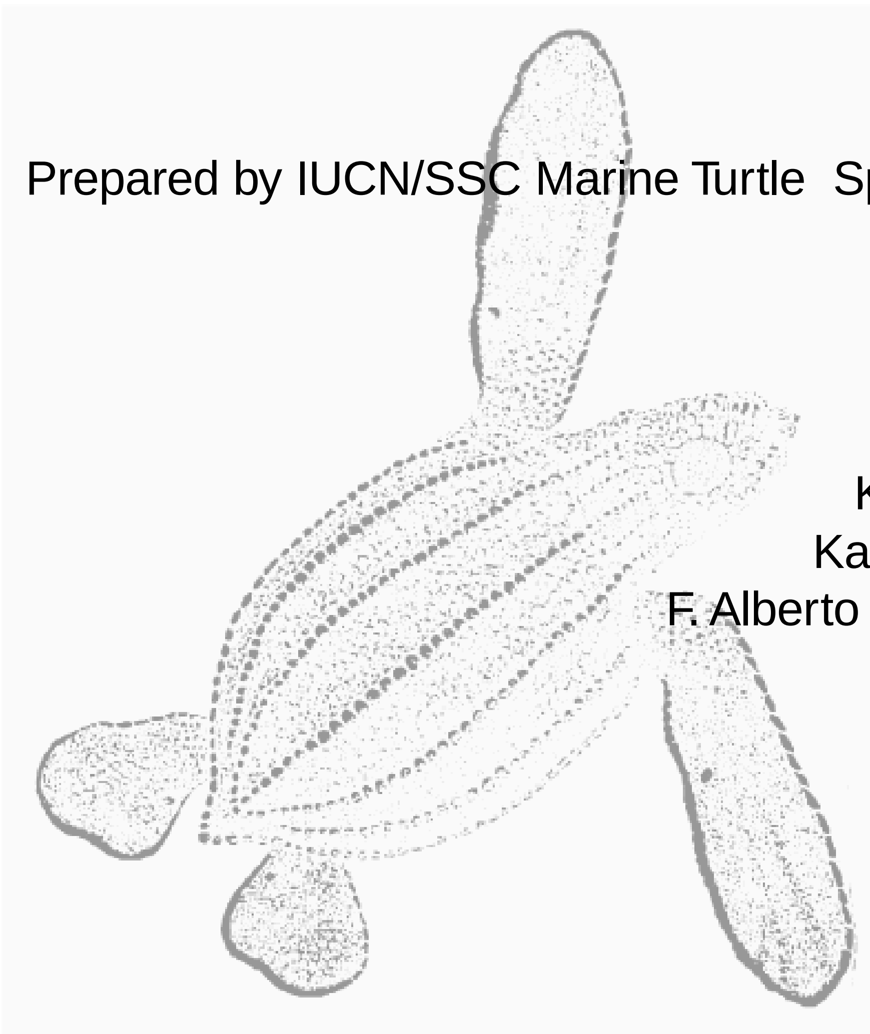
Research and Management Techniques for the Conservation of Sea Turtles



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Development and publication of *Research and Management Techniques for the Conservation of Sea Turtles* was made possible through the generous support of the Center for Marine Conservation, Convention on Migratory Species, U.S. National Marine Fisheries Service, and the Worldwide Fund for Nature.

ISBN 2-8317-0364-6

Printed by Consolidated Graphic Communications, Blanchard, Pennsylvania USA

Cover art: leatherback hatchling, *Dermochelys coriacea,* by Tom McFarland

This publication should be cited as follows: Eckert, K. L., K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (Editors). 1999. *Research and Management Techniques for the Conservation of Sea Turtles.* IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

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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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Acknowledgements

True to the spirit and structure of the Marine Turtle Specialist Group (MTSG) of the World Conservation Union (IUCN), this ma (nual is the result of the collaborative efforts of scientists and policy-makers from around the world. The Editors are deeply grateful for the support and encouragement of these colleagues, and for their willingness to share data, experience, and expertise. We are especially indebted to the more than 60 authors who made this manual possible, and to all those who participated in the peer-review process.

The following persons contributed substantively to the overall quality of the manual by participating in its peer-review: Ana Barragan (Facultad de Ciencias, Universidad Autónoma de México); Anna Bass (University of Florida, USA); Miriam Benabib (Instituto de Ecología, Universidad Autónoma de México); Alan Bolten (University of Florida, USA); Annette Broderick (University of Wales Swansea, U.K.); Deborah Crouse (Fish and Wildlife Service, USA); Andreas Demetropoulos (Ministry of Agriculture and Natural Resources, Cyprus); Peter Dutton (National Marine Fisheries Service, USA); Scott Eckert (Hubbs-Sea World Research Institute, USA); Nat Frazer (University of Florida, USA); Jack Frazier (CINVESTAV, México); Marc Girondot (Université Paris 7-Denis Diderot, France); Brendan Godley (University of Wales Swansea, U.K.); Hedelvy Guada (WIDECAST, Venezuela); Julia Horrocks (University of the West Indies, Barbados); George Hughes (KwaZulu-Natal Nature Conservation Service, South Africa); Naoki Kamezaki (Sea Turtle Association of Japan); Rhema Kerr (Hope Zoological Gardens, Jamaica); Jeffrey Miller (Queensland Department of Environment and Heritage, Australia); Jeanne Mortimer (Conservation and National Parks, Republic of the Seychelles); Wallace J. Nichols (University of Arizona, USA); Joel Palma (World Wildlife Fund-Philippines); Claude Pieau (Institut Jacques Monod, Paris, France); Henk Reichart (STINASU, Suriname); Rodney Salm (IUCN, Eastern Africa Regional Office); Laura Sarti M. (Facultad de Ciencias, Universidad Autónoma de México); Barbara Schroeder (National Marine Fisheries Service, USA); Jeffrey Sybesma (Faculty of Law, University of the Netherlands Antilles); Robert van Dam (Institute for Systematics and Population Biology, The Netherlands); Alessandra Vanzella-Khouri (United Nations Environment Programme, Jamaica); and Jeanette Wyneken (Florida Atlantic University, USA).

The manual could not have been undertaken without financial support from the center for Marine Conservation, the Convention on Migratory Species, the World Wide Fund for Nature, the U.S. National Marine Fisheries Service, and the Florica Cooperative Fish and Wildlife Research Unit (USGS, Department of the Interior, Research Work Order 172).

We also extend our gratitude to Tom McFarland ("Tom's Turtles") for his artistic contributions to the chapter on taxonomy, external morphology and species identification as well as the manual's cover. His dedication to precision ensures that manual readers have access to clear and accurate diagnostic illustrations. These beautiful drawings improve the survival prospects of sea turtles in a very real way, since effective conservation action depends on accurate data, including species identity. Rose Bierce and Deb Smith designed the layout, and Deb did a marvelous job of transforming dozens of individual chapters into a usable product. In all, the project drew on the talents of more than 100 people around the world. To everyone, our heartfelt thanks!

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Overview

Research and Management Techniques for the Conservation of Sea Turtles
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Introduction to the Evolution, Life History, and Biology of Sea Turtles

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Seven species of sea turtles representing two families, Cheloniidae and Dermochelyidae, are the only living members of what has been a large and diverse marine radiation of cryptodiran turtles. These seven species include the loggerhead (*Caretta caretta),* green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), Kemp's ridley (*Lepidochelys kempii*), olive ridley (*Lepidochelys olivacea),* flatback (*Natator depressus*), and leatherback (*Dermochelys coriacea*) turtles. An eighth species, the black turtle or East Pacific green turtle (*Chelonia agassizii),* is recognized by some biologists, but morphological, biochemical, and genetic data published to date are conflicting, and the black turtle is currently treated as belonging to *Chelonia mydas*. (See Pritchard and Mortimer, this volume).

Sea turtles inhabit every ocean basin, with representatives of some species found from the Arctic Circle to Tasmania. Hawksbills are perhaps the most confirmedly tropical of the sea turtles, whereas leatherbacks are known to make forays into colder, sometimes polar, waters. With the exception of Kemp's ridley and flatback turtles, sea turtles are cosmopolitan in distribution. Kemp's ridley is restricted principally to the Gulf of Mexico and the eastern seaboard of the United States, with some individuals occasionally found along the shores of the United Kingdom and western Europe. The flatback is endemic to the Australian continental shelf.

The living sea turtles are a monophyletic group (derived from a common ancestor that has not given rise to other living turtles) of the suborder Cryptodira. This suborder includes those turtles that close their jaws by contracting muscles over a cartilage on the otic chamber (Gaffney, 1975). In all living cryptodires, the head is retracted in a vertical plane and assumes an S-shape between the shoulder girdles (Gaffney and Meylan, 1988). Living sea turtles have a reduced ability to retract their heads compared to other living cryptodires, but thick, nearly complete skull roofing confers additional protection to the head. The oldest members of this sea turtle radiation date back 110 million years to the early Cretaceous (Hiryama, 1998). An earlier (late Jurassic) lineage of cryptodiran sea turtles, the family Plesiochelyidae, is considered to be independent of that which produced the living forms (Gaffney and Meylan, 1988).

Sea turtles are considered highly derived morphologically and have many adaptations for life in the sea. All species share features such as paddle-shaped limbs, in which all movable articulations between the distal bony elements are lost and three or four digits of the hand are markedly elongate. Lacrimal, or tear, glands are remarkedly enlarged and modified to remove excess salts from body fluids; the salts are derived mostly from drinking sea water. Sea turtle shells are characterized by a reduced amount of bone. Sea turtles are also streamlined to various degrees, which improves their hydrodynamic efficiency. An enlarged shoulder girdle with a markedly elongate coracoid serves as an attachment site for the well-developed pectoral muscles which are used for swimming.

A generalized life-history model (Hirth and Hollingworth, 1973; Carr *et al.*, 1978) developed with data from the green turtle, and elaborated upon by numerous other authors, provides a framework for understanding and refining the life histories of all species of sea turtles. Although each species diverges from the model in significant ways, the phenomenon of seasonal and ontogenetic shifts in habitat occupation appears to explain much of the observed movements and migrations. Upon leaving the nesting beach as hatchlings, green turtles, loggerheads, and hawksbills begin a pelagic (open ocean) phase that is believed to last at least several years. They are often found at sea in association with weed lines or drift lines that exist near frontal boundaries near major currents. Passive drifting with currents has been demonstrated in the immediate post-hatching period. The flatback seems to be an exception to this pattern; hatchlings remain in coastal waters and apparently lack a pelagic phase (Walker and Parmenter, 1990). The habitats of post-hatchling leatherbacks and ridleys remain unknown.

This early pelagic phase, originally referred to as the "lost year" by Archie Carr, varies in duration among species and among populations. Western Atlantic loggerheads, for example, remain in the pelagic environment until they are well over 40 cm in straight carapace length, whereas Atlantic green turtles, hawksbills, and Kemp's ridleys 20-30 cm in carapace length are commonly found in shallow-water habitats.

Carr *et al.* (1978) discussed the concept of "developmental habitats" and defined them as places where immature sea turtles commonly occur but where adults of the same species are rarely, if at all, found. These may consist of one or a series of habitats (generally coastal feeding grounds) through which turtles pass as they grow to adult size. Entry into, and departure from, developmental habitats appear to occur at predictable sizes for some species. Individual turtles are often caught repeatedly in the same area over intervals of several years, implying residency in these developmental habitats. The amount of time the various species remain resident in any particular developmental habitat before moving on to the next is poorly known.

Estimates of growth rates in wild individuals indicate typically slow growth, with age-to-maturity ranging 15 to 50 years or more, depending on the species and geographic area (Balazs, 1982; Bjorndal and Zug, 1995). Adult turtles spend most of their lives in the adult foraging ground (with or without immatures), an area that is usually separate from the nesting beach. Adult foraging grounds may be fixed in space, such as seagrass beds, or transitory, such as areas in the ocean with seasonably predictable blooms of jellyfish or benthic invertebrates. During the reproductive season, adult turtles travel to the vicinity of the nesting beach, where they may remain for up to several months. Mating takes place along the migratory corridor, at courtship or breeding stations, and in the vicinity of the nesting beach. During the reproductive season, both males and females may be found in the longshore waters off the nesting beach, also called the internesting habitat.

Different species of sea turtles share many behaviors, especially those involved in reproduction. For this reason, methodologies for studying or managing sea turtles at the nesting beach are very similar for all the species. Female sea turtles typically nest more than once per reproductive season; most do not nest in consecutive years. Nesting behavior is highly stereotypic. Species-specific differences exist in parameters such as nesting habitat preference, nesting strategy (aggregated vs. solitary), size at first reproduction, average clutch size, and details of the nest size and construction. One highly divergent reproductive behavior is that of nesting in huge aggregations over a period of a few days. These mass arrivals, or *arribadas*, are formed only by Kemp's and olive ridleys.

All sea turtles appear to exhibit migratory behavior at different times in their lives. Reproductive migrations between feeding grounds and nesting beaches are the best documented because of the ease of tagging adult females on nesting beaches. Journeys spanning many thousands of kilometers are known to occur. The seasonal movements of sea turtles in search of food may also be considered as migrations. For example, leatherbacks nesting in the Wider Caribbean region return to jellyfish-rich waters in the northern and eastern Atlantic basin after breeding. Immature turtles travel between successive developmental habitats, which may be separated by hundreds or thousands of kilometers. What has been gleaned about migratory behavior of sea turtles from recaptures of tagged turtles has been greatly augmented in recent years by the use of molecular genetics (to identify the nesting beach origin of turtles captured at sea) and satellite telemetry. The latter yields information about the actual course of travel, rather than point-to-point capture data.

Because of their wide-ranging migratory nature, sea turtles require international cooperation to ensure their survival. All seven species of sea turtles are included on the IUCN Red List of Threatened

Animals (Baillie and Groombridge, 1996): Kemp's ridley and the hawksbill are considered Critically Endangered; loggerheads, green turtles, olive ridleys, and leatherbacks are listed as Endangered; and flatbacks are considered Vulnerable. These categories reflect the global status of whole taxa and are based on criteria such as population level, population trends, extent of occurrence, and probability of extinction in the wild.

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Designing a Conservation Program

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Whether one defines conservation as "preservation" or as "management for sustained utilization," there can be little doubt that sea turtles are in need of stringent conservation measures. While short- and long-term objectives, as well as specific methodologies, will necessarily differ among conservation programs, none can hope to realize its full potential without prior planning. Information gathering, the involvement of stakeholders (which, in the case of sea turtles, may include multilateral constituencies), and the recruitment of sufficient human and financial resources are fundamental to program success. Prior planning benefits all levels of program application, from single nesting beaches or foraging grounds (serving one or more nesting or foraging assemblages) to international initiatives encompassing multiple range states, recognizing that cooperative mechanisms are requisite in the management of shared migratory populations.

The overall goal of any conservation plan for sea turtles is to promote the long term survival of sea turtle populations, including the sustained recovery of depleted stocks and the safeguarding of critical habitat, integrated with the well being and needs of human communities with which they interact. Specific objectives will differ, but should include: (i) identification of populations; (ii) assessment of the conservation status of the population throughout its range and identification of key recruitment areas *(e.g.,* breeding and nesting sites); (iii) regular monitoring of populations (to assess trends); (iv) calculation or estimation of annual mortality; (v) effective protection of important nesting beaches, feeding areas, and known or suspected migratory corridors; (vi) implementation of a sufficient regulatory framework; (vii) regulation of domestic and international commerce in parts and products; and (viii) achieving and perpetuating public support for program goals and objectives.

Guidelines and Criteria

Population Size and Trends

The foundation upon which all management decisions are based must include an accurate assessment of population size, including a determination of whether populations are stable, increasing, or declining. Index habitats (intensive study areas designated to include major nesting and foraging grounds) should be monitored at intervals consistent with the determination of population dynamics over the period of at least one generation, a period of time which may range from little more than a decade for *Lepidochelys* to three decades or more for the slower-growing herbivorous *Chelonia*. Data collection should include the number of females reproductively active, the number of nests laid, the number of eggs/ nest, and the number of young hatched on an annual basis; annual and inter-annual nesting periodicity; estimates of growth, maturity, and longevity; and an evaluation of survivorship among life stages.

Critical Habitat

An assessment of the distribution and status of critical habitat *(i.e.,* habitat critical to the survival of sea turtle populations), and the protection of such habitat from both existing and anticipated threats is fundamental to the conservation of sea turtles. Major threats to nesting beaches include shoreline development *(e.g.,* the direct effects of roads and built structures, as well as the indirect effects of increased traffic and inadequate waste disposal), artificial lighting, coastal sand mining, and beachfront stabilization structures. Major threats to foraging grounds and migratory corridors include industrial and agricultural discharges (point and non-point sources), destructive fishing practices, petroleum industry activities *(e.g.,* exploration, production, refining, transport), seabed destruction *(e.g.,* dredging, anchoring), and other forms of marine pollution, including persistent marine debris. Index habitats should be protected to the highest degree practicable. Strategies for the protection of habitats important to sea turtles should be fully incorporated into local, national, and regional (international) integrated coastal zone management initiatives.

Sources of Mortality

A conservation plan must identify and quantify important sources of mortality, both direct and indirect *(e.g.,* capture which is incidental to other commercial fishing operations), in all life stages. Mitigating solutions must be designed and implemented. These should encompass, where appropriate, strengthening existing national legislation and international agreements (including making fines and other penalties commensurate with product value), promoting multi-sectoral public awareness *(e.g.,* urban consumers, rural stakeholders, coastal landowners, government), adopting fisheries-related management actions *(e.g.,* gear modifications, time and area closures, alternative livelihoods), closing market loopholes, and fielding efficient and motivated law enforcement units. Identifying illegal and clandestine threats to sea turtle populations, including addressing sensitive socio-political issues, is an important consideration in any national or regional conservation plan. Identifying ways to convert users and other stakeholders to stewards, as a means to reduce mortality, should be a priority.

Research and Data Management

Research and inquiry should be encouraged; notwithstanding, the mere accumulation of information is insufficient to meet the needs of a competent conservation program. Standard record-keeping procedures, trained field and analytical personnel, and centralized and appropriately accessible databases are crucial to program success. Research is needed both to define the extent of the conservation challenge, and to evaluate the effectiveness of a potential intervention or management response. Recommendations for intervention should be based on appropriate research, and designed to respond to a defined threat. Popular forms of intervention, including egg hatcheries, head-starting (the rearing and subsequent release of yearlings), and predator control, may not address in any meaningful way the underlying threat(s) facing the target population. The significance of research, including routine population and habitat monitoring, is lost without conscientious data management.

Public Awareness and Education

Including environmental concern in the consciousness of the average citizen is crucial to the sustained survival of both human residents and wildlife, especially endangered wildlife. Sea turtles are particularly good candidates for public education campaigns. They are easily cast as symbols of the health of the coastal zone, both marine (coral reefs, seagrass) and terrestrial (sandy beaches, littoral forest). Coastal peoples in particular have observed sea turtles in one setting or another, and the connection between protecting sea turtles and protecting large segments of the economic base *(e.g.,* fisheries, tourism) can often be clearly articulated to both rural and urban audiences. Finally, sea turtles are integral to the folklore and cultural history of many peoples around the world and as such have an added potential for capturing the imagination and emotion of a citizenry. Public awareness campaigns should accompany conservation action, target relevant stakeholders (specifically or collectively), and embrace all available avenues of communication, including print and electronic media, school curricula, extension programs, public displays, and local gatherings *(e.g.,* festivals, political events, town meetings).

Other Considerations

To validate proposals of sustainable use, PBR (potential biological removal) or other appropriate models should be presented, based on current abundance estimates and determinations of maximum intrinsic rates of increase, together with sources of mortality and their predicted trends. Because all sea turtle populations have extended geographical ranges *(i.e.,* distributions comprising multiple range states), proposed domestic use should not compromise the status of the population elsewhere in its range. Prior to the initiation of any harvest, long distance tracking *(e.g.,* using satellite telemetry) and genetic studies should be undertaken to determine both the full range of the target population and the genetic composition of the locally occurring assemblage from which the harvested animals will be drawn. Predetermined threshold values of population trends and changes in status, mortality, or habitat should be articulated such that the passing of these thresholds would automatically trigger the suspension of harvest(s) and the initiation of appropriate conservation measures.

For a variety of reasons (including relatively slow growth, delayed maturity, high juvenile mortality, wide-ranging movements and migrations, the importance of long-lived adult age classes, and a dependence on vulnerable coastal ecosystems), the biology of sea turtles confounds attempts at defining sustainable take. To maximize accuracy in the underlying estimations of population size and population dynamics, and the consequent interpretation of what might constitute sustainable take, assembling the necessary data would require decades of careful field work in multiple range states. Notwithstanding, advances in remote sensing, genetic technologies, and computer simulations can assist managers to make informed decisions based on databases that span several years, rather than several decades. In any case, the outcome will depend on the quality of the data assembled. All data collection should be done by trained personnel, rely on standard methodologies, and be subjected to rigorous peer-review.

Concluding Remarks

Few sea turtle populations currently occupy their full historical range or approach their historical abundance. Some of the largest breeding assemblages of sea turtles that the world has ever known have gone extinct (or nearly so) over the course of little more than a century. The specific attention of government and nongovernmental entities to the design and implementation of scientifically sound conservation plans is, therefore, urgently needed. Moreover, the notion that species- or population-level sea turtle conservation, management, or recovery can be defined based on the unilateral actions of governments hosting specific nesting assemblages or foraging aggregations of sea turtles is obsolete. In recent years, managers and government officials have come to recognize that sea turtles are shared resources, and that shared resources require shared responsibility. For a conservation program to succeed, every effort must be made to involve all relevant sectors and stakeholders in planning and, ultimately, in implementation.

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Priorities for Studies of Reproduction and Nest Biology

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An understanding of reproduction and nest biology is essential for recovery and management of sea turtle stocks. Without this knowledge, well intentioned but ignorant conservation efforts can be detrimental to sea turtles. A case in point: removing sea turtle eggs from the beach to incubation boxes placed within protective storage was an accepted management practice for many years until the effect of incubation temperature on sex was determined. As a result, an unnatural preponderance of male turtles may well have been produced by this "conservation" measure. Research on incubation temperatures at nesting beaches proved to be necessary for wise conservation guidelines involving the protection of sea turtle eggs.

The nesting beach provides a narrow but important window of opportunity for studying reproduction and nest biology. Essential information may be obtained with proper focus and commitment, particularly within the areas of demographics, hatchling recruitment, and nesting habitat quality. Until quite recently, the most dependable source of information on population numbers and the changes in these numbers over time was derived almost exclusively from beach studies of reproduction and nesting biology. This chapter will focus on research and management needs on the nesting beach, including studies of the adult females, eggs, and hatchlings.

Guiding Principles

Choose a project with importance for management and recovery of sea turtle populations. Will the proposed study improve the conservation picture for sea turtles, their reproductive success, or the quality of their nesting habitat? Will project results enhance local management capability, as well as regional cooperative efforts that are often international in perspective? Each study should be evaluated on a periodic basis for its benefit to the recovery of the species, in addition to satisfaction derived by the investigator. Periodic review of purpose should be adopted by every investigator studying reproduction and nest biology on beaches.

Think in terms of time. Sea turtles are long-lived organisms with delayed age of first reproduction and many years of potential reproductive activity. Hatchling production will not provide recruitment to the adult nesting population for decades following departure of the hatchlings from the nesting beach. Numbers of actively nesting females vary enormously from year to year for environmental reasons not well understood. Thus, a priority for certain reproductive studies is the capacity to design and support long term monitoring programs of a decade or more in duration. As this may require teams of field biologists extending over several human generations, there must be the capacity and technical knowledge for database management and computer analysis that does not live and die with the individual observer. Reproductive studies of long duration benefit from a team effort.

Be sensitive to the turtles' welfare. Balanced against the need for professional studies on nesting beaches is a strong moral and scientific imperative to minimize the negative impact of research on the sea turtles being investigated. Research frequently involves inevitable harassment of the animals, such as tagging, weighing, clutch relocation, and even hatchling release. Studies of threatened and endangered animals must always insure that the benefits of the research for management and recovery of the species outweigh the costs inflicted upon the research subjects. Furthermore, if the behavior of the turtle is affected adversely, this fact may invalidate the data gathered and ruin the scientific credibility of the study.

Using tagging as an example, we know that marking nesting females with flipper tags and internal passive integrated transponder (PIT) tags is an important technique for life history studies. However, tagging, even when done correctly, can be disruptive to nesting females. As is true for any manipulation, tagging should not be done unless absolutely necessary. Tagging is a research tool, a means to an end, and not a priority unto itself. When appropriately applied (and especially if sample sizes are large), benefits may include sufficient tag returns to evaluate migration patterns, foraging locations, and the causes and intensity of mortality away from the nesting beach, with special reference to harvest levels. Faithful, intensive coverage of the nesting beach for many years provides an opportunity for measuring population recruitment and annual survival. In order to achieve credible results, rates of tag loss should be measured, tag records must be error free, and tagging databases should be accessible to any serious student of sea turtle behavior who may intercept a tagged animal and need to know the location of her nesting beach.

Eggs and hatchlings should be handled with caution and only where needed. Manipulation of eggs often reduces hatching success, and its effect on the viability of the hatchlings is largely unknown. Natural dispersal of hatchlings from nest site to offshore pelagic habitat represents a critical process involving a progression of behavioral responses obviously sensitive to disruption. Hatchlings should not be detained following their emergence without a very specific purpose.

Research Priorities

Inventory Nesting Beaches

Long term conservation of sea turtles will depend on the availability and condition of nesting beaches. Where is the suitable nesting habitat, and is there historic and/or current evidence of nesting? Nesting beaches should be inventoried by area, habitat type, ownership, and conservation status. Records should be maintained regarding the loss or degradation of nesting beaches due to natural or anthropogenic causes, and decisions made concerning which areas of greater nesting activity deserve regular, methodical monitoring.

Document Nesting Activity

Document when and where nesting activity is taking place, the species involved, and the intensity and trends of nesting. Surveys need not be strictly nocturnal. Excellent nesting surveys can be achieved with trained personnel on daytime patrols, if some nighttime measurements are available to calibrate daytime observations. Conduct surveys with methodical design, so that survey results are comparable between seasons, study sites, and observers. Design and commit to a program with the capacity for many years of replicate surveys. Train observers in standardized data gathering and archiving procedures. Positive evidence of *no* nesting is also important from a management standpoint.

Calculate Hatch Success

Small, seemingly marginal nesting beaches may provide optimum nesting opportunities, while some wilderness beaches may suffer near zero reproductive success. It should be a management priority to identify beaches with high nesting activity and estimate hatch success (including likely causes of low hatch rates) at those sites. Conservation efforts should be focused at sites where high levels of reproductive success can be realized.

Define Genotypic Variation

Genetic identification of nesting populations is a priority, both at nesting beaches and on the foraging grounds. Ultimately, successful global mapping of genotypic variation among nesting assemblages will depend on the cooperation of beach studies located throughout the world. Sampling one egg from each clutch, saving a dead, unhatched embryo, or collecting a small biopsy from the rear flipper of a nesting female represent disturbances that are justified by the knowledge gained in identifying the genetic signature of a nesting population. On the other hand, drawing blood samples from nesting females is a difficult procedure with severe harassment potential, and should be done only by trained personnel.

Measure Population Parameters

Population parameters measured with accuracy and precision are crucial for developing predictive models needed for management decisions. Beach studies for this purpose might include measuring annual mortality and recruitment to the nesting population, immigration and emigration to the nesting population, average fecundity (eggs laid) per female, sex ratio, and the proportion of population fecundity realized as hatchlings entering the water. Understanding annual variation in numbers of nesting females requires comprehensive beach coverage for most of the nesting season (as many as 100-200 days/year) and surveys that extend over many years. Very little error in measurement of annual survival and recruitment of adults and age to reproductive maturity can be tolerated by population models, as opposed to clutch size and hatching success that can be measured with less accuracy. Presence or absence of each female on the nesting beach and her absolute number of clutches laid are specifics that need to be known with certainty. Population studies also require intensive tagging (with accurate estimates of tag loss) and careful maintenance of voluminous, error-free field records.

Investigate Relevant Conservation Issues

A broad range of important studies may be included here: people and pets and their effects on nesting behavior and the survival of eggs and hatchlings; perturbation or manipulation of the beach environment and its effect on nesting adults and hatchlings, including problems associated with beach lighting, sand mining, vehicle and foot traffic with resulting sand compaction, exotic vegetation, and coastal development; toxic materials and the chemical and physical quality of beach sand for embryonic development; effect of beach nourishment on hatching success; the effect(s) of feral animals and exotic pests. If hatcheries are essential, then research and improve on the methods. To ignore conservation issues or fail to measure adequately their importance to sea turtle reproductive success is management negligence.

Design Objectives

A successful project starts with clearly defined objectives, a knowledge of what needs to be measured to meet those objectives, and a research plan that, among other things, takes into account the number of seasons or decades of seasons required to achieve accurate estimates of the relevant parameters *(e.g.*, presence of nesting activity on a nesting beach, hatch success, number of reproductively active females, recruitment and mortality of adult females). Equally important is defining the portion of the nesting population being studied. Based on knowledge from genetic markers, a management unit or MU of nesting females can be defined and the geographic scope of its nesting activity can be defined. This may be distributed over many nesting beaches on many islands or along a mainland beach many kilometers in length. An investigator should know whether the chosen study of reproduction and nest biology needs to consider the MU. Studies of hatch success, for example, may be applied to a beach (narrowly) or an MU (broadly). Studies of population parameters at selected sites must consider the movement of animals between nesting sites within the MU, lest estimates of mortality and recruitment of the adults become meaningless.

As a profession, we are at a stage with beach studies where much has been learned, but obvious gaps remain in our understanding. Studies that improve the survival outlook for sea turtles are worthy efforts. Studies that minimize unnecessary disturbance of the animals are worthy efforts. Replication of facts without design is not a priority. Anecdotal observations on individual turtles is not a priority. Reinventing (or "rediscovering") what we already know is not a priority. Our collective focus should be to achieve comparable, replicative results with accuracy and precision. Studies of reproduction and nesting biology provide the greatest return for the conservation of sea turtles if they are comparable to other similar studies. This manual provides excellent guidance toward standardized "best practices." Finally, we should seek to invest in each other and in our collective capacity to conserve sea turtles by sharing our results and publishing our data in a timely fashion.

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Priorities for Research in Foraging Habitats

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Research on sea turtles on their foraging grounds has lagged far behind research on nesting beaches. Although sea turtles spend at most 1% of their lives in or on nesting beaches—in the form of embryos, hatchlings, and adult females that emerge to deposit their eggs—approximately 90% of the literature on sea turtle biology is based on nesting beach studies. Clearly, the reproductive period is a critical one, but the uneven distribution of research effort is not due to this fact alone. Generally, research on nesting beaches is less expensive and has higher ratios of turtle encounters to human effort than does research on foraging grounds. Anyone who has spent days on rough seas searching for turtles and finding them at a rate of one per day cannot help but think wistfully of the colleague working on the nesting beach who, during a pleasant evening stroll, is certain to encounter many more turtles. The bias toward nesting beach research also reflects the fact that many biologists who work with sea turtles were trained in programs that stressed terrestrial, rather than marine, habitats. This terrestrial bias influences not only the choice of habitats, but also the way in which questions are asked. One can only wonder if the "lost year" stage of all but two sea turtle populations would still be lost if more marine-oriented scientists were involved in sea turtle biology.

Role in the Ecosystem

The role of sea turtles in the structure and function of ecosystems has been largely unstudied and should be addressed as a high priority. An understanding of their capacity to affect ecosystem structure and function can be viewed as the ultimate integration of our knowledge of sea turtle biology. In addition to this excellent goal of basic biology, such studies have important implications for the management and conservation of sea turtles and marine ecosystems. Under the pressure of increased demand, priority for access to conservation resources is shifting to those species that have critical roles in the functioning of ecosystems. Are sea turtle species central to and essential for healthy ecosystem processes or are they relict species whose passing would have little effect on ecosystem function? To answer this question, the roles of sea turtles as predators and prey, as competitors with other species, and as conduits for substantial energy and nutrient flows within and between ecosystems must be elucidated. Necessary analyses range from simple studies of feeding habits—tremendous gaps still exist in our knowledge of sea turtle diets—to evaluation of complex interspecific interactions, such as of hawksbills in a coral reef habitat.

Pelagic Studies

High priority must be given to the early pelagic stage that occurs in most sea turtle species. Undoubtedly the poorest known life-stage, the location of this stage is only known for two populations—the North Atlantic and North Pacific loggerhead populations. Thus, studies of these two pelagic populations are of prime interest, and efforts must be made to locate the early life-stages of other populations.

In addition to the early pelagic stage, increased emphasis is needed on the pelagic stage in those species—primarily the leatherback and olive ridley—that remain in pelagic habitats as sub-adults and adults. In general, these two species are the least studied, largely because of their pelagic distribution.

Population Identification, Migrations, and Abundance

Identification of sea turtle populations throughout their life cycle is another area of research that deserves high priority. Most sea turtles undertake complex developmental migrations that carry them through a number of habitat types and many different national jurisdictions. These complex migrations and variable residence times result in enigmatic distribution patterns with turtles from various nesting populations intermingling on foraging grounds. These characteristics significantly increase the difficulties of developing effective management plans for sea turtle populations. Three approaches are currently employed in these investigations: passive tags (both external and internal), satellite telemetry, and genetic markers. Each of these techniques has advantages and disadvantages, and the resolution of movement patterns and population identification undoubtedly will result from an integration of these three approaches as well as techniques not yet developed.

The lack of reliable methods to estimate population levels in foraging habitats hampers our ability to monitor population trends over time. Development of reliable techniques of population estimation, either relative or absolute, should be a high priority. Such techniques would allow us to monitor the effects of human activities on sea turtle populations and the success or failure of management policies.

Closely related to the elucidation of distribution and migratory patterns and population abundance is the identification of critical habitats—other than nesting beaches—that must be protected to ensure that minimum habitat requirements of sea turtle populations are met. These habitats will include pelagic and benthic foraging areas, mating and internesting habitats, and migratory corridors that are used by turtles when moving among these habitats.

Population Structure and Regulation of Productivity

Quantitative descriptions of population structure and measures of critical demographic parameters such as somatic growth rates, age at first reproduction, survivorship, recruitment, migration, and sex ratio are essential for the development of population models. Growth rates and residence times also provide invaluable bioassays for habitat quality and population health. Studies that address these priority parameters are underway, but many more are needed that represent the complete range of habitat types and species. Genetic structure of populations can be integrated with the more standard measures of population structure to give important new insights into this field.

Descriptive assessments of demographic parameters, however, cannot be the end point. Priority should be given to studies that go beyond the descriptive level and evaluate the regulatory mechanisms that control these demographic parameters. Such studies would examine the roles of nutrition, hormones, genetics, physiology, disease, and behavior in the regulation of productivity (growth and reproduction). Research in this area would address such questions as why green turtles grow at different rates in different foraging grounds and why intervals between breeding seasons appear to be consistently longer in some geographic regions than in others for the same species. Only by understanding the regulation of productivity can we gain the ability to predict how sea turtle populations will respond to perturbations in their environment from such factors as global climate change or various human activities.

Anthropogenic Effects

Knowledge of the effects of human activities on sea turtles in foraging habitats are clearly a high priority for the management and conservation of sea turtles. Current levels of directed take of sea turtles on foraging grounds and the effect of these harvests on population stability should be assessed. The opinion that sea turtle populations can sustain harvests on their foraging grounds as long as they are protected at their nesting beaches reflects a lack of understanding of just how unrelenting and efficient such harvests can be.

Also critical is the quantification of indirect effects on sea turtle populations such as incidental capture in fisheries, potential for competition between humans and sea turtles for food, and effects of pollution and debris. Degradation of foraging habitats through pollution, siltation, and destructive fishery practices is much more difficult to monitor than that of nesting habitats, but no less important. Degradation of habitat quality may have widespread effects by suppressing the immunological system of sea turtles and making them more susceptible to disease and other stressors.

Human activities must be assessed not only for lethal effects on sea turtles, but also for sub-lethal effects. The latter are often more difficult to discern, but their cumulative effect of lowering growth rates and reproductive output can have a greater population effect than that of direct mortality. Measures of human impacts should be incorporated into sea turtle population models to evaluate their overall effect on sea turtle populations. Development of mitigation measures should be given high priority.

Conclusion

The research described in this section requires substantial investments of time, efforts, and funds. Resources are not available to support such studies on all populations of all sea turtle species. Thus, a high priority should be given to the development of predictive methods that employ more readily available data to address these research needs. Examples of such methods are the use of size-frequency data to estimate growth rates or the use of remote sensing to predict current-mediated movements of young, pelagic-stage sea turtles. Once validated, such techniques can have wide application. Also, representative populations should be selected for intensive studies and long-term monitoring. By focusing on such "index" populations, resources can be used most effectively.

Of course, the value of any of the above studies is only realized when the results are analyzed and published. Timely publication of research results should always be a high priority. Methods—such as regional databases—should be established so that data of regional significance can be shared, and interdisciplinary studies, which can focus broad areas of expertise on individual questions, should be encouraged.

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Community-Based Conservation

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Why Conserve Sea Turtles?

Sea turtles have been used since time immemorial for food (oil and protein) and other commodities (bone, leather, oil and shell). Their importance in trade dates back millennia, whether it was calipee, leather, live turtles, meat, oil or tortoise shell that was trafficked. Recently, sea turtles have become important for non-consumptive uses: tourism, educational and scientific research, activities that provide opportunities for employment and information services, as well as other economic gains.

A less apparent, but irreplaceable value is as ecological resources. These reptiles are unique components of complex ecological systems, the vitality of which is linked to exploitable products (including fishes, mollusks and mangroves) as well as to "ecosystem services" *(e.g.,* stabilizing coastal areas). Because they migrate thousands of kilometers and take decades to mature, turtles serve as important indicators of the health of coastal and marine environments on both local and global scales. In addition to their value as material resources, these animals have immeasurable worth as cultural assets. Diverse societies have traditionally held sea turtles as central elements in their respective customs and beliefs. Traditionally, to be an Arawak in Guyana, a Bajun in Kenya, a Concaac ("Seri") in Mexico, a Miskitu in Nicaragua, a Tahitian in Tahiti, or a Vezo in Madagascar, is to hunt and exploit sea turtles. In industrialized societies these reptiles also serve special functions; with their charismatic nature and intriguing life cycle, they are ideal for educational and research activities.

This means that marine turtles are model flagship species for both local and international conservation; by conserving these animals and their habitats, vast areas of the planet have to be taken into consideration, and managed adequately. In a word: conserving sea turtles means protecting the seas and coastal areas, which in turn means protecting a complex, interconnected world on which human societies depend.

Biological Conservation: What Needs to be Managed?

Wildlife management and biological conservation are as much managing people as managing wildlife: in the end, they are politics—not biology. Marine turtles have persisted for eons, prospering without protected areas, conservation laws, action plans, research manuals, and other accouterments of conservation programs. It is when people are involved, with over-exploitation and habitat perturbation, that biological conservation becomes essential. Anyone who benefits from sea turtles (either in consumptive or non-consumptive practices), or from their marine and coastal habitats, is a "stake holder," for they have vested interests in the condition of the resources. A basic necessity is that beneficiaries of resources be the stewards of those resources; it is to their advantage that these resources endure, and along with the rights of use, they have the responsibility of collaborating in conservation activities (see Marcovaldi and Thomé, this volume).

As a rule, "top-down" management is ineffective: no amount of laws, decrees, protected areas, action plans, lists of endangered species, or research projects will assure the conservation of an animal or its habitat—especially if it migrates over half the planet and takes decades to mature. Clearly, there must be norms regulating the use of common resources, but it is imperative that resource users be aware and supportive of these measures. Realistic conservation practices must be integrated with, and supported by, the communities that interact with the turtles and their habitats. It is fundamental to appreciate that the condition of the environment is intimately related to the status of human communities, and in many cases community-based conservation (CBC) is considered part of community development. CBC has become fashionable, but with good reason: it is essential for realistic, long-term conservation of shared resources.

CBC: A Philosophy and a Challenge

CBC is more a philosophy than a technique: there are few standard procedures, but instead a gamut of approaches to similar problems. Conceptual as well as material challenges are common: financial and other resources are rarely adequate, but these deficits are not specific to CBC. Perhaps most limiting are human resources: people who are trained, competent, interested, and available to make long-term commitments to CBC are themselves rarer than most endangered species. Conceptual issues are diverse, complex, and often foreboding. Because CBC is fashionable, many people will be attracted to it, some for less than honorable reasons. True CBC is not simple to accomplish. Developing "bottom-up" management is not only time-consuming, but often this process is resisted, undermined, or co-opted by people in power (PIP). A chronic problem is the difference between local interests for development and conservation, and those of PIP. Rarely do PIP comprehend the complex issues at the level of individual communities; indeed, their priorities are traditionally the concentration of power and control—not promoting democracy and empowerment. To begin with, traditional rights and responsibilities involved in resource use are rarely reflected in the legal structures of modern states, but instead exist as unwritten, even implicit, understandings at the community level, with culturally relevant forms of transmission and control.

