. Linton *et al*. (1987) and Rutala (1990) provide useful information. Washing in hot water with a strong detergent is useful for sanitizing instruments. Sodium hypochlorite (bleach solution diluted 1:10) is an excellent and inexpensive disinfectant, but it is corrosive and rapidly deactivated by organic debris. Glutaraldehyde solutions or formalin are effective sterilants but residues are very toxic. Chlorhexidine solutions and povidone iodine solutions are effective and less toxic to tissues, and can be used to disinfect skin as well as surfaces. Alcohol is not an effective disinfectant unless instruments are flamed or soaked for very long periods of time. Whichever disinfectant is used, adequate contact time must be allowed for effect. When caustic or toxic compounds are used, instruments should be rinsed thoroughly prior to contacting living tissues.

The Future

As marine turtle resources and marine ecosystems become more intensively managed, with individual turtles and populations being manipulated within and possibly moved between natural habitats and artificial enclosures, the potential impact of infectious diseases will become more and more apparent. Health monitoring will become an important part of overall management so that new potentially devastating diseases can be discovered before they threaten management efforts and so that diseases already having such effects can be monitored and controlled. Presently, much of the diagnostic work performed on marine turtles is performed in retrospect, at necropsy or in the face of a disease outbreak. It will be important to make population health monitoring more prospective by developing and using mass screening diagnostic tests for disease agents of concern.

Serodiagnostic tests are highly sensitive and specific for a particular pathogen and are important components of prospective population health monitoring. Development of serodiagnostic tests for marine turtles are in the early stages. Significant progress has occurred with the production of monoclonal antibodies specific for green turtle immunoglobulin classes (Herbst and Klein, 1995). Several of these monoclonal antibodies can be used with other marine turtle species also. With these reagents, antibody responses of marine turtles to any foreign antigens, including infectious agents and toxins, can be detected. The limitations on applying these reagents widely in standardized tests has been the paucity of antigens.

While the monoclonal antibodies provide half of the requirement for reliable, repeatable, standardized serodiagnostic tests, we do not yet have reliable sources for well characterized, standardized test antigens with which to monitor any disease. Although some preliminary immunodiagnostic tests have been produced (Herbst *et al.,* 1988), they require further development and refinement before they are available for wide application. Nevertheless, it must be emphasized that plasma specimens should be collected and archived now, because each collection of samples provides a snapshot in time of the disease exposure of a turtle population. All field biologists who are handling marine turtles for other purposes are urged to consider collecting plasma to archive for future testing. This recommendation points to the obvious need to establish a registry or plasma bank to curate these samples.

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