Integration

CBC requires contributions from many disciplines, much wider than biology. This is not simply a matter of assembling a group of assorted specialists; a common language and conceptual foundation must be worked out, often beginning with disparate, fragmented, and isolated—even antagonistic—viewpoints. There must be a long-term commitment on the part of these "facilitators," who need to form a team among themselves, but also establish a partnership of mutual respect and understanding with the citizenry, for they must understand the capacity, limitations, needs, and desires of local inhabitants. This requires social integration and cultural sensitivity. Yet, facilitators must not beguile themselves into thinking that they are natives, and hence understand all the intricacies *(e.g.,* cultural, economic, familial, historic, political, social) of a community. It is normal for communities to be divided along diverse sociological axes, and internal conflicts are usual. At times it is unclear who are the members of a community. Thus, consent is not easy to achieve, and CBC requires full-time, long-term commitments, with unlimited patience on the part of the facilitators, essential for building confidence and consensus.

Considerations of Time

As a result, it is critical that sufficient time be allotted to CBC: to cut short a program, or the follow-up activities, presents a grave risk not only of misunderstandings and failure, but of long-term rejection of future conservation and development activities. At the same time, it is essential that facilitators be realistic and honest in regard to the duration and nature of their involvement with the community, and not lead people to believe that they will be there forever, solving problems. Paternalism must not be confused with true development: ***the goal of true CBC facilitators is to work themselves out of a job***. The romanticism about bucolic communities being "in balance with nature" must also be avoided, just as much as sanctioning poverty and under-development in the name of preserving "traditional lives" and "noble savages." Merely being rustic, or marginalized by modern society, is not the same as abiding by customs of environmental protection, nor being in favor of the long-term conservation of one's own resources. Hence, because CBC results are long in coming, some critical conservation issues need other approaches: CBC is rarely appropriate for quick resolutions of urgent issues.

Community and Participation

Participation by diverse sectors of the community is imperative to CBC, bearing in mind that the term "community" is a simplification, for any population will be divided into sectors and interest groups. While the involvement of all sectors is fundamental, the act of participating is a political process, and great care must be taken to insure that the participation process does not lead to distortions in power and access to resources. All members of the community must feel that there is an "open-door" policy to participate in

CBC activities, and that all negotiations and transactions be guileless and transparent. Guaranteeing full grassroots participation does not necessarily imply interacting with every single person in the community all the time; facilitators must respect the social structure, working through local leaders, organizers, and other principal actors. However, it is critical to distinguish true leaders and local "experts" from political appointees and "good scouts" who are seeking favors and advantages. Not all natives are native experts, and not all local "leaders" are accepted by their communities; some locals—just as many company executives and politicians—are skilled at self-projection by conforming to preconceived stereotypes and convincing outsiders of their importance.

Contemporary Challenges

Rural communities usually cause less destructive impacts on the environment than do urban populations; yet rural peoples are commonly caught between traditional-valued cultures and consumer-oriented social pressures. Societies, their cultures and traditions, are dynamic and evolve in time and in response to changes. However, contemporary communities are exposed to unprecedented alterations, both rapid and profound: human populations are expanding as never before, yielding burgeoning competition for resources; tentacles of the global market are everywhere, resulting in rampant resource depletion, global contamination, and environmental perturbation, with the consequent lack of access to basic resources, along with cultural homogenization. As a result, traditional practices, although relevant to former conditions, may be inappropriate to contemporary situations; alternatively, there may be acculturation and the loss—or even rejection—of traditional knowledge and values, which are appropriate for guiding the relationship between humans and the environment.

A primary objective in CBC of marine turtles is developing culturally acceptable practices that protect turtles and their habitats, and at the same time benefit coastal communities. Where exploitation and other activities that affect the turtles and their habitats are traditionally involved, this will ordinarily call for profound modifications to established practices. Clearly, if sea turtle populations have declined and their nesting and feeding areas are heavily perturbed, while at the same time human numbers are burgeoning, along with increases in per capita consumption, there is no way that turtle exploitation can be carried out as it was "in the old days." This is especially problematic in this age of "neoliberalized" and globalized economies: converting locally produced and consumed resources into commodities for world markets, while facilitating unrestricted access to resources and markets, rarely provides adequate compensation to the producers.

Developing Alternatives

The search for, and implementation of, "alternatives" is standard for CBC, and here there are more challenges. Alternatives must be acceptable to the people who are to use them; users must know what is involved, have the technical capacity to accomplish what is necessary, and the results must be beneficial to them, as well as meeting their expectations. Moreover, community leaders and authorities must be in accordance with the alternatives. There must be true collaboration in the development of conservation activities, empowering people as full participants with responsibility, not just as witnesses (or worse, ignoring, or even deceiving and/or dominating them). Just as important: the alternatives must be ecologically sound. For example, "ecotourism" is frequently offered as a "quick-fix" for solving conservation and economic problems of disadvantaged communities, but there are many considerations—both social and ecological—that must be resolved before this can be implemented as a viable alternative.

Even when both social and ecological requirements are met, community development projects do not exist in a steady state; changes in both socio-cultural and environmental aspects are common, often as a direct result of the conservation/development program. Since both societies and environments are dynamic in space and time, it could not be any other way! Each community has its own idiosyncrasies: historic, cultural, economic, political, and environmental, so there is no one formula or model for CBC or the development of alternatives.

The Challenge of Autonomy

While community self-sufficiency and self-rule are noble goals, facilitators must be realistic, and objectively appraise levels of social cohesion, as well as experience at administration and political organization. There are basic social and political requirements to be able to exist independently of the politically and economically ruthless systems which beset today's coastal communities. It is no trivial challenge for a group of relatively inexperienced, powerless people to resist the social and economic pressures of much larger and better-financed industrial and political entities, in which success is measured in terms of unlimited growth and conquest. It is usually necessary to facilitate the link between members of communities and PIP who operate in political and economic spheres with different—or foreign—cultural values. On the other hand, it is irresponsible to implement *everything* that is proposed by a community, just in the name of self-rule, particularly when there is sound evidence for long-term, negative consequences. Hence, community members must have access to fundamental information, as well as time and assistance in interpreting it and reflecting upon its relevance to their lives and families.

Training and Learning

Customarily, some form of capacity building is required so that community members can use newly acquired, or modified, alternatives and meet their needs and expectations—without causing environmental and social damage. Whenever possible and appropriate, local traditions and practices should be included, or rehabilitated, in conservation plans and actions; this is particularly true for environmental education. It is crucial to understand both local lore and basic sea turtle biology to be able to integrate indigenous knowledge and beliefs with scientific explanations: it is also essential to have the objectivity and humility to listen to and learn from unlettered people. However, it is just as important to facilitate social and political organization in the community, which in the end means the development of leaders and political structures. The distinction between true CBC and king-making is very gray, and great caution and integrity are required in this arena; the institution of clear processes for accountability is critical. As facilitators are both sources and conduits to limited resources *(e.g.,* money, information, and PIP), the distribution of their services must take into account the heterogeneity of the community.

Priorities

As there is no end of social and environmental problems, the focus of CBC must be toward solving root problems and not treating symptoms. Hence, the community chosen, the geographic area, the social dilemmas to be grappled with, and the conservation issues to be resolved *(e.g.,* species and ecosystems) should be objectively evaluated, so that the limited resources invested in the project will have the greatest conservation and social impact in both time and space. Making full use of the “multiplier effect” is fundamental, in which competent teachers and leaders train more of their respective types.

Conclusions

The goal of CBC is to integrate community development with the conservation of culture and traditions, while simultaneously protecting the environment and resource base; this entails promoting the use of resources without reducing their long-term value, in economic, social, and ecological terms. Success can be evaluated by the availability of exploited resources as well as ecosystem services, the persistence of species *(e.g.,* exploited, keystone, or endangered) and the maintenance of culturally important landscapes. It also means a greater degree of self-sufficiency and self-determination for the community on all fronts: economic, social, cultural, political, etc. In the end, the common long-term motivation for communities to conserve their common resources is the fate of their future generations. Despite the enormity of the challenge of CBC, it provides unique rewards and satisfaction to those who nurture the process.

Summary

Despite the need to develop case-by-case actions for CBC, several generalities can serve as specific steps: define the problem (bearing in mind the social and political ramifications); construct realistic goals, together with means of objective evaluation for both short and long term; identify local stake holders as well as other key players; evaluate attitudes, and appraise agendas (stated and hidden) of all players; appraise gains and losses of different parties (both measurable and unmeasurable but perceived); develop realistic strategies and alternatives working through consensus, keeping in mind the challenges of integration, time constraints, etc.; develop forms of communication and symbols that are relevant and effective, including capacity building; keep the process open and participatory; avoid romanticism and paternalism.

Acknowledgments

This synthesis has benefited from an incomparable diversity and wealth of experiences, ideas, concepts, and criticisms shared during annual Reunions of Latin American Marine Turtle Specialists. In addition, Margarita Alba, Ma. Dolores Cervera M., Betty B. Faust, Brian Miller, Charles Tambiah, Peter Richardson, and Chris Wemmer made valuable comments on earlier versions.

2

Taxonomy and Species Identification

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Taxonomy, External Morphology, and Species Identification

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Scientific and Vernacular Names

There are a great many vernacular names for most sea turtle species, although relatively few for the more restricted species *(Natator depressus, Lepidochelys kempii)*. In this section, only three examples (English, French, and Spanish) are given for each species.

Family Dermochelyidae	
Dermochelys coriacea:	Leatherback (E); Tortue luth (F); Tortuga laúd (S)
Family Cheloniidae	
Chelonia mydas:	Green turtle (E); Tortue verte (F); Tortuga verde (S)
Chelonia mydas / C. agassizii / C. m. agassizii [1]:	Black turtle (E); Tortue noire (F); Tortuga prieta (S)
Natator depressus:	Flatback turtle (E); Chelonée à dos plat (F); Tortuga aplanada (S)
Eretmochelys imbricata:	Hawksbill (E); Tortue imbriquée (F); Tortuga de carey (S)
Caretta caretta:	Loggerhead (E); Caouanne (F); Caguama (S)
Lepidochelys kempii:	Kemp s ridley (E); Chelonée de Kemp (F); Tortuga lora (S)
Lepidochelys olivacea:	Olive ridley (E); Chelonée olivâtre (F); Tortuga golfina (S)

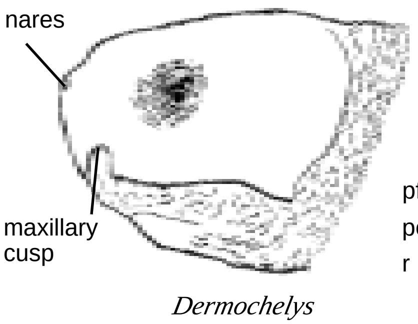
1 *Authors Note:* Valid arguments can be presented both in favor and against the designation of the Black turtle as a full species within the genus *Chelonia*; namely, *Chelonia agassizii*. On balance, we support the full species concept because we believe it meets the traditional criteria of degree of morphological divergence and probable existence of reproductive isolation mechanisms, and because the science of objective interpretation of revealed differences in genotype and their relationship to systematics is still evolving. Others disagree. For insight into the continuing debate the reader is referred to Pritchard (1996, 1999), Bowen and Karl (1996) and Karl and Bowen (1999).

Editors Note: The systematic status and nomenclature of the Black turtle or east Pacific green turtle, sometimes referred to as *Chelonia agassizii* or *C. mydas agassizii*, remains uncertain. Recent genetic evidence supports an Atlantic-Mediterranean vs. Indian-Pacific grouping, while morphological and behavioral data suggest an east Pacific species or subspecies. Cognizant of the unfinished scientific debate and aware of the fact that the IUCN does not at the present time recognize the Black turtle as a species (or subspecies) of *Chelonia*, this manual adopts a conservative *status quo* position; namely, that there are seven species of sea turtle and the agassizii type is embraced within the global *Chelonia mydas* complex. At the present time the MTSG has no formal position on the ongoing debate, but is strongly supportive of research in this area.

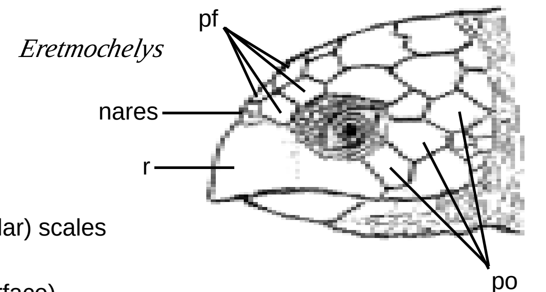
Illustrations: Tom McFarland provided the illustrations for Figures 4-11 and 13. Figures 1, 2, 12 and 14 were modified by J. Mortimer from original illustrations by T. McFarland. The authors are most grateful for T. McFarland s contribution to this chapter.

External Morphological Structures and Taxonomic Characters

Figures 1 and 2 illustrate some of the external morphological structures used to identify sea turtles to species. These structures can also be used to reference a specific point on the body of a turtle—such as the exact location of an injury, scute anomaly, etc. Where a series of multiple scutes or scales each have the same name *(e.g.,* vertebral, marginal, etc.) individual scutes can be differentiated by numbering them from anterior to posterior and by noting right or left side of the body *(e.g.,* sixth right marginal scute).



pf = prefrontal scales
po = postorbital (postocular) scales
r = rhamphopheca (tomium is biting surface)



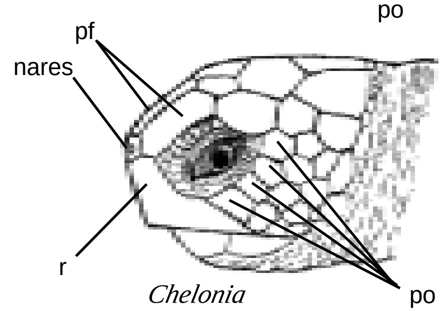


Figure 1. Anatomical features of sea turtle heads noting the location of the prefrontal and postorbital scales which are diagnostic in the identification of some species. Note two pairs of prefrontals in *Eretmochelys* and one pair in *Chelonia* and three pairs of postorbitals in *Eretmochelys* and (usually) four pairs in *Chelonia.* Adult *Dermochelys* lack head scales.

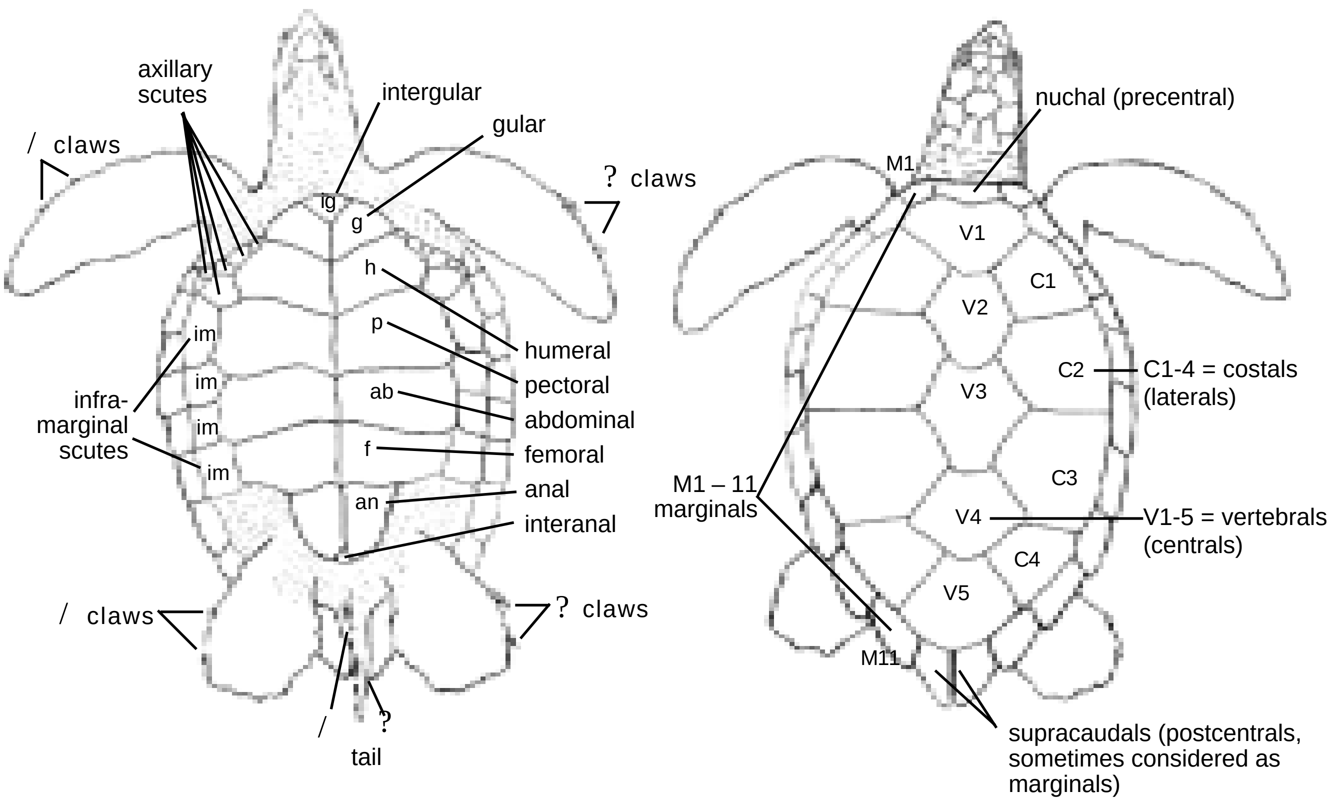


Figure 2. An illustrated guide to external morphological features of sea turtles, including scutes of the plastron (lower shell) and carapace (upper shell). Where scutes have more than one name, alternative names are provided in parentheses. The secondary sexual characteristics indicated are visible only in adult turtles. Note that inframarginal scutes span the distance between the marginal and large plastron scales (h, p, ab, f).

Simplified Key to Adult and Subadult Turtles Viewed in the Wild

The following key is designed to identify subadult or adult turtles spotted briefly at the ocean surface from a boat or an airplane, or seen by a diver underwater. To further assist such identifications, Figure 3 depicts dorsal silhouettes of the various sea turtle species.

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1. Leathery, scuteless black or spotted carapace, posteriorly pointed and with prominent longitudinal ridges; carapace length to about 180 cm; all oceans, temperate or tropical .... ***Dermochelys coriacea***

1'. Carapace hard with large scutes, rounded or elongate but not posteriorly pointed; carapace length less than 120 cm .... see 2

2. Carapace wide and almost circular; head width to about 15 cm; dorsal coloration gray to olive-green, unmarked; maximum carapace length to about 70 cm .... see 3

2'. Carapace not so wide as to be almost circular; coloration variable; maximum carapace length to 120 cm .... see 4

3. Carapace very flat and wide, coloration relatively light, juveniles gray, circular in outline; maximum carapace length 72 cm; Gulf of Mexico, eastern USA, vagrant of western Europe .... ***Lepidochelys kempii***

3'. Carapace relatively steep-sided, especially in eastern Pacific; typically dark olive; juveniles gray, circular in outline (similar to *L. kempii);* maximum carapace length 72 cm; Pacific, Indian and South Atlantic Oceans (Trinidad to Brazil; West Africa) .... ***Lepidochelys olivacea***

4. Head very large (width up to 28 cm in adults); carapace broadest anteriorly, elongate, and posteriorly narrowed, with a hump at the fifth vertebral scute; color uniform reddish-brown; maximum carapace length 105 cm; usually temperate waters of all oceans, including Mediterranean and US Atlantic, occasionally in tropics ... ***Caretta caretta***

4'. Head not very large (width to 12-15 cm in adults); carapace not broadest anteriorly, lacking hump at fifth vertebral scute; color variable, carapace often boldly marked, typically with dark brown or black streaks, or plain olive; tropical seas .... see 5

5. Head small, anteriorly rounded; carapace heart-shaped .... see 6

5'. Head either very narrow and anteriorly pointed or medium and broadly triangular; carapace either relatively narrow or broadly oval .... see 7

6. Carapace smooth and wide (modest incurving above hind limbs), coloration variable but usually with radiating streaks, or spots in some large adults; maximum carapace length 120 cm; tropics and subtropics, all oceans .... ***Chelonia mydas***

6'. Carapace typically narrowed by strong incurving above hind limbs, color almost black, plain or spotted; carapace length to 90 cm, usually less; eastern Pacific, with rare vagrants further west .... ***Chelonia*** sp. (Black turtle)

7. Head narrow, with pointed bird-like beak (head width to 12 cm); carapace relatively narrow and lacking upturned sides, often well marked, scute borders obvious and overlapping, posterior margin of carapace usually strongly serrated; carapace length to about 90 cm; tropical waters, all oceans .... ***Eretmochelys imbricata***

7'. Head broadly triangular and relatively flattened (width to 15 cm); carapace broadly oval, very flat with upturned sides, without markings, scute borders often indistinct, and edges of shell smooth; carapace length to about 100 cm; tropical Australia .... ***Natator depressus***

Identification of Sea Turtles Available for Close Examination

If the turtle is in hand, otherwise constrained, or stranded (dead) on the coast, it is appropriate to utilize the more detailed descriptions on the following pages in Figures 4-11 to confirm the identification. On very rare occasions, cheloniid turtles of different genera may hybridize. Typically, the offspring are morphologically intermediate between their parents.

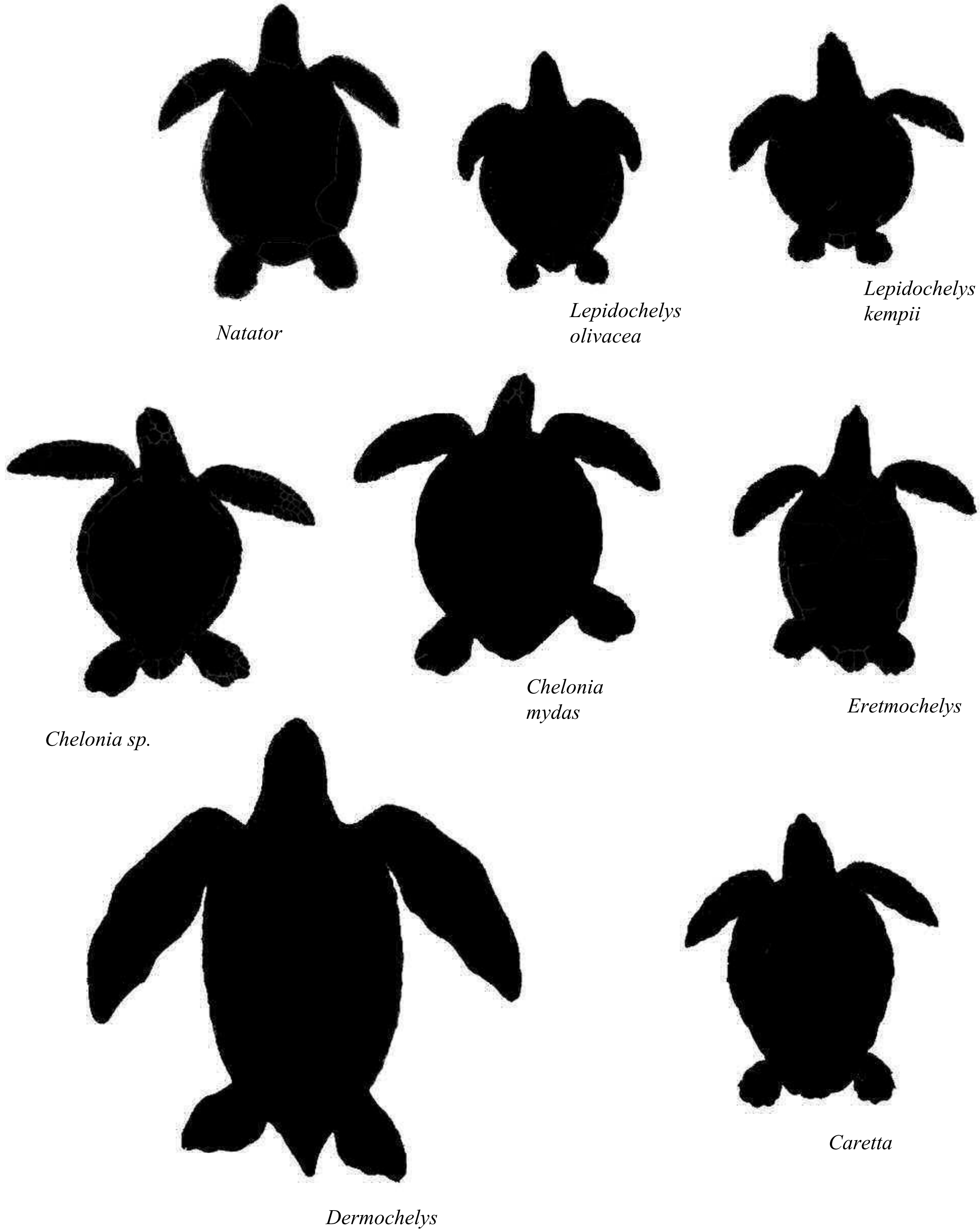


Figure 3. Sea turtle silhouettes viewed from a distance; sizes are relative for adult turtles

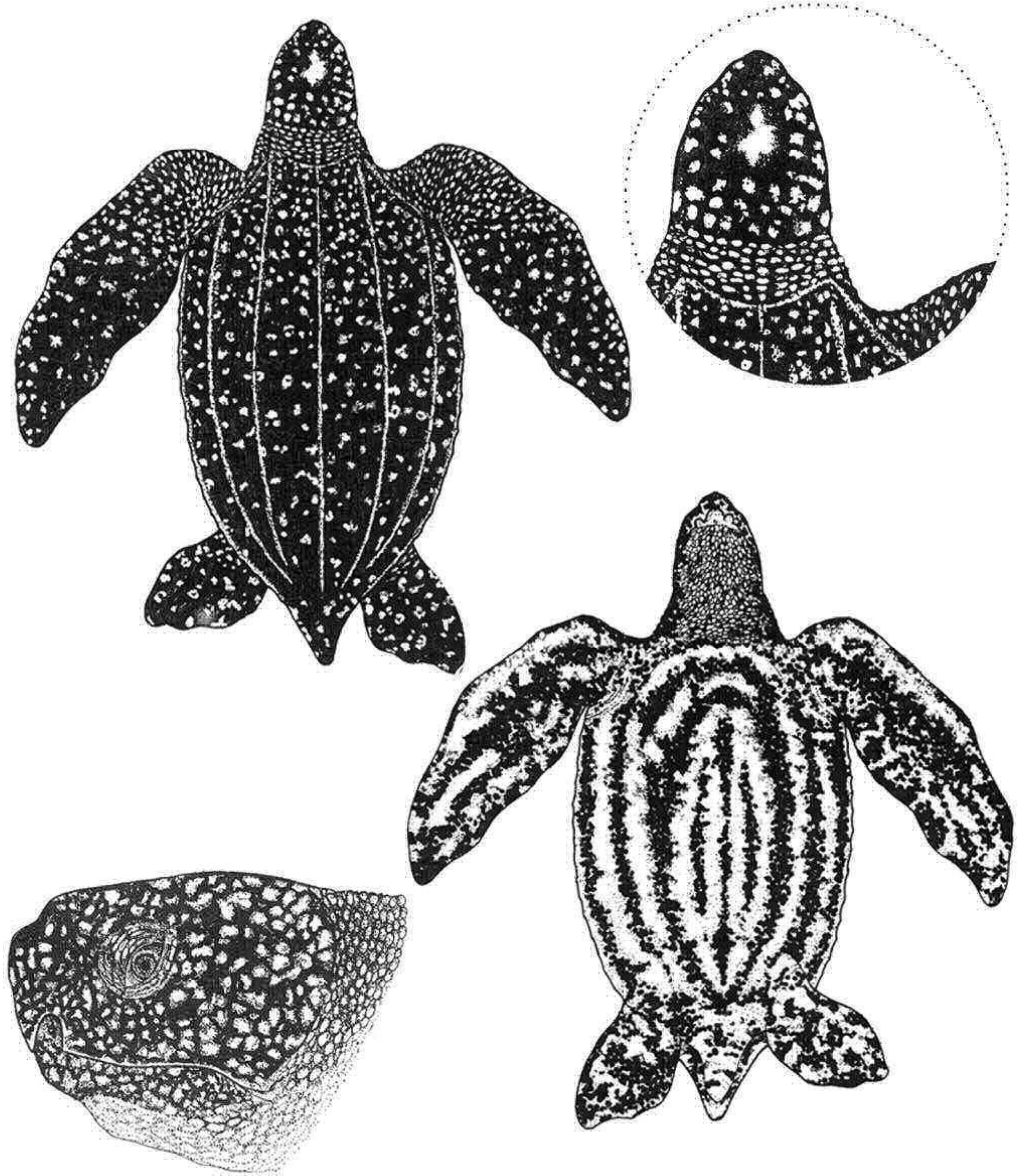


Figure 4. Leatherback turtle (*Dermochelys coriacea*).

Carapace: elongate with seven prominent longitudinal ridges (keels); scutes always absent; adults with smooth skin, but hatchlings covered with small bead-like scales; straight carapace length (SCL) to 180 cm (to 165 cm in east Pacific). **Head:** shape broadly triangular; width to 25 cm; two prominent maxillary cusps, covered with unscaled skin in adults. **Limbs:** forelimbs extremely long; unscaled skin in adults; all limbs clawless. **Coloration:** dorsally predominantly black, with variable degrees of white or paler spotting; spots may be bluish or pink on neck and base of flippers; light pigment predominating on plastron. **Plastron:** relatively small, distensible (with very little bone). **Distribution:** all oceans, sub-arctic to tropical. **Weight:** adult females to 500 kg in the western Atlantic, less in eastern Pacific.

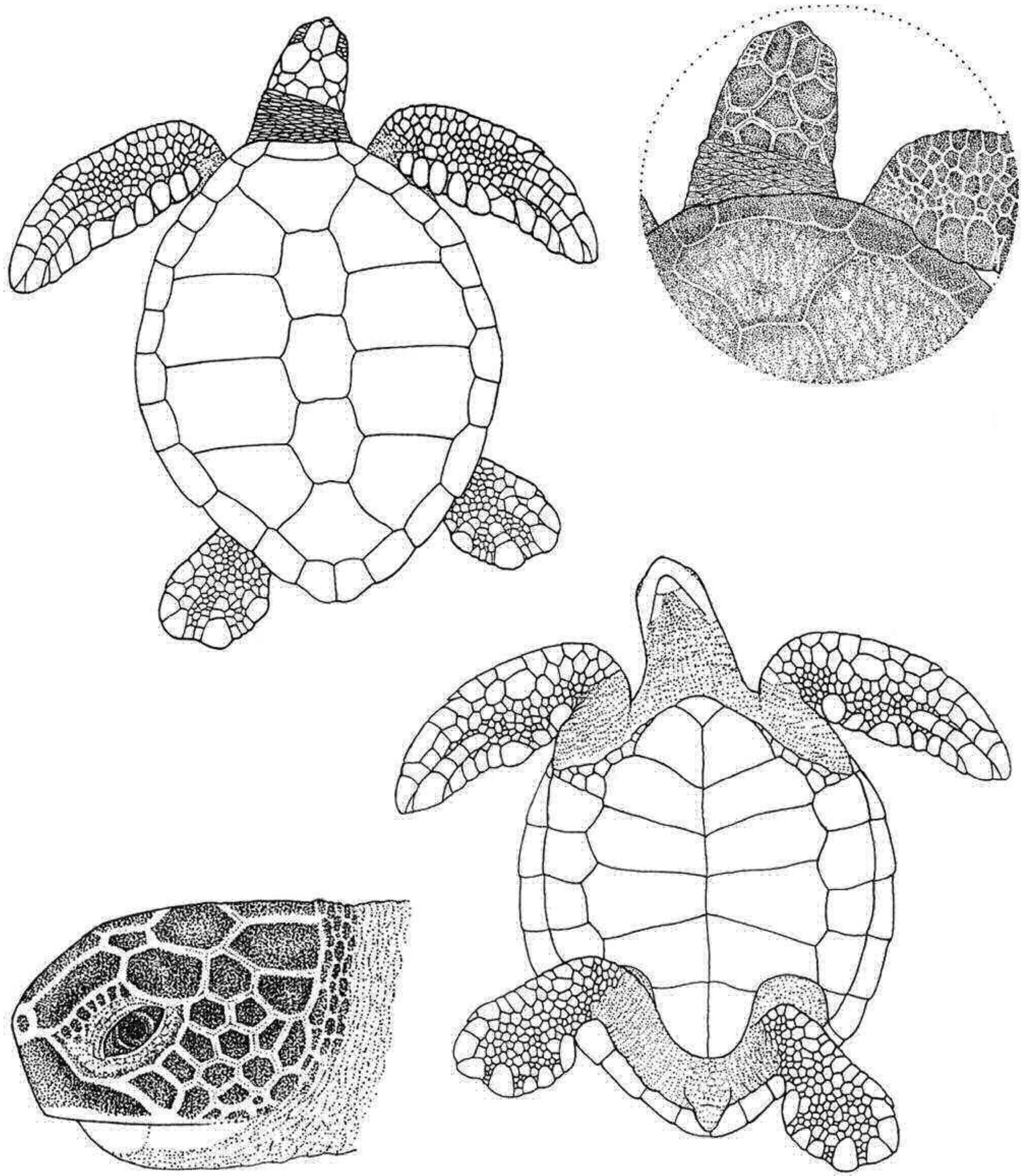


Figure 5. Green turtle (*Chelonia mydas*).

Carapace: broadly oval, margin sometimes scalloped but not serrated, and not incurved above hind limbs; four pairs of costal scutes; straight carapace length (SCL) to about 120 cm. **Head:** anteriorly rounded; width to 15 cm; one pair of prefrontal scales; four pairs of postorbital scales. **Limbs:** single claw on each flipper (rarely, two in some hatchlings). **Coloration:** dorsally black in hatchlings, becoming brown with radiating streaks in immatures, very variable in adults (generally brown, buff, and other earth tones; plain streaked or spotted); underside white in hatchings, yellowish in adults. **Distribution:** all sub-tropical and tropical seas. **Weight:** to about 230 kg in the Atlantic and western Pacific Oceans, less in the Indian Ocean and the Caribbean.

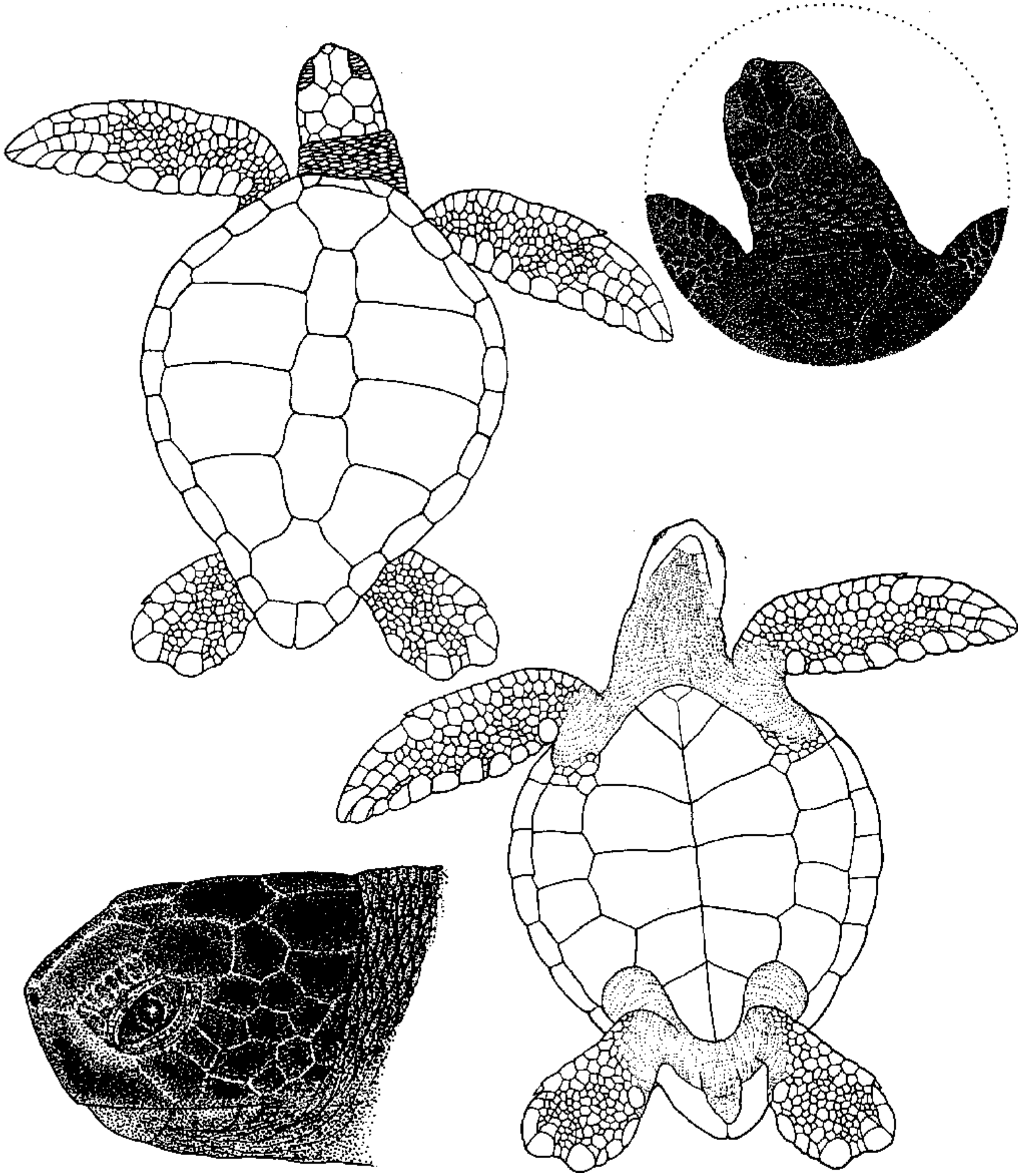


Figure 6. Black turtle ***(Chelonia sp.).***

Carapace: heart-shaped and posteriorly tapered in adults; not serrated; often tectiform (tent-shaped) and flat-topped in anterior profile; four pairs of costal scutes; straight carapace length (SCL) to about 90 cm. **Head:** anteriorly rounded; width to 13 cm; one pair of prefrontal scales; four pairs of postorbital scales most common (followed by three pairs). **Limbs:** limbs may be relatively longer than in other *Chelonia* populations; single claw on each flipper. **Coloration:** dorsally black in hatchlings, remaining dark throughout life; adults may be uniformly black above or with black spots or other markings on a greyish background; underside white in hatchlings but within a few weeks or months becoming infused with gray pigment. **Distribution:** East Pacific Ocean. **Weight:** to about 120 kg (average adult about 70 kg).

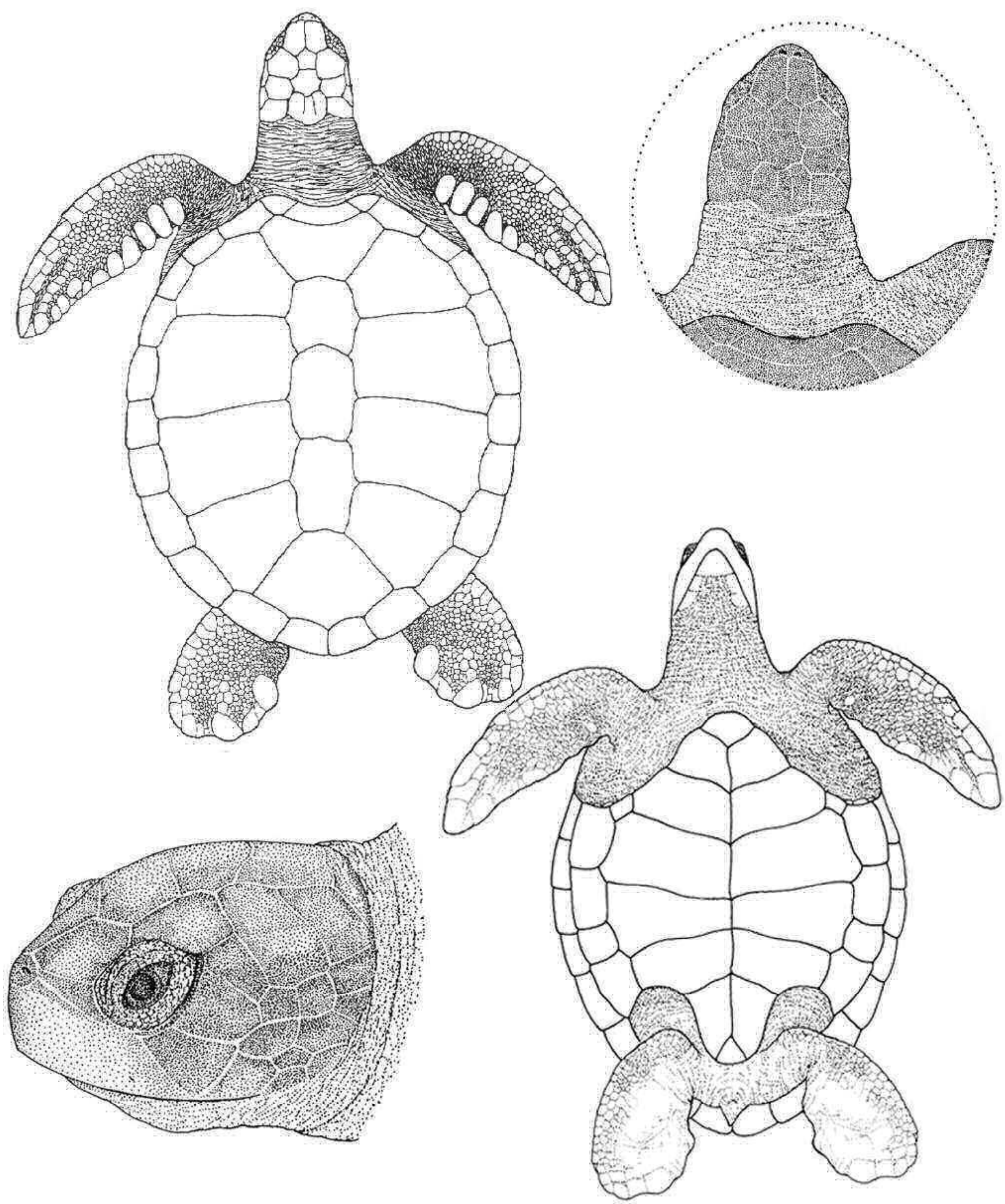


Figure 7. Flatback turtle *(Natator depressus).*

Carapace: very broad and rounded, with upturned lateral margins; four pairs of costal scutes; scutes very thin and with a softer texture than in other cheloniid turtles, with seams often disappearing in old adults; curved carapace length (CCL) to about 100 cm. **Head:** wide, broad, flat and subtriangular in shape; width to 13 cm in adults; three pairs of postorbital scales; one pair of prefrontal scales. **Limbs:** large scales present only on the edges of the front flippers, with most of the flipper covered by wrinkled skin or very fine scales; single claw on each flipper. **Coloration:** dorsally uniform olive-green in hatchlings and adults; yellowish ventrally. **Distribution:** confined to waters of tropical Australia and possibly southern New Guinea. **Weight:** to about 90 kg.

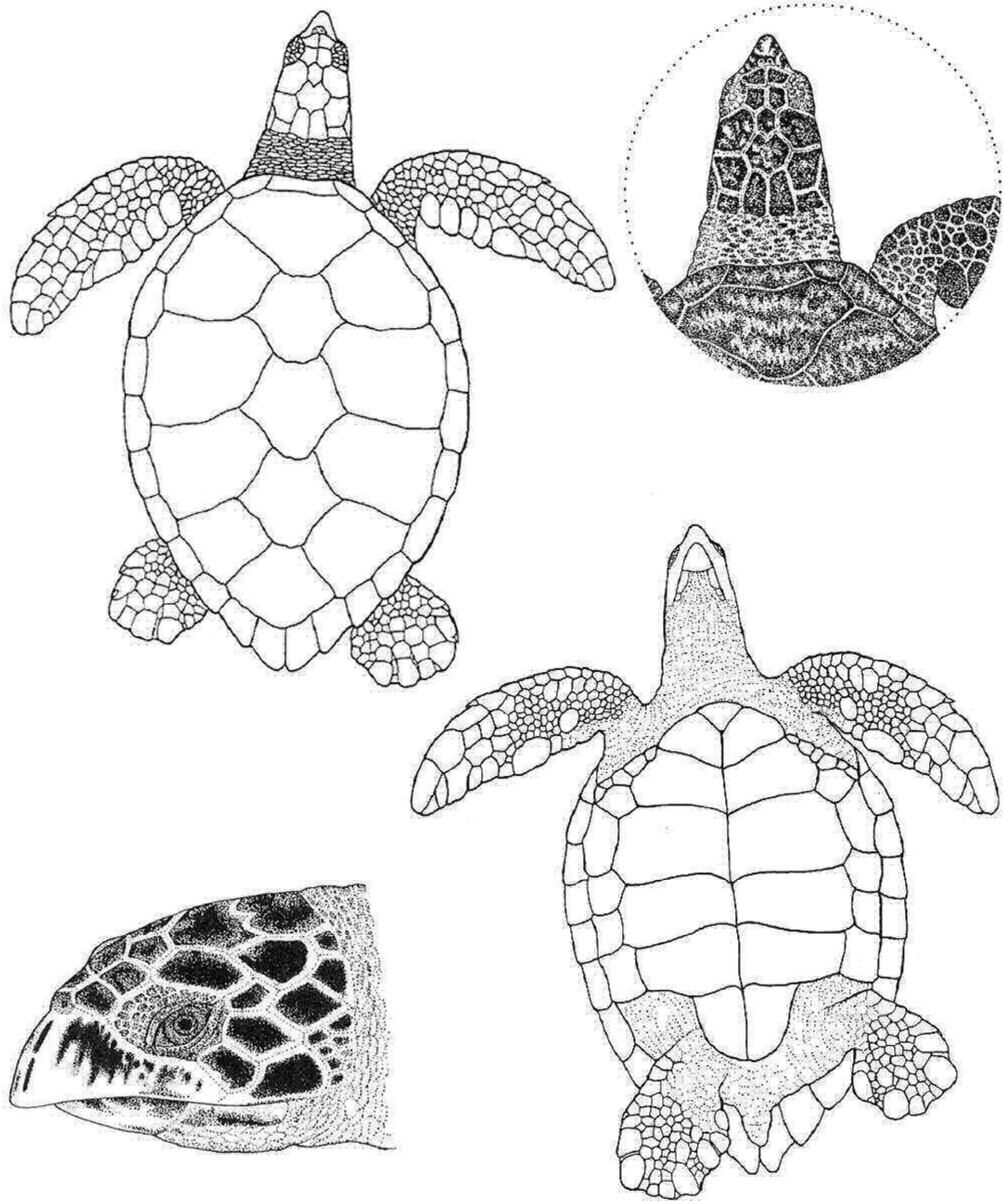


Figure 8. Hawksbill turtle *(Eretmochelys imbricata).*

Carapace: oval, with a strongly serrated posterior margin and thick overlapping (imbricate) scutes (except in hatchlings and some adults); four pairs of costal scutes, each with a slightly ragged posterior border; straight carapace length (SCL) to about 90 cm. **Head:** relatively narrow; width to 12 cm; with a straight bird-like beak; two pairs of prefrontal scales. **Limbs:** front flippers are medium length compared to other species; two claws on each flipper. **Coloration:** dorsally brown (dark to light) in hatchlings, often boldly marked with amber and brown variegations in juveniles and younger adults; underside light yellow to white, sometimes with black markings (especially in Pacific specimens). **Plastron:** four pairs inframarginal scutes. **Distribution:** all oceans, tropical waters. **Weight:** to about 80 kg (average about 60 kg).

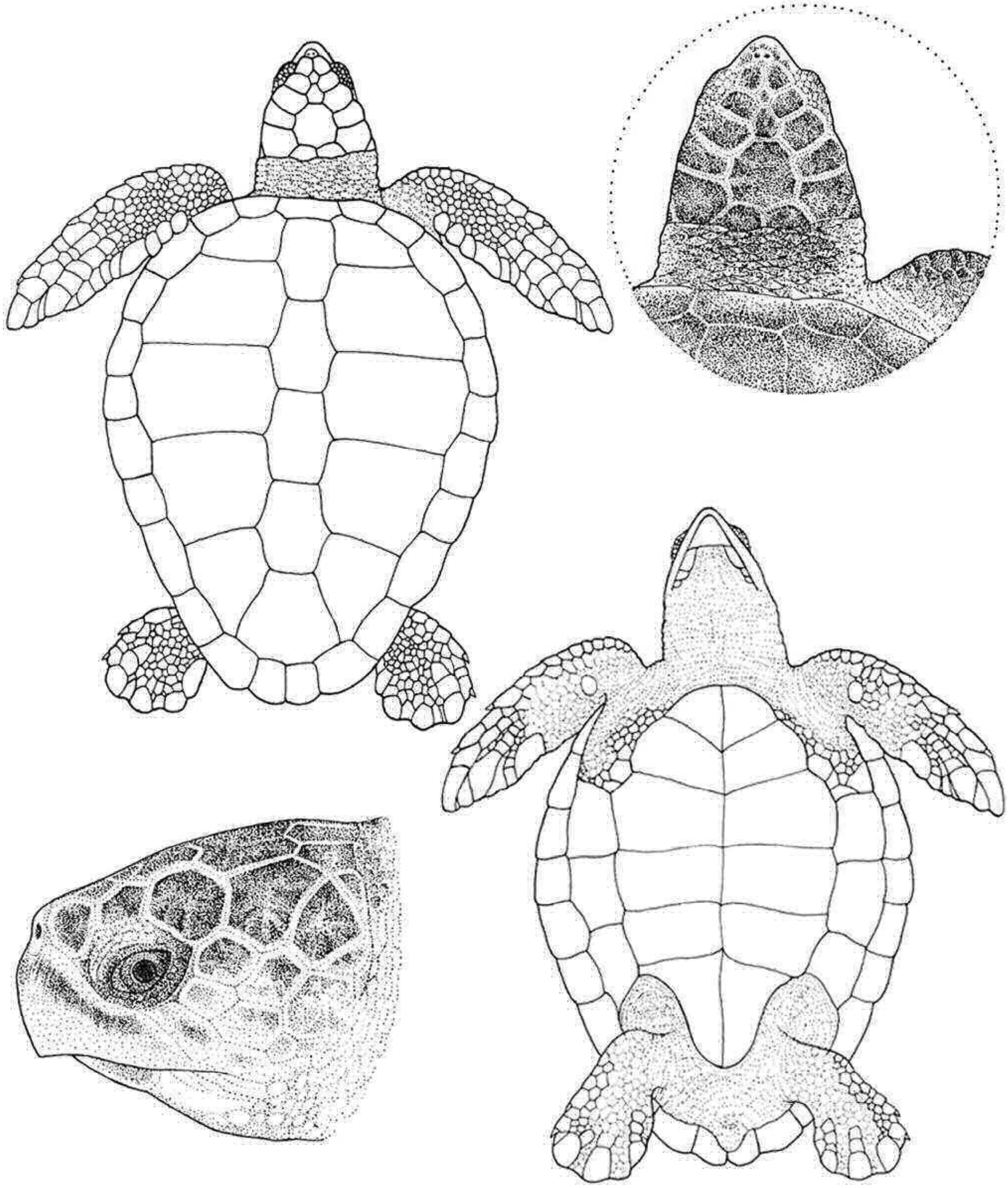


Figure 9. Loggerhead turtle *(Caretta caretta).*

Carapace: moderately broad; lightly serrated posterior margin in immatures; thickened area of the carapace above the base of the tail (at the fifth vertebral) in subadults and adults; five pairs of costal scutes, the first (anterior) pair the smallest; straight carapace length (SCL) to about 105 cm in northwestern Atlantic, smaller in some other areas, the smallest adults being in the Mediterranean (to about 90 cm). **Head:** large and broadly triangular in shape; width to 28 cm; two pairs of prefrontal scales. **Limbs:** front flippers relatively short compared to other species; two claws on each flipper. **Coloration:** dorsally light to dark brown in hatchlings, generally unmarked reddish-brown in subadults and adults; underside brown in hatchlings, yellow to orange in subadults and adults. **Plastron:** three pairs inframarginal scutes. **Distribution:** all oceans, usually temperate waters, sometimes subtropical and tropical. **Weight:** to about 180 kg in the western Atlantic and to about 150 kg in Australia; less than 100 kg in the Mediterranean.

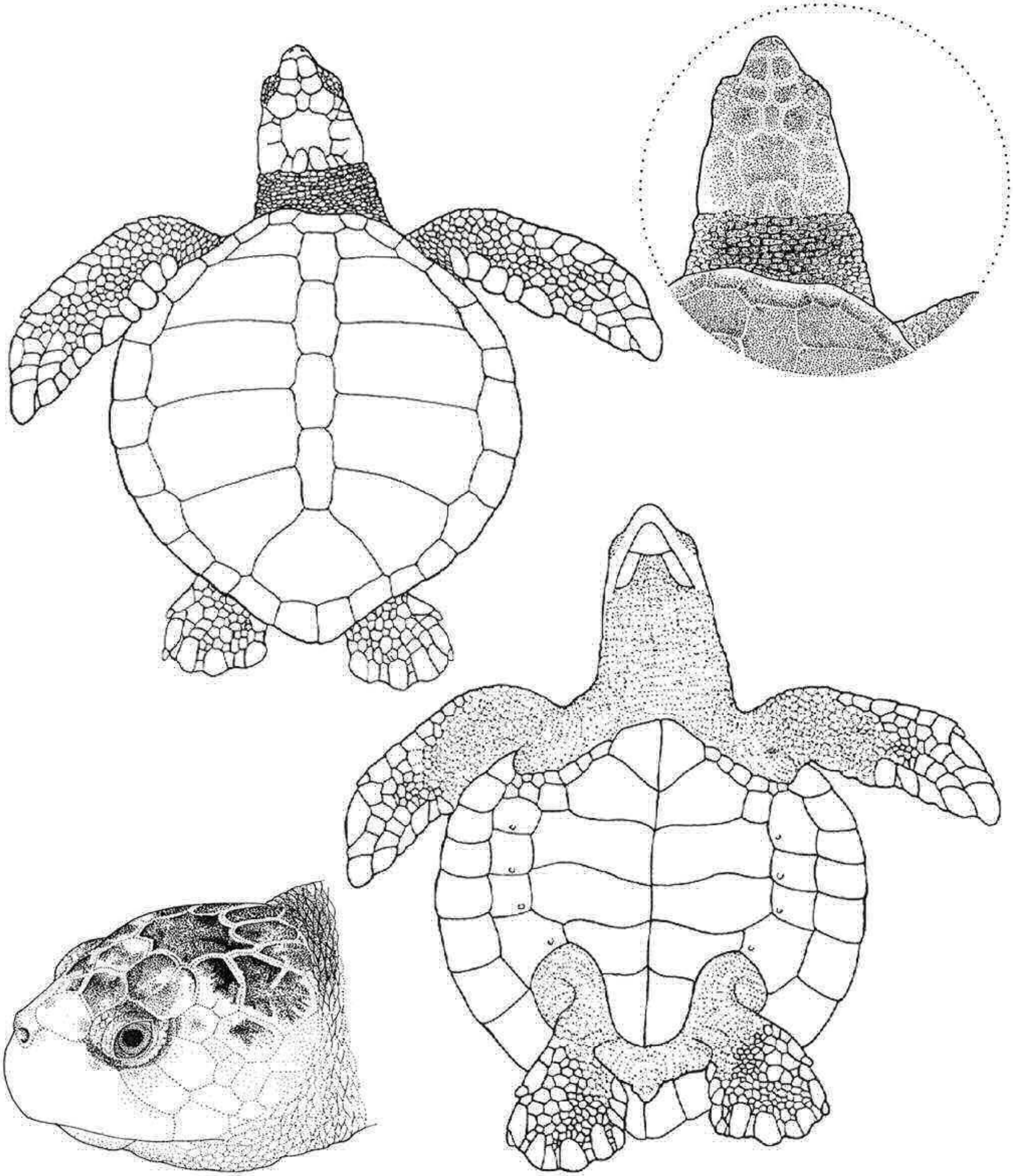


Figure 10. Kemp s Ridley turtle *(Lepidochelys kempii).*

Carapace: relatively short and wide, almost circular (wider in adults than that of *L. olivacea);* modest marginal serration or scalloping; high vertebral projections in juveniles, but carapace smooth and low in adults; carapace scutes slightly overlapping in immatures, and non-overlapping in adults; five pairs of costal scutes; straight carapace length (SCL) to 72 cm. **Head:** relatively large, subtriangular with convex sides; width to 13 cm; two pairs of prefrontal scales. **Limbs:** two claws on each flipper (some adults may lose the secondary claw on the front flippers). **Coloration:** dorsally grey in immatures, light olive-green in adults; underside white in immatures, yellow in adults. **Plastron:** a distinct, small pore near rear margin of each of the four inframarginal scutes. **Distribution:** Gulf of Mexico, eastern USA, occasionally western Europe. **Weight:** typically 35-50 kg.

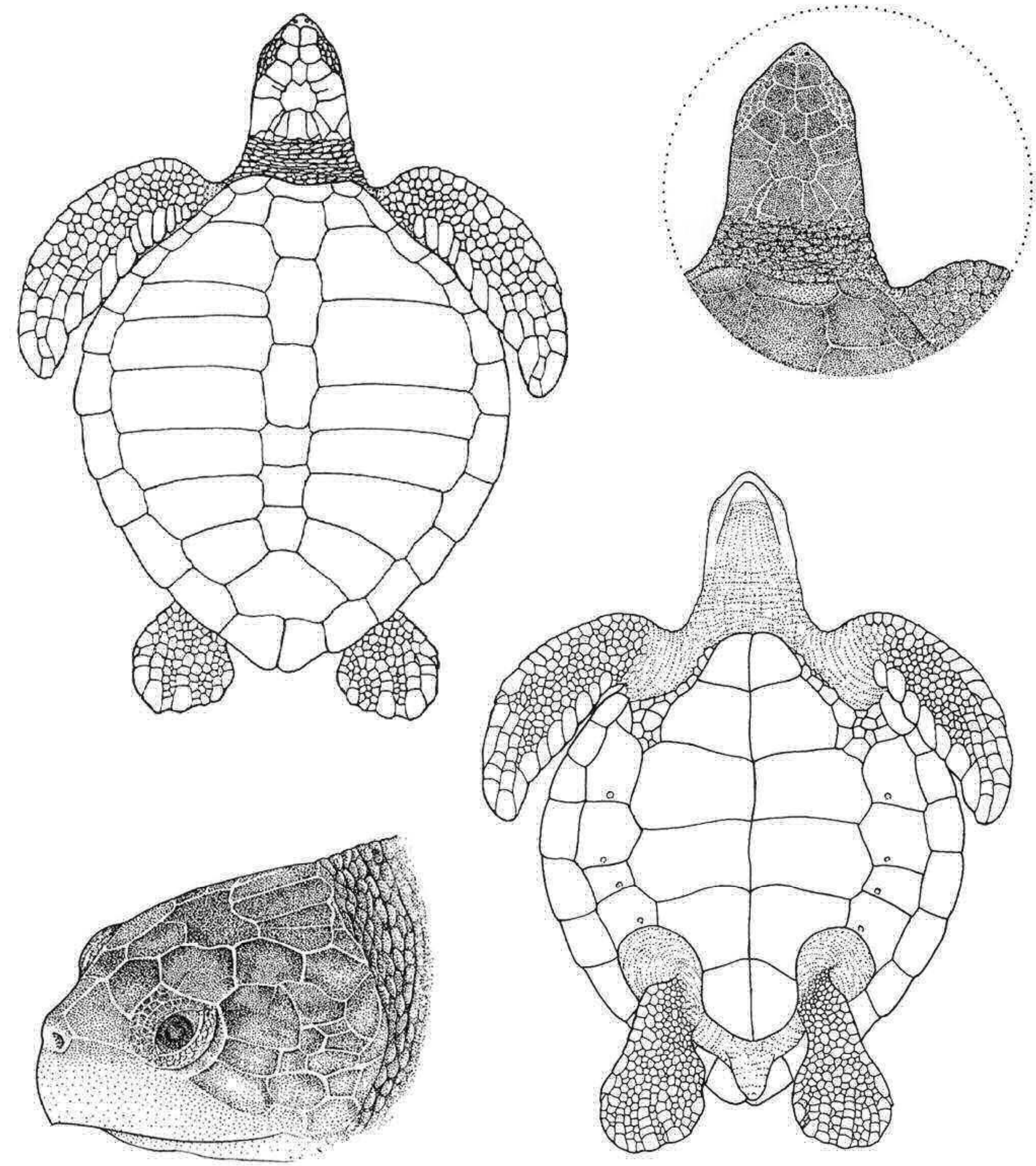


Figure 11. Olive Ridley turtle *(Lepidochelys olivacea).*

Carapace: short and wide, but narrower and higher than in *L. kempii;* high vertebral projections in juveniles; carapace smooth but elevated and somewhat tectiform (tent-shaped) in adults (especially in the East Pacific); five to nine pairs of costal scutes (usually six to eight) often with asymmetrical configuration; carapace scutes slightly overlapping in juveniles, non-overlapping in adults; straight carapace length (SCL) to 72 cm. **Head:** relatively large, triangular from above; width to 13 cm; two pairs of prefrontal scales. **Limbs:** two claws on each flipper (some adults may lose the secondary claw on the front flippers). **Coloration:** dorsally grey in immatures, mid to dark olive-green in adults; underside white in immatures, cream-yellow in adults. **Plastron:** a distinct, small pore near rear margin of each of the four inframarginal scutes. **Distribution:** tropical waters of Pacific, Indian and South Atlantic Oceans. **Weight:** typically 35-50 kg.

Key to Identification of Hatchlings

The key characters for identifying hatchlings (apart from color) are similar to those used for subadults and adults, although samples of hatchlings show greater variation in the numbers and configuration of the carapace scutes. Following is a species identification key for hatchling turtles. The composite drawings in Figures 12 and 13 portray relative differences in size and color among the sea turtle species as well as other diagnostic features.

---

1. Carapace covered with large horny plates; longitudinal carapace ridges, if present, not more than three in number and not of contrasting color; forelimbs much shorter than length of carapace; plastron color uniform or nearly so; carapace usually less than 60 mm (except in *Natator)* .... see 2

1'. Entire surface of the animal (carapace, plastron and extremities) covered with small, soft, polygonal scales; seven longitudinal carapace ridges (including edges of shell) boldly outlined in white against a black background; forelimbs extremely long (almost as long as the carapace); plastron mottled black and white; typical carapace length (SCL) 60 mm (range 55-63 mm) .... ***Dermochelys coriacea***

2. Ventral coloration light; costal scutes four pairs .... see 3

2'. Ventral coloration dark; costal scutes four to nine pairs .... see 5

3. Overall coloration light: dorsum light olive-yellow and plastron white with a peripheral yellow band; broadly oval carapace; postorbital scales three pairs; relatively large size, typical carapace length (SCL) 61 mm (range 56.5-65.5 mm); tropical Australia .... ***Natator depressus***

3'. Carapace black or blue-black, typically with a white margin; plastron white; heart-shaped carapace (some posterior narrowing); postorbital scales usually four pairs (but sometimes three); typical carapace length smaller than *Natator;* tropical and subtropical seas including Australia .... see 4

4. Forelimbs outlined in white; head scales blackish with narrow light (whitish) borders; postorbital scales usually four pairs; plastron pure white; typical carapace length (SCL) 49 mm (range 46-57 mm); distribution tropical and subtropical, not East Pacific region .... ***Chelonia mydas***

4'. Forelimbs and head scales sometimes outlined in white, but white edges may be reduced or absent; postorbital scales typically four pairs (but sometimes three); plastron initially white, but soon darkens; typical carapace length (SCL) 47 mm (range 41-52 mm); distribution Galapagos Islands and Meso-America ...... ***Chelonia*** sp. (Black turtle)

5. Color brown (dark to light) above and below; inframarginal scutes typically three or four pairs .... see 6

5'. Color very dark gray to black above and below; inframarginal scutes typically four pairs .... see 7

6. Costal scutes four pairs; oval carapace; inframarginal scutes typically four pairs; typical carapace length (SLC) 42 mm (39-46 mm) .... ***Eretmochelys imbricata***

6'. Costal scutes five pairs; carapace broader in shoulder region than in *Eretmochelys;* inframarginal scutes typically three pairs; typical carapace length (SLC) 45 mm (38-50 mm) .... ***Caretta caretta***

7. Costal scutes usually five pairs; typical carapace length (SLC) 43 mm (38-46 mm); expected distribution of hatchlings Tamaulipas, Vera Cruz and South Texas (rare strays in southeastern USA) .... ***Lepidochelys kempii***

7'. Costal scutes usually six to nine pairs (sometimes five); typical carapace length (SLC) 42 mm (38-50 mm); distribution circumtropical, mostly mainland shore, not Gulf of Mexico and east USA .... ***Lepidochelys olivacea***

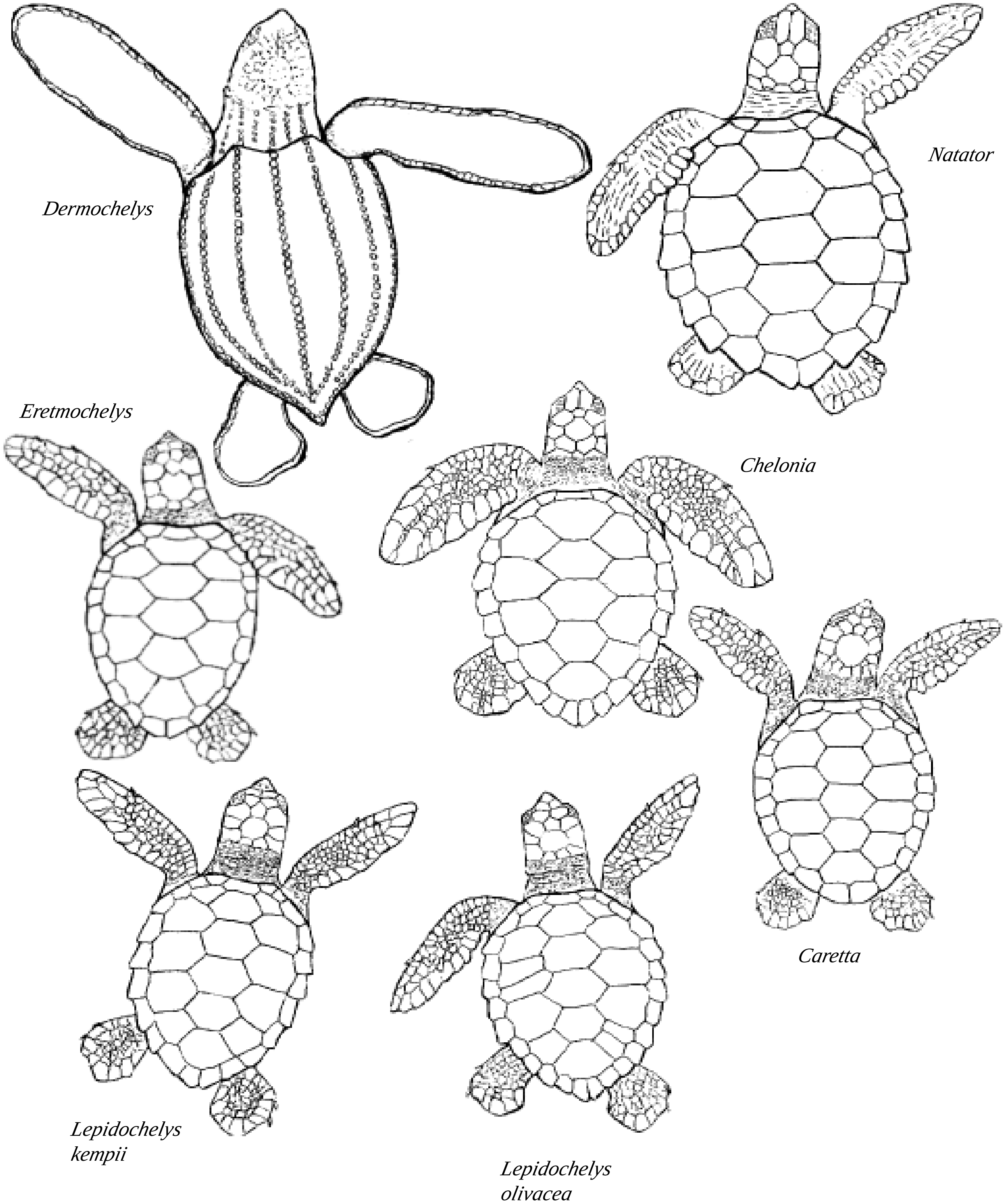


Figure 12. Sea turtle hatchlings of seven species. Sizes are 80% of actual size.

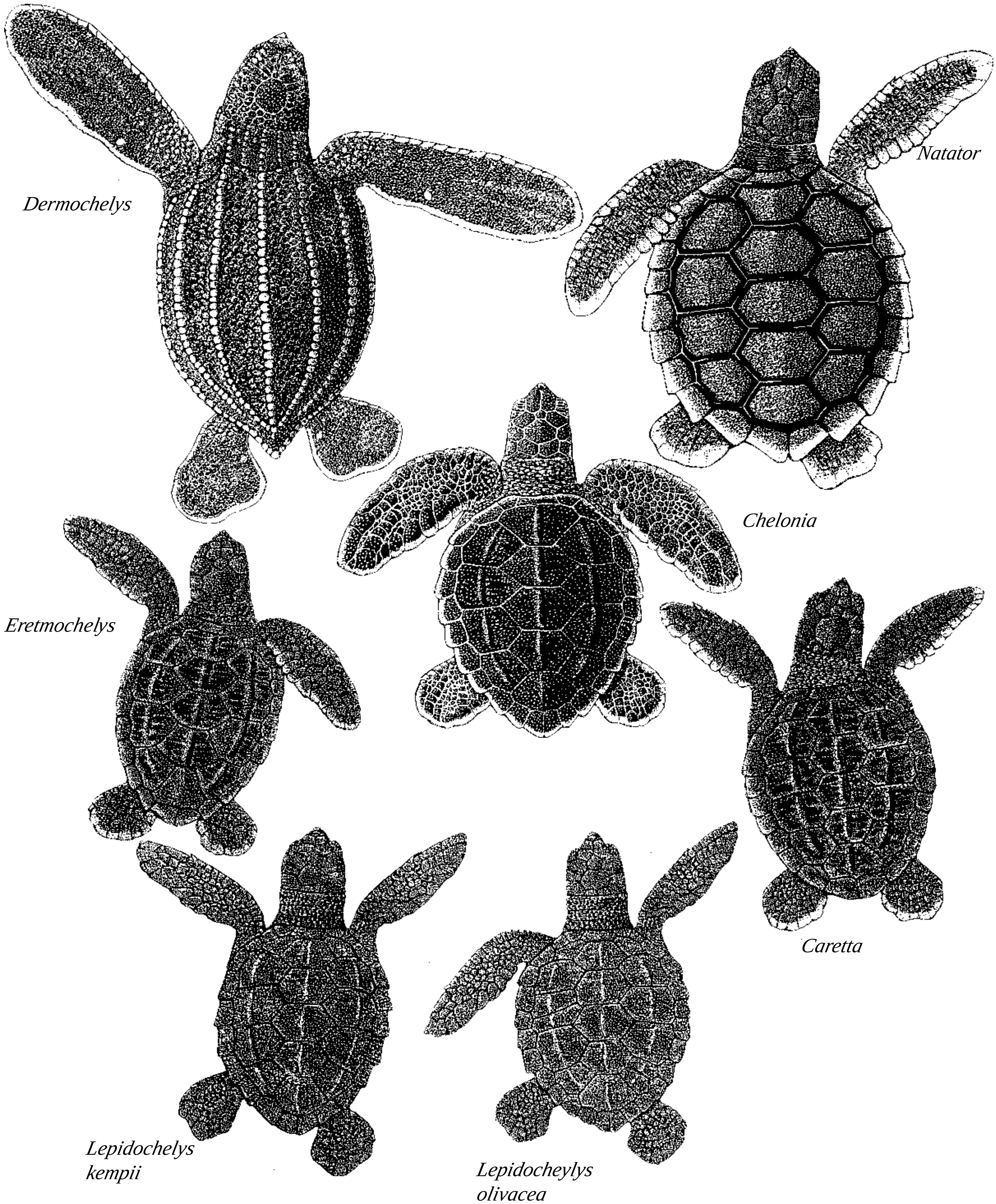
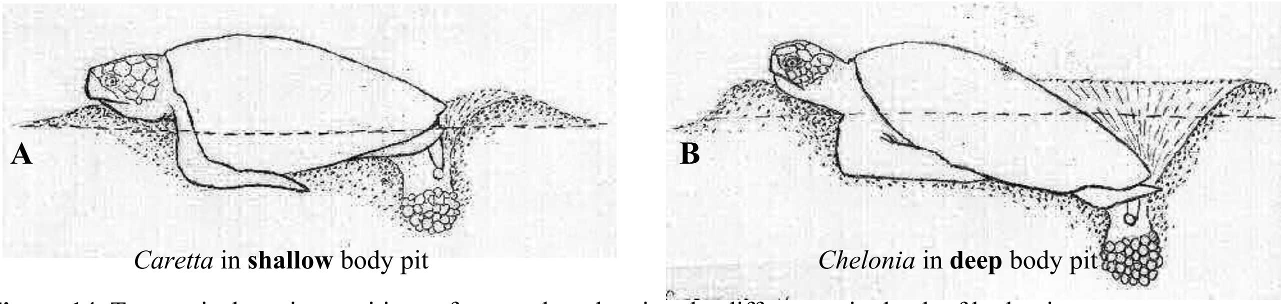


Figure 13. Sea turtle hatchlings of seven species. Sizes are approximately 80% of actual size.



Caretta in **shallow** body pit

Chelonia in **deep** body pit

Figure 14. Two typical nesting positions of sea turtles, showing the differences in depth of body pits.

Identification of Tracks and Nests

The following descriptions of tracks and nests typical of each species will serve as a guide for field workers trying to identify the tracks they encounter. Nevertheless, the tracks of different species can be difficult to tell apart especially those of *Caretta, Lepidochelys,* and *Eretmochelys.* Differences in beach substrate can alter the appearance of tracks made by the same individual, and morphological variation *(i.e.,* body size, flipper length, etc.) between individuals or populations of the same species can produce differences in track widths. Thus, we encourage field workers to spend time watching nesting turtles and to note the characteristics that distinguish the tracks made by local populations of each species.

Important diagnostic features used to differentiate turtle tracks by species include track width (cm), body pit depth (deep vs. shallow), and whether the diagonal marks made by the front flippers are symmetrical or asymmetrical. Some turtles *(Caretta, Eretmochelys, Lepidochelys and Natator)* dig little or no body pit (Figure 14A). Others *(Dermochelys, Chelonia)* leave conspicuous body pits (Figure 14B) after the female has displaced large amounts of sand while constructing and covering her nest. A symmetrical track is formed when the front flippers move together synchronously to pull the turtle over the surface of the sand, resulting in a track in which the right and left halves are almost mirror images. An asymmetrical track is formed when the front flippers move alternately (right, left, right, left, etc.) to carry the turtle forward.

Various other large animals sometimes leave tracks on the beach. Crocodiles, monitor lizards, and iguanas leave toe and claw prints and heavy tail drags. Because fresh water turtles are usually smaller than adult sea turtles, they walk with their plastron clear of the ground. Thus they tend to leave narrow tracts (less than 50 cm wide) comprising a series of isolated foot prints and no drag mark. In the Galápagos islands, Hawaii, and a few other places seals or sea lions may leave tracks that are superficially similar to those of sea turtles.

Species Tracks and Nest Descriptions

Dermochelys coriacea

Track width: 150-230 cm (less in the eastern Pacific than elsewhere).

Type of track: very deep and broad, with symmetrical diagonal marks made by the forelimbs, and usually with a deep incised median groove formed by dragging the relatively long tail.

Preferred beach type: wide, long, tropical beaches with steep slope, deep rock-free sand, and an unobstructed deep water or soft mud bottom approach.

Egg size and number: diameter of full-size (yolked) eggs averages 51-55 mm. Clutch size averages 80-90 eggs throughout most of the range but only 60-65 in the eastern Pacific. Few clutches exceed 120, not including a variable number of yolkless undersized eggs found in every nest.

Geographic location of nesting beaches: isolated mainland beaches in tropical (mainly Atlantic and Pacific; few in Indian Ocean) and temperate (south west Indian Ocean) oceans. Some low density nesting on islands (Greater and Lesser Antilles, Solomon Islands, and islands of the Bismarck Sea).

Chelonia mydas

Track width: typically about 100-130 cm but variable.

Type of track: deeply cut, with symmetrical diagonal markings made by the forelimbs. Straight, central tail drag marks present, either as a solid or a broken line.

Preferred beach type: ranges from large, open beaches to small cove beaches; preferably with an open offshore approach.

Egg size and number: egg diameter typically 40-46 mm. Clutch size averages 110-130.

Geographic distribution of nesting beaches: large colonies nest on both mainland beaches and remote oceanic islands. Tropical and occasionally subtropical beaches in all oceans (Atlantic, Pacific, and Indian oceans; Mediterranean and Red seas).

Chelonia sp. (Black turtle)

Track width: 70-90 cm.

Type of track: relatively deeply cut, with symmetrical diagonal markings made by the forelimbs. Straight, central tail drag marks present, either as a solid or a broken line.

Preferred beach type: small to intermediate sized mainland and island beaches; may use beaches with rocky outcrops or rocks exposed by low tide.

Egg size and number: egg diameter typically 40-45 mm. Reported mean clutch size ranges from 66-75 in Mexico to 81 in the Galápagos Islands and 87 in Pacific Costa Rica.

Geographic distribution of nesting beaches: principal nesting grounds in Michoacan (Mexico), Pacific coast of Costa Rica, and the Galápagos Islands (Ecuador).

Natator depressus

Track width: about 90 cm.

Type of track: relatively lightly cut, with either symmetrical or alternating marks made by the forelimbs.

Preferred beach type: fairly large open beaches, on mainland or large islands; reef habitat avoided.

Egg size and number: egg diameter typically 50-52 mm. Clutch size averages about 50-55 eggs.

Geographic location of nesting beaches: northern Australia.

Eretmochelys imbricata

Track width: typically 70-85 cm.

Type of track: shallow, with alternating (asymmetrical), oblique marks made by the forelimbs. Tail drag mark may be present or absent. Nests and tracks can be difficult to distinguish from those of ridleys, but the two species prefer different beach-types, and rarely nest together. Hawksbills frequently nest under overhanging vegetation (unlike ridleys which usually nest in open areas) and often wander extensively before nesting. Individual flipper prints of hawksbills are deeper than those of ridleys.

Preferred beach type: almost exclusively tropical; often use narrow beaches on islands or mainland shores with reefs obstructing offshore approach. Hawksbill nesting habitat is often separated (spatially or temporally) from that used by other turtle sea species.

Egg size and number: egg diameter typically 32-36 mm. Average clutch size varies from 70-90 in the Arabian peninsula to 110-180 elsewhere.

Geographic location of nesting beaches: tropical mainland and island beaches in the Atlantic, Pacific, and Indian oceans, and Red Sea. Nesting colonies worldwide are depleted from over-exploitation. The largest remaining populations occur in Australia, Mexico, Seychelles, and Indonesia.

Caretta caretta

Track width: typically 70-90 cm.

Type of track: moderately deeply cut, with alternating (asymmetrical) diagonal marks made by the forelimbs. Typically no tail drag mark.

Preferred beach type: generally extensive mainland beaches and barrier islands; moderately steep beach profile preferred.

Egg size and number: egg diameter typically 39-43 mm. Average clutch size ranges from about 90-110 in the Mediterranean to 100-130 elsewhere.

Geographic location of nesting beaches: nests most abundantly in subtropical and temperate areas (southeast USA, Oman, temperate Australia, South Africa, eastern and southern Mediterranean, Japan, southern Brazil), occasionally in the tropics (Belize and Colombia), and sometimes on islands (New Caledonia, Solomon Islands).

Lepidochelys kempii

Track width: 70-80 cm.

Type of track: very lightly cut, may be quickly obliterated by wind; alternating (asymmetrical) oblique marks made by the forelimbs. Tail drag mark lacking or inconspicuous.

Preferred beach type: wide, extensive, and continuous beaches with scrubby dune vegetation on mainland shores and barrier islands.

Egg size and number: egg diameter typically 37-41 mm. Average clutch size is 104 eggs.

Geographic location of nesting beaches: primarily near Rancho Nuevo, Tamaulipas, Mexico; occasional nesting in Veracruz and Campeche Mexico, in southern Texas, and rarely elsewhere. Formerly highly aggregated in nesting groups known as arribadas, but over-exploitation and incidental mortality in trawl nets has reduced *arribada* sizes to dozens or a few hundred rather than thousands.

Lepidochelys olivacea

Track width: 70-80 cm.

Type of track: similar to that of *L. kempii* (above).

Preferred beach type: tropical mainland shores and barrier islands, often near river mouths.

Egg size and number: egg diameter typically 37-42 mm. Average clutch size ranges from 105 to 120.

Geographic location of nesting beaches: eastern Pacific (Baja California and Sinaloa, Mexico to Colombia), south Atlantic (Guyana to Brazil and West Africa), northern Indian Ocean (especially Orissa India), and western Pacific (Malaysia and Thailand). Nesting often solitary or in small groups; but in India, Costa Rica, and Mexico arribadas of many thousands of animals may come ashore at once.

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Population and Habitat Assessment

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Habitat Surveys

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There are vast tracts of coastal domain potentially suitable for sea turtle nesting and foraging, but for which little is known about the presence (seasonality, distribution, abundance) or activity patterns of these increasingly rare creatures. When faced with identifying and/or prioritizing management actions, such as declaring a protected area or regulating territorial development or resource use, it is useful to know whether and how sea turtles might be affected by management decisions. Moreover, in regions where comprehensive sea turtle research or conservation programs are desirable but lacking, managers are faced with identifying potential study sites in the absence of comprehensive field data.

The purpose of this chapter is to illustrate and summarize various techniques which may be used to characterize nesting and feeding habitats potentially suitable for, or in use by, sea turtles. With the resulting baseline data in hand, more efficient allocations of effort and resources can be achieved than would otherwise be the case. Once preliminary investigations have identified potentially important habitat, population assessment methodologies described in subsequent chapters of this manual should be followed.

Nesting Habitats

The existence of many kilometers of sandy beaches does not guarantee the existence of suitable nesting habitat. In this section we discuss generalized techniques that may help to characterize and identify potential nesting beaches, without actually observing gravid turtles. The techniques are presented in the same order as they would be applied in the field.

Interviews

For our purposes, we assume that there are few or no formal reports of sea turtles nesting in the area(s) under investigation. In this case, the most cost-effective place to begin is by interviewing coastal residents. Although they may not necessarily be fishermen, residents are likely to have some knowledge of the major fauna inhabiting their surroundings. A series of questions addressing observations on sea turtles, such as the occurrence and seasonality of mating, egg-laying, or the appearance in markets of egg-bearing turtles, should be posed to obtain basic information. Care must be taken not to bias the responses of interviewees (see Tambiah, this volume).

Preliminary Surveys

On the basis of information gleaned by interviews, coastal areas reported to have nesting or some related activity, such as the consumption or marketing of sea turtle eggs, should be visited during the appropriate season. The most obvious confirmation of nesting is the presence of crawls, nesting pits, or egg shells on the beach. These should be recorded to species if possible and characterized as fresh or aged (see Pritchard and Mortimer, this volume; Schroeder and Murphy, this volume). Predominant threats, if discernible, should be noted *(e.g.,* slaughter of turtles, depredation of eggs, erosion or inundation of nests).

Relevant physical features should also be noted, such as dominant vegetation types, beach composition *(e.g.,* origin [calcareous, volcanic], grain size, sand compaction) and profile, typical wave conditions, and the presence of rivers or estuaries. There are several studies that have characterized nesting beaches based on features such as profile, vegetation and/or grain size (see Hirth, 1971; Márquez *et al.*, 1976; Balazs, 1978; Carr *et al.*, 1982; Mortimer, 1982; Corliss *et al.*, 1989; Márquez, 1990) and in the absence of any direct evidence of nesting, this literature should be examined for insight into potential nesting grounds.

While care should be exercised in interpreting results, generalizations are possible. For example (see Mortimer, 1982), leatherbacks prefer deep and unobstructed underwater access and a relatively steep (often windward) beach profile, while hawksbills routinely traverse shallow, coral strewn habitat to reach more heavily vegetated, low profile beaches. Leatherback and green turtles tend to nest in open habitat, whereas hawksbills often create obscure nesting pits in the littoral forest *(e.g.*, beneath *Suriana maritimia*, *Cocoloba uvifera*, or *Eterocarpus erectus* in the insular Caribbean). Olive ridley turtle nesting areas commonly occur on beaches separated from the mainland by coastal lagoons or estuaries.

In remote, uninhabited areas (where interviews are not practicable), preliminary surveys should be done by boat or, even better, by low flying aircraft. While aerial surveys are relatively expensive, they are also the fastest and most efficient method of covering long continental coastlines or otherwise inaccessible island groups. Able partners should be sought, such as the Coast Guard, missionary groups, or charter supply corps with access to air transport in outlying areas. For details on how to conduct an aerial survey, see Schroeder and Murphy (this volume).

Follow-up Techniques

Once preliminary assessments are complete and areas with a high potential to support nesting have been identified, field teams can be deployed to undertake a more detailed analysis. One or two two-week periods of ground patrol (preferably nocturnal) during what is believed to be the peak nesting or hatching season should be sufficient to confirm nesting, estimate nest density, verify species, and gain some degree of insight into any major threats. With these data in hand, a manager is well positioned to move forward with the design and implementation of more specific conservation or management action.

Foraging Habitats

Sea turtles spend most of their lives in underwater habitats, both coastal and oceanic. Identifying and assessing potential foraging habitat is fundamental to the success of any conservation or management program. Although studying sea turtles in the water is much more difficult than studying them on land, an increasing number of published studies illustrate a variety of proven methods *(e.g.*, Ehrhart, 1983; Balazs *et al.*, 1987; Collazo *et al.*, 1992; Limpus, 1992; see also Ehrhart and Ogren, this volume). In this section we discuss generalized techniques that may help to characterize and identify potential feeding grounds, without necessarily observing resident turtles. The techniques are presented in the same order as they would be applied in the field.

Interviews

As in the case of beaches (above), we assume that there are virtually no baseline data available. Again, the most cost-effective place to begin is by interviewing potentially knowledgeable residents, including fishermen, ferry or supply ship crews, Coast Guard or other marine patrol officers, and divers. A series of questions addressing observations on sea turtles, such as the presence of adults or juveniles in nearshore or offshore areas, patterns of seasonal movement, fisheries statistics, or the marketing of sea turtle products, should be posed to obtain basic information. As always, care must be taken not to bias the responses of interviewees (see Tambiah, this volume).

Preliminary Surveys

On the basis of information gleaned by interviews, areas where sea turtles are routinely or predictably observed should be targeted for further investigation. These areas should be visited by survey personnel using snorkel or SCUBA gear. Relevant biotic and abiotic characteristics (algae, corals, flora and fauna) should be recorded together with accurate locations of the sites using a Global Positioning Systems (GPS) device, if possible. For example, evidence of nibbling on sea grasses (by green turtles) or sponges (by hawksbills) might be discernible (Vicente and Tallevast, 1995; van Dam and Diez, 1997). Ecological data, including water temperature, current flows, depth, and obvious geological structure (significant rock formations, crevices, vertical walls) should also be recorded.

As a prerequisite to field investigation, a literature search should be undertaken to review pertinent features of sea turtle foraging habitats and habits, including dominant prey items and feeding patterns (Casas-Andreu and Gómez-Aguirre, 1980; Mortimer, 1981; Ogden *et al.*, 1980, 1983; Dodd, 1988; Limpus, 1992).

In many cases, key species or species indicators are food items; these include sponges, seagrasses, algae, and crustaceans. For example, encrusting sponges *(e.g., Chondrila, Chondrosia, Niphates, Cynachira, Geodia, Ricordia*) are the most common food items for hawksbill turtles which forage in coral reefs, rocky outcrops or some mangrove-fringed bays and estuaries of the Caribbean. Loggerhead and olive ridley turtles aggregate to feed on red crab, *Pleuroncodes planipes*, concentrations in upwelling areas of the Eastern Pacific; green turtles forage on sea grasses *(e.g., Zostera, Thalassia*) and algae *(e.g., Gelidium, Cracillaria*) in typically relatively shallow, protected waters.

Some organisms can also be considered as indicator species. For example, barrel sponges *(Xetospongia muta)*, some boxfish *(e.g., Lactophyrys)*, angelfish *(e.g., Holacanthus, Pomacanthus)*, and hard corals such as *Plexaura* sp., *Agaricus agaricites*, and brain coral *(Colpophyllia natans)*, are also typically found in areas of the Caribbean where hawksbills forage, although they are not known to be hawksbill prey items. Aerial photos and marine resource atlases may help to identify important benthic types *(e.g.,* coral reefs, seagrasses).

Foraging sea turtles surface regularly during bouts of feeding, so that surface observations can provide useful information. Species can sometimes be predicted from information available about the benthos. Hawksbills appear to be obligate spongivores, green turtles are herbivores, and loggerheads, ridleys and flatbacks are omnivores with a penchant for crustaceans and mollusks. Leatherbacks are mainly oceanic medusae feeders and, while there are seasonal exceptions in some parts of the world, this species is not likely to be routinely encountered in coastal waters (with the exception of gravid females in their internesting habitat).

In uncharted or less traveled areas where preliminary data are unavailable from interviews or marine resource atlases (the latter serving to identify where important foraging grounds might be located, based on the distribution of coral reefs, estuaries, or sea grass meadows), there is little recourse but to visit representative habitats by boat and examine the area first hand using snorkel or SCUBA gear. Standard methods, such as linear transects, should be employed for rapid assessment of potential areas (Rogers *et al.*, 1983; Sullivan and Chiapponne, 1993; Chiappone and Sullivan, 1994, 1997; Bolten *et al.*, 1996). In some cases, especially when large areas are involved, preliminary information can be gained from aerial surveys (see Henwood and Epperly, this volume).

Follow-up Techniques

When preliminary assessments indicate that a particular area constitutes potential foraging habitat, more detailed underwater appraisals should be undertaken. In-water studies *(e.g.,* capture-recapture studies) can provide insight into the distribution, abundance, size classes, and species of sea turtles present (see Ehrhart and Ogren, this volume). Food items can be quantified through the use of linear transects, quadrants, or other standard methods (Weinberg, 1981). Permanent grids should be considered for the purpose of monitoring changes in the habitat over the long term.

Sighting networks should be established to provide information to managers on an ongoing basis (see Shaver and Teas, this volume). As more and more information is assembled, managers are able to refine their conservation and management priorities, as well as to enact specific habitat protection measures to safeguard important foraging grounds and migratory corridors.

In all cases (terrestrial and marine) where follow-up initiatives are planned, priority should be placed on research design (see Congdon and Dunham, this volume), thereby ensuring an efficient allocation of human and financial resources, as well as maximizing the usefulness of data collected.

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Research and Management Techniques for the Conservation of Sea Turtles
K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, M. Donnelly (Editors)
IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Population Surveys (ground and aerial) on Nesting Beaches

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Introduction

Nesting beach surveys are the most widely implemented monitoring tool in use by the global sea turtle community and are an important component of a comprehensive program to assess and monitor the status of sea turtle populations. These assessments are necessary to evaluate the effects of recovery and conservation activities which are being implemented at all life history stages. Appropriately designed nesting beach surveys, in concert with studies of nesting females (see Alvarado and Murphy, this volume; Balazs, this volume; Owens, this volume) and nest success (see Miller, this volume), can provide information relative to the number of nests deposited annually, the number of nesting females that are reproductively active annually, and annual nest productivity. Nesting beach surveys, as discussed in this chapter, refer to ground and/or aerial surveys conducted to gather information on the number of nesting and non-nesting emergences occurring on a non-*arribada* beach. Readers contemplating population surveys on mass nesting beaches should consult Valverde and Gates, this volume.

Nesting beach surveys range from highly structured standardized sampling to "snapshots" of nesting activity within a nesting season. While nesting surveys are currently widespread, the variability in techniques, along with inadequate documentation of methods used or assumptions made, often hampers our ability to make meaningful assessments of the status of nesting populations. The principal purpose of this chapter is to provide a simplified strategy for nesting beach monitoring which may prove useful in designing a valid monitoring program for previously unsurveyed beaches, or for modifying an existing program. The extent to which nesting beach surveys are conducted will depend on many factors including, but not necessarily limited to: remoteness and geography of the survey area, available personnel and equipment, and nest density. This chapter does not discuss the identification of nesting crawls to species, a critical component of any nesting beach monitoring program (see Pritchard, this volume, for guidance).

In order to be of value over a long period of time, surveys on nesting beaches must be cost-effective, reproducible, quantitatively rigorous, and easily taught to others who will continue the surveys. Two nesting beach monitoring methodologies are generally employed—patrolling the beach on foot or by vehicle (ground surveys) and patrolling the beach by aircraft (aerial surveys). This Chapter will review both methodologies and provide the information needed to implement a new nesting beach monitoring program, or, if one is already in place, may provide the reader with suggestions for improvements. This chapter is divided into three general sections: (1) an overview of survey methodologies and aspects of nesting beach surveys common to both methodologies, (2) ground surveys, and (3) aerial surveys.

Which Methodology Should be Used?

The determination of whether to employ ground survey or aerial survey techniques will depend on several factors. Chief among these is the geographic extent of the survey area, beach type, and the resources (money, equipment, and personnel) available. Ground surveys, conducted either by foot or by vehicle, allow close scrutiny of crawls for identification and counting, and are preferable where: (1) other aspects of nesting beach activities require personnel to regularly traverse nesting beaches *(e.g.,* nest monitoring, predator control); (2) the beach is accessible and the survey area is relatively short; (3) the structure of the beach is difficult to survey by air from the standpoint of aircraft maneuverability and/or; (4) crawl signs are obscure due to the beach type *(e.g.,* pebble beaches, nesting under heavy vegetation). Aerial surveys are preferable for reconnaissance of large geographic areas to ascertain relative use of nesting beaches (or presence/absence) and to patrol nesting beaches that are inaccessible by foot or vehicle. Either methodology is useful for long-term standardized surveys provided they are appropriately designed.

Once the survey platform (aerial or ground) has been selected, the next step is to determine the specific methodology that will be employed. There are two methods that have been successfully employed when conducting ground or aerial surveys. The first type requires differentiating between nesting and non-nesting emergences and assessing only "fresh" crawls *(i.e.,* those made the previous night for morning surveys, or the night of the survey, for night-time ground surveys). The second methodology involves counting all crawls, or in some cases, all crawls with visible body pits, without differentiation between nesting and non-nesting emergences, and may or may not involve the enumeration of "fresh" vs. "old" crawls. The methodology chosen will depend on an assessment of the variables explained below and both methodologies require appropriately designed ground-truthing. The authors do not recommend surveys that attempt to differentiate nesting emergences from non-nesting emergences *in situ* when "old" crawls are mixed with "fresh" crawls in the survey counts.

Aspects of Nesting Surveys Common to Both Techniques

Variables Affecting Data Collection

In any nesting survey, the detectability of a nesting event, and hence, the accuracy of the survey, is influenced by many factors. These variables are important regardless of whether the survey employs ground or aerial techniques. The most critical component of both types of surveys is the implementation of appropriately designed ground-truthing on a subsample of beaches. Ground truthing provides verification of the data collected by survey personnel and enables the development and application of appropriate corrections in the final data analyses. The major variables associated with identifying, differentiating, and enumerating crawls on the nesting beach are: observer/surveyor accuracy, turtle species, nesting density, beach type, time-of-day (position of sun), wind, rainfall, and human activity on the beach.

1. Observer/Surveyor Accuracy: Intrinsic observer error can heavily influence survey accuracy. A comprehensive nesting survey program must include observer training and ground-truthing (see below, this chapter).
2. Turtle Species: Some species exhibit nesting behaviors that inordinately complicate the identification and differentiation of crawl sign. For example, hawksbills generally prefer to nest in heavy vegetation and may traverse rock or coral rubble leaving little or no crawl sign. In contrast, leatherback crawls generally result in extensive beach disturbance which can equally confound the differentiation of nests from non-nesting emergences. Nesting behavior variability among species must be taken into account in planning and implementing nesting surveys.
3. Nesting Density: Nesting beaches that support high density nesting may not be good candidates for use of the aerial survey technique. The sheer number of crawls, often overlapping each other, can make it extremely difficult, if not impossible, to accurately assess crawls from the air. Aerial surveys are best suited for nesting beaches that support low to moderate levels of nesting activity, unless a helicopter is available for surveys on high density nesting beaches.
4. Beach Type: Variations in beach type may affect the reliability of crawl counts. Beaches can have fine sand, coarse sand, coarse sand mixed with some shell, and very hard packed areas composed almost entirely of shell. On the latter, impressions made by the flippers are indistinct. Variability in beach profiles can affect the width and symmetry of crawls and complicate species identification and/or differentiation of nests from non-nesting emergences.

5. Time-of Day (Position of Sun): The low sun angle in the early morning casts a deep shadow behind the tracks and makes them highly visible. By mid-morning, this shadow effect is lost and crawls are more difficult to see. For aerial surveys, glare becomes more of a factor later in the survey. Overcast days eliminate the shadow effect on tracks and make it more difficult to discern nest field signs. It is recommended that surveys be conducted at the same time each day, preferably in the early morning, to eliminate one of the variables affecting survey accuracy.
6. Wind: Crawls may be weathered or obliterated depending upon wind intensity, duration, and direction. The moisture content of the sand moderates the effects of the wind to some degree and the portion of a track in damp sand may remain more distinct than the portion in dry sand. Crawls weather differently depending upon the wind's direction relative to the orientation of the beach. One part of the beach might contain clear, distinct crawls, while crawls made the same night in another area may appear faint or older.
7. Rainfall: Rainfall obscures crawls and confounds crawl identification to varying degrees. Emergences before a rain generally appear older than emergences that occur after a rain. This same effect can occur during a survey if rain falls on all or part of the survey beach. Under these conditions, when employing surveys that require the differentiation of "fresh" crawls from "old" crawls it is essential to rely on the crawl's relationship to the intertidal zone to determine the age of the crawl (provided the survey beach has an obvious tidal fluctuation). Crawls are generally still visible after slight or moderate rainfall, but heavy rainfall will often obliterate crawls completely. Aerial surveys, and to a lesser extent ground surveys, are of little value after nights of widespread, prolonged rain or strong winds.
8. Human Activity on the Beach: Human activity obscures crawls, body pits, and other nest field signs. On-going nest protection projects also destroy field signs when screening or relocating nests. It is important to have an understanding of the level of human activity on your survey beach, including nest protection efforts, and to ensure that this is taken into account when planning both ground and aerial surveys.

Data Collection Forms

Data collection forms for ground and aerial surveys of nesting beaches should be simple and straightforward and all surveyors should use the same data form for a particular beach. The reader may wish to consult researchers having established nesting survey programs for examples of data collection forms. These can serve as models, however, forms should always be tailored to your particular beach and include all relevant information (see Appendices 1 and 2).

Partitioning the Nesting Beach

One of the most important components of establishing a long-term nesting beach monitoring program is defining the survey area. In order to make year to year comparisons of nesting beach survey data, the survey area must be known, must be consistent, and must be measured. It is useful to partition the beach into equal segments or zones, so that data are available at a resolution finer than the entire survey length. The ability to analyze survey data by zone is particularly useful when evaluating or assessing the effects of habitat alteration on nesting success *(e.g.,* artificial lighting, coastal armoring). The maximum distance recommended for survey zones is 1.0km. Zones can be demarcated by using marked stakes or posts, however, because these markers are usually temporary, a more permanent record of the zone endpoints should be made, either by referencing a direction and distance from more permanent features when they exist *(e.g.,* buildings, rivers, or inlets) or through the use of Geographic Positioning System (GPS) units if available. Since data will be collected by survey zone, it is important that all personnel involved in the survey are knowledgeable about the start and endpoints of the various zones.

Examining Survey Error – Ground Truthing

Inherent in all nesting beach surveys is a level of survey error. Track sign can be difficult to interpret and errors will be made in separating nesting emergences from false crawls, discerning "fresh" crawls from "old" crawls (for surveys that require differentiation), and differentiating one species crawl from another. Errors may be more pronounced on moderate or high density nesting beaches when the magnitude of crawls complicate nesting field sign. In the case of ground or aerial surveys designed as total crawl counts, correction factors must be developed to estimate nests and false crawls. A critical component of ground or aerial surveys is assessing the magnitude of these errors. Ground-truthing should take place several times throughout the course of the season, on a sub-sample of the entire survey area under varying tidal and weather conditions, and on all beach types within the survey area, in order to assure an unbiased sample. In the case of aerial surveys, ground-truthing is conducted for every flight. Ground-truthing must employ techniques that confirm the presence or absence of eggs. The only way to confirm the presence of eggs is to see them, either during egg deposition, or later by excavation or probing, or as a result of the activities of predators. The former method involves directly observing the activities of nesting females (without interfering with their behavior), marking the resultant crawls with numbered flags or stakes, and using these data to check the survey information gathered by surveyors on the nesting beach the following day. Alternatively, as described below, crawls can be excavated or probed within the ground-truth area(s) to verify the presence or absence of eggs and the authors differ on which is the preferred method. The first method involves slow and methodical localized digging (use small diameter test holes and dig with the hands only—no implements!) to confirm that eggs are present or absent. The second method involves the use of a small, narrow diameter probe stick which is gently inserted into the sand to test for the softened area of sand directly above the clutch. Extreme care must be exercised when probes are used so that eggs in the clutch are not punctured. Either technique should be used only by experienced, well trained, and properly permitted personnel. Care should always be taken to ensure that clutch "finding" techniques are not taught (either directly or by indirect observation) to persons who may illegally poach nests. Where ground truthing is conducted to calibrate aerial survey data, additional information collected on the ground survey which may be helpful includes a description of the crawls observed, their sequence, and their location relative to the same landmarks used by aerial observers (see Partitioning the Nesting Beach above).

Regardless of the ground-truthing methodology employed, for either ground or aerial surveys, the results will yield an estimate of survey error(s) which must be applied as a correction factor(s) in the final data analyses.

Determination of Nesting vs. Non-Nesting Emergences

As described above, under "Which Methodology Should be Used?", certain ground and aerial surveys are designed to differentiate nesting and non-nesting emergences at the time of the survey. This type of survey employs methods used to assess nesting activity that do not require the direct confirmation of eggs at every nest site. Under certain beach conditions and for certain species, field or "crawl" signs can be used to determine whether an emergence has resulted in egg deposition provided the observer has sufficient training and experience. As noted above, appropriately designed ground-truthing must be implemented to assess the accuracy of all survey techniques so that correction factors can be developed and applied in the final data analyses. Although each species has certain characteristics which result in unique crawl sign, many of the characteristics are highly similar (for species specific crawl and nest descriptions see Pritchard, this volume). You must gain experience with the crawl sign nuances of all of the species nesting on your survey beach, thus reducing survey error. Certain terminologies, which may or may not be familiar to the reader, are regularly used to describe and interpret crawl sign. It is valuable for the reader to have a through understanding of this terminology before proceeding further. The following glossary and discussion of crawl sign, primarily based on loggerheads, may be useful and will be applicable to a certain extent to all species of sea turtles:

Backstop: An approximately 45° incline made in the sand as sand is pushed back with the rear flippers during the excavation of the primary body pit. Such a steeply inclined backstop is not present in the secondary body pit.

Crawl: Tracks and other sign left on a beach by a sea turtle.

Egg Chamber: The cavity excavated by the rear flippers into which the turtle deposits a clutch of eggs.

Escarpment: The perimeter of the secondary body pit where the front flippers have cut away a small cliff into the surrounding sand.

False Crawl: A crawl resulting from an abandoned nesting attempt (a non-nesting emergence).

Nesting Crawl: A crawl resulting from a nesting attempt in which eggs were deposited.

Primary Body Pit: The excavation made by a turtle on the beach just prior to digging the egg chamber.

Secondary Body Pit: An excavation made by a nesting turtle primarily using the front flippers following the deposition of eggs. The spoil from the secondary body pit generally covers the primary body pit and the egg chamber with sand.

Nesting Crawl Field Signs

The first step is to identify the emerging and returning crawls by observing which direction the sand is pushed—as a turtle crawls it will push sand backward with each flipper stroke (Figure 1, note arrows). Noting the direction of travel will help in understanding the behavior of the turtle, which results in the crawl sign. Follow the path taken by the turtle and look for evidence of front flipper covering—sand misted or thrown back over the emerging track (Figure 1 (B)), a secondary body pit and/or escarpment (generally a crescent shaped cliff), and sand thrown in the vicinity of the secondary body pit (Figure 1 (C)). The shape of the secondary body pit may be somewhat circular or oblong, depending on the location of the nest site. The sand which is misted or thrown during body pitting and covering generally has a higher moisture content than the dry sand on the beach surface and this difference can be helpful in understanding and evaluating crawl sign.

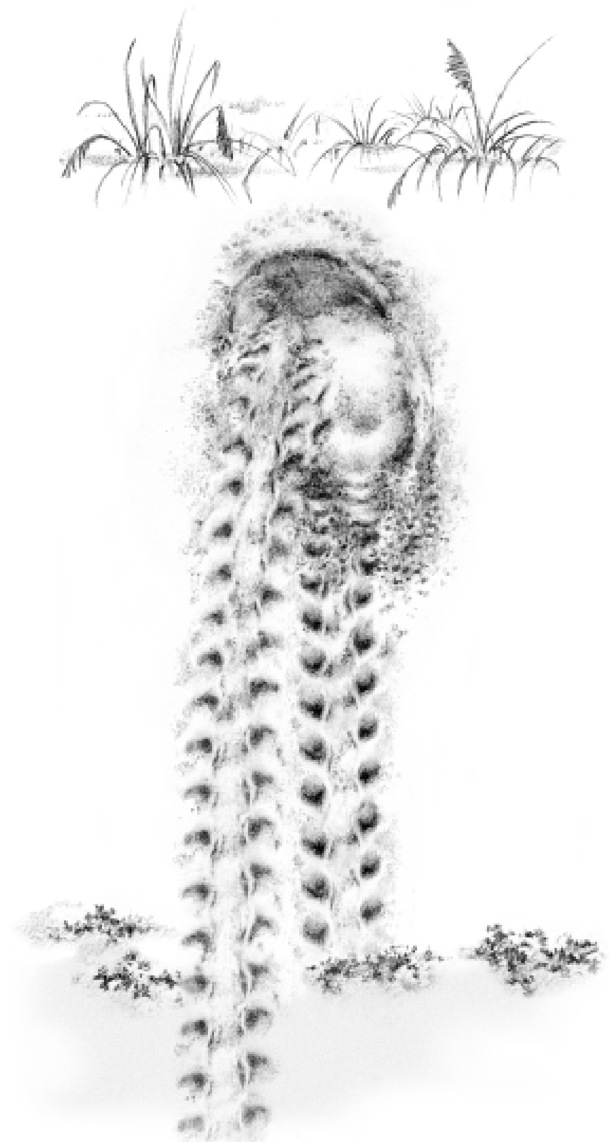

Figure 1. Stages of successful loggerhead *(Caretta caretta)* nesting, with emerging crawl (A); sand misted or thrown back over the emerging track (B); a secondary body pit and escarpment, with sand thrown in the vicinity (C); and returning crawl (D). (E) marks the high tide line.

During aerial surveys that are designed to differentiate nesting from non-nesting emergences and "fresh" from "old" crawls, the relative lengths of the emerging and returning crawls can also be an indicator of nesting, on survey beaches with obvious tidal fluctuations, but this differentiation technique should only be used in the absence of any corroborating evidence and when the apex of the crawl is obstructed from view. If the emerging track is considerably shorter than the returning crawl, this provides evidence that the turtle spent a considerable time on the beach and may have nested. However, it is important to ensure that the turtle was not simply wandering on the beach or making repeated attempts at nesting.

False Crawl (Non-Nesting Emergence) Field Signs

Observe the entire crawl carefully and look for any of the following signs: (1) very little or no sand disturbed, other than the crawl itself which is most commonly U-shaped or a simple arc, but which may include moderate to extensive wandering (Figure 2 (A) and (B)); (2) a backstop with sand pushed back (not thrown) over the emerging crawl, typically between two mounds of sand piled by the front flippers during construction of the primary body pit (Figure 2 (D)); (3) considerable sand disturbed from a digging effort, but with the crawl exiting the disturbed area and returning toward the ocean (4) considerable sand disturbed from a digging effort, but with a smooth-walled or collapsing egg chamber (devoid of eggs) and no evidence of covering (Figure 2 (C)). Note that a depredated nest will generally be characterized by eggshells or partially consumed eggs littering the nesting site. Depredated nests must be counted as nesting emergences during the survey, but should be noted as depredated if quantifying nest success. During aerial surveys, some crawls may be classified as "unknown,"

Figure 2. Examples of false crawls (non-nesting emergences) made by loggerheads *(Caretta caretta)* include extensive wandering with no body pitting or digging (A); U-shaped crawl to the high tide line (B); considerable sand disturbance and evidence of body pitting and digging and no evidence of covering (D); and considerable sand disturbance, evidence of body pitting and digging with a smooth-walled egg chamber and no evidence of covering (C). (E) marks the site of a crawl where the relative lengths of the emerging and returning crawls are the same. (F) marks the high tide line.

for example, when the face of the dune collapses on the apex of the crawl (obscuring the crawl sign) or when the apex of the crawl is obscured by dune vegetation and the relative lengths of the emerging and returning crawls are the same (Figure 2 (E)).

Ground Survey Methodology

Equipment Needed

If the survey is to be conducted on foot, no extensive equipment is needed beyond a sturdy hat and sunscreen. If vehicles are to be used they should be small, three or four-wheeled, all terrain vehicles (ATVs). ATVs are relatively lightweight and have large balloon-style high pressure tires which leave low-relief tire tracks and which do not exert extensive force over incubating nests, which may be traversed (though never intentionally) during the course of the nesting season. ATVs are ideal for surveys that are geographically extensive, however, they must be regularly maintained to protect against the wear and tear resulting from daily exposure to sand and saltspray. Regardless of the survey mode, the only other equipment necessary are data sheets, writing utensils, and a camera for any unusual findings.

Periodicity and Timing of Ground Surveys

In many cases nesting beach surveys are conducted daily in conjunction with other sea turtle conservation activities such as nest protection efforts (see Boulon; Miller; Mortimer, this volume). Complete counts of nesting and non-nesting emergences require daily monitoring throughout the nesting season. However, daily monitoring is not always necessary or logistically possible and data from intermittent surveys can be used as an index to total nesting, provided there are baseline data available and provided the survey is appropriately designed to periodically sample throughout the nesting season. Intermittent surveys may be designed in two ways: those that count only fresh crawls *(i.e.,* those made the previous night) or those that count all crawls regardless of age. In the latter case, data on track longevity must be collected and incorporated as a correction factor in the final data analyses.

Where daylight survey techniques are employed, nesting surveys should begin just after sunrise for the best viewing of crawls. Track sign begins to deteriorate as the sun dries out the sand, and the crisp shadows that facilitate track identification are lost as the sun rises in the sky. Additionally, on beaches that are visited by humans for recreational purposes, foot traffic and beach activities will obliterate nesting crawls. Daytime nesting surveys are the recommended approach as they do not require repeated traversing of the survey area which can cause disturbances to nesting females and because it is easier to accurately discern crawls in the daylight. However, in some cases, it may be necessary and/or preferable to conduct night-time surveys when other research activities necessitate night-time patrols or when nest protection efforts must be carried out during the night.

Ground Survey Techniques

The following discussion describes how to conduct daytime ground surveys designed to differentiate nesting from non-nesting emergences and “fresh” from “old” crawls. Surveyors should move along the beach at the level of the latest high tide line. Upon discovery of a crawl, the surveyor must first determine that the crawl is fresh. Depending on weather conditions crawls can persist for days or even weeks. The only completely reliable method to ensuring that only fresh crawls are counted is to traverse the survey beach, by foot or vehicle, above the expected high tide line, on the day prior to the day the survey will be conducted. In this way, the impressions from all fresh crawls will cross over the vehicle track or will not have been previously “marked” by sweeping the feet across the track. An alternative method of discerning fresh crawls from old crawls, described under the section of this chapter dealing with aerial surveys, will depend greatly on the tidal conditions at the nesting beach and is slightly less reliable. The surveyor must next visually determine whether or not the crawl is a nesting or non-nesting emergence (see above) in addition to determining which species of turtle made the crawl (see Pritchard, this volume). Surveyors must have the experience necessary to accurately make these determinations based on the characteristics of the crawl. All crawls are enumerated and recorded on the data sheet. After each crawl is evaluated and recorded, the track should be “marked” *(e.g.,* effacing the upper part of the track) to avoid duplicate counting on subsequent survey days. Regardless of the method used to “mark” the crawl, it should be consistent among and familiar to all survey personnel to avoid duplicate counting.

Training

All surveyors should be fully trained prior to conducting surveys on their own. A comprehensive training program will include a classroom session and field sessions. Classroom training should include slides or photographs of various types of crawls for each of the species that are known to nest on the survey beach. A thorough understanding of the nesting behavior of each species is critical to accurate interpretation of nesting track sign. New surveyors should be introduced to these nesting behaviors by observing nesting turtles with an experienced surveyor who can explain each part of the nesting process and how each behavior influences track sign. New surveyors should work side-by-side with experienced surveyors until they are fully confident of their ability to identify nesting emergences from false crawls and distinguish among the species using the survey beach.

Aerial Survey Methodology

Equipment Needed and Technique

Helicopters have the best visibility, adjustable speed and the capacity to hover. These aspects are especially useful when training new observers. However, in most cases they are expensive and may not be readily available for regular survey flights. Single engine, wing-over-cockpit aircraft are generally more readily available and most frequently employed. The following variables must be taken into consideration when using aircraft to survey nesting beaches.

1. Speed: Crawls will obviously be missed if the aircraft’s speed is too fast. The speed of the aircraft should be adjusted to the track density. On low density beaches (<1 track/km/flight) crawls can be recorded accurately at 100 knots. On moderate density beaches (1 to 5 crawls/km/flight) it is possible to accurately count crawls at 80 knots. Where track densities are higher, (>5 crawls/km/flight) it is necessary to fly at 60 knots.
2. Altitude: Flying too low causes a similar prob-

lem as flying too fast. Objects are in the field of view for a shorter time, causing increased eye movement which results in lowered accuracy and observer eye fatigue. Survey altitude is dependent on species. For example, loggerhead beaches in the U.S. are best surveyed at 60m, while leatherback beaches in Mexico are best surveyed at 250m. Before undertaking a survey it is prudent to test the best speed and altitude by making a few test flights with simultaneous ground surveys. Choose the speed and altitude that results in the lowest percent error.

3. Position: The position of the aircraft is important in order to accurately assess crawls. The most important aspect is to gain the best view of the area where most of the nests are located (often the upper beach) while still allowing good viewing of crawls that terminate lower on the beach *(e.g.,* many false crawls). The best position for the aircraft is the one that maximizes crawl viewing, taking into account the relative angle of the sun on the beach, which enhances shadowing and the discernment of field signs. As with speed and altitude, a pre-survey test flight can help determine the optimal track-line.
4. Pilot: The importance of the pilot in maintaining the correct speed, altitude, and position of the aircraft, while maintaining safety, cannot be overemphasized. Working with the same pilot on multiple surveys is recommended whenever possible.
5. Fatigue: Fatigue causes a loss of observer concentration. This aspect generally becomes noticeable after about three hours of flight time or where long sections of coastline have few crawls. Surveys should be designed to minimize observer fatigue.

Timing and Periodicity of Aerial Surveys

Aerial surveys are best flown beginning at dawn**.** The relatively low angle of the sun creates a shadow effect and enhances crawl sign, sand thrown by the turtle is still moist resulting in fresher crawl sign, and human activity is minimal. Aerial surveys are, by their nature, intermittent or periodic surveys, whereas ground surveys may be daily or intermittent. The interval between surveys is important to the accuracy of the overall survey. Aerial surveys designed to enumerate only fresh crawls should be scheduled to maximize monthly tidal cycles, on beaches where there are obvious tidal fluctuations. In the southeast U.S., for example, the twice monthly spring tides wash the widest area of the intertidal zone and remove the lower portion of old crawls. Scheduling flights on the morning *after* the optimum tide (one that peaks just at dark), the morning *of* that optimum tide and one day *prior to* this tide prevents most of the errors in aging crawls and provides a three-day window for the surveys. Flying three consecutive days tends to smooth out daily variability in turtle activity. The accuracy of counting only fresh crawls is affected by: the time of the evening high tide relative to the time at which the turtles crawl, and the relative height of this tide on consecutive days. The difference of one hour in the tidal cycle can result in track aging errors if the tide peaks after dark. Aerial surveys designed as total track counts that rely on ground-truthing to develop correction factors for estimating nests and false crawls need not rely on the methods explained above regarding tidal fluctuations.

Aerial Survey Techniques

During aerial surveys designed to differentiate nesting emergences from non-nesting emergences, and enumerate only "fresh" crawls, the observer should scan the rack line created by the previous night's high tide. Ignore any crawls that **do not** extend below this line regardless of how "fresh" they may appear. If a crawl extends below the most recent high tide line, the eye should follow the crawl to its apex. Examine the track for the field signs related to species identification (see Pritchard, this volume) and examine the area for the field signs described above under "Determination of Nesting vs. Non-Nesting Emergences". If identification of the track type (nesting vs. non-nesting) cannot be made, based on characteristics at the apex of the crawl, examine both legs of the track to determine if they were of equal or unequal lengths. The difference in these lengths may be helpful in identifying crawls (see above). The assessment of crawls is rapid and observers must take into account all available field sign to make the best assessment at each crawl. Aerial surveys that are designed as total crawl counts do not involve differentiation of each crawl.

Training

Observer experience is an important variable in any aerial survey. It is rare, though not impossible, to have total agreement between the aerial observations and the ground truth data. In order for new observers to improve their aerial survey techniques, it is important for them to recognize the types of errors that can be made. Depending on the specific aerial survey methodology employed, observer errors include: missed observations, misidentification of track type, misidentification of species, and mis-aging. Inexperienced observers should be made aware of the types of errors that they are making (determined from ground-truthing) so that they can improve their accuracy based on objective criteria. This type of observer training requires mapped ground-truthing during the training period.

Data Analyses and Interpretations

The goal of nesting beach surveys is to determine the abundance of nests on a beach over a specified time interval. There are multiple approaches to survey design that have been successfully employed by researchers at different nesting beaches across the globe. The published literature is generally lacking in detailed accounts of methodologies for nesting beach surveys. In some cases, methodologies are under development and the reader is encouraged to further his/her knowledge by communicating with experienced researchers to remain abreast of new developments and to ascertain which methodologies will best fit their particular circumstances.

Well designed nesting surveys can and do provide short-term information that is integral to management and recovery programs. However, it is important to recognize that population trends at nesting beaches take many years to accurately discern, thus emphasizing the value of long-term standardized surveys. Most sea turtle species take well more than a decade to mature. Therefore, the population-level effects resulting from management efforts may not be evident for many years, especially if impacts are occurring that reduce population size at the early life history stages. Sea turtle biologists, managers, and enthusiasts should be cautious in interpreting nesting beach survey data collected over a short time frame. Year-to-year fluctuations are common and should be thoughtfully reviewed and considered rather than immediately construed as absolute indications of the health or status of the population. Equally important is the caution that nesting survey data from one area cannot be extrapolated to unsurveyed areas as nesting densities can vary greatly from one stretch of beach to another. Neither can nesting survey data be assumed constant in a temporal sense, data collected over a specified time interval cannot be extrapolated to large, unsurveyed time intervals. The real value in nesting beach surveys lies in establishing a standardized, repeatable, and statistically rigorous long-term record of nesting activity to monitor population status.

Appendix I
[SAMPLE] Nesting Beach Ground Survey — Daily Report Form

Date of Survey ______________________ Beach Name ____

Observer(s) ____

Time Start ____________ AM PM Time End _____________ AM PM

Beach Zone or LAT/LONG	Species 1 (e.g., *Caretta)*		Species 2 (e.g., *Chelonia)*		Species 3 (e.g., *Dermochelys)*	
	#Nests	#False Crawls	# Nests	#False Crawls	#Nests	#False Crawls
A						
B						
C						
D						
E						
F						
G						
etc/						
Total						

Comments

[SAMPLE] Nesting Beach Aerial Survey Data Collection Form

Date of Aerial Survey ____________________ Observer(s) ____________________

Recorder ____________________ Pilot ____________________

Survey Beach Name ____________________

Time Start Survey __________ AM PM Time End Survey __________ AM PM

Type of Aircraft ____________________ Speed __________ Altitude __________

Current Weather Conditions ____________________

Previous 24-Hour Weather Conditions ____________________

Beach Zone or LAT/LONG	Species 1 (e.g., *Caretta)*			Species 2 (e.g., *Chelonia)*			Other
	#Nests	#False Crawls	Total	# Nests	#False Crawls	Total	Strandings, Vessels, etc.
A							
B							
C							
D							
E							
F							
G							
etc/							
Grand Total							

Comments ____________________

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Ground Truth Survey Made ____________________

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Research and Management Techniques for the Conservation of Sea Turtles
K.L. Eckert, K. A. Bjorndal, F.A. Abreu-Grobois, (eds.)
IUCN/SSC Marine Turtle Specialist Group publication #4 1999

Population Surveys on Mass Nesting Beaches

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Overview

The olive ridley turtle (*Lepidochelys olivacea*) is considered the most abundant sea turtle in the world (Limpus, 1995). This is due largely to a circumglobal distribution and the fact that this species nests in larger numbers than any other species of sea turtle. Mass nestings are observed in the Eastern Pacific, Indian Ocean and South Atlantic, where olive ridleys have been estimated to lay from 5,000 to as many as 150,000 clutches of eggs over the course of just a few nights (reviewed by Cornelius et al., 1991). This mass nesting behavior (also called *arribada* after its name in Spanish) is characterized by large numbers of nesting females, as well as by high nesting density and the, as yet unexplained, nesting synchrony of participating individuals.

In spite of the relative abundance of olive ridleys with respect to other sea turtles, historical population ranges have been severely reduced in some areas by over-exploitation, poor hatching success, and the incidental capture of adults in shrimp nets (Cornelius et al., 1991; Valverde et al., 1998). Long term conservation efforts focused on ridley mass nesting populations may have been successful in some cases (Márquez. et al., 1996), but the current status and recovery potential of these species can only be assessed using the long term monitoring of demographic variables, and by employing robust and reliable statistical methods for data analysis (e.g., see Carr, 1980; NRC, 1990; Meylan, 1982). The use of short term survey records to evaluate the status of a population is inadequate and ill-advised because of the natural variability of inter-annual nesting numbers (NRC, 1990; Limpus, 1995).

The survey of olive ridley populations is complicated by the fact that standard ground and aerial survey techniques (see Schroeder and Murphy, this volume) are ineffective when faced with overwhelmingly high *arribada* densities. While surveys of nesting females have been conducted at all known *arribada* beaches, providing at least rough estimates of demographic parameters for the most important olive ridley populations in the world, population estimates are largely based on biased or inadequate methodology (see Valverde et al., 1998, for a discussion).

A general criticism of all previous estimation methods is that they fail to provide a measure of variability in the parameters estimated, including their associated confidence intervals. This constraint makes it impossible to determine the reliability of the resulting estimates. Further, because the mathematical relationship among the estimates obtained by the different methods is unknown, direct comparison of data from the different rookeries is difficult at best. Finally, these various methods have never been validated against *arribada*s of known size. Until this is done, the accuracy of the estimates will remain uncertain.

The objective of this chapter is to describe a universal method designed specifically to estimate in the field the number of nesting females that participate in individual *arribada* events. Because this method is unbiased and has a minimal number of assumptions, it is expected to yield reliable and comparable estimates among different *arribada* rookeries. The technique has been fully described in a recent paper (Gates et al., 1996), to which the reader is referred for a more technical and detailed description.

Methodology

The technique described here is referred to as the "Strip Transect in Time Method". Although other approaches are available (see Gates et al., 1996), this method appears to be the easiest to implement. For simplicity, we will refer to the strip transects as, simply, "transects".

At least two transects, but no more than five, should be established per 100 m stretch of beach. At Gahirmatha, India transects are set up above the high tide mark (and are thus unaffected by tidal changes) by burying 1 m of a 2.5 m long, thick (~10 cm in diameter) stick. Three sticks are established along the longitudinal limits of the transects, one set per side, every 5-10 m between sticks depending on the extent of the available nesting area. A thread is tied on the sticks waist-high so as to join the three sticks along one side of the transect (contributed by Bivash Pandav, Wildlife Institute of India). The width of the transect can be fixed as desired. We suggest a transect width of 2 m. Because turtles may obliterate the high tide mark when crawling *en masse* up the beach, the approximate distance between this point and the closest transect stick can be noted prior to the *arribada*. This will allow surveying the same transect length every time.

We estimate that an observer should not survey more than 20 transects (at a rate of 2 transects/100 m) during one *arribada*, particularly on beaches exceeding 2 km in length. We recommend that the survey of all 20 transects/km take no longer than 45 minutes. On long beaches where turtles are known to shift laterally from *arribada* to *arribada*, transects should be established to include the entire stretch of beach known to accommodate nesting. Although surveys must be conducted regularly in the area of higher utilization during a given *arribada*, quick inspection of the transects outside the main area should be performed to ensure that no turtles are nesting there.

It is absolutely essential that only egg-laying females be counted during the transect surveys. To satisfy this condition for every female participating in the survey, egg deposition must be verified (such as by carefully excavating into the nest chamber). If no eggs are present, the animal should not be included in the tally. Only egg laying animals whose carapace centers are within the borders of the transects can be counted. The average time required for egg-laying (i.e., the elapsed time between the release of the first and the last eggs of a single clutch) must also be determined. At Nancite Beach the value is approximately 15 minutes. It is important that this parameter be determined for each *arribada* until no significant variability is observed among *arribada*s. At this point, the same value can be used for future mass nesting events. An initial sample of some 30 individuals may suffice to accomplish the task.

Counts within the transects should begin at the onset of the *arribada*. The purpose of defining the beginning of the *arribada* is to avoid initiating the surveys on a night of heavy solitary nesting. There appears to be considerable variability among biologists as to when the onset of the *arribada* occurs. For the purpose of standardization, we suggest that the onset of the *arribada* be defined when 100 or more turtles are estimated to be on the beach simultaneously (Cornelius et al., 1991). It is unnecessary to predefine the end of the *arribada*.

Counts should always be initiated at one extreme of the nesting area and finished at the opposite end. Before initiating the surveys, a fixed time interval between counts must be established. This interval should remain constant throughout the duration of the *arribada*. If no nesting occurs within the transects, zeroes must be recorded. We suggest surveying the transects every two hours to allow surveyors time to examine all the transects and still have time to carry out other chores. The interval between surveys cannot be shorter than the effective time of nesting to avoid counting the same turtle more than once. An important modification from the original method we proposed (Gates et al., 1996) is that transect surveys need not be conducted during non-nesting hours (usually daylight hours). The reason for this is that zeroes are ignored when computations are done.

The available nesting area on the beach must be calculated; for example, by measuring the length of individual transects and multiplying their average value by the length of the stretch of beach used by the gravid females. Transect sticks can be used as a reference to define the beginning and the end of each transect. This will facilitate the measurement of the transects during or after the *arribada* has concluded.

Information collected during the survey should be organized to facilitate data processing. We recommend that a table including at least the parameters listed in Table I be utilized for recording data. The example given in the table illustrates the use of the formulas for one session of a three session *arribada*. Here, a "session" is defined as the consecutive hours in which turtles emerge synchronously to nest within a 24-hour stretch of an *arribada*. Usually, this period occurs at night as olive ridleys tend to nest during dark hours. With all the necessary information collected, an estimate of the number of nesting turtles and various parameters can be obtained using the following equations (again, for details see Gates et al., 1996):

$$M = \frac{AH}{2wtl.} \cdot \frac{n..}{\bar{h}}$$

with a variance of the estimated number of nesting turtles in the *arribada*:

$$v(M) \approx M^2 \left[\frac{v(n..)}{n..^2} \right]$$

If transects are approximately of equal length:

$$v(n..) = \frac{m \sum \sum n_{ij}^2 \sum_{i=1,t} n_i^2}{m-1}$$

If transects are not of equal length:

$$v(n..) = \frac{m^2 \sum_{i=1,t} \left[L \sum_{j=1,m} l_j n_{ij}^2 - \left(\sum_{j=1,m} l_j n_{ij} \right)^2 \right]}{L^2 (m-1)}$$

The associated 95% confidence interval is given by the approximation:

$$M \pm M \sqrt{\frac{v(n..)}{n..^2}}$$

The coefficient of variation is given by:

$$CV(n..) = \sqrt{\frac{v(n..)}{n..^2}}$$

The length of the session can be calculated using the simple equation:

$$k = t * r$$

Where:

M = estimated number of nesting females;
A =total available nesting area (m^2);
H =duration of the *arribada* (min);
w =half-width of transect (m);
t =number of sampling periods;
$\sum l_j = l. = L$ =sum of the lengths of all transects (m);
$n..$ =sum total of egg laying turtles counted;
$\bar{h}$ =average time spent by turtles laying eggs (min);
$v(M)$ =estimated variance of estimate;
$v(n..)$ =estimated variance of the total number of egg-laying females;
m =number of transects;
n_{ij} =number of egg laying turtles in the i^{th} period and the j^{th} transect;
$n_{i.}$ = sum total of turtles in all transects in the i^{th} period;
k= length of session (min);
r= inter-sampling interval (min).

When analyzing the *arribada*, the statistics must be calculated independently on each individual session of continuous monitoring as variances and other parameters are not directly additive. Nonetheless, the estimates of number of females can be summed to provide a global estimate of number of nestings.

We recommend that standard errors and confidence intervals be reported, rather than the variances, which tend to be large. In addition, the units of the different variables included herein are different from those of our previous publication (Gates et al., 1996). This modification is intended to facilitate manual calculations in the field. Alternatively, a computer program has been written and transformation of the units may be necessary. Copies of the program, as well as complementary information on its use, are freely available and may be obtained from the author (CEG). The program includes a simulation option that permits a comparison of standard errors obtained from real data with those of an idealized population.

Limitations

The method described here is easy to implement, involving few logistical hurdles. Nevertheless, the method may not be suitable to estimate number of nesting females under all conditions. For example, low numbers of nesting animals, such as are found on beaches were solitary nesting occurs, reduces the accuracy of estimates. As a general rule, the method is only applicable in circumstances where more than 1,000 turtles nest synchronously. In cases of lower densities, more orthodox surveying approaches (e.g., Schroeder and Murphy, this volume) may be advisable. Our method, however, is self-compensating since very large confidence intervals (probably encompassing zero) will inevitably result when sampling very small populations, thus rendering the estimates highly dubious.

A drawback of carrying out computations per session is that the number of turtles during some sessions may be too low to allow statistically sound results. Thus, the value of the analysis per session as shown in this paper is to provide an example of the correct use of the formulas and to manually generate estimates in the field with the assistance of a hand calculator where power may not be available.

Sources of Error

Errors result from the erroneous classification of turtles. For example, wrongly presuming a turtle to have nested (laid eggs) or prematurely including turtles engaged in the construction of an egg chamber. These errors are difficult to quantify. The best solution is to insist that surveyors stringently conform to the rule that the presence of eggs must be verified in every case. Errors result from the incorrect measurement of nesting and sampling areas (transects). Because of the expansion factors included in the formulas used (see above), these mistakes may induce significant deviations from accurate population estimates. Again, efforts must be made to ensure proper measurements.It is important that the inter-sampling interval be maintained constant throughout the *arribada* to ensure consistency in the collection and processing of data.

Lastly, determining the exact time of the onset of an *arribada* can be problematic. Large deviations may have significant effects on the estimates obtained. It is advisable that experienced observers determine these times. Nonetheless, under most conditions, a deviation of one or two hours from the true value of this parameter may not have a significant impact on the final estimations.

Acknowledgments

The authors acknowledge the contributions of Jocelyn Peskin, Claudette Mo, Jorge Ballestero, and Ana Chaves in the collection of data and field support. Our gratitude is extensive to Róger Blanco, Sigifredo Marín and other personnel of the Santa Rosa National Park, Guanacaste Conservation Area, Costa Rica for their continued logistic support during the development of this manuscript. We also thank Alberto Abreu for his valuable criticism of the final draft of the paper. Part of the research whose results are included here was funded through grants from the U.S. National Science Foundation to Dr. David W. Owens (NSF-IBN-9124014).

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Table 1. Strip Transect in Time Method. An analysis of the first session from a hypothetical survey are shown to illustrate method.

Observer:		**Total available nesting area (m^2)**	**24,200**	***M***	**9,652**
Average egg-laying time (min)	**13.17**	**Half-transect width (m)**	**1**	***S.E.*=**	**2,068**
Total duration of arribada (min)	**360**	**Total length of transects (m)**	**376.96**	***U.C.L.*** $_{95\%}$ **=**	**13,786**
Number of sampling periods	**3**			***L.C.L.*** $_{95%}$ **=**	**5,515**

< **Transect Number** >

Session	Day	Time	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	$n_{i..}$
I	1	12:00 AM	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	2
		2:00 AM	0	0	0	0	0	0	0	0	2	5	3	2	3	2	2	2	0	2	0	0	23
		4:00 AM	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	1	0	1	1	8
		6:00 AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		8:00 AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10:00 AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		12:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		8:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
II		10:00 PM	0	0	0	0	0	0	0	1	4	4	1	2	1	0	2	1	0	0	2	0	18
	2	12:00 AM	0	0	0	0	0	0	0	1	0	2	1	3	2	1	0	0	0	1	0	0	11
		2:00 AM	0	0	0	0	0	0	0	0	1	1	2	0	0	1	0	0	0	0	1	0	6
		4:00 AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6:00 AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		8:00 AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10:00 AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		12:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		8:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
III		10:00 PM	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2
	3	12:00 AM	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2
		2:00 AM	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
		4:00 AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
																						***n..*=**	**74**

Calculations for Session I data

$$M = \frac{AH}{2wtl.} * \frac{n..}{\bar{\bar{h}}} = \frac{(24{,}200\ \text{m}^2)(360\ \text{min})}{2(1\ \text{m})(3)(376.96\ \text{m})} * \frac{33\ \text{turtles}}{13.17\ \text{min}} = 9{,}651\ \text{turtles}$$

$$v(n..) = \frac{m\sum\sum n_{ij}{}^2 - \sum_{i=1,t} n_{i.}{}^2}{m-1} = \frac{20(1^2+\ldots+1^2)-(2^2+23^2+8^2)}{19} = 50$$

$$v(M) \approx M^2\left[\frac{v(n..)}{n..^2}\right] \approx (9{,}651)^2\left[\frac{50}{(2+23+8)^2}\right] \approx 4{,}276{,}483$$

$$S.E. = \sqrt{v(M)} = \sqrt{4{,}276{,}483} = 2{,}068$$

$$U.C.L._{95\%} = M + 2M\sqrt{\frac{v(n..)}{n..^2}} = 9{,}651 + 2(9{,}651)\sqrt{\frac{50}{1{,}089}} = 13{,}786$$

$$L.C.L._{95\%} = M - 2M\sqrt{\frac{v(n..)}{n..^2}} = 9{,}651 - 2(9{,}651)\sqrt{\frac{50}{1{,}089}} = 5{,}515$$

$$CV(n..) = \sqrt{\frac{v(n..)}{n..^2}} = \sqrt{\frac{50}{1{,}089}} * 100 = 21.4\%$$

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Studies in Foraging Habitats: Capturing and Handling Turtles

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Introduction

The foraging habitats of marine turtles vary greatly in their physical and biotic attributes. Water depth; bottom type; presence/absence of tidal flow, currents and/or surge; wind velocity; and water clarity are among the factors that must be accounted for in planning in-water surveys. Despite the spectrum of conditions and circumstances with which researchers have had to deal, one general kind of equipment, the so-called “tangle net,” has been used relatively effectively in many places. Technical specifications, deployment methods, soak times, net lengths, net tending methods, anchor attachment, and other considerations all have varied according to circumstance, but the basic implement is a large-mesh tangle net soaked for periods of time in the waters of the foraging habitats. The situation in which such a net works best is that of a protected but relatively open, relatively shallow bay, sound, or lagoon with little or no movement of water except that driven by the wind at the surface. We intend to adopt such a situation as an arbitrary standard—a starting point for which we can lay down the basics of net specifications, deployment styles, tending methods, etc., then follow that with comments about the modifications of these items that are warranted under different environmental conditions. It is advisable for researchers contemplating the use of net-capture and hand-capture methods to gain experience by visiting established projects where they have been used successfully.

Some researchers capture turtles by hand and, although the precise procedures are generally locality-specific, we provide a synopsis of hand-capture techniques following the sections on net-capture.

Net Specifications

A typical net consists of webbing (mesh) hung from a braided polypropylene (0.635cm in diameter) top line that is suspended at the surface by floats. The webbing is made of 18-gauge twisted nylon twine and the mesh size is 40cm stretch (knot to knot). Such a net is said to be 20cm “on the bar,” which means that each side of a mesh square is 20cm long. Some researchers use nets made of 50 lb-test monofilament line with satisfactory results, but there is more tendency to cut the skin with such a net. Other mesh sizes up to 50cm stretch can be used if there is reason to believe that turtles smaller than about 40cm straight carapace length (SCL) will not be encountered. Where there is the possibility that there will be turtles smaller than about 32cm SCL (such as some known assemblages of juvenile green turtles) a smaller mesh size (usually 30cm stretch) can be used. See the section below on net tending for a discussion of the benefits and liabilities of smaller mesh size. One method of float attachment involves fastening individual “bullet-shaped” Styrofoam floats with spring clips at approximately 10m intervals at each deployment. The bullet-shaped floats stand up and “dance” when a turtle is tangled in the net near them. Other workers prefer to have a larger number of smaller, round floats with center holes permanently arranged along the top line. Still others have used a top line that is uniformly impregnated with foam along its entire length. The latter two methods are satisfactory and may be preferred in certain situations, but they do not provide as much information to the observer as to what is happening beneath the surface as the bullet-shaped floats do. For best results the bottom line of the net should be made of No. 30 continuous lead core line.

Tangle netting is generally not undertaken in water deeper than about 4m and so most nets are not more than 4m tall (wide). It may be possible to have taller nets constructed for specific circumstances, but one should understand that such nets work best when some of the webbing is lying loosely on the bottom, not stretched out to maximum depth in the water column.

Net Deployment

Net deployment begins with the rigging of an 8-kg Danforth-type anchor. A 1.5m section of 0.8cm chain should be shackled to the ring on the anchor shaft. Another shackle should be used to tie a 15m length of 1cm nylon line to the chain. The other end of the line should be tied to one of the free ends of the top line of the net. Some readers may question anchoring the top line, but experience has shown that to be the correct manner. The net can be paid out from the uncovered bow of an outboard boat operated in reverse or from the stern of a boat specially equipped with a net platform and the engine mounted off-center on the transom. The bow (or stern) should be free of all cleats and other hardware that would interfere with the deployment, tending, and retrieval of the net. The anchor is lowered to the bottom as the boat moves away, and the entire length of anchor line is paid out. At that point it is tested to assure that the anchor has penetrated the bottom and is holding. That having been done, a float is attached to the top line at the point where the webbing begins and the net mesh begins to enter the water. Two or three workers attend the net as it is paid out, making sure that the bottom line does not get twisted over the top line and attaching floats at 10m intervals. The time at which the mesh first begins to enter the water and at which the last of the mesh is soaked should be recorded. Another anchor, rigged in the manner described above, is tied to the other free end of the top line when the last of the webbing is deployed. A worker holds onto the second anchor until the line is taut and then lowers it overboard. Net deployment should begin at the upwind end of the netting site and the boat operator should set a course that is at about a 45-degree angle to the wind. This will assure that the wind will keep the stern and propeller away from the net during subsequent net tending.

The length of net to be soaked varies with conditions and the experience of the researcher. It is not wise to soak more than 100-150m of net in any new situation. With experience it should be possible to gradually increase the length of net soaked to as much as 450m in a protected, shallow bay, or lagoon. Ordinarily that would be the maximum that could be tended effectively by a crew of four to five people in one boat. In most other situations, soaking half as much net, or less, is prudent.

Tending the Net

In the sort of situation that we have adopted as our standard (shallow, protected bay or lagoon) the net can be checked by hand over hand elevation of the top line from the bow of a boat. The level of intensity of net tending varies with mesh size and with the minimum size of turtles in the population. Turtles larger than about 40cm SCL have the bulk and strength to rise to the surface to breath even when well entangled. Smaller turtles, especially those smaller than 35cm, can get both anterior flippers thoroughly enmeshed and have difficulty reaching the surface. The net should be tended continuously in any new situation, and it is a good idea to keep the net in view at all times. Where experience has shown that there are no turtles under about 40cm SCL and where 50cm stretch mesh is used, attention to the net can be somewhat less assiduous. However, in those situations where turtles in the 30-35cm size range (or smaller) are a possibility and a mesh size of 40cm (stretch) or smaller is used, the net should be tended continuously. Heightened awareness and more intensive tending are warranted whenever 30cm mesh is used and where smaller turtles are likely to be captured.

Departures from the Standard

Netting Over Near-Shore Oceanic Reefs

The problems here involve the movement of water (the surge and flow of tidal change and sea state), poor water clarity, and the tendency for the net to become caught on the jagged surfaces of reef structures. These factors preclude checking the net from the bow of a boat and require that free divers snorkel the top line continuously. The use of SCUBA gear is not advisable because divers are constantly caught up in the net by their regulators, octopuses, buckles, and snaps. The crew must be sufficiently large that there can be two snorkelers in the water for every turtle that is captured (one to handle the turtle, one to pull snagged mesh off the bottom). A crew of six, in one boat, should not set more than about 200m of net (half of that or less in new situations) and nets should not be deployed when visibility does not exceed the depth of the net.

Cuts and Channels

Turtles of several species are often concentrated in cuts and channels that interrupt large areas of grass flats, oyster reefs, and other shallow water habitats. These cuts or tidal channels are characterized by strong currents associated with the ebb and flood of the tide onto and from the inshore flats. Tangle nets are difficult to set in such situations, but they can be fished by drifting or anchoring a relatively short length of net (30-40m drift net; 50m set net). In the former method the net is tied between two boats riding the current from one end of the cut to the other. Two free divers are required to disentangle turtles and bring them to the boat.

The latter technique ("set net") requires that the net be anchored across the cut or channel. The net should be deployed during the slack tide period to facilitate positioning it perpendicular to the current. Care should be taken not to allow the webbing to "knot up" while paying it out of the boat. The anchors must be set firmly in the bottom and bridles at each end of the net pulled tight with the anchor warps (lines). Strong tidal currents can pull the anchors loose, causing the net to drag downdrift and/or become fouled on the bottom. This is especially true in areas of limestone bottoms and oyster reefs.

If the channel is wider than the net or is a poorly defined depression or slough, netting efficacy can be enhanced by anchoring a second net 3 to 5m downdrift from the first net. It should block that part of the channel not covered by the first net and overlap the end of the first net by a few meters. Turtles using these channels sometimes avoid or escape from the first net they encounter, only to become entangled in the second.

Other departures from the standard set described above that are relevant to cuts and channels, involve mesh size and depth (width) of the net. The larger mesh size (50cm stretched) may be preferable because it avoids, to some degree, the incidental capture of large numbers of fishes, especially sharks and rays, that are commonly found in these habitats. Also, if more meshes (about 20) are hung in between the float line and lead line, the surplus or slack webbing tends to entangle small turtles when they contact the net as they surface to breathe. Another modification to the standard net involves use of a larger anchor, a Northill type weighing at least 15kg, and attached to bridles at each end of the net. The bridles (3-4m lines attached to the ends of the top and bottom lines that come together at a single anchor line) should be rigged so that the bottom leg to the lead line is one meter longer than the upper leg attached to the float line.

Ports, Basins, and Other Partial Enclosures

Marine turtles of several species sometimes use deep, man-made basins as foraging habitats. Such basins are usually far too deep to allow the use of tangle nets throughout most of their extent, but some have shallow shelves around their perimeters where nets can be deployed in much the same manner as in bays and lagoons. In this case the net is deployed parallel to the shore at a distance of 5-10m and long-handled dip nets can be used to augment the capture of turtles that come up into the shallows to feed. The net is checked by hand-over-hand top line elevation from the bow of a boat that slips quietly through the narrow zone between the net and shore. Attempts can be made to dip turtles seen in shallow water near the shoreline. When that fails, the turtles move toward the deep water and are often caught in the tangle net deployed near the edge of the shelf. Admonitions relative to net length and attention to the net are essentially the same as for the standard situation (bays, lagoons, etc.).

Marshes and Tidal Creeks

Along some low-energy shorelines characterized by salt marshes and tidal creeks marine turtles (mostly green turtles in this case) are known to move into the creeks on the rising tide. With experience and local knowledge it is possible to deploy a relatively short length of tangle net across the mouth of these creeks and capture turtles as the tide falls. The method is similar to the "set net" procedure described above under "cuts and channels." As in the case of netting over oceanic reefs, it is advisable for any researcher contemplating surveys of such habitats to gain experience by visiting and participating with a seasoned practitioner.

"Striking" the Net

In areas where surface and bottom conditions permit and where numbers of turtles can be seen at the surface, an anchored barrier net can be drawn in a circle around small groups of turtles. Once turtles are enclosed by the net, free divers enter the water to catch them by hand and bring them to the boat. This is usually done a number of times in rapid succession and is referred to as "striking."

Hand-Capture

Specific procedures by which researchers hand-capture marine turtles are as varied and sundry as the places and circumstances where this method is used. Our attempt here is to provide a succinct, ordered, but necessarily subjective approach to the subject. Most hand-capture techniques fall into one of three arbitrary categories: those in which boats are directly involved in the pursuit and capture; those in which barrier nets of some kind are used; and those involving the use, primarily, of SCUBA and/or snorkeling gear. This method generally requires clear water with good visibility.

Diving with Active Watercraft Pursuit

In some cases this involves pursuing the animal until it begins to tire and then diving on it from the bow or gunwale of a boat. In other cases the turtle is simply followed at relatively slow speed until it stops or slows down or quiescent turtles on the bottom are searched for; then (in either case) the dive is made. The diver enters the water hands first, aiming just slightly in front of the turtle. Momentum carries the diver beneath the surface where, if fortune prevails, he/she grasps the turtle by the nuchal and posterior marginal scutes and guides it to the surface. In the case of small turtles stationary on the bottom, the diver usually pins the animal against the sand to make the initial capture, then grasps both shoulders prior to making the ascent.

Diving to Tend a Barrier Net

In places where turtles occupy small coves between prominences of land or shallows, it is possible to capture them by deploying nets across the mouths of the coves. Turtles that move seaward encounter the net and usually dive to the bottom where they can be procured by free divers.

Free Diving and Use of SCUBA

Turtles sleeping or resting quietly on the bottom can, in some instances, be approached stealthily and captured directly by a free diver or one employing SCUBA. Usually, however, two divers are required; one to approach the animal from the front and distract it while the other diver approaches from above and behind, makes a quick, final descent, and grasps the turtle at the insertion of the anterior flipper (small turtles) or by the nuchal and posterior marginal scutes (turtles greater than about 45cm). A modification of this method used at night involves shining a bright diver's light on the bottom in front of the turtle, distracting or disorienting it to the extent that another diver can move in from above and behind and grasp it.

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Aerial Surveys in Foraging Habitats

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One proven method of obtaining at-sea information on sea turtle distribution and abundance is the use of trained observers aboard aircraft. This technique is applicable to sea turtles because they must surface periodically to breathe, and a fraction of the turtles in an area will be on the surface and available for counting at any given time. This fact is well supported by radio, sonic and satellite tracking experiments where surfacing behavior of several turtle species has been documented. The applicability of this technique for population estimation is dependent upon overall objectives, funding levels, area size, target species, turtle size, turtle abundance, observer experience, and a number of other factors.

Theory

Every biologist has at some point been exposed to the concept of sampling to estimate the total population. With aerial surveys, this sampling is in the form of transects through an area during which all sightings are recorded. Sightings from each transect are converted to sightings per unit area and extrapolated to estimate the population for the total study area. In the case of sea turtles, this estimate is for turtles on the surface, not the total population. To estimate the total population, it is necessary to determine the proportion of turtles on the surface and correct the surface densities accordingly.

Two commonly used analytical approaches for estimating the area covered along a transect are line-transect and strip-transect. Both have been used for analyzing aerial survey data; if the distance of each sighting from the transect is measured, either method of analysis can be used for analyzing the data. For additional discussion on the strengths and weaknesses of the two approaches refer to Buckland *et al.* (1993), Cormack *et al.* (1979), and Epperly *et al.* (1995). For a discussion of the analysis of the data, see Gerrodette and Taylor (this volume).

Methods

Aircraft selection is important in planning an aerial survey. A single engine aircraft may be adequate for low budget operations in nearshore waters (within gliding distance of land). Larger twin-engine aircraft are recommended for offshore operations. Plexiglass bubbles on the sides or in the nose of the aircraft providing forward, aft, and downward trackline visibility are essential to meet the assumptions of line-transect theory.

Aircraft should be equipped with a Global Positioning System (GPS) or other navigational system which ideally is interfaced with an onboard laptop computer for continuous position recording. Flight altitude and airspeed should be constant within a study and depend upon primary objectives of the survey and variables such as species of turtle, size, sex, behavior, study area, and a number of other factors. For studies of sea turtles, altitudes should be about 150m (500ft) or less and airspeed should be 150 to 225km/hr.

The perpendicular distance of each sighting from the transect can be determined using clinometers and/or interval marks on plexiglass bubbles, window frames, wing struts, or other fixed aircraft parts. For all sightings, the location, time, environmental parameters, distance from trackline, turtle species, and associated species are normally recorded. The survey team usually consists of two or more observers and a data recorder to ensure constant viewing from both sides of the aircraft.

In theory, the minimum distance between transects is determined by the maximum swimming speed of the target species, so that multiple counts of the same individual do not occur. In reality, however, transect spacing generally relates to practical considerations of how much effort can be devoted to an area to accomplish the overall survey objectives; usually it is available effort that limits the number of transects. To maximize the effectiveness of individual surveys, transect length should be selected on the basis of area to be surveyed, available time, aircraft, and survey objectives. Transects are usually parallel to each other (primarily for logistical reasons) and are perpendicular to gradients (such as depth) that may affect turtle density. The more transects flown, the more accurate the estimation of density, assuming that transects are spaced far enough apart to avoid multiple counts of the same individual.

Environmental conditions influence whether a flight should be conducted. Safety is of utmost importance. Safety equipment, such as a life raft, survival kit, flares, and VHF radio, should be carried on all over-water flights. Survival suits should be standard equipment when flying over cold water. Secondly, sea state influences the ability of observers to detect turtles on the surface and also may affect turtle behavior. Ideally, flights should be conducted only when sea states are less than 0.6m with no or few whitecaps *(e.g.*, Beaufort Sea State -2). Lastly, glare is a confounding factor. Flights should be conducted as close to noon as possible to minimize glare. Researchers should consider issuing polarized sunglasses to all observers to standardize for glare as much as possible.

The ability to determine turtle species depends on observer experience. Experienced observers comment that color, rather than silhouette, is most important in identifying sea turtle species from the air. When a determination of species cannot be made, it is sometimes useful to indicate whether the sighting represents a leatherback (*Dermochelys*) turtle or a hard-shelled species; in this case, the silhouette is diagnostic.

Discussion

Aerial surveys are probably most appropriate when very little is known about turtle distributions and abundance over relatively large areas. In such a case, the aerial survey would be used to determine turtle distribution and abundance and to identify "hot spots" for future in-water studies. Aerial surveys are also appropriate for documenting seasonal or annual variations in distribution and abundance patterns.

Anyone contemplating the use of this technique should carefully consider the types of data that can and cannot be obtained from the air. No biological information *(e.g.*, size, weight, sex, condition, age, growth, tags) can be acquired from aerial sightings. This type of information must be obtained from in-water studies (see Ehrhart and Orgren, this volume) that should be conducted in conjunction with aerial surveys for purposes of ground-truthing. In addition, some level of radio and sonic tracking (see S. Eckert, this volume) is essential to determine the proportion of time spent at the surface by turtles within the study area. The major advantage of aerial surveys rests in the fact that they are a relatively fast way of obtaining a quasi-synoptic picture of turtle distribution and abundance over broad study areas.

Aerial surveys are not something that can be accomplished easily. Observer experience is critical to the success of an aerial survey. Untrained and/or inexperienced observers often have difficulty seeing turtles from an aircraft. Skill in sighting and identifying turtles improves with time, and every effort should be made to ensure that a survey is not dominated by inexperienced observers.

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Estimating Population Size

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Introduction

Estimating population size is important for several reasons. An estimate of population size is critical for science, conservation, and management. Many threats to turtle populations cannot be evaluated unless we have an estimate of population size. For example, if we know that 100 turtles per year die in fishing nets, is this a serious threat? If population size is 1,000 turtles, the deaths of 100 turtles per year is indeed a very serious and immediate threat, but if population size is 1,000,000 turtles, the threat is much less serious. Estimating population size is also important for assessing the risk of extinction or extirpation. Small populations are more likely to become extinct than large ones.

Because of sea turtle life history characteristics, it is nearly impossible to estimate total population size directly for any sea turtle population. Instead, we estimate the size of only one part of the population, such as adults (typically, adult females). Because juvenile turtles are pelagic, dispersed over a wide area, and difficult to detect in the water, it is extremely difficult to estimate the size of this part of the population. Therefore, when discussing population size, it is important to be clear about which part of the total population is being estimated and the assumptions underlying any extrapolation to the total population *(e.g.,* 1:1 sex ratio among adults).

Another important distinction is the difference between relative and absolute population size estimates. Absolute population size is the actual number of animals. Relative population size, also called an index of population size or an index of abundance, is a number proportional to absolute population size. Unless the factor of proportionality is known, there is no way to convert an estimate of relative abundance to an estimate of absolute abundance. Nevertheless, estimates of relative abundance can be very useful. The most common example is the use of nest counts as an index of abundance. Such data can be extremely valuable as a way of detecting trends in abundance over long periods of time. Estimates of relative population size are usually simpler and less expensive to obtain than estimates of absolute population size. However, estimates of relative population size also require more assumptions; if these assumptions are violated, the estimates may be biased.

Bias and Precision

The quality of any estimate has two measures: bias and precision. It is important to know the distinction between these terms. Consider an analogy of shooting at a target (Figure 1). When Figures A and B are compared, we see that both are precise *(i.e.,* the shots are not widely scattered), but the shots in B tend to be too low. In statistical terms, the shots in B are (negatively) biased. Consider the challenge of estimating the number of turtle nests when the beach is not walked frequently enough to tally every nest. We may know that a few were missed and that our count is therefore negatively biased, but that our count is still very close to the true number. To improve accuracy, we could adjust the sights of our "estimation gun" by applying a correction factor. Now consider two guns: a pilgrim's musket and a sniper's rifle. The rifle shoots with great precision and is equivalent to an abundance estimate with very low variance, such as a nest count in an intensely surveyed area. Even an expert marksman, however, would be considerably less precise with the musket; repeated attempts with the musket result in a more diffuse pattern than with the rifle (Figures C and D). Statisticians measure the precision of an estimate by its variance; thus, the shots in C and D have high variance (low precision) relative to A and B. This poor precision is equivalent to abundance estimates made for aerial surveys of turtles at sea where they are rare, hard to see, and some unknown proportion is beneath the surface. If we did not correct for the proportion not visible, the result would be an estimate that was both imprecise and biased (Figure D).

When decisions are made using estimates, we should consider the quality of the estimate. Therefore, each estimate of any quantity, such as population size, should be accompanied by a consideration of its bias and an estimate of its variance. Variance is important because it is a measure of the certainty (precision) of the estimate. If an estimate of population size has high variance, it means that we are not very certain of its value, and any management decisions based on it should be made cautiously. In their seminal paper on management of living resources, Holt and Talbot (1978) advocate that the less precise the data are, the more conservative the management decisions should be. For example, suppose a population of turtles is declining. If our estimates of abundance have high variance, it is likely that we will not be able to detect that decline statistically. Without an estimate of variance, the data could be interpreted as indicating no decline, and consequently no management action would be taken. On the other hand, if we do have an estimate of variance, we can calculate the probability of being able to detect the decline (Gerrodette, 1987, 1993; Taylor and Gerrodette, 1993).

The importance of bias depends on the question under consideration. For example, if we are interested in trends in the abundance of adult females, a relative index of abundance may suffice (bias is unimportant as long as it is constant). On the other hand, if we want to know if a certain mortality level is too high *(i.e.,* unsustainable at the population level), we would certainly want to remove bias and have an estimate of absolute abundance.

Methods of Estimating Population Size

Estimating Population Size from Beach Counts

From the number of nests, the number of adult turtles (male and female) can be estimated as

$$\hat{N} = \begin{pmatrix}\text{number}\\ \text{of nests}\end{pmatrix} \div \begin{pmatrix}\text{no. nests}\\ \text{per female}\end{pmatrix} \div \begin{pmatrix}\text{proportion of}\\ \text{females nesting}\end{pmatrix} \div \begin{pmatrix}\text{proportion}\\ \text{of females}\end{pmatrix} \div \begin{pmatrix}\text{proportion of}\\ \text{beaches covered}\end{pmatrix}.$$

Obviously this involves the estimates of many separate quantities. The estimation of each factor in

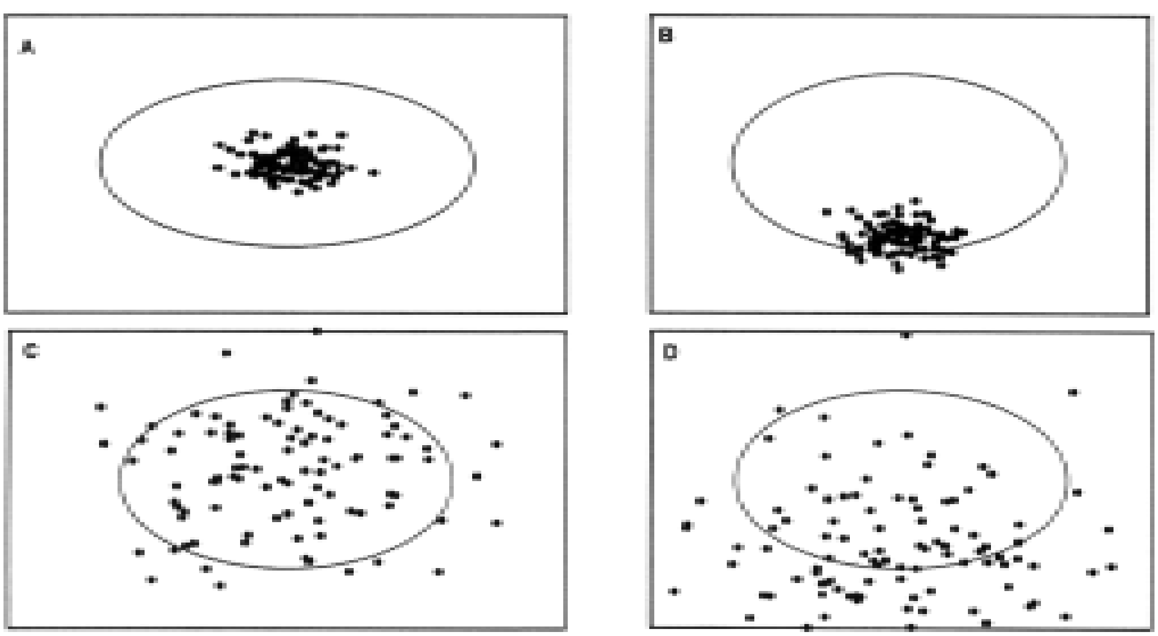

Figure 1. Shot patterns on targets demonstrating (A) precise and accurate (non-biased) shots, (B) precise but inaccurate shots, (C) imprecise but accurate shots, and (D) imprecise and inaccurate shots. (Source: White et al., 1982).

the equation is covered in other sections of this manual. The variance of $\hat{N}$ is a sum of the variance of each factor (assuming independence). The bias in $\hat{N}$ similarly depends on the biases of each of the factors in this equation. However, some are more likely to contribute bias than others. For example, if a complete nest count is attempted, the count is likely to be slightly negatively biased because a few nests will be missed. On the other hand, incorrectly estimating the proportion of beaches covered could contribute large positive or negative biases.

Estimating Population Size from Transect Surveys

Estimating population size from transects is a widely-used method in wildlife studies. A standard reference is the monograph by Seber (1982). Small aircraft can be used to count nests on beaches (see Schroeder and Murphy, this volume). Sighting surveys from planes or ships can also be used to estimate turtle abundance at sea. Because turtles tend to occur at low density and are hard to see, such surveys will tend to produce few sightings, but they can be conducted in conjunction with surveys for other species such as sea birds or marine mammals. To estimate absolute abundance, such surveys must also correct for turtles which are submerged at the time of the survey and are not available to be seen.

There are two basic types of transect surveys. Strip transects assume that an area of a certain width has been surveyed and that no turtles within a certain distance of the trackline have been missed. This will not be true unless the width of the strip is very narrow, in which case the survey will cover only a small area and not be very efficient. Line transects (Buckland *et al.* 1993), a newer and superior method, have largely replaced strip transects. Line transects make efficient use of all sightings, the statistical models are well developed, and free software is available (see footnote). However, line transects require a minimum of about 30 sightings, and preferably more than 50, to estimate population size. Also, while strip transects simply require the number of turtle sightings that occur along a transect, line transects also require that the distance of each sighting from the trackline be measured. That is, line transects require extra information, but if this extra information is available, better estimates are possible.

For transect surveys, the number of turtles is estimated by

$$\hat{N} = \frac{n}{2wlg} A,$$

where n = number of turtle sightings, l = length of transects, w = width of transect on one side of trackline, g = fraction of turtles visible, and A = size of the study area. The fundamental difference between line- and strip-transect surveys is that, in a strip transect, the width w is simply chosen, while in a line transect, w is estimated from the data and is called an "effective strip width." The variance of $\hat{N}$ is estimated from the variance of replicate transect lines, by assuming some distribution for n (usually Poisson), or by a computer intensive technique called boot strapping (Efron and Tibshirani, 1993).

Estimating Population Size by Mark-Recapture

Mark-recapture is another common technique for estimating abundance in wildlife studies. Several comprehensive papers explain mark-recapture theory, discuss assumptions, and demonstrate the technique (Cormack, 1979; Seber, 1982; Pollock *et al.*, 1990). In this context, "marking" or "tagging" means any method of identifying individual turtles and "recapture" means any method of re-identifying a marked individual at a later time. Individual turtles might be "tagged" and "recaptured" photographically, for example, by unique patterns on carapaces or heads. Tagging is widely used in sea turtle studies, mostly to obtain information on growth, movement, and population dynamics (Chaloupka and Musick, 1997).

Mark-recapture models come in a variety of forms. "Closed" models assume that no births, deaths, immigration, or emigration occur during the period of study, and so are applicable only for discrete populations of turtles within a relatively short period of time (within one nesting season, for example). "Open" models, on the other hand, allow populations to change in size during the period of study. Open mark-recap-

Authors' note: Software referred to in this chapter may be obtained at no cost by writing to the Colorado Cooperative Fish and Wildlife Research Unit, Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, CO 80523, USA. Software may also be obtained through the Internet at http://nmml.afsc.noaa.gov/distance/map.htm for line-transect analyses and at http://www.cnr.colostate.edu/~gwhite/software.html for mark-recapture analyses.

ture models, often referred to as Jolly-Seber-Cormack models after the original developers, estimate survival rates as well as abundance. There may be a single or multiple periods of tagging, and a single or multiple periods of recapture. In general, population size is estimated by assuming that the proportion of marked animals in a sample is the same as the proportion of marked animals in the population. The original simple estimator, proposed by Petersen 100 years ago for a closed population with a single period of tagging and a single period of recapture, is

$$\hat{N} = \frac{nM}{m} ,$$

where $\hat{N}$ is the number of animals tagged in the first period, and *M* is the number of animals captured in the second period, of which *m* are tagged. More complicated models involve the simultaneous estimation of population sizes and survival rates in each year, and no simple equation can be written for the estimator of population size. However, free software is available for carrying out such analyses, including the variances of the estimates (see footnote).

The general assumptions of mark-recapture analysis are: (1) there are no births, deaths, immigration, or emigration during the period of study (although this assumption can be relaxed for open population models, as noted above); (2) all animals have the same probability of being tagged; (3) tagging does not affect the probability of being recaptured; (4) tags are not lost, and tags, when present, are always detected; and (5) recaptured animals are a random sample of the population.

When applying mark-recapture analysis to sea turtle populations, there are several important issues. One is tag loss (assumption #4). Any kind of "tag" may be lost, and estimating rate of tag loss is an important part of a mark-recapture analysis. Of course, it is good if tag loss is low, but it is more important that tag loss be consistent. The interpretation and analysis of mark-recapture data is far more difficult if, over the years, different kinds of tags have been used, tags have been applied in different positions, and tagging has been carried out by different people with varying skill and experience. With long-lived animals such as sea turtles, these kinds of variation are inevitable, but the importance of keeping this variation to a minimum cannot be overemphasized. To interpret mark-recapture data properly, specific studies need to be carried out to estimate tag loss *(e.g.*, McDonald and Dutton, 1996).

Another important issue is the randomness of samples (assumptions #2 and #5). At the beginning of a study, it is important to define the population that is to be estimated, and to take steps to tag and resample the population randomly. If turtles are tagged on a certain beach, for example, is the population being estimated restricted to that beach? If turtles are visiting other beaches, and turtles from other beaches are occasionally coming to the beach being studied, then the population being estimated is not for that beach only, but for a larger area. Also, is the beach under study a random sample of the whole population? It is important to consider these questions, and to test them if possible. Unlike transect studies, it is not important that effort in mark-recapture studies be constant. The tag and recapture sample sizes can be different. Methods of capturing turtles during the tagging and recapture phases can be different. In fact, there may be some advantage in having different methods during the two phases because there may be slightly different biases. The most important thing is to obtain a random sample. Simply tagging and recapturing a large number of turtles gives meaningless data (at least for abundance estimation) unless the assumptions of the analysis are fulfilled.

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Research and Management Techniques for the Conservation of Sea Turtles
K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, M. Donnelly (Editors)
IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Population Identification

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In the last decade genetic techniques have illuminated several aspects of marine turtle life history. For example, do female turtles return to nest on their natal beach? Do males provide an avenue for gene flow between nesting colonies? Does more than one male contribute to a clutch? What are the evolutionary relationships among sea turtle species? Can DNA "fingerprints" be used to trace marine turtle migrations? All these questions have yielded to molecular genetic studies in recent years (reviewed by Bowen and Avise, 1995; Bowen and Karl, 1996).

While all aspects of natural history are relevant to conservation, perhaps the most powerful genetic tools for marine turtle management are those which can identify discrete breeding populations on the nesting beaches and in corresponding feeding habitats. Resolution of populations (or stocks) in marine turtles is confounded by the extensive migrations made by most species as juveniles and as breeding adults. These migrations highlight the need to identify the geographic range of feeding habitats that support a specific breeding population and, conversely, to assess proportions of different breeding populations present in a particular feeding ground or harvest.

This chapter reviews the practical framework for using genetic information to identify breeding populations of marine turtles. Two fundamental themes underlie our discussion: (i) proper use of genetic information requires that the goals of the study are unambiguous and that the appropriate sampling design and molecular markers are employed; and (ii) molecular data are most informative when integrated with field studies, especially tag-recapture studies.

This chapter provides a brief description of the molecular approaches and protocols for sampling (Appendix 1), but not for the individual genetic methods. The latter are detailed in Hillis *et al.* (1996) and their applications to marine turtles are reviewed in Bowen and Witzell (1996) and Bowen and Karl (1996). For a discussion on the identification of breeding populations and evolutionary units see Moritz *et al.* (1995). For a description of population genetic processes, see Hartl and Clark (1997).

Choice of Molecular Markers

Mitochondrial DNA (mtDNA) has proved particularly effective for detecting population structure in marine turtles. The resolving power of mtDNA assays is technique-dependant; several studies have reported enhanced population discrimination using the rapidly evolving control region rather than whole-genome restriction fragment (RFLP) analysis (Table 1). For this reason, the control region is recognized as the mtDNA segment of choice for nesting beach surveys. The general conclusion from these surveys is that female turtles typically return to their region of origin to breed (natal homing behavior) but that breeding populations may encompass several adjacent nest-

Table 1. Molecular markers used to identify marine turtle populations

Marker	Inheritance	Population Variation[1] within/among
Nuclear genome		
protein electrophoresis	biparental	low/low
anonymous single-copy	biparental	low/low
microsatellites	biparental	high/low-moderate
Mitochondrial genome		
restriction fragments	maternal	low/low-high
control region sequences	maternal	low-high/moderate-high

[1] Relative variation within and among regional assemblages of rookeries.

Note: For more complete reviews, see Bowen and Karl (1996), Bowen and Witzell (1996).

ing habitats, separated by as much as 100-400 km (Norman, 1996; Bowen and Avise, 1995).

The mtDNA molecule is maternally transmitted, meaning that male offspring inherit their mother's mtDNA but do not pass it on to subsequent generations. In many circumstances, female-inherited markers offer a distinct advantage because they provide perspectives on female reproductive behaviors that are paramount to species survival (Bowen and Avise, 1995). On the other hand, mtDNA does not yield a complete picture, and can prompt a misleading interpretation of isolation between populations if there is some form of male-mediated gene flow, as is likely for green turtles (Karl *et al.*, 1992; FitzSimmons *et al.*, 1997a,b). For this reason, studies of nuclear DNA variation are highly desirable to complement mtDNA studies and to provide a more complete understanding of population genetic structure.

Population studies of nuclear DNA typically use segments of the genome that do not code for specific protein products. These non-coding regions accumulate mutations more rapidly than protein coding regions, and thereby provide greater sensitivity (Table 1). The nuclear DNA segments that are appropriate for sea turtle population studies include anonymous single copy nuclear DNA (ascnDNA; Karl *et al.*, 1992), minisatellites (Peare and Parker, 1996), and microsatellites (FitzSimmons *et al.*, 1997a). Minisatellite and microsatellite techniques, popularly known as DNA fingerprinting, have also been used to assess pedigrees and the possibility of multiple paternity in marine turtle nests (FitzSimmons, 1998). The latter approach is gaining acceptance as a standard tool in conservation genetics, and may be widely used for population studies of marine turtles in the next decade. The array of such nuclear DNA technologies is rapidly developing, so it is likely that additional assays will become available in the future, including direct sequencing of nuclear DNA segments (Karl, 1996).

Glossary of Genetic Terms

mtDNA-mitochondrial DNA in turtles is passed from the mother to her offspring, and from her female offspring to the next generation. Variants are typically called haplotypes, and when several haplotypes are present among populations, information is revealed about the structure of female lineages.

nDNA-nuclear DNA is inherited from both parents and thus studies using nuclear markers provide information about gene flow among populations as influenced by both females and males.

ascnDNA-anonymous single copy nuclear loci. These are unique (i.e., single copy) regions of nuclear DNA that can be useful genetic markers in marine turtles due to mutation events that have generated multiple alleles (Karl et al., 1992).

Microsatellite loci- regions of nuclear DNA defined by the presence of a repetitive segment of DNA in which the repeated unit is 1-6 base pairs long. These regions have high mutation rates that generate alleles of different lengths which can be useful as genetic markers for fine scale population resolution and pedigree studies.

Restriction Length Fragment Polymorphism(RLFP)- Digestion of a segment of DNA (or the whole mtDNA genome) by restriction enzymes produces fragments of particular lengths depending upon the location of restriction sites (e.g., the *Mse*I enzyme cuts at all 'TTAA' sites). A mutation at a restriction site would prevent enzyme digestion, thus different fragment lengths would be generated.

Stock Assessment of Nesting Populations

In interpreting the distribution of genetic variation, researchers are essentially using a one-way test. If significant divergence is observed between nesting populations, then we can infer that gene flow is low and that nesting cohorts constitute isolated breeding populations. However, the converse conclusion does not invariably hold. If genotype frequencies are not significantly different between two nesting areas, then we cannot be certain that these sample sites are united in a single, random mating population. This may be the case, but there are three reasons why it may not be. First, it could be that the test lacked statistical power because of small sample size (Baverstock and Moritz, 1996). Second, it could be that the populations have only diverged recently and genetic differences have not yet accumulated. Third, relatively few migrants *(e.g.,* 10 per generation or less) are sufficient to homogenize allele frequencies, yet 10 migrants per generation would have an insignificant impact on demographic processes in most nesting populations. Thus, rookeries that are genetically homogeneous could still effectively be demographically independent.

Stock Assessment in Feeding Grounds and Harvests

The finding of genetic differences between nesting populations makes it possible to determine which rookeries contribute to a particular feeding area or harvest. For example, loggerhead turtle samples from the two primary nesting areas in the Pacific Ocean, southern Japan and Queensland, Australia, are characterized by a fixed difference in control region sequences. Hence every loggerhead in the Pacific region carries a natural mtDNA tag which indicates country of origin with a high degree of confidence. These markers have been used to determine which nesting colonies are impacted by loggerhead turtle mortality in drift net fisheries (Bowen *et al.*, 1995). This approach, known as mixed stock analysis is now being used to assess stock composition in a variety of harvests and feeding grounds for several marine turtle species (Broderick and Moritz, 1996; Bowen *et al.*, 1995). The power of this approach, however, depends upon the extent to which all the potentially contributing stocks have been characterized. This requires a comprehensive sampling of regional nesting populations, a process that is now well underway for most species of marine turtle. However, even without complete coverage it may be possible to provide qualitative advice on which breeding populations are represented in migratory pathways and feeding habitats. We expect this application will be a significant management tool.

Sampling Strategies and Sample Size

Molecular genetic studies have been revolutionized by PCR technology, which allows amplification of specific genes from minute amounts of DNA. Prior to the advent of PCR technology, genetic analyses required fresh or frozen tissues, a considerable logistical handicap when the study organism occupies isolated tropical habitats far from the nearest laboratory. With PCR methodology, tissues can be stored for extended periods without refrigeration (Appendix 1). Partially degraded tissues, such as might be obtained from dead turtles, cooked meat, or processed turtle products, can often be analyzed.

PCR-based methods require specific primers, short pieces of synthetic DNA, to direct the enzyme-mediated reaction. Several such primers have now been developed that work on nuclear DNA and mtDNA from most or all species of marine turtle (Table 2). One of the commendable features of marine turtle population studies has been that most labs have used the same sets of primers, allowing direct comparisons of genetic information at homologous loci across the range of globally distributed species. We hope that this trend will be continued.

What constitutes an adequate sample size? The answer depends on the technique, level of underlying genetic difference, and the question under consideration. To define reproductive populations with mtDNA, the minimum sample size for statistical comparisons is 6-8 *where there are strong differences*, although N = 20 is recommended for most population assessments. If mtDNA data from the rookeries is intended as a basis for feeding ground assessments, then samples of N > 30 may be desirable to obtain more accurate estimates of allele frequencies. For nuclear DNA surveys of nesting populations, particularly with microsatellites, larger population samples (N = 30-50) are desirable because of the greater numbers of alleles detected. To establish the geographic scale of a breeding population, a hierarchical sampling scheme is appropriate, wherein samples encompass multiple nesting habitats within a region *(e.g.,* a few hundred kilimeters), and then multiple regions separated by hundreds to thousands of kilimeters.

Sample sizes from the feeding grounds or harvests (for mixed stock assessment) depend on the number of candidate source populations and the level of differentiation between nesting colonies (Broderick and Moritz, 1996). A typical feeding ground sample should include at least 100 individuals (although a smaller sample may be informative in a qualitative sense) and it may be appropriate to stratify samples according to age, sex, and year. Samples of N>100 are justified when several candidate rookeries may contribute cohorts or there are large numbers of alleles, as may be the case for microsatellites (see Chapman, 1996). Pilot studies combined with simulations of maximum likelihood estimates *(e.g.*, Broderick and Moritz, 1996) are important to assess (i) whether the questions posed are answerable within logistic constraints, and (ii)what sample sizes will be required.

Synergy between Genetic Surveys and Tagging Studies

We have tried to summarize the major strengths and limitations of molecular data for stock assessment. From the above, it should be obvious that we do not regard genetic assays as a quick fix or panacea for population identification. Yet, with appropriate sampling and integration with ecological studies (see below), these methods can provide valuable insights.

Genetic data and information from tag returns can interact in three ways. First, tagging studies generate hypotheses about migration patterns that are testable with genetic data. In several sea turtle species, hypotheses about the reproductive migrations of sea turtles, formulated on the basis of tag-recapture studies, have been evaluated with genetic surveys (Bowen *et al.*, 1992, 1994; Broderick and Moritz, 1996; FitzSimmons, 1997a). Second, tagging data can be used to test whether nesting populations that appear to be united by extensive gene flow (based on genetic data) also show frequent exchange of nesting females on a contemporary scale. For example, recapture data confirm frequent exchange of female turtles among adjacent nesting habitats that are genetically homogeneous (Limpus *et al.*, 1992; Norman, 1996). Third, molecular data can provide novel perspectives that can be tested subsequently through tagging programs. For example, genetic data may indicate that a breeding population extends beyond the borders of intensive tagging studies — this inference can be tested by extending mark-recapture across a broader geographic scale. Finally, genetic data may demonstrate rare long-distance colonization events which are difficult to document by tagging alone (Bowen *et al.*, 1992, 1994; Dutton, 1995).

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APPENDIX 1.
Sampling Protocols for Genetic Analysis via PCR

Nesting females, hatchlings, and turtles captured at sea can be safely sampled for blood or tissue, taking care to avoid infection of individuals or cross-contamination of samples. For live animals the surface where blood or tissue will be removed should be cleaned with a detergent solution, 70% ethanol, or isopropanol. Instruments must be cleaned thoroughly between successive sampling (or discarded), and sample tubes should be new (not reused), clean and clearly labeled.

Collection of Blood

Blood usually is removed from the dorsal cervical sinuses on either side of the vertebral column in the neck, following the protocol of Dutton (1996). In adult turtles this sinus may be 1-3 cm. below the surface of the skin. Sampling is easier if the animal is positioned at a slight angle to enhance blood flow to the head region, and the head is pulled to stretch and relax the neck muscles. Although blood sampling is a simple and robust technique, there are some cautions. First, this technique should not be attempted by inexperienced personnel, as errors could lead to damage of blood vessels or nerve tissue in the vertebral column, especially in hatchlings. Second, obtaining blood from nesting females is limited to the egg laying interval (or as she returns to the sea) and it may be difficult if her head is uphill and blood flow is reduced. For leatherback turtles, blood can alternatively be obtained from the rear flippers (Dutton, 1996).

Materials

- Lysis buffer: 100 mM Tris-HCl, pH 8; 100 mM EDTA, pH 8, 10 mM NaCl; 1.0% (w/v) SDS (sodium dodecyl sulfate)
- needle and syringe (or vacutainer apparatus) without anticoagulant treatment.
- labeled screw-cap tubes or other sealed storage containers

Methods

1. Collect blood in a new syringe as described in Dutton (1996), using a new needle for each sample. The amount of blood taken and needle size should be scaled to the size of the turtle: *i.e.*, for adults take 0.5-1.0 ml blood using a 20-22 gauge x 38mm needle, and for hatchlings take 0.02-0.1 ml blood using a 28-30 gauge 12.7mm needle. For leatherbacks an 18 gauge x 76mm needle is recommended (Dutton, 1996).
2. Add blood immediately to a labeled tube with lysis buffer: approx 1:10 ratio blood to lysis buffer.
3. Gently invert the tube several times to mix ingredients.
4. Samples can be stored at room temperature for at least 1 year. Avoid exposure to heat or sunlight.

Note: Lysis buffer is nontoxic and can be stored for extended periods at room temperature.

Collection of Other Tissues

Tissue samples of 0.1-0.2 gram may be removed without risk to an adult animal, provided that sterile techniques are observed. Dutton (1996) recommends removing tissue plugs from the dorsal surface of the rear flippers, and other researchers have obtained good results from skin samples (<1cm^2) taken with a scalpel or biopsy tool from the neck/shoulder region . If sampling hatchlings, it is also possible to get reliable DNA samples from a small notch (2mm) removed from the trailing edge of the carapace with a scalpel blade (FitzSimmons, unpubl. data). In collecting samples from dead animals, we recommend taking muscle tissue from underneath the skin. Tissues that have been previously frozen are acceptable. Dried tissues and even bone may also work.

If eggs are the source of tissue, either the entire embryo or a sample of soft tissues from advanced embryos may be preserved. For very young embryos, the blastula or developing embryo can be used. If freshly-laid eggs are collected, we recommend allowing the eggs to develop for a few days until a blastula can be identified. If this is not possible, then a portion of the yolk membranes may provide sufficient DNA.

Materials

- DMSO preservative solution: 20% DMSO (dimethyl sulfoxide) in water saturated with salt (NaCl).
- Labeled screw-cap tubes or other sealed storage containers
- Razor blade, scalpel, or biopsy punch
- Disposable gloves (recommended)

Methods

1. Collect a tissue as appropriate. Clean all instruments thoroughly between sample collections to avoid cross-contamination of samples.
2. Chop the tissue a few times with a razor blade to increase penetration of buffer.
3. Add tissue to labeled tube with DMSO solution. The tissue/buffer ration should be between 1:5 and 1:10.
4. Samples can be stored at room temperature for at least a year. Avoid exposure to heat or sunlight.

To make one liter of saturated salt/DMSO solution:

1. Add NaCl (about 200 g) to 750 ml of distilled water, until salt no longer dissolves.
2. Add 200 ml DMSO.
3. Add distilled water as needed to make up a 1 litre volume. The presence of precipitated salt indicates a saturated solution.

Note: Care should be taken in handling DMSO because it soaks rapidly into skin and can be an irritant to the skin, eyes, and respiratory system. The saturated salt/DMSO solution is nonflammable, and can be stored indefinitely at room temperature. Some salt may come out of solution during storage. This does not indicate that the preservative has expired.

Alternatives

Tissues can be stored successfully in 70-95% ethanol, or a similar concentration of isopropanol, rather than DMSO. In the absence of other preservatives, samples can be cut into small (< 0.5 cm) pieces and packed in salt. Sun-dried material may also work.

Sampling and Project Design

Nesting Colonies

For nesting colonies, care should be taken to collect only one sample from a given female. This may constitute a blood sample from the nesting female, or a single egg or hatchling sample from a nest. Since females typically lay more than one nest per season, samples should all be taken within a re-nesting interval; *i.e.*, within two weeks, or females should be tagged to prevent repeat sampling.

Pedigree and Multiple Paternity

For analyses of pedigrees or multiple paternity, a pilot project is recommended which would include sampling 10-20 offspring per single clutch from 5-10 females. More extensive sampling might include 10-20 females and up to 50% of the offspring in a clutch, including unhatched embryos, and multiple clutches from individual females (FitzSimmons, 1998).

Feeding Ground Samples

Turtles captured at sea should be sampled following the blood or tissue protocols, the size and sex recorded, and tagged prior to release. This will diminish the possibility of re-sampling the same animal, and may provide important recapture data to corroborate findings based on genetic markers.

Table 2. Primers used for amplification of DNA sequences in marine turtles.

Primer Approx.	Sequence 5'-3'	Species[1]							Approx. Length (bp)
		Cc	Cm	Dc	Ei	Lk	Lo	Nd	
mtDNA control region									
TCR5[2]	TTGTACATCTACTTATTTACCAC	++	++	++	++	+	+	++	380
TCR6[2]	CAAGTAAAACTACCGTATGCC								
LTCM1[3]	CCCAAAACCGGAATCCTAT	-	++	-	-	-	-	-	510
HDCM1[3]	AGTGAAATGACATAGGACATA								
scnDNA[4]									
Cm-12R	AGCTGAAGCCAATGAAGAAGAA	+-	++	—	+-	+-	+-	+-	1380
Cm-12L	GCTCAGGTTTAGCTCGAAGGT								
Cm-14R	TAAGCATTATACGTCACGGA	+-	++	—	+-	+-	+-	+	930
Cm-14L	AGTATTTGGGCAGAACAGAA								
Cm28R	TAAATGCCAGGTATGTAACTC	+-	+-	+-	+-	+-	+-	+-	1400
Cm28L	GATTGCTGGTCTCTGGAAGGCT								
Cm-39R	TGCTAGTTTTGTTAGTTCTGGT	+	++	—	+	+	+	+	1350
Cm-39L	ATAGTGGATTGGAGAAGTTGTT								
Cm-45R	CTGAAAGTGTTGTTGAATCCAT	+-	++	+-	+-	+-	+-	+-	1000
Cm-45L	CCGCAAGCAAAACATTCTCT								
Cm-67R	GAATATAAGATTTCATACCCCA	-	++	-	-	-	-	-	1160
Cm-67L	TTTAATTCTGAAAACTGCTCTT								
microsatellite									
Cc7-F[5]	TGCATTGCTTGACCAATTAGTGAG	++	—	-	-	-	-	++	180-190
Cc7-R[5]	ACATGTATAGTTGAGGAGCAAGTG								
Cc117-F[6]	TCTTTAACGTATCTCCTGTAGCTC	++	++	++	++	-	++	++	210-270
Cc117-R[6]	CAGTAGTGTCAGTTCATTGTTTCA								
Cc141-F[7]	CAGCAGGCTGTCAGTTCTCCA	++	—	-	-	-	-	+-	180-210
Cc141-R[7]	TAGTACGTCTGGCCTGACTTTC								
Cm3-F[6]	AATACTACCATGAGATGGGATGTG	+-	++	++	++	-	+-	++	140-200
Cm3-R[6]	ATTCTTTTCTCCATAAACAAGGCC								
Cm58-F[6]	GCCTGCAGTACACTCGGTATTTAT	+-	++	++	++	-	+-	++	120-150
Cm58-R[6]	TCAATGAAAGTGACAGGATGTACC								
Cm72-F[6]	CTATAAGGAGAAAGCGTTAAGACA	++	++	+-	++	-	++	++	230-300
Cm72-R[6]	CCAAATTAGGATTACACAGCCAAC								
Cm84-F[6]	TGTTTTGACATTAGTCCAGGATTG	++	++	++	++	-	++	++	310-370
Cm84-R[6]	ATTGTTATAGCCTATTGTTCAGGA								
Ei8-F[6]	ATATGATTAGGCAAGGCTCTCAAC	++	+-	++	-	++	++	++	170-250
Ei8-R[6]	AATCTTGAGATTGGCTTAGAAATC								
DC99[8]	CACCCATTTTTTCCCATTG	-	-	++	-	-	-	-	120-140
	ATTTGAGCATAAGTTTTCGTGG								

[1]+ amplifies, unknown variability, +- amplifies, invariant, ++ amplifies and is variable, - unknown, —no amplification

[2]Norman et al. 1994

[3]Allard et al. 1994

[4]Karl et al. 1992, Karl 1996

[5]FitzSimmons 1998

[6]FitzSimmons et. al. 1995

[7]FitzSimmons et. al. 1996

[8]Dutton 1995

4

Data Collection and Methods

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Defining the Beginning: The Importance of Research Design

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A proverb: "Success is like a turtle climbing a mountain. Failure is like water running down hill."

Introduction

The probability of success of a research project is greatly enhanced when the "beginning" is correctly defined as a precise statement of goals and justification. Having accomplished this, the sequential steps necessary for writing a research plan and then successfully executing a research project are easier to identify and organize. Therefore, the message of this chapter is: by the time the laboratory is prepared or the first datum collected in the field, substantial effort should already have been invested in the conceptual and logistical framework of a project. This chapter discusses the steps that should result in a well-designed and integrated research plan.

A research plan consists of two general areas: research concepts and context (Table 1) and research logistics (Table 2). How well a research project is planned and how well the steps in the plan are integrated can make the difference between success or failure. The process of writing a research plan should start as soon as possible in the development of research ideas. For assistance with the process of writing research plans and research proposals, see Reis-Lehrer (1995).

Table 1. An outline of the concepts and context sections of a research plan.

1. Literature survey
   a. Become familiar with the literature to identify a research problem and to explore the areas of biology that may impact the research plan.
   b. Explore ways that enhance the integration of the study into broader biological disciplines and ways to collect, analyze, and present your data so that they are useful to others (e.g., for comparison among populations, or to use in models).
2. Carefully and clearly state the problem in form of concise questions or as working or null hypotheses.
3. Make a list of possible scenarios related to your research questions and then rank the most probable ones.
4. Discuss all aspects of the research proposal with colleagues as you develop them. Investigators that work in the same or closely related fields are valuable sources of information.
5. Write a detailed research proposal.

Table 2. An outline of the logistics section of a research plan.

1. Identify what information needs to be collected and how it will be collected.
   a. Develop a logistical and quality control plan for the data collection, handling, and storage procedures, including who will be responsible for each procedure.
   b. Design data sheets that prompt the person collecting data for each measurement. Data sheets should also be made with data recording, entry into computer files, editing and data analyses in mind.
   c. Prior to actual data collection, carefully "walk through" as many of the techniques and procedures as feasible to detect problems with protocols and equipment.
   d. Evaluate incoming data for recurring errors; frequent review may reveal unsuspected patterns that, if identified and responded to quickly, provide opportunities to improve research protocol or direction.
2. Talk to other investigators currently working with similar types of studies about logistics, research protocol, quality control plans, and types of data analyses.
3. As soon as possible following data collection, the data should be entered into computer files and then reviewed to detect problems associated with data sheets, data recording and entry, and with computer files.
4. Order supplies as far in advance as reasonable and funds are available.
5. Plan for the unexpected (equipment failure, accidents, illness, unusual weather).

Research Concepts and Context

The Literature

The ultimate quality and success of research are often a reflection of the time and effort invested in developing research ideas and concepts, a stage of planning that includes becoming familiar with the literature. Because the probability that a research idea will arise in a vacuum is vanishingly small, the probability of having a good research idea is higher for an investigator with experience and knowledge of the literature than it is for a novice.

The immediate goal of a literature survey is to determine if the research idea is worth pursuing (the research may have been done, or new publications may have revealed problems with the research idea). A second goal is to maximize the usefulness of research results by integrating them into the specific research topics *(i.e.,* the results of others working in the same area) and into broader biological topics and disciplines *(e.g.,* life history, reproductive effort, parental investment, kin selection, conservation).

Placing a specific research project in a broader context requires a familiarity with: (1) literature outside of a specific research topic, (2) knowledge of current projects of other investigators, and (3) an historical perspective of the research problem and general topics. Scientific libraries at universities and individual reprint libraries are sources of review articles, book chapters on research topics and techniques, and articles on related topics of interest. To get started with sea turtle research, consult the Recent Papers section of the *Marine Turtle Newsletter.*

Research Questions

If the research idea is still viable after reviewing the literature, it is time to develop the research questions or hypotheses to be tested. The process of developing these details increases the probability of asking the right questions, and therefore, collecting the appropriate data. No amount of time spent, hard work, or elegant statistical methods will overcome the damage caused by a poorly framed question. Because research questions determine what, where, when and how data are collected, these questions represent an important link between the conceptual and logistic aspects of planning a research project.

An enlightening *a priori* exercise entails listing all possible answers to specific research questions (this list should not be influenced by whatever biases you may bring to the research). From the list of possible answers, rank those that appear the most probable answers or outcomes. By definition, the list of ranked answers should be shorter than the list of possible answers. The list and rankings should be kept with the research plan and then examined for possible surprises when the project is completed.

Hypotheses

Specific hypotheses or research questions depend on the context of a study. Testing a scientific hypothesis (a statement that attempts to predict how a particular feature of nature works), or answering a scientific question, almost always involves testing at least one, and often several, statistical hypotheses. A statistical hypothesis is a statement that attempts to predict the parameters of one (or more than one) probability distribution; for example, that the means of two distributions are not different. Both experimental and observational studies should be designed with the ultimate statistical tests in mind.

Clear statements of statistical questions or hypotheses should be made early in the process of planning research and should be formulated so that they are concerned with the parameters of well-defined statistical populations. The statistical populations must be the population sampled, or the methods of statistical inference will not apply. Consultations with statisticians about experimental design, data collection, and statistical analyses should be made early in the process of developing the research design. Consultations with a statistician will be more profitable if the research questions or hypotheses are clearly stated and if some prior effort has been made to understand experimental design.

Sample Size

One area that is often overlooked in the design of ecological studies concerns the amount of data to collect. Data collection is often difficult, expensive, and may involve unavoidable destructive sampling of animals. Obtaining sufficient data to provide robust statistical tests of hypotheses may often conflict with logistical and ethical considerations concerning data acquisition. In such cases, sample sizes need to be large enough to provide adequate tests of important experimental effects, but they should not be unnecessarily large. Excellent sources for details of experimental design and sampling protocol can be found in Manly (1992), Sokal and Rohlf (1995), Winer (1971).

Statistical Power

The probability of rejecting a null hypothesis when it is false is termed the power of a statistical test, and calculation of sample size necessary to detect effects of a particular magnitude is called power analysis. If lack of consideration of statistical power results in inadequate sample sizes, confidence intervals about parameter estimates will be too wide to provide support for a conclusion that a null hypothesis was not rejected.

All research programs should include calculations of the power of statistical tests or width of confidence intervals (or both) that will result from planned sample sizes. Data necessary for these calculations (the variation expected for a given parameter) may not be available for the system that the researcher wants to study. Pilot studies or estimates based on previous studies of similar systems may provide the data for power analysis. Details of how to conduct power analyses for various statistical tests and sampling designs are complex and beyond the scope of this chapter; however, detailed treatments may be found in most texts on experimental design (Winer, 1971; Montgomery, 1984; Manly, 1992; Sokal and Rohlf, 1995).

Development of the Research Plan

Two steps should be ongoing in the development of both the conceptual and logistic areas of a research plan. First, informal conversation with colleagues should be undertaken. Each colleague will bring a different viewpoint to proposed research that can improve research questions and generate new ones. In addition, many logistical problems that may hamper a new research program have already been experienced and solved by others. Because formal reviews are time consuming and are a courtesy, requests for these reviews should not be made until all conceptual and logistic steps in the research design are completed.

Second, the early development of a research plan should include the beginning of a detailed written research proposal. The process of writing a detailed proposal will help identify problems with research concepts, questions, and logistics and will enhance integration of various aspects of the proposed research. Project design and management programs are available for personal computers that assist project organization and time budgeting; these programs can be of value organizing research and writing the research plan.

Research Logistics

Research Quality

Once the questions or hypotheses have identified the data that are necessary, a plan should be developed for data collection (Table 2). Even though the precise statement of the research goals has identified the correct data needed to answer research questions, the quality of data collected depends on consistency of collection procedures, completeness, and accuracy of measurements. Therefore, each step of the logistical portion of the research plan should be based on how to assure the quality of the data collected (Table 3). The goal should always be to obtain the best data possible; however, each step in a research plan should consider the safety of the investigators and the welfare of the study organisms.

Table 3. Topics for the data acquisition and quality control section of a research plan.

1. Data collection.
2. Data recording.
3. The number of people making measurements and recording data.
4. The number and kinds of quality control measures necessary to verify the accuracy and consistency of data collected.
5. Data entry into computer files.
6. Storage of original and copies of data sheets and computer files.
7. Assignment of tasks and responsibilities.
8. Data analyses.

Data Sheets

Data sheets should be designed to complement the data collection process and minimize mistakes and omissions. If data are collected in a sequence (date, time, location, animal identification, sex, body length, body mass), data columns should be organized in the same order to minimize recording errors. In addition, errors made during transfer of recorded data to computer files will be minimized if the structures of the data files are in the same order as the data sheets.

Quality Control

Many research efforts are beyond the scope of one investigator and some require many field and laboratory assistants. If more than one person is involved in making measurements or observations, and recording data, it is important that the results are consistent and repeatable among personnel. The accuracy (how close measurements are to the actual dimensions of the object) and repeatability (how close measurements are to each other if taken by more than one person) required for a particular parameter measurement will determine how much training of personnel is necessary and how frequently instrument calibration will be necessary.

Consistency of measurements among personnel is critical for reliable data collection because statistical detection of differences depends to a major degree on the variability of the parameter being compared among treatments, years, or sites. For example, the validity of comparisons of population numbers among years or between two areas depends on: 1) whether the level of effort and consistency of data collection were similar for each sample period or area, 2) whether techniques used to obtain the data and data analyses were similar, and 3) whether the same investigators or investigators with similar training collected the data each year. In addition, the reliability of statistical detection of differences depends on the degree of variability of parameters that are compared among years or between sites.

Data Management

If at all possible, a personal computer should be used to store, edit and manage data. A spread sheet program or a relational data base management program (there are many versions of both on the market) should be used. Some programs allow the user to develop a computer screen that looks just like a data sheet, a feature that can help reduce data entry errors. Data management programs also offer error detection procedures and data manipulation features such as data sorting or indexing (arranging data in specific ways), data queries (counts or displays of categories of data), and the ability to build in custom programs that summarize data automatically (means, minimums, maximums, and standard deviations). Many of these programs also contain some data graphing procedures that allow visual inspection of data. The internal features of these programs greatly assist management of data, and provide options for transferring data into other formats for use with statistical packages and sophisticated scientific graphing programs.

A master data file should be identified and should contain the latest data and represent the most recent editing changes. Entry of data should never be made directly into the master file, and data analyses should never be performed on the master file. Data should be entered into a separate file, edited, and then appended to the master file. Backups of the master file should be made and stored in separate locations. In addition, the person responsible for data editing, management, and storage of backups should be clearly identified.

Personnel

Personnel represent a major expense for many research projects. Hiring self-motivated and goal-oriented people and giving them adequate training and guidance has obvious benefits to a research effort. However, recognizing those characteristics in potential field assistants can be difficult. One exercise that conscientious people practice, or can be trained to conduct, is to ask themselves the following questions before beginning a task: 1) Do I clearly understand both the immediate and long-term goals of the task? 2) Do I know how to accomplish the goals? 3) Do I have the necessary supplies and equipment to complete the task?

If each person (including the principal investigator) involved in a research project asks themselves these questions each day, the time wasted will be greatly reduced. After a task is completed, another question should always be asked: 4) Am I communicating appropriately and adequately with others involved with the project about problems, results, and decisions I made while completing the task? Lack of communication among investigators is one of the most widespread problems in research conducted by a team.

Summary

The value of a research project is determined not just by the new data obtained, but how the research complements previous investigations and contributes to our understanding of broad biological topics or to tests of broad ecological theories, concepts, or general problems in conservation and management of biodiversity. Just as individual research questions influence the quality of data collected, suites of related questions within a research project influence the quality of extended research goals such as synthesis of general topics *(e.g.,* causes of population regulation and dynamics; sources of variation in growth rates among individuals or populations) and development of new questions and hypotheses that will guide future research.

Acknowledgments

Previous versions of this chapter were improved by comments from Kurt Buhlmann, Nat Frazer, J. Whitfield Gibbons, Nancy Dickson, Mark Komoroski, and Roy Nagle. Writing was supported by Financial Assistance Award Number DE-FC09-96SR18546 from the U.S. Department of Energy to the University of Georgia Research Foundation.

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Data Acquisition Systems for Monitoring Sea Turtle Behavior and Physiology

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The development of microchip and microprocessor technologies, along with improvements in battery design, has enabled researchers to expand the study of marine turtle biology in ways that could only be imagined a few years ago. New technologies allow acquisition of data on behavior, physiology, habitat use, and migratory movements at a reasonable cost and without once formidable logistical requirements. This chapter describes some of these methods, how and when to use them, and how to avoid misusing them.

Very High Frequency (VHF) Telemetry

VHF telemetry is probably the oldest and simplest electronic technology that has been applied to marine turtles. Generally the objective is to determine turtle location at distances too far for visual confirmation, or to "home in" on a turtle for visual verification of position. In its most basic form, the system consists of a fixed frequency radio transmitter, a receiver capable of detecting the transmitter's frequency, and a directionally sensitive antennae. Usually a compass or compass rose (where the perimeter of the disk is divided into graducules representing compass direction) is associated with the antennae to indicate where the transmitter is located relative to the receiver. The tracking technician simply rotates the antennae and records the bearing giving the strongest signal. Each transmitter is set to a specific frequency and individuals are identified by these unique frequencies; or, alternatively, by the signal repetition rate (the latter method is uncommon). Two bearings are recorded simultaneously (or nearly so) from two receivers at separate locations; the intersection of these bearings estimates a turtle's location. A number of published studies report results of VHF turtle tracking *(e.g.,* Dizon and Balazs, 1982; Mendonça and Ehrhart, 1982; Mendonça, 1983; Keinath, 1986; Chan *et al.,* 1991) and from these, as well as studies of other taxa, information on commercial providers of equipment can be gleaned.

The advantages of the VHF technique include its simplicity, relatively large number of reference materials, and comparatively inexpensive cost. The greatest disadvantage (and the most frequently overlooked problem) is its relatively poor accuracy. Few skilled technicians exceed a ± 5° accuracy. Consequently, the resulting "location" is not exact but rather falls within a polygon whose borders are determined by the angle of the bearing and the accuracy of the individual technician. For example, the precise location of a turtle 15-20 km from a receiving station with a 5° measurement error lies within a polygon whose area is 16 km^2 (Figure 1). Technicians should be thoroughly versed in methods for correcting inherent errors (see White and Garrott, 1986, 1990; Zimmerman and Powell, 1995). A second disadvantage is that transmitters must be on the surface to be detected. For some species of turtle, surfacing may occur for only a few seconds once each hour, greatly reducing any opportunity for triangulation. When this is the case, a rapid repetition rate *(e.g.,* 0.25-0.5 pulses per second) is recommended. Battery life is reduced more quickly in this case, but the technician receives more pulses per unit of time, enhancing his/her ability to locate the signal. Finally, daily variation in abiotic factors *(e.g.,* rain, humidity, radio interference) can degrade signal quality.

Sonic Telemetry

In many ways *(e.g.,* sensing and triangulating a signal), sonic tracking is similar to VHF tracking. In contrast to VHF telemetry, which relies on airborne radio waves, sonic signals are transmitted underwa-

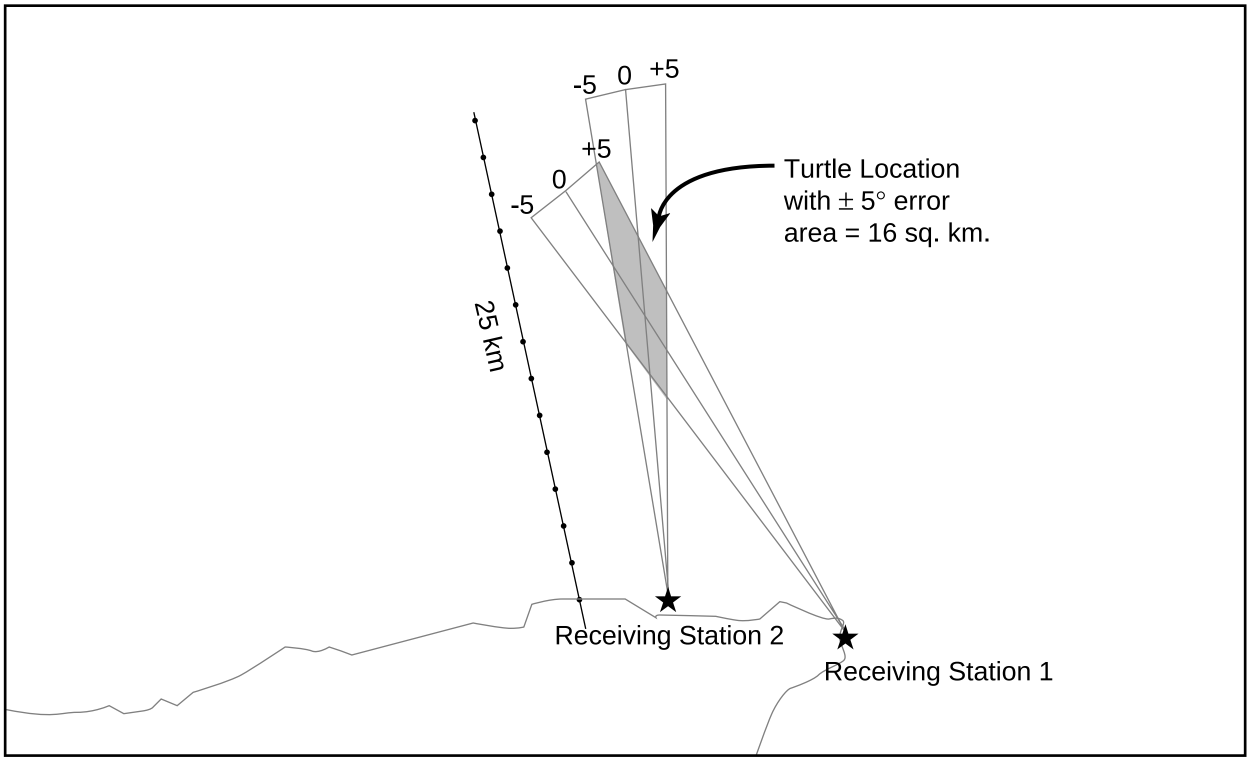

Figure 1. Location polygon established using VHF telemetry with a 5 degree measurement error.

ter, and instead of a directional antennae, a directional hydrophone is used and turtles are tracked beneath the surface. The technique is well developed, quite reliable and, while generally more expensive than VHF telemetry, still reasonably priced. Sonic pingers can encode data, such as temperature or depth, into the signal. Finally, sonic telemetry is often more accurate relative to the degree of error associated with the bearing; however, there are still a large number of potential errors that must be addressed (see Collazo and Epperly, 1995). The primary disadvantages are that range is limited and studies must be conducted from a boat. Moreover, sonic signals are more susceptible to interference and bounce than are VHF radio waves; thus, environmental conditions can strongly affect results. Since there is inherently more noise interference (biotic and abiotic) underwater, receivers must incorporate effective filters. Sonic signals can be degraded by heavy particulate loading in the water and blocked altogether by submarine structures. It is advisable to purchase the best receiver possible, thus taking advantage of superior noise reduction technologies.

Satellite Transmitters and Satellite Linked Data Recorders

Satellite telemetry provides a superior means of monitoring long distance movement, as well as various behavioral parameters, and has been used successfully by a number of researchers *(e.g.,* Hays, 1993; Plotkin *et al.*, 1995; Morreale *et al.*, 1996; Beavers and Cassano, 1996; Eckert and Sarti, 1997; Eckert, in press).

Currently, ARGOS CLS provides the only Earth-orbiting satellite system capable of establishing daily global locations of transmitters attached to wildlife. The system consists of two TIROS-N satellites in low Earth polar orbits with on-board radio receiver and transmitter units, a series of Earth based receiver stations, and several Earth based Global Processing Centers (GPCs). [N.B. At the time of writing, a third satellite has been put online but its future is uncertain.] Each satellite makes one orbit in 101 minutes, crossing the equator at a fixed time each day. The ground-track covered during each pass is about 5,000 km wide and overlaps 2,100 km with the previous pass at the equator. The amount of overlap increases with latitude so that satellite coverage (from two satellites) at specific locations increases from six satellite overpasses per day at the equator to 28 passes per day at the poles. The satellite is within radio view of any point on the earth for about 10 minutes. All transmitters utilize the same frequency, 401.65 MHz, with effective transmission power output between 0.25-1.0 watts. Repetition rate is limited by ARGOS to 40 seconds. Encoded in each transmission is an identification signal, as well as sensor data from each transmitter.

Transmitter locations, which are reported as latitude and longitude, are calculated by ARGOS using Doppler shift. As the satellite approaches the transmitter, the frequency of the transmitted signal rises; as the satellite moves away, the frequency falls. By comparing these values to the known frequency of the transmitter, distance (and subsequently the angle of the transmitter relative to the satellite) can be calculated. Since the zone of reception from each satellite is cone-shaped, the cone intersects any particular elevation on the Earth at two points. These two points are reported as the two possible locations of the transmitter. Locations presented by ARGOS are of variable accuracy and are classified by ARGOS as 3, 2, 1, 0, A, B or Z with “3” the most accurate and “0” the least accurate. A number of factors affect location quality, including numbers of uplinks, elapsed time between uplinks, and signal quality. A, B and Z location classes (LC) rarely have locations assigned to them. LC 3, LC 2 and LC 1 are reported by ARGOS to be accurate to <150 m, <350 m and <1000 m, respectively, while LC 0 is accurate to >1000 m.

The accuracies reported by ARGOS represent probabilities and can vary. Thus, it is prudent for investigators to field test individual transmitters prior to deployment. Routine post-analysis of reported locations is essential; any unrealistic locations should be discarded. The criteria used in editing the database should be reported in any published results. For a further discussion of satellite location accuracy, the reader is referred to Keating *et al.* (1991) and Stewart (1997). Since ARGOS is continually improving the accuracy of its reported locations, earlier critiques are obsolete.

The biggest advantage of this technique is the ability to transmit data other than location. Some satellite transmitter manufacturers equip transmitters with sensors capable of reporting data on water temperature, dive depth, dive duration, and other information. With an on-board microprocessor to control data acquisition and compile the data for transmission, the only limitation is that data acquisition is limited to the capacity of the ARGOS platform to handle the data stream. The biggest disadvantage is cost. Transmitters are priced at US$ 1,800-4,200 each and satellite usage time approaches US$ 4,000 per year per transmitter (although rate discounts are available). Moreover, data analysis requires a skilled technician. Notwithstanding, the potential to monitor the movements and behavioral patterns of multiple turtles for a year or more at great distances outweighs the disadvantages since, for example, attempting to gather equivalent data by other means *(e.g.,* tracking by boat) would be far more expensive and would likely yield poorer quality data.

Hybrid and Advanced Telemetry Systems

The development of the Global Positioning System (GPS), which utilizes a system of geosynchronous satellites and a land-based receiver, holds great promise for wildlife tracking. Off-the-shelf receivers are relatively inexpensive (< US$ 100 for simple designs) and have a typical accuracy of 100 m (accuracy can be improved to a few meters in areas where differential reception is available). There is widespread interest in adapting GPS to wildlife tracking, several companies and laboratories are currently developing instruments, a number of configurations are being tested, and a few prototypes have been deployed on terrestrial species. One style of GPS receiver simply stores locations at predetermined intervals for later recovery, a second style retransmits that information over VHF (or other short-range) radio frequencies, and a third style transmits location data via the ARGOS satellite system. The advantage of the latter style is that it allows accurate position referral on a single transmission as opposed to the 3-5 transmissions (spaced at least 40 seconds apart) currently required by ARGOS to establish a position. At the time of writing, none of these systems were available commercially for marine wildlife telemetry.

Geolocation Tags

Originally developed for marine mammals (Delong, 1992), this data logger utilizes day length and sunrise and sunset times to estimate its latitude and longitude. The instrument consists of an accurate clock and a microprocessor with sensors to measure pressure, temperature and light level. Accuracy is usually to 1°, except during Equinox periods when it may be more variable. Two configurations are available. The first simply stores a location at a preprogrammed interval (usually daily) until the tag is recovered, and the second (which should soon be available commercially) is coupled to a satellite transmitter and timed-release mechanism. In the latter case, the tag detaches itself and surfaces to transmit its stored location data set via the ARGOS satellite platforms. Currently these tags are in use on studies of marine mammals and migratory fish. Advantages include reasonable cost (ca. US$ 1300 for the basic tag; US$ 3,000-4,000 for the self-releasing tag) and self-locating ability irrespective of surfacing duration. The primary disadvantage is its relatively coarse resolution (1°) and the need to recover the tag.

Time-Depth-Recorders (TDR)

TDRs are electronic data loggers, often microprocessor controlled, that utilize pressure transducers to monitor pressure (depth) and store the data at predetermined intervals. The results can be integrated over time to determine dive depths and durations, ascent and descent rates, bottom time, and other behavioral variables. TDRs have been successfully utilized on diving marine mammals, birds and sea turtles *(e.g.,* Kooyman *et al.*, 1983, 1992; Eckert *et al.*, 1996). The cost is relatively low considering the resolution of the data; however, instrument recovery is required. Accuracy is typically good but variable among manufacturers. Some form of transducer error correction must be incorporated into the TDR (or into the data processing software) to account for calibration shifts that may occur during deployment. Further, most TDRs have resolutions relative to their maximum range (though there have been significant improvements recently from some manufactures). For example, one manufacturer reports a resolution of 0.25 m for a TDR with a range of 0-500 m, 0.5 m for a range of 0-1000 m, and 1.0 m for a TDR with a maximum range of 2000 m. It is important to choose a TDR configuration that is most suitable for target species and the research objective. Analysis of surfacing intervals and dive times should take the instrument resolution into account.

Other Data Loggers

The basic data logger design utilized with the TDR can be adapted to record other information such as temperature, swim speed, distance traveled or even compass direction. Many of the same caveats relative to understanding measurement and accuracy limitations apply as described for TDRs (above). As is always the case, an understanding of the basic biology of the target species is requisite. For example, if a velocity (swim speed) logger has a minimum startup speed (the minimum speed at which the impeller begins to turn) of 1 m/sec, then it would not be useful for turtles with average swim speeds below 1 m/sec. Further, care must be taken in data analysis to note that "0" speed records may actually represent periods when the turtle's speed was below the stall speed of the recorder; the turtle may not have actually been stopped.

Heart Rate Counters (HRC)

HRCs are a special type of data logger. Two varieties (analog recorders and digital counters) are available. The analog recorder is essentially an electrocardiogram (ECG) recorder that (in most cases) uses a magnetic tape to record a data trace. Sampling rate is usually high (in excess of 60 samples per second) and the unit has all the advantages and disadvantages of standard ECG traces, including an inability to avoid interference from myogenic sources. Probably the most significant disadvantages are size (when housed for underwater deployment) and the fact that most are capable of recording for only a few days. Digital counters attempt to count only the R-wave portion of the ECG signal and integrate that count over time. The advantage is that it only stores information on heart rate (and not the entire ECG signal) and therefore can be deployed in a small, entirely electronic package. The disadvantage is the difficulty these units have in distinguishing interference signals from the R-wave; as a result, they are highly prone to providing spurious data which often cannot be detected during analysis. In their current configuration (as counters), their use is not recommended. However, as technology improves, digital ECG recorders (as opposed to counters) should become available and the new technology may resolve many of the accuracy problems inherent in the digital counters.

Instrument Packaging and Attachment

Paramount to the success of any telemetry experiment is the packaging and attachment of the data acquisition instrument. A number of design parameters must be considered. First and foremost, the instrument must not interfere with the behavior or wellbeing of the turtle. This rule is inviolate, for it is important both ethically and scientifically. If the study animal is disturbed by an attached instrument (constrained by excess drag or hindered by painful attachment configurations), it will not behave “normally” and the resulting data will be erroneous. Instrument manufacturers are often hesitant to custom design an instrument package, due to cost factors and concern that a new package may reduce instrument performance. Therefore, it is the responsibility of the investigator to propose new instrument packages and to adequately field test them.

To minimize potential behavioral disturbance, instrument packages must be hydrodynamically clean. The profile should be as low and smooth as possible, and should slope both to the front and rear (the design of the posterior end of the tag is almost more important than the front, due to the effect of turbulence on hydrodynamic performance). Approximating a teardrop, or half teardrop, shape is wise (tapering to the rear). Size and weight of the transmitter package are also important, but less so than hydrodynamic form. In some cases there are advantages to choosing a comparatively larger package if it is neutrally weighted in water, and efficiently designed. Consideration should also be given to swim speed; hydrodynamic design is far more critical to the study of fast swimmers than it is to slower swimmers. Placement of the package on the turtle is also important. Attachment points on the posterior one-third of the carapace are preferred, but this is often not practical for transmitters which require reasonable antennae exposure.

Hydrodynamics should also be strongly considered when designing floating instrument packages. Too often floats are designed and tested in a tank, with little consideration to their performance when towed behind a turtle. The result can be a tag with unacceptably high drag and/or poor behavior in the water. A common mistake is to use spherical or bullet-shape floats. When pulled, a spherical float often spirals and creates high drag; a bullet-shaped float tends to dive below the turtle where it can foul more easily on the bottom. A final and oft overlooked hydrodynamic problem is biofouling. Biofouling increases drag and reduces instrument performance. For long term (> 3 weeks) deployment, the instrument package (including attachment) should be covered with a good quality antifouling paint. The potential for biofouling increased instrument drag is high.

For most species, attachment by polyester resin or epoxy adhesives are adequate. Beavers *et al.* (1992) provide guidance when studying hard-shelled turtles. There are a wide variety of adhesives that secure an instrument to a turtle. Care should be taken with quick setting epoxies. The heat of curing a quick setting epoxy can burn the tissue underlying the affected scute, causing it to peal off in a few days. For short term deployment with small instruments, small holes can be drilled through the outer edges of the marginal scutes; the instrument is then wired and glued in place. If marginal scute abrasion is a concern, it is prudent to mount the instrument under the carapace margin rather than on top. The use of screws to anchor the instrument to the carapace is not recommended because of the possibility of penetrating the lungs. A sea turtle’s lungs are attached directly beneath the carapace and can occupy a surprisingly large area. In the case of leatherback turtles, attaching the instrument package to a flexible harness is recommended. In this case it is particularly important to consider biofouling. The harness should be constructed to allow break-away if the turtle becomes entangled, and it should have some means of self-release if the turtle is never recovered (see Eckert and Eckert, 1986).

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Research and Management Techniques for the Conservation of Sea Turtles
K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, M. Donnelly (Editors)
IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Databases

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Because of sea turtle life cycle characteristics, trends in the population dynamics of sea turtles are analyzed from essential data accumulated over many years, typically for more than a decade and, ideally, should be based on information for all life stages. Even so, most databases are comprised of data gathered from nesting females; that is, by beach monitoring programs. These data, when properly collated, can still provide a meaningful and representative evaluation of population dynamics. The scenario becomes progressively more complicated, however, when individuals comprising a specific management unit nest at multiple beaches, necessitating monitoring at more than one site. To obtain an accurate representation of population dynamics in this case, data need to be shared and integrated, rapidly and efficiently, among localities and sometimes across national borders. Raw data by themselves have little value. Only through proper collecting, organizing, processing and presentation do they become meaningful. Properly managed and structured databases enhance the efficiency of information archival and transfer.

This chapter describes a model database system for GIS-compatible information. The model is applicable for the management of long-term data from a single sea turtle project and, if adopted by a multi-rookery program with information exchange between data-gathering programs and management agents, the model can bolster integrative collaboration within or among nations. In a multi-program application, a high degree of standardization in methodology, terminology, etc. is required to permit the exchange and comparison of data across space and time. The design presented here borrows extensively from a system employed for Indo-Pacific information management (Limpus, 1995), as well as from a national sea turtle database in México (Briseño-Dueñas and Abreu-Grobois, 1994).

In general, managing sea turtle information through a database should enable: (1) updated and sufficient information for the purpose of monitoring the conservation status of individual management units (breeding stocks) and assessing conservation and management programs; (2) long-term storage and retrieval of data related to sea turtle biology; (3) rapid transfer and exchange of standardized information among research and/or monitoring programs; and (4) the accumulation of long time-series of population parameters useful in robust analyses of population dynamics. There are two organizational levels to be considered: the organization of participants (especially if the database will manage data from more than one project) and the organization of the data, or database structure.

Organizing a Database

Organizing Participants

For regional or multinational applications, participants will be drawn from a variety of organizations and jurisdictions. Such heterogeneity in the membership will undoubtedly require delicate prior agreements on the role each party will have in the management of the database. Agreement must be reached on the rights and obligations of each participant, the custodianship of the database, and proper standards for the complete information cycle. Most likely there will be government agencies, universities, NGOs, and (sometimes) private enterprises involved in data-gathering activities. Despite issues of general interest *(e.g.,* recovery of sea turtle populations, maintenance of quality in the data), short-term commitments and requirements may vary widely among program partners. Management authorities, for example, will need timely periodical reports to assess the success of their programs; research scientists will require high standards in data quality and assurances of intellectual property protection. Most everyone will want to safeguard the proper acknowledgment of data authors.

Issues to Consider

Individual and collective legal requirements must be reconciled, particularly when the project includes a complex array of participants. In such a case, establishing a coordinating group (sometimes referred to as a "hub") is useful and should be comprised of representatives entrusted with decisions related to the management process. The hub might also handle inquiries, archive key documents and publications for distribution, and maintain backup copies of selected data sets for increased security. Further issues to consider include: (1) rights of access to the data, both by users participating in the database development project as well as by outsiders; (2) protection of intellectual rights and proper acknowledgment; (3) appropriate uses of the data; (4) custodianship; and (5) validation standards for data. Control over data access in modern computer software is relatively easy to achieve. In multi-user contexts, an array of user names and passwords can be designed to give access solely to accredited parties. If desired, each user can be controlled by differing degrees of "privileges" in data access. For example, access restrictions (designated as full, limited, or none) can be implemented, depending on the user, to whole data sets or even to individual records.

Intellectual Property Rights

Perhaps the single most delicate point in a database involving many organizations, particularly one in which academic institutions are involved, is the need to ensure adequate regard for intellectual property rights. Clear guidelines on appropriate use are essential, particularly when data gathered by one party are required by another. In the case of data sets released for general consultation by accredited users, it may be enough to guarantee that the source is acknowledged whenever the data are used. But in cases where participants provide data that are considered sensitive, safeguards are prudent; in particular, due regard must be afforded to the privileges of academic researchers to publish original findings. One solution is to specify access constraints over a specified time period and dictated by the data provider for specific records or data sets. The constraints (data present but not available for consultation) could be sustained over a length of time *(e.g.,* 1-2 yr.) considered by the parties reasonable.

Custodianship

Implementing custodianship alleviates several potential problems, and helps to ensure stability and quality in the database. In the absence of designated custodianship, important management tasks might be duplicated, neglected or omitted. Custodianship entails a strong commitment to guarantee various aspects, including (from WCMC, 1996): (1) advising users on potential database use(s), including permitted and forbidden usage (uncertainties or ambiguities could be pointed out on specific data sets); (2) ensuring that publication, information products and all outputs derived from the database acknowledge data sources and protect intellectual property; (3) coordinating the coding of parameters applied to major referential variables *(e.g.,* project sites, organizations, personnel, geographic reference grid); (4) ensuring that the database is up-to-date, adequately documented, and maintained in such a way as to be accessible to users; (5) undertaking periodic updating, safe backups and adequate virus protection; and (6) proposing occasional changes to structure and content as needs arise. Monitoring of tagged turtles produces information needing special consideration since the data are useful to both the tagging and recapturing parties. The custodian's responsibilities should include the facilitation of this access, again with respect to intellectual property rights.

Designation of custodianship may not be easy. Legal considerations may dictate that the custodianship be awarded to a government agency, yet greater continuity and quality can sometimes be achieved if the task is given to a reputable academic organization. In general, custodianship should be conferred on the organization most familiar with the history, management characteristics, and potential uses of the database. Broad consensus may be necessary when several groups contest the claim. Principally, the custodian group must be technically capable, inspire confidence in the users, and have an acceptable long-term stability. Organizations with stable financial resources and a prior track record in the field are good prospects as long as they are also considered to be impartial (lacking conflict of interest).

Organizing Data

The organization or structure of the database to be adopted should both contain enough fields to hold information on key parameters of sea turtle population dynamics and be distributed in such a way as to avoid redundancy of space usage, while facilitating searches (or queries) and data retrieval. Further, two levels of sea turtle monitoring capability must be recognized and provided for in a database of broad applicability. Programs with sufficient expertise and resources generate high-resolution data, where information on individual turtles is available through nesting or capture/tagging monitoring. Programs characterized by more limited resources oftentimes do not monitor individual turtles; nevertheless, basic data are collected on key parameters from global, whole-nesting beach surveys. The majority of sea turtle monitoring programs fall somewhere between these two types as it is often impossible in practice to locate, tag and monitor even the majority of turtles nesting on a single beach, particularly where the population is abundant.

Validation

Validating information from field sheets is essential to database quality. Unfortunately, in many database applications this aspect is weakly observed. For efficient data analysis, formats for each data attribute and input must be adhered to strictly and with total consistency. Use of numerical coding for repetitive data helps to avoid typographic errors. Validation should be done at the point of origin, before transferring to a global database. Although some programs provide automatic checking for the simplest mistakes *(e.g.*, verifying that all required data fields are filled, cross-checking locations given against a master catalog, ensuring that repetitive and constant data are in corresponding fields), only direct revision on hard copies of entered data should be considered reliable for the detection of error(s). Temporary files can be implemented to hold data that only after full validation get transferred into master files.

Compatibility, Software and Hardware

Above all, compatibility in the process of data exchange needs to be ensured so all parties have access to the information. While standardized methods for data gathering are basic, compatibility in software and hardware is also important. Even though modern software has facilitated converting data from one format to another, compatibility is far from perfect between products of different manufacturers. Choice of software is thus critical and, particularly for multi-user applications, selecting a single product is highly recommended, particularly when further analysis of the data with sister-applications will be sought.

There are ample choices in database management software. Consideration should be given to: (1) volume capacity (is it capable of managing the volume of data and number of users?); (2) expansion (can it cope with future increases in user volume? does it contain easy to learn facilities for developing application; *e.g.*, entry screens, querying tools, reporting facilities?); (3) is the product likely to continue being supported and enhanced?; (4) does the program have sufficient experience for the development of applications and maintenance of the software? Most often, a choice of software will hinge between selecting a popular package affording ease of use, a quick learning curve, and sufficient power for modest single project applications (the simplest representatives cost

Table 1. Structure for NESTING BEACHES CATALOG (considered essential)

elements of table	fields	notes
	— each record to contain descriptive data for a single nesting site —	
· ***date***	- data entry date	
· ***descriptors of site***	- ***beach code*** - beach name, abbreviation - other local names - total size - actual length protected	preferably defined by national or international coding system
· ***location***	- latitude, longitude - country, state, municipality - reference landmark (natural or town)	
· ***source of data***	- name of person, affiliation, ***personal code, institutional code***	coding system could **link** to national/international international database of personnel and institutions
· ***others***	- other significant parameters	ecosystems, land use/tenure, management authority, significant developments, disturbance factors
· ***notes***	- complementary observations	

Table 2. Structure for ANNUAL BEACH SURVEY (considered essential)

elements of table	fields	notes
	— *each record to contain data from a single nesting site for a given year* —	
· ***date***	- date of data entry	
· ***descriptor of locality***	- ***beach code***	**links** with NESTING BEACHES CATALOG
· ***year/season***	- ***nesting season***	may prefer to use format such as 95/95 or 95/96 to allow for nestings spanning more than one calendar year
· ***locality/season***	- ***beach-season code*** (concatenated)	**links** with ANNUAL NESTINGS BY SPECIES TABLE
· ***survey coverage***	- extent of beach protected during given season	permits estimation of variations in survey coverage
· ***responsibility***	- management authority or organization (govt, international instit. university, etc.)	
· ***source of data***	- name of person, affiliation, ***personal code, institutional code, literature citation code***	coding system could **link** to national/international database of personnel and institutions or bibliography
· ***notes***	- complementary observations	

Table 3. Structure for NESTING Table (high-resolution)

elements of table	fields	notes
	— *each record to contain observations from a single nest* —	
· ***date***	- date nest laid	
· ***event registration number***	- ***registration number***	id for observation, corresponds to reg. number on field data sheets; use incremental numbers, starting every year
· ***event code***	- composite ***event code*** (made from concatenating site+season+registration number codes)	unique record id **links** with CAPTURE TABLE if nester seen; facilitates ordering and flagging of records by site of origin
· ***clutch data***	- nest number - total number of eggs laid - complete or partial clutch - which clutch of the season - number of eggs incubated - yolkless eggs - multiyolked eggs - quantification of other descriptors of embryo (e.g., partial development)	
· ***egg data***	- egg diameter, weight	best if summarized as mean, std. dev., range and sample size; can use individual measurements but will need separate tables
· ***hatching data***	- number of eggs hatched - number of dead hatchlings - number of deformed hatchlings - number of hatchlings released - number of females	can derive estimates of hatching success from these these data sex ratio can be estimated
· ***nest data***	- depth to top egg, to bottom - nest location in beach	
· ***fate of clutch***	- relocation code	final incubation site (e.g. *in situ*, beach hatchery, incubation house)
· ***applicable restrictions***	- apply restrictions yes/no - specifications of restrictions - length of time data to remain restricted	
· ***data source***	- name of person, affiliation responsible for the data ***personal code, institutional code, literature citation code***	coding system could **link** to national/international database of personnel and institutions, or bibliography if data are obtained from publications
· ***notes***	- complementary observations	

US$ 100-1,500; *e.g.*, Access, Paradox, dBase) and a sophisticated system with specialized database engines designed for efficient simultaneous multi-user, multi-platform and rapid remote-access by 50 users or more. At the latter end, databases can contain more than 10^6 records and Unix-based platforms are recommended (the most expensive software can cost >US$ 10,000; *e.g.*, SQL Server, Oracle).

The selection of a computer type *(e.g.*, PC-IBM, Macintosh, UNIX-based) has almost become a moot question, as manufacturers steer towards greater integrating capacities, particularly between and within networks interconnected locally or remotely. When a large database project is envisaged, only specialized servers should be considered. Connectivity to enable rapid exchange of data between individuals and organizations is now possible and economical with current electronic communication among local area networks (LAN) or between remote stations employing the Internet (either e-mail for batch queries, or interactive consultations through a Wide World Web interface). All modern software have capability for remote data searching via any of these. In all cases, expert advice is recommended particularly since integration of software and the development of queries and further analysis of data will require some degree of programming.

Documenting the Database

Often existing information is underutilized, largely because its location, content and applications are unknown. To avoid this, databases should include adequate documentation, providing descriptions of the structure, name, format and fields (data dictionaries) together with information about location and any policies regarding data access. As a whole, these metadata ("data on data") can clarify to users and outsiders the content, functions, and management of a database. Collaborative work and further consultation for management practices are enhanced; sharing, linking and improving existing databases are also facilitated.

Table 4. Structure for ANNUAL NESTINGS BY SPECIES Table (considered essential)

elements of table	fields	notes
	—— *each record to contain data for a single species nesting at site for a given year* ——	
· ***date***	- date of data entry	
· ***locality/season***	- ***beach-season code*** (concatenated)	**links** with ANNUAL BEACH SURVEY TABLE
· ***species***	- species, ***species code*** - management unit nesting here, ***MU code***	species and management units may be given numerical codes to facilitate queries; **links** with species and MU tables (optional)
· ***species season census data***	- time span of survey, dates - estimated time span of nesting activity, dates	permits estimation of variations in survey coverage from season to season
	- total females counted	stipulate if actual or estimated
	- total dead turtles found	specify raw or processed
	- total nest count	specify raw or processed
	- count of nests destroyed	specify raw or processed
	- count of eggs protected	poached nests, nests destroyed by natural causes
	- estimate of eggs lost	
	- count of hatchlings released	
	- estimation of total females, nests, eggs	
	- methodology code	extrapolation to full extension in cases when only partial surveys are possible; specify which
· ***annual rookery size***	- estimated size (females, nests) - methodology of derivation	value should include conf. limits statistical basis
· ***conservation significance***	- significance coding by species	rating for each species: e.g., rookery size relative to global size of management unit
· ***source of data***	- name of person, affiliation, ***personal code, institutional code, literature citation code***	coding system could **link** to national/international database of personnel and institutions or bibliography
· ***notes***	- complementary observations	

Database Application

The database model presented here employs a relational design, with basic entities (tables) holding data in a set of rows (the records) and columns (the fields). As the name indicates, tables are "related" or "linked" operationally through common fields. For simplicity, field formats (name, type, size) are not specified and descriptions of content appear in a generic manner that will require final assignment by someone experienced with database construction (assignments should suit individual needs). Copies of a documented working database adopting the same basic design can be obtained from the authors by request to exemplify a working application.

The parameters considered essential for sea turtle conservation goals are distributed among separate

Table 5. Structure for CAPTURE Table (high-resolution)

elements of table	fields	notes
	—— each record to contain observations on a single turtle ——	
· ***primary tag number***	- ***number of primary tag***	**links** with TAGS CATALOG; if this is a recaptured turtle, primary tag number needs to be confirmed from tag catalog; if secondary tag seen, entry should be replaced with primary tag number
· ***tag***	- presence/absence of tag - status (tag applied for first time, recapture, old tag replaced) - position of tag - presence/absence of tag scar(s)	tag status should be coded to enable flagging
· ***date***	- date of observation	
· ***event registration number***	- ***registration number***	id for observation, from field data sheet. If turtle laid nest, will be the same number as in NESTING TABLE (**link**)
· ***descriptors of turtle***	- ***composite event code*** (concatenation of site code+year+registration number) - maturity - sex - species	same as in NESTING TABLE (**link**) if capture is in nesting beach, otherwise parallel non-nesting capture site coding to be used
· ***turtle measurements***	- carapace length (curved or straight) - carapace width (curved or straight) - turtle weight - tail lengths (from carapace, plastron, vent) - head lengths (length, width)	type of measurement needs to be standardized (can use more than one but in separate fields)
· ***primary activity of turtle***	- activity	in the context of the turtle's life cycle
· ***capture method***	- capture method	
· ***health and condition of turtle***	- health, condition, stranded	several fields may be necessary and could include information on stranded turtles
· ***additional experimental actions on turtle***	- experiments	optional, related to specific projects
· ***location***	- latitude, longitude if turtle caught in open sea, or, sector in a national/regional grid system - ***beach code*** if found on nesting site	**link** to NESTING BEACHES CATALOG
· ***reproductive history of turtle***	- remigration interval - total number of clutches for the breeding season	
· ***applicable restrictions***	- apply restrictions yes/no - specifications of restrictions - length of time data to remain restricted	
· ***data source***	- person, affiliation responsible for data, ***personal code, institutional code, literature citation code***	coding system could **link** to national/international database of personnel and institutions, or bibliography if data are obtained from publications
· ***notes***	- complementary observations	

Table 6. Structure for TAGS CATALOG (high-resolution)

elements of table	fields	notes
	—— each record to contain data for single tag ——-	
· ***primary tag***	- ***number of primary tag***	this is the first tag number applied and should be used to identify turtle throughout its life; when additional tags are applied, the number is repeated for cross referencing; may be convenient to separate alpha-numeric prefix and numeric portions of number into separate fields
	- "return to" data (institution/address to be contacted) - tag type and/or material - tag position on body of turtle	
· ***secondary tag***	- number of applied tag - "return to" data (institution, address to be contacted) - tag type and/or material - tag position on body of turtle	replacement or multiple additional tags
· ***date***	- date of data entry	
· ***special tags***	- tag number, position	description of type
· ***source of data***	- name of person, affiliation responsible for the data ***personal code, institutional code***	coding system could link to national/international database of personnel and institutions
· ***observation code***	- ***event code***	**links** with CAPTURE TABLE
· ***notes***	- complementary observations	

tables following a logical, thematic organization. This modular design ultimately avoids redundancy while facilitating use of some of the tables depending on the immediate needs (or limitations) of the project and extending into other parameters as capacities increase. Parameters considered essential are contained in fields in the NESTING BEACHES CATALOG, ANNUAL BEACH SURVEY, and ANNUAL NESTINGS BY SPECIES tables (Tables 1, 2, and 4, respectively). Minimally, a monitoring program should generate data for these, which can be complemented by data found in historical or current publications. As capabilities are extended, rigorously collected high resolution data *(e.g.,* based on monitoring of individual, tagged turtles) may be added to the database by implementing the remaining NESTING, CAPTURE, and TAGS CATALOG tables (Tables 3, 5, and 6, respectively).

The annual registers should be viewed as important means with which to summarize the major parameters that facilitate an evaluation of conservation results on a beach-by-beach basis, incorporating important pieces of information on factors such as beach survey coverage and mortality (poaching, strandings, natural disasters, etc.) that are normally assessed on an overall basis. For example, the compilation of data through use of the ANNUAL tables can be used to assess conservation status in connection with recovery goals or benchmarks *(e.g.,* target number of nests per season).

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Factors to Consider in the Tagging of Sea Turtles

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Introduction

Sea turtles are tagged to achieve the recognition of individuals or cohorts for research purposes. Tagging is most often conducted to obtain information on reproductive biology, movements, strandings, residency and growth rates. This chapter will cover the use of external and internal tags, exclusive of remote sensing techniques (sonic and radio transmitters; see S. Eckert, this volume), naturally occurring genetic markers (see FitzSimmons *et al.*, this volume), data logging devices that require the electronic down-loading of stored information (see S. Eckert, this volume), and the injection of tetracycline or other substances to mark skeletal components.

Tagging of sea turtles as defined for this chapter includes: the external attachment, usually to the flippers, of a metal or plastic tag inscribed with numbers and words; the insertion into the body of a wire tag or microprocessor that can be detected with a electronic device; the marking of the carapace or other body part with paint, or by engraving or minor surgery to remove or alter tissue to form a recognizable external mark.

Historically, tagging has been the single-most valuable activity in advancing our understanding of sea turtles and their conservation needs in relation to complex life cycles, reproductive migrations, slow growth rates (for some species), and delayed sexual maturation. In many cases, a commitment to years of systematic tagging may be necessary to achieve certain objectives. However, in some instances the tagging of even a few turtles, particularly at nesting beaches where tagging has never been conducted, can yield valuable insight into migrations and the locations of resident foraging areas.

Unfortunately, current technologies and techniques for effectively tagging sea turtles are less than perfect. The degree of success from tagging, in terms of tag retention and maintaining recognition of a turtle, can be highly variable due to multiple factors that can include the following: the type of tag used and where and how it is applied to the turtle; the species of turtle and size class tagged; the geographical location and character of the marine environment; the skill of the person doing the tagging; the condition of the tagging gear; and the number of tags applied to each turtle.

Few of these elements have been carefully studied and quantified. Consequently, the researcher initiating a tagging program must make decisions based on uncertain and often confusing information, realizing that the outcome may not be apparent for years or even decades. An important objective of this chapter is to provide the reader with a basic understanding of what factors must be considered, and what options are available, to optimize the success of a tagging program in terms of the objectives that need to be accomplished.

The length of time the tag is expected to stay on the turtle to achieve the program's objectives is a fundamental consideration. The longer the desired time, the more uncertain the outcome. Hence, the first goal of a tagging program must be to minimize tag loss to ensure that recognition is retained, while not causing any lasting harm to the turtle from the tagging process. The second critical goal is to measure the extent of tag loss in order to correctly interpret resulting data and to adjust tagging techniques accordingly.

A realization of the above factors and limitations is essential to a new tagging program or improving an existing one. A methodology that may be successful at one location, under a certain set of circumstances, may be inadequate elsewhere. In short, tagging sea turtles at present can be considered partly science, partly art and partly guesswork. Having provided this warning, there are nevertheless an array of guidelines and options that can be set forth to help conduct an effective and productive tagging program.

Externally Applied Tags

The most commonly used tags on sea turtles are made of metal or plastic that attach to the posterior edges of the flippers. Some workers have constructed tags that attach through the edge of the carapace, but detailed information on their level of success is not yet available.

Plastic Flipper Tags

Plastic tags most often used on sea turtles consist of two pieces that require a special applicator to snap the sides together. Once in place they can not be taken apart without destroying the tag. An additional tool, such as a leather punch or pointed object, is usually needed to pierce a hole in the flipper prior to using the tag applicator. The resulting locked tag consists of two rotating parallel plates joined at the end where the tag passes through the flipper.

Plastic tags, such as the Jumbo Tag (45 x 17 x 10 mm) made by Dalton Supplies Ltd., England (fax 441-491-419-001) can be ordered in different colors with numbers and lettering embossed on both the internal and external surfaces of the tag's plates.

As with all tags applied to sea turtles, researchers in different areas have reported varying levels of success using plastic tags. Plastic may be liable to increased wear, brittleness and breakage depending upon the type of plastic, the behavior of the turtle, and the characteristics of the marine habitat where the tagging will occur. Also, unlike the completely closed design of metal tags after application, the open-ended shape of most plastic tags makes them liable to entanglement in gill nets. This in turn can result in an increased risk of mortality to the turtle from forced submergence and/or greater tag loss from tearing.

Some manufacturers of plastic tags have made claims as to the superior nature of their product for use on sea turtles. Before purchasing any tag, it is recommended that a researcher supplement the company's information with independent inquiries in order to obtain a balanced viewpoint.

Metal Flipper Tags

Metal tags commonly used on sea turtles are made of pure titanium (Stockbrands Company, Mt. Hawthorn, Australia, fax 619-444-0619) or blends of metals known as alloys that have enhanced physical characteristics. Monel 400 and Inconel 625, trademarks of International Nickel Company, are two alloys used to make tags for sea turtles by the National Band and Tag Company (NBTC) of Newport, Kentucky, USA (fax 001-606-261-8247).

Metal tags require a special applicator for proper attachment. However, except for the tough front flippers of leatherbacks, pre-punching is usually not needed due to the self-piercing design of the tag. When the applicator is squeezed, the sharp point of the tag pierces through the flipper and passes into a hole in the opposite end of the tag, where it bends over and locks into place. The resulting shape of the tag is rectangular or oval with no parts that can easily entangle in a net. This simplified locking mechanism exists on NBTC tag style 681C (25 x 8 x 9 mm) and style 1005-1 (8 x 2.5 x 2.5 mm). Style 1005-1 tags are small enough to be used on hatchlings, but are only available in Monel. The style 681C tag is produced in Inconel or (as style 1005-681) Monel. NBTC also offers a style 1005-49 tag (40 x 10 x 11 mm) in Monel only, but the locking mechanism is more complex involving an internal bridge that the point bends around. In some cases this lock has been the site of accelerated corrosion when the tags have been used on sea turtles.

Stockbrands' titanium tags (40 x 11 x 10 mm and 17.5 x 6 x 4-6 mm tapered) also have the simplified point-through-the-hole locking design. [Note that the latter measurement of each tag dimension listed is the gap within the tag after application.]

Difficulties in applying metal tags are sometimes experienced that involve incomplete sealing of the tag's point or the point prematurely bending over before passing through the hole. Some researchers using titanium tags have found it necessary to scrutinize and slightly bend each tag prior to application to make sure the point aligns with the hole. Similar malfunctions with Inconel and Monel tags often seem to be related to the manner in which the tag is snapped into the applicator by the researcher. NBTC has recently updated its instructional literature in an effort to lessen this problem.

Malfunctions when applying metal tags can also result from the use of applicators that are rusted, clogged with sand or other debris, or are worn from heavy use. All tag applicators must be inspected and cleaned on a routine basis and discarded when they cease to function properly. The timely replacement of worn applicators is an essential part of any tagging program. Stainless steel applicators available from

Stockbrands for titanium tags are more resistant to the wear that contributes to malfunction.

Malfunctions of Inconel and Monel tags can also result from slight differences in the manufacturing process. A company should be asked to test each applicator purchased with an order of tags to ensure they will seal properly. Additionally, applicators that function well with one batch of tags may not always do so with tags ordered at a later time. Testing and re-testing of applicators and tags prior to use in the field is an absolute necessity.

All metals corrode in sea water, but it is their rate of corrosion that must be of concern to sea turtle researchers. Prior to the availability in the late 1970's of tags made of Inconel and titanium, Monel tags were commonly used on sea turtles dating back to the early 1960's. Monel tags have exhibited highly variable rates of corrosion, both between geographical locations and on different turtles tagged at the same study site. For example, Monel tags applied to green turtles in the Hawaiian Islands and recovered 2-4 years later were found pitted and deteriorating from corrosion. Tag loss from this factor alone was estimated to be at least 90%. In sharp contrast, a few of the Monel tags used in Hawaii have been recovered in excellent condition 20 or more years later. Unpredictable variations in the quality of the Monel used to produce different orders of tags may also be a factor in their rate of corrosion.

Titanium and Inconel are equivalent in their superior resistance to corrosion in sea water. Tags for sea turtles made of these metals are recommended, unless one can be absolutely certain that Monel will not corrode at a rate unacceptable for the purpose of the research at the site where the work will occur. For example, Inconel tags have shown no visible signs of corrosion after being attached for 21 years to adult green turtles in captivity at Sea Life Park Hawaii.

Tag Sizes

Both plastic and metal tags are available in different sizes. The size of the tag selected for use on a particular size-class of sea turtle rests with the judgement of the researcher. No data exist to offer clear guidance.

The size of the tag used should seem appropriate for the size of the turtle, keeping in mind that tags on immature turtles must provide sufficient space during the growth process. However, this issue is complicated by the fact that the position of a tag on a flipper can alter over time as the turtle grows. This change may result in the tag's piercing site ending up too close to the posterior edge of the flipper, hence making it more liable to tearing and loss. Or, if the piercing site ends up at a more anterior location (farther away from the posterior edge), the tag can become overgrown or the gap within the tag can become crowded with tissue. The latter problem is of less concern with plastic tags that have one end open and two plates that rotate freely.

The ideal, of course, when tagging immature turtles is to have the piercing site and the tag remain in the same relative position on the flipper as growth takes place to an adult size. However, achieving this goal is difficult.

Tag Numbers and Message

Externally applied metal and plastic tags can be inscribed by the manufacturer with an address or other visible message, as well as identification numbers and letters. The size of the tag used will dictate the length of these two components. Some companies are able to imprint very small characters that allow more information to be included. The manufacturer's literature may not always show this option, so it is always wise to make personal contact with a company representative to discuss specific needs.

On metal tags the letters and numbers are formed by a high pressure stamping process. The manufacturer should be instructed not to stamp close to or directly on parts of the tag where the metal must bend when applied to the turtle. These areas can be weakened by stamping and, for certain alloys like Monel, can cause increased corrosion and tag loss. On titanium tags fissures and breakage may be more likely due to the brittle nature of this metal.

Careful thought must be given to the message that will be used on the tag. It is highly desirable that a concise mailing address, or other positive and practical means of notification, be used that will remain valid indefinitely or at least for the life of the project. For messages written in English the inclusion of words like "notify" or "write to" may be helpful to a lay person in determining what action should be taken when encountering a tagged turtle. In contrast, the use of words such as "return to" or "send" may result in a tag being removed from a live turtle and mailed to the specified address.

A decision will also have to be made whether or not to offer a reward for reporting the recovery of a tag, and if such wording should appear on the tag as an incentive for reporting. If a monetary reward is offered, the future availability of the funds must be assured or at least considered. Some researchers feel that offering and advertising a reward will motivate fishermen to take turtles that are already endangered due to over-hunting and other reasons. Other researchers feel that this factor is of minor concern and that the benefits of increased tag reporting are worth the risk. If a reward is deemed necessary, then compensations such as t-shirts, caps or posters with turtle designs can be given as an alternative to money.

Careful attention must be given to the identification numbers ordered from manufacturers of external tags. The use of the same number series by a company can occur when filling orders from different researchers (or even from the same researcher). A company can not be depended upon to monitor and notify a researcher when tags are being ordered with a number series that has previously been produced. A new tagging program has the responsibility to find out what identification numbers have been and are currently being used in the region in order to lessen the chances of duplication. The duplication of a number series is not a problem when ordering passive integrated transponder (PIT) tags. It is not possible to order a specific number series for PIT tags, since a 10-element alphanumeric unique identification is coded into each tag without duplication between manufacturers.

Numbers are usually stamped on metal and plastic tags in a consecutive manner. The ordering of duplicate numbers on two or more tags, so that all tags attached to a turtle will bear the same number, is not recommended as being practical or necessary. Duplicate tag numbers also increase the potential for different turtles to be accidentally tagged with the same tag number.

Tagging Sites

External tags used on the front flippers should always be attached at a proximal location, where the swimming strokes will cause minimal up-and-down movement of the tag. Figure 1 illustrates the preferred proximal front flipper tagging site used by many researchers. Tags have also been applied with success to the hind flippers of both immature turtles and nesting females (especially nesting leatherbacks) at the location shown in Figure 2.

Some workers use additional or alternate tagging sites that are between the large scales along the posterior edges of the front flippers, or directly through one of the scales. Care must always be taken to ensure that the gap within the tag is wider than the thickness

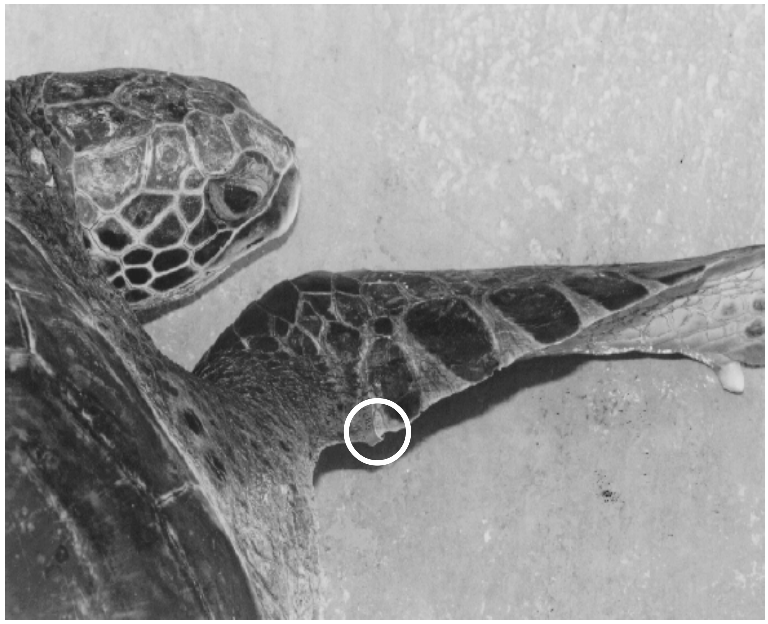

Figure 1. Style 681C Inconel tag attached to the proximal front flipper tagging location used by many researchers. The tag's piercing site is proximal of and adjacent to the first large scale on the posterior edge of the flipper.

Figure 2. Style 681C Inconel tag attached to the hind flipper of a juvenile green turtle. The tag's piercing site is proximal of and adjacent to the first large scale. This tagging location seems to work well on nesting females. Discomfort to the turtle from applying the tag here is much less than when applied to a front flipper.

of the flipper where tagging will occur.

Metal tags with their closed design should never be attached too far onto the flipper in a manner that inhibits the full range of free movement of the tissue within the tag; *e.g.*, the movement that takes place when both front flippers stretch forward when digging a body pit and during the nest covering process. To reduce injury from tag abrasion, metal tags used on the front flippers of leatherbacks should be applied so that the point that locks the tag ends up on the dorsal surface of the flipper.

Fouling of Tags

Barnacles, algae and other fouling organisms can grow on metal and plastic tags attached to turtles living in certain marine environments. Algae is harmless except for needing to be scraped off in order to read the tag inscription. However, if barnacles become excessive they will produce drag and tearing that contributes to tag loss. The tag "scar" that results from this sort of loss will often be a healed slit or v-notch. However, there is no certainty that a scar will be formed and remain detectable following the loss of a tag from any cause.

Ways to Reduce Problems

The following additional suggestions can help to minimize difficulties when using metal tags:

1. Mark one jaw of the applicator with colored paint as a reminder of the correct way to insert the tag.
2. Use durable tape to keep the tags from falling off the cardboard or plastic strip that they are loosely attached to when delivered from the factory. Groups of tags can also be strung together in a secure consecutive fashion with monofilament fishing line for convenient use in the field and to prevent loss.
3. Gain experience in tagging by applying tags to a piece of cardboard. Several tags from each new order should always be tested in this manner with each applicator. It should be noted that metal tags are designed to pierce something in order to work properly. Don't seal a tag for testing purposes without attaching it to cardboard or other similar material that mimics the flipper.
4. Tags that fail to lock when applied to a turtle are difficult, frustrating and sometimes impossible to properly correct, even when using additional tools. A tag that malfunctions should be removed, recorded as being destroyed and replaced with a new tag.
5. There are two distinct motions involved in applying metal tags. The first step is to squeeze the applicator so the tag point pierces the flipper. The second step a moment later involves applying substantially greater force to drive the point through the hole and make it bend over completely. The handles of the applicator should always be gripped as far back as possible to gain maximum leverage. Some taggers may find it helpful to use both hands to complete the second step.
6. After attachment, feel the tag with your finger and visually inspect to make sure the point has bent over into a fully locked position. Metal tags can pop open and be lost if not securely sealed.

Tissue Grafts and Other External Marks

Contrasting pigmented marks can be created by the surgical exchange (or autografting) of small pieces of tissue between the carapace and plastron. These marks, sometimes called "living tags," are retained and increase in size as a hatchling or young turtle grows to an adult. By doing the grafts on different scutes, the marks can be used to distinguish year-classes. The marks will appear in older turtles as spots or streaks, depending upon the site selected for the graft. An awareness by researchers and the public that turtles have been marked in this manner is essential for recognition and reporting to occur. The grafting procedure requires some skill, patience and practice but once mastered it can be carried out on hatchlings quite rapidly.

The notching of a marginal scute or combination of scutes by minor surgery can also be used to identify year-classes of hatchlings. However, these marks may become confused with natural injuries as a turtle grows larger. Small holes drilled in various combinations through the marginal scutes of juvenile to adult turtles appear to be retained for many years and can also be used for marking purposes.

Any marking procedure that involves cutting tissue, as described above, should involve consultation with a veterinarian and the exercising of proper precautions to prevent the transmission of disease.

Paint and other substances, including two-part resins, can be used to form identifying characters on the carapace. These marks are often short-lasting due to abrasion and the natural process of cellular shedding and regrowth. Numbers or other marks can be created on adult females that will stay readable throughout a nesting season. This can be accomplished by the shallow engraving of a scute using a portable Dremel Mototool with a No. 131 cutting bit and then applying paint to the grooves.

Internal Tags

Wire Tags

Small 2 mm wire tags made by Northwest Marine Technology (Shaw Island, Washington USA, fax 001-360-468-3844) can be inserted into the flipper of hatchlings or larger turtles to identify year-classes. Tags may be magnetized by passing a magnet over them either before or after implantation, if a magnetometer is used for detection. X-ray equipment can be used to detect the tags in either their magnetized or unmagnetized state. Portable magnetometers are available for field use. However, the possibility has been raised by some researchers that a turtle's navigational ability might be harmed by using magnetized tags.

Wire tags are sold with notch-coding that permits numerous year-classes to be identified. However, a coded tag must be removed from a sea turtle in order to decode it.

PIT Tags

Passive integrated transponder or PIT tags are small inert microprocessors sealed in glass that can transmit a unique identification number to a hand-held reader when the reader briefly activates the tag with a low frequency radio signal at close range. PIT tags used on sea turtles range in size from 11.5 x 2.1 mm to 20.0 x 3.2 mm. Even larger ones are manufactured that have been used on domestic livestock. Larger PIT tags can be read from greater distances than smaller PIT tags.

PIT tags have been inserted into the shoulder muscle of sea turtles or under the scales or between the digits of a front or hind flipper. PIT tags are a relatively new innovation in sea turtle research. The disadvantages of PIT tags include their higher cost, the cost of the readers, and the inability of someone without a reader to detect that a turtle has been tagged. In addition, PIT tags can sometimes migrate within body tissue making it necessary to carefully scan the entire area where implantation occurred. PIT tags have the advantage of being encased in glass and positioned inside the turtle where loss or damage over time from abrasion, breakage, corrosion or tearing should be virtually non-existent. PIT tags therefore offer the promise for reliably retaining the identification of individual sea turtles for decades, something that is not considered possible with externally applied tags. PIT tags may prove especially valuable for tagging leatherbacks due to the high loss of external tags applied to this species.

Until long term PIT tag retention is proven, it is always advisable whenever possible to apply two or more external tags to each turtle, as well as one or more PIT tags. Multiple tagging in this manner will help to reduce the chance of losing a turtle's identity. The use of two or more tags on each turtle also provides the basis to compute the probability of tag loss in a tagging program.

PIT tags are available from several companies including Avid (Norco, California, USA, fax 001-909-737-8967), Destron-Fearing (South St. Paul, Minnesota, USA, fax 001-303-444-1460), and Trovan Ltd. (Koln, Germany, fax 49-221-395-893).

PIT tags are made in two different transmitting frequencies (125 and 400 khz), but the readers that can readily detect 400 khz are being phased out. Also, the readers made by one company may not always be capable of reading tags produced by another company. Efforts toward better industry standardization and compatibility are underway.

Other Important Considerations

When to Tag

The decision of when to tag relates mainly to nesting females. To the extent possible, turtles emerging to nest should be allowed to lay their eggs before tagging takes place. Some researchers feel the best time to tag is immediately after egg deposition when backfilling of the egg chamber starts with the hind flippers. If tagging must occur prior to this time, some turtles will prematurely return to the sea but will usually emerge again to successfully nest on a subsequent night.

Cost of Tags

The cost of buying tags and applicators and shipping them to the study site is an important consideration. Again, the goals and finances of the tagging program will be guiding factors to the researcher. Tags that under some conditions may be more liable to loss are less expensive than ones that may have a longer retention time. For example Monel tags, which have been known to corrode rapidly in some cases, cost about US$300 per 1000, while tags made of Inconel and titanium cost US$750 and $2200 per 1000, respectively. Plastic tags cost US$400 per 1000. The applicators for metal and plastic tags range from US$15-70 each. PIT tags cost US$4-10 each. The more expensive ones are sterilized and include a disposable injector. Lower per unit prices may be available when metal, plastic or PIT tags are ordered in large quantities. PIT tag readers cost US$300-1250. The more expensive readers have greater sensitivity in their ability to detect tags. Readers that use easily obtained disposable batteries are recommended over ones that have a built-in rechargeable battery.

For many projects the cost of tags, even the more expensive ones, may end up being only a small percentage of the overall budget when taking into account personnel salaries, travel, living expenses at the study site, and post-fieldwork data analysis, report writing and publication. In view of the great importance of the tag to most work with sea turtles, it is recommended that funds for tags be budgeted first and foremost, rather than last, in order to obtain the "best" tag in ample quantity for the project to be conducted.

Storage of Tagging Data

Inherent in conducting a sea turtle tagging program is the need to accurately record and store for future retrieval the tag numbers, return address, tag type, tag size, date and place of tagging, and all data collected for the tagging event. The principal value of tagging results from the recovery and recognition of a turtle at some later date. The archiving of all tagging information should occur, with duplicate copies stored separately as a safeguard against catastrophic loss.

Regional data bases are sometimes established to provide a centralized location for storage of tagging and tag recovery data collected by multiple researchers. Regional data bases offer several advantages if they are operated properly with long term funding support. These advantages include accurate archiving of data, protection against loss, timely retrieval of tag information, and the capacity to analyze data on a regional basis to facilitate regional management of sea turtles. Regional data bases sometimes supply standardized tags and tagging gear at no cost. Individual tagging projects may come and go, but the regional repository will ideally remain intact.

A regional tagging data base should never be started without the assurance of longevity. Before contributing data to a regional entity, the researcher should determine and find acceptable the conditions for future ownership of the data, agreements for publication and any other aspects, including restrictions and obligations that may exist now and possibly in the future. All agreements and conditions should be confirmed in writing with the authority in charge.

Tag Recovery

Except for certain kinds of short term census work, a turtle that is tagged, and then never seen again, will not yield its full potential for research. Recovery is therefore a vital factor. The three means of recapturing a tagged turtle include intentional capture efforts by researchers, accidental or intentional capture by fishermen, and the chance encounter by the public such as finding a tagged turtle stranded ashore. Directed efforts can be carefully planned to increase the possibility of recovering tagged turtles. Other means are mostly a matter of luck and the willingness of persons to report the tag.

Old tags present on recaptured turtles that are unreadable due to corrosion or being imbedded with tissue should be removed and replaced with a new tag. If a turtle with a tag from a different program is re-tagged, the original tagger should be informed of the change.

Disease Precautions

Precautions need to be taken to prevent the spread of infectious diseases during tagging. Tag applicators and piercing equipment, such as used for plastic tags, must be disinfected after coming into contact with blood or other body fluids. Two complete sets of tagging gear are recommended; one kit for turtles that are diseased and the other kit for apparently healthy turtles. Used tagging gear should never be transferred between projects in different regions. Pre-sterilized PIT tags with disposable injectors should be used in areas where disease may be an issue. The used PIT tag injector needles should be placed into proper disposal containers.

Some researchers apply Betadine, 70-90% alcohol, antibiotic ointment, or other agents on the flipper where the skin will be pierced by the tag. Metal tags in particular must be cleaned prior to use to remove lubricating oil or other reside resulting from the manufacturing process. Soaking the tags in alcohol or other agent as a final step may also be advisable.

Discomfort to the Turtle

The application of external or internal tags will produce some level of pain to the turtle. The discomfort displayed is usually short and highly variable between individuals. Most turtles barely seem to notice, while a few others exhibit a marked response. Topical anesthetics, such as ones sold over-the-counter for human sunburn, can be applied prior to tagging. This may help to demonstrate compassion on the part of the researcher where the public routinely views tagging activities.

In some instances the jaws of the tag applicator may pinch the turtle and cause as much discomfort as the actual tag-piercing process. In such cases it may be possible to alleviate the problem by grinding off certain unnecessary portions of the jaws.

The small wound-site resulting from a tag properly applied to the flipper should heal completely in a short time, similar to what happens when a person's ear is pierced for an earring. However, healing may not occur if a tag is applied too tightly, or the tag corrodes and releases copper and nickel oxides, as can sometimes happen with Monel tags.

The issue of possible adverse effects from tagging, especially when tagging females on a nesting beach, has been raised in the past and must be briefly addressed here. There is no basis to believe that the tagging experience or presence of tags will cause lasting harm or alter a turtle's long term behavior. When females were first tagged decades ago some researchers were concerned that this might cause the turtles to nest elsewhere, since none returned to nest the year after initially being tagged. This misunderstanding was eventually dispelled with the knowledge that most sea turtles have multiple-year nesting cycles.

Hazards to the Researcher

There is an element of risk to the researcher when tagging large turtles on a nesting beach. Powerful, fast and unexpected swings of the front flippers can inflict painful blows. Tag applicators not gripped firmly may be turned into hazardous projectiles as the result of violent flipper movements. Sand on a nesting beach can be flung by the flippers with incredible force creating a danger to the researcher's eyes if caution is not exercised. Durable shoes are advisable to protect against foot injury from a nesting turtle that suddenly decides to crawl while being tagged. Some turtles attempt to bite when handled during underwater capture efforts and when brought out of the water to be tagged.

The sharp point of a metal tag and the injector needle of a PIT tag are also hazardous and can easily puncture a finger or other body part if care is not taken. Repetitive motion injury can occur to a researcher's hand and forearm from squeezing a tag applicator multiple times when tagging turtles over months or years.

Acknowledgments

I am grateful to the following individuals for their helpful review, comments, and assistance in the preparation of this chapter: George Antonelis, Karen Bjorndal, Alan Bolten, Charles Caillouet, Donna Dutton, Peter Dutton, Karen Eckert, Denise Ellis, Sheryan Epperly, Brendan Godley, Fred Haas, Kevin Haas, Colin Limpus, Joanne Braun McNeill, Jeanne Mortimer, Shawn Murakawa, and Laura Sarti.

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Research and Management Techniques for the Conservation of Sea Turtles
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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Techniques for Measuring Sea Turtles

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Sea turtles are measured to accomplish a number of objectives, and there are many technique and equipment options. The objectives may dictate different levels of accuracy and precision (see below) as well as the appropriate methods and equipment to be used. Sea turtles are measured on nesting beaches to relate body size to reproductive output, to determine minimum size at sexual maturity, and to monitor nesting female size for a particular rookery. Sea turtles are measured on foraging grounds to determine frequency of size classes of turtles present as well as to monitor growth rates. The size frequency of a population is an important parameter of that population's demographic structure. Analyses of growth rates can indicate habitat quality and physiological status.

Accuracy and Precision

Project objectives will determine the levels of accuracy and precision necessary to accomplish the project and thus the equipment to be used. "Accuracy is the nearness of a measurement to the actual value of the variable being measured. Precision is not a synonymous term, but refers to the closeness to each other of repeated measurements of the same quantity" (Zar, 1984).

There are few studies of precision in sea turtle measurements (Bjorndal and Bolten, 1988, 1989; Frazier, 1998; Shoop and Ruckdeschel, 1986; van Dam and Diez, 1994). In all reports and publications, measurement precision should be included. One method of reporting measurement precision is to present the mean, standard deviation and/or standard error, and range of the absolute difference between pairs of repeated measures on a series of turtles (sample size should be reported) that span the size range of turtles in the study population (Bjorndal and Bolten, 1988, 1989; van Dam and Diez, 1994). Precision may vary for each type of measurement (Bjorndal and Bolten, 1988, 1989; Shoop and Ruckdeschel, 1986). Precision can be increased by having one individual take all measurements. If that is not practical, the precision of members of the research team should be compared over time (Bjorndal and Bolten, 1988, 1989; Shoop and Ruckdeschel, 1986).

Measurements should be made in metric units; conversion to metric units (necessary for publications) from other systems results in misrepresentation of degrees of accuracy and precision. For example, converting measurements taken to the nearest eighth of an inch to millimeters does not correctly represent the level of accuracy of the measurements. Calipers, tape measures, and scales should be calibrated frequently.

Scute Nomenclature

There is inconsistency in the nomenclature used to describe the elements of the carapace (for a discussion see Pritchard and Trebbau, 1984). The nomenclature suggested by Pritchard and Trebbau (1984) is recommended and can be summarized as follows. Scutes are cornified plates forming the surface of the shell. Vertebral scutes are the large scutes along the midline of the carapace. Costal scutes are the large scutes forming a longitudinal series on each side of the vertebrals. Marginal scutes are the numerous small scutes around the edge of the shell, except the median scute on the midline anterior to the vertebrals (which is the nuchal scute) and the paired posterior marginals (which are the supracaudal scutes).

Measurement Procedures: Linear Measurements

Linear measurements can either be taken with calipers (straight-line measurements) or with a flexible tape measure (curved measurements). The decision is one of accuracy, precision, cost, and convenience. Curved measures tend to be less accurate and less precise (Bjorndal and Bolten, 1989; Frazier, 1998; Pritchard *et al.*, 1983; Shoop and Ruckdeschel, 1986) because of irregularities and epibionts on the surface of the turtle's shell. Also, in the juveniles of some species, the vertebrals are keeled, and the posterior carapace in some species has a steep change in slope which make curved carapace length difficult to measure with accuracy and precision. However, flexible tape measures are significantly less expensive than calipers and are significantly more convenient to carry and maintain.

There are great differences in the quality of calipers, not only in the mechanism and fit of the arms sliding on the main rail, but also in the calibration and scale of measurements *(e.g.,* centimeters versus millimeters). Tree calipers that have very long arms should be modified so that the arms are only as long as necessary. Excess arm length makes calipers more cumbersome to use and reduces both accuracy and precision. Both arms of the calipers must be of equal length so that when measurements are taken, the measurement endpoints on the turtle are at the same distance from the main rail of the calipers. Calipers should be selected for appropriate accuracy and precision and should have metric units.

Flexible, fiberglass tape measures are better than metal tape measures for curved measurements because they more closely conform to the shape of the shell and do not corrode. Cotton tape measures should be avoided because they stretch easily. Tape measures should have metric units.

To ensure accuracy and precision, length of the calipers or the tape measure should exceed the maximum expected length of the turtles in the study population so that reported lengths are the result of a single measurement rather than sums of partial measurements. Epibionts interfering with measurements should be removed when the accuracy of the measurements is important. If measurements are affected by injuries or deformities these irregularities should be noted, and perhaps the measurements should not be included in analyses, depending on the project's objectives.

Five standard linear measurements are presented: carapace length (Figures 1 and 3), carapace width, tail length (Figure 2), head width, and plastron length. For many studies, carapace length may be the only measurement needed.

Linear Measurements of Hard-shelled Turtles

Straight Carapace Length

At least three different straight carapace lengths have been used by sea turtle researchers (Pritchard *et al.*, 1983).

- Minimum straight carapace length (SCLmin; Figure 1a) is measured from the anterior point at mid-

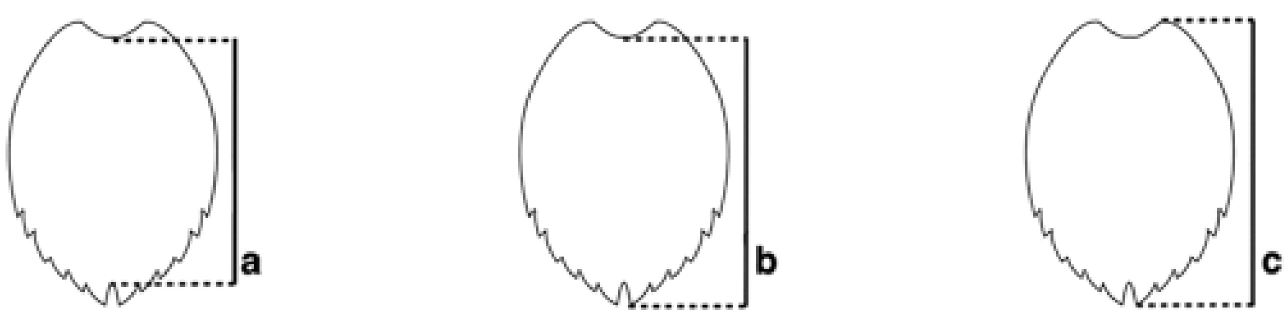


Figure 1. The anterior and posterior pairs of anatomical points for three carapace length measurements. (a) Minimum straight carapace length (SCLmin) and minimum curved carapace length (CCLmin) are measured from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supracaudals. (b) Straight carapace length notch to tip (SCLn-t) and curved carapace length notch to tip (CCLn-t) are measured from the anterior point at midline (nuchal scute) to the posterior tip of the supracaudals. (c) Maximum straight carapace length (SCLmax) is measured from the anterior edge of the carapace to the posterior tip of the supracaudals. Anterior and posterior locations must be on the same side of the carapace.

line (nuchal scute) to the posterior notch at midline between the supracaudals.

- Straight carapace length notch to tip (SCLn-t; Figure 1b) is measured from the anterior point at midline (nuchal scute) to the posterior tip of the supracaudals. Often the tips of the supracaudals are not symmetrical; for consistency, the supracaudal that yields the longer SCLn-t should be used.
- Maximum straight carapace length (SCLmax; Figure 1c) is measured from the anterior edge of the carapace to the posterior tip of the supracaudals. Anterior and posterior locations must be on the same side of the carapace. For consistency, the side that yields the longer SCLmax should be used.

The recommended straight carapace length measurement is SCLmin (Bjorndal and Bolten, 1989; Gerosa, 1995). SCLmin is a better measurement because the posterior tips of the supracaudals are frequently broken in juveniles or worn away in adults. If time permits, both SCLmin and SCLn-t can be measured so that comparisons with other data sets can be made. To avoid confusion, measurements should always be clearly defined on data sheets and in publications.

Curved Carapace Length

The lack of clearly defined starting and ending points may contribute to the variance in precision of curved carapace lengths (Shoop and Ruckdeschel, 1986). Because of the curvature (and thickness) of the nuchal scute, the junction of skin and scute should be used as the anterior point. The posterior point should be the posterior tip of the dorsal surface. Two different curved carapace lengths have been used.

- Minimum curved carapace length (CCLmin; Figure 1a) is measured from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supracaudals.
- Curved carapace length notch to tip (CCLn-t; Figure 1b) is measured from the anterior point at midline (nuchal scute) to the posterior tip of the supracaudals. Often the tips of the supracaudals are not symmetrical; for consistency, the supracaudal that yields the longer CCLn-t should be used.

The recommended curved carapace length measurement is CCLmin (Bjorndal and Bolten, 1989; Shoop and Ruckdeschel, 1986). There is greater variability in CCLn-t because of the unpredictable way that the tape measure deviates from the midline.

Carapace Width

Carapace width is measured at the widest point; there are no anatomical reference points. Straight carapace width (SCW) is measured with calipers; curved carapace width (CCW) is measured with a flexible tape measure. For each turtle, the anatomical location on the carapace where SCW and CCW are measured may not be the same. There must be consistency in the orientation of the turtle when SCW is measured, particularly with juvenile turtles, to avoid an additional source of variation. If the turtle is lying on its carapace (plastron up), the mass of the turtle tends to spread the carapace thus increasing the width of the carapace. Also, carapace width changes as the turtle inhales and exhales. For consistency, because CCW must be measured with the turtle lying on its plastron, SCW should be measured with the turtle also in this orientation.

Tail Length

Figure 2 shows two tail measurements. Total tail length (TTL) is the distance from the midline of the posterior margin of the plastron to the end of the tail following the curvature of the tail. Postcloacal tail length (PTL) is the distance from midcloacal opening to the end of the tail following the curvature of the tail. For both TTL and PTL, the turtle is positioned on its carapace, and a flexible tape measure is used to obtain the measurements. In sea turtles, tail length is a secondary sex characteristic; mature males develop long tails and females have short tails. In mature male turtles, the ratio of TTL to PTL is greater than in mature females. In juvenile sea turtles, tail length does not indicate an individual's sex.

Head Width and Plastron Length

Head width (HW) and plastron length (PL) are less frequently measured in sea turtles than are carapace length and width. HW is measured at the widest point with calipers. PL should be measured with calipers along the midline. Some variation in measurement is introduced because frequently the anterior and/or posterior edges of the plastral scutes do not completely overlay the anterior and/or posterior edges of the underlying bone. PL should be measured along the midline from the anterior edge to the posterior edge of the underlying bone when it extends beyond the scutes. PL measurements are less precise than SCLmin and SCLn-t (Bjorndal and Bolten, 1988).

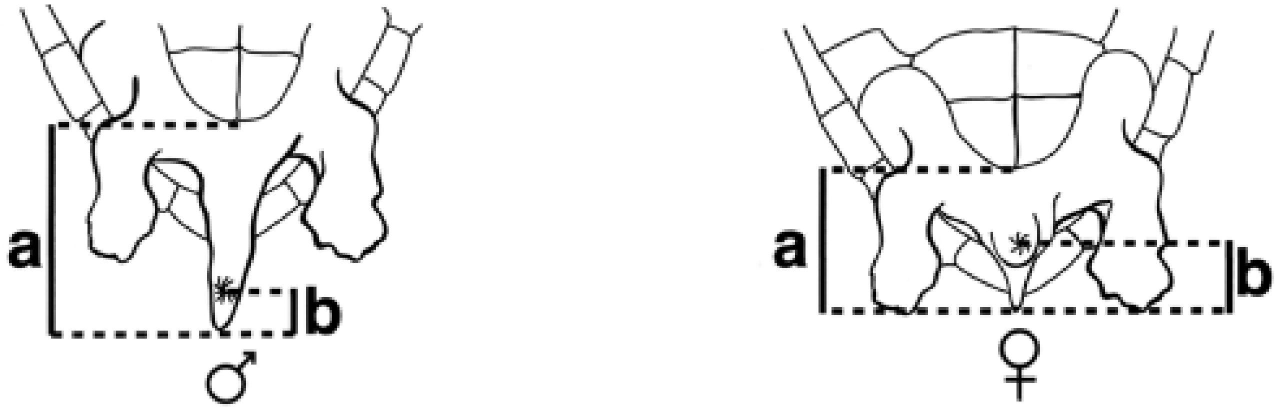


Figure 2. Two tail length measurements: (a) total tail length (TTL) is the distance from the midline of the posterior margin of the plastron to the end of the tail following the curvature of the tail; (b) post-cloacal tail length (PTL) is the distance from mid-cloacal opening to the end of the tail following the curvature of the tail.

Linear Measurements of Leatherback Turtles

Carapace Length

Both straight carapace length (SCL) and curved carapace length (CCL) are measured from the nuchal notch (anterior edge of the carapace at the midline) to the posterior tip of the caudal peduncle (Figure 3). If the caudal peduncle is asymmetric, for consistency, measurements should be made to the longest point. Straight measures are recorded using calipers. Curved measures are made alongside the midline (vertebral) ridge. Curved length is not measured along the crest of the ridge because of irregularities in the ridge and the difficulty of keeping the tape on the ridge. The end of the tape measure should be securely positioned at the junction of skin and carapace at the midline ridge, and the tape pulled taut to the caudal peduncle, allowing the tape to follow a "natural" position alongside the ridge.

Carapace Width

Carapace width is measured at the widest point; there are no anatomical reference points. Straight carapace width (SCW) is measured with calipers. Curved carapace width (CCW) is measured with a flexible tape measure; the tape measure does not follow the curvature of the ridges, but rather spans from ridge crest to ridge crest. For each turtle, the anatomical location on the carapace where SCW and CCW are measured may not be the same.

Tail Length, Head Width, and Plastron Length

Head width is measured at the widest point with calipers. Turning an adult leatherback onto its carapace for the purpose of measuring tail and plastron length is not desirable. If juveniles are encountered, tail length should be measured as described for hard-shelled turtles and plastron length should be measured with calipers along the midline from the anterior edge to the posterior edge.

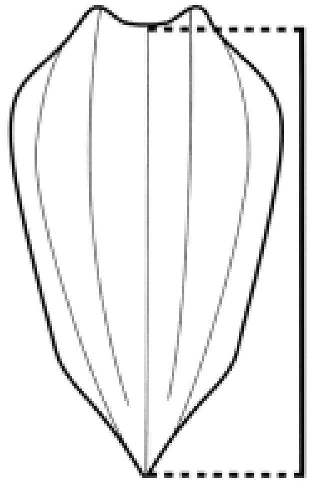
Linear Measurements of Hatchling Turtles

Hatchlings should be measured with small calipers following procedures for straight-line measurements described above. Because the shells of hatchlings are very flexible, care should be taken not to distort the shape of the shell when taking measurements.

Measurement Procedures: Mass Measurements

Body mass is a more biologically significant measure of body size than are linear measurements because physiological parameters scale to mass. However, body mass is more difficult to measure and is more variable because of reproductive state and nutritional status *(e.g.,* extent of gut fill). After a series of mass measurements and linear measurements have been collected for a population, a regression equation

Figure 3. The anterior and posterior anatomical points for straight carapace length (SCL) and curved carapace length (CCL) in leatherback turtles. In both cases, length is measured from the nuchal notch (anterior edge of the carapace at the midline) to the posterior tip of the caudal peduncle.



can be used to estimate body mass from carapace length (Bjorndal and Bolten, 1988; Boulon *et al.*, 1996). However, over time the relationship of mass to length can change with changes in habitat quality and density-dependent effects (Bjorndal *et al.,* in press).

Sea turtles can be weighed using a spring scale. The appropriate scale for the size range of turtles should be selected. In general, the absolute accuracy of a spring scale decreases with increasing total capacity. To reduce trauma when weighing sea turtles, a net or mesh support should be used to cradle the turtle. A portable tripod with a pulley system can be constructed to lift the turtle. Hatchlings can be placed in a small clean bag (obtain tare first) and weighed on a spring scale. Greater accuracy can be achieved by weighing hatchlings on a triple-beam balance or electronic balance.

Sources of Supplies

Supplies for measuring turtles *(e.g.,* tree calipers, tape measures, spring scales, and waterproof notebooks) are available from forestry supply companies. Anthropometer calipers (available from scientific or medical suppliers) are very accurate but are expensive.

Conclusions

There are a number of ways to measure turtles. Methods appropriate for the study should be selected and used consistently. Recommended methods for measuring sea turtles have been described in this chapter. However, whatever measurements are used, the specific measurements should be clearly defined. The precision of the measurements should be determined and included in any project report and publication.

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Nesting Periodicity and Internesting Behavior

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Nesting Periodicity

Females of all sea turtle species deposit multiple clutches of eggs over the course of a reproductive lifetime (one or more reproductive years). An "inter-nesting interval" is the period, in days, between a successful nesting and the first attempt at a subsequent nesting by an individual turtle during a single nesting season (=reproductive year). "Remigration interval" is the period, in years, between nesting seasons for an individual female. Estimating the size of an annual nesting population (the number of individual turtles nesting each year) is critical for any conservation or management strategy. In the absence of a saturation tagging project (which reveals precisely how many turtles nest each year), one key element in obtaining a reliable estimation is accurate information about the population's nesting periodicity.

Internesting intervals range from 12-15 days for *Caretta, Chelonia, Eretmochelys* and for *Lepidochelys* solitary nestings; 13-18 days for *Natator;* and 9-10 days for *Dermochelys.* In *Lepidochelys, "arribada"* (mass nesting) intervals are longer and more variable because their occurrence is apparently also influenced by environmental factors such as wind and tide. Calculating the internesting interval requires a representative sampling of turtles nesting at least twice during the reproductive season (N of 100 or more is recommended). The closer the size of the sample is to the total number of nests laid, the more reliable the estimate will be. Turtles must be tagged (that is, known as individuals) and, in the case of each individual, the number of days between a successful nesting and subsequent nesting attempt must be counted. (Note that the internesting interval is *not* defined as the number of days between nests, but rather from one successful nesting to the first landing of the next nesting cycle.) From this sampling, a population average can be calculated. It is useful to report the average, as well as range (minimum and maximum) and standard error.

To aid in visualizing the internesting interval, it is helpful to represent the data graphically in histogram form, partitioning the x-axis in 2-day intervals (5-7 days, 7-9 days, 9-11 days, and so on). Data derived from nightly beach coverage and a comprehensive tagging program will illustrate a primary peak within a broadly defined species average *(e.g.,* 9-10 days for *Dermochelys),* with secondary peaks representing multiples of the average interval; in the case of *Dermochelys,* 20 days, 30 days, 40 days, and so on. In studies of *Caretta*, *Chelonia* and *Eretmochelys*, observed internesting intervals exceeding 25 days should be discarded in any calculation of the internesting interval, as should observed intervals exceeding 18 days for *Dermochelys.* In these cases, intervening nests are likely to have been laid unobserved, and data should be treated as described in the following paragraph.

At a monitored site where nesting females are tagged and the average internesting interval is, say 12 days, **clutch frequency** (average number of clutches laid by individual turtles during a single nesting season) should be estimated not from observed nests alone, but rather based on the assumption that all nesting occurs at regular 12 day intervals. A nest chart with nesting dates for (tagged) turtles enables an investigator to visualize gaps in the records for each individual's nesting sequence. These gaps will manifest themselves as internesting intervals of multiples of the regular period (24, 36, or more days), most likely reflecting the occurrence of nestings unobserved by the monitoring staff and should be taken into account for more precise calculations. Tallying observed and unobserved (missed) nests from this procedure, and dividing the total by the number of tagged turtles provides a more reliable estimation of clutch frequency than would be the case if the number of observed nests alone were divided by the number of tagged turtles. The result should be considered an underestimate, however, since gaps in the nesting record do not, by definition, appear when the missed nest was either the first or last nest laid by a particular female.

An indirect method of estimating the number of nesting females per year is derived from the total number of nests (defined as the successful deposition of eggs) recorded during the same time period. The total number of nests is divided by clutch frequency, defined as the average number of nests laid per female per year (ranging from 2-3 for *Lepidochelys* to as many as seven or more for *Dermochelys)*. Clutch frequency varies somewhat from year to year, as well as geographically. Once the value is known for a particular species and area (based on data collected at a monitored study site where nocturnal coverage includes a comprehensive tagging program), then estimates of population size can be obtained at sites where only crawl counts are available. For this, additional knowledge of **nesting success** must also be available. If, for example, 200 crawls are counted during a nesting season, yet only 160 of them are judged to be nests (that is, to contain eggs), and the average clutch frequency is known to be four, then the number of nesting females can be estimated at 40 (160/4).

Having an estimate of internesting intervals, clutch frequency and nesting success at a monitored study site allows calculations of population size at unmonitored sites where only crawl counts are available. In the absence of 100% beach coverage, which is difficult to achieve even in the most comprehensive monitoring programs (the case for most nations which cannot afford to comprehensively monitor all nesting beaches on a nightly basis) this information is fundamental to management. However, at least one comprehensively (nocturnally) monitored site is a requisite to obtain the basic data.

Since only a proportion of the adult population reproduces each year, it is necessary from a management standpoint to look beyond annual estimates of nesting females to estimates of the adult population at large. Information on the **remigratory interval** (inter-seasonal nesting periodicity) is essential to estimating the total number of mature females in the population at large. Assuming strict site fidelity among years and assuming that each female in a population nests every year, then the total number of nesting females observed per year would be equivalent to the total number of sexually mature females in the population at large. However, with the possible exception of *Lepidochelys,* no sea turtle population studied so far is characterized by all, or even a majority, of its mature females nesting every year. Generally, when a turtle completes a reproductive season, two, three, four or more years will pass before she is seen on the nesting grounds again.

To convert the number of nesting females per annual season to the total number of reproductively active females in the population at large, the average remigration interval must be known. With the exception of *Lepidochelys,* remigration intervals are not well known. It is possible for turtles to skip many years (perhaps a decade or more in some cases) between reproductively active seasons; thus, there are few databases with the longevity to measure this parameter accurately. Literature values for remigration intervals range from 2-3 years for most species, but these should be used with caution as they are constructed, for the most part, from as yet short-term (less than a decade) tagging studies and most have not taken into account the variable of tag loss.

To determine the remigratory interval, nocturnal beach coverage must include the tagging of nesting females for periods exceeding one decade, must maintain accurate tagging records, and must take into account calculations of tag loss. When a tagged female is encountered, her period of absence from the nesting beach can be calculated from tagging records documenting her last recorded nesting. At the end of each nesting season, a range of remigratory intervals will be evident in the database. These will be constrained by the number of years in which tagging has been conducted; that is, it is not possible to document a remigration interval which exceeds the number of years tagging has taken place. Over time, primary and secondary intervals can be documented for the study population. It is essential to obtain data over many reproductive seasons to obtain robust estimates of remigratory behavior.

Encountering Turtles and Collecting Data

Biological data essential for determinations of reproductive periodicity are obtained from nightly (or daily in some *Lepidochelys* populations) nesting beach patrols during the reproductive season. Crews inspect each turtle encountered and, typically after egg-laying, proceed with evaluations of injury, ectobiota and other items of interest, as well as measurements (see Bolten, this volume) and tags (see Balazs, this volume). If the turtle is tagged, tag numbers are registered. The presence of potential tag scars should be noted regardless of whether the turtle is to be tagged or not. If no tag is present, single or multiple tags are applied, depending on the standard practice. Whether or not eggs were deposited should be noted. If eggs are laid, the nest should be given a reference number and its location registered. Nest location can be estimated from reference points or stakes set up at predetermined spots along the length of the beach and separated by no more than 0.5 km.

Turtle behavior from her emergence onto the beach until her return to the sea can be documented in discrete phases, such as: a) emerging from the sea, b) crawling from the point of emergence to the nesting zone, c) selecting a specific nesting site, d) creating a body pit, e) excavating an incubation chamber, f) depositing eggs, g) covering eggs, h) camouflaging the nest, and i) returning to the sea.

Typically a track is observed before the turtle is seen. Species identification should be ascertained from the track, and a determination made as to whether or not the track is “fresh.” Fresh tracks are detectable in the wettest portion of the beach, below the tidal line or immediately above it. They are very dark in the wet portion of the beach, and very pale in the dry zone. Fresh tracks are generally clear; that is, they are not marred by footprints, crab tracks, etc. Older tracks, on the other hand, are found exclusively above the high water mark, and are generally overlaid by animal tracks *(e.g.,* crabs, sea birds, domestic or feral animals). Older tracks are comparatively faint and appear the color of the surrounding beach.

Ascertaining whether the track is an “up” (ascending) or “down” (descending) track will facilitate locating the turtle and/or nest (see also Schroeder and Murphy, this volume). Once the turtle is found, the phase of the nesting cycle should be determined (see above) and recorded. Meanwhile, precautions must be taken when approaching a nesting turtle to avoid the use of any artificial light. Females, especially during the early phases of the nesting cycle (prior to laying eggs), are typically cautious and can be easily frightened by disturbance, particularly artificial light or extraneous noises. These can cause an abandonment of the nesting process. Turtles should be approached from behind and only after the body pitting process has begun. This stage can be detected by the presence of wet (darker) sand surrounding the turtle, and by the motion of all four flippers (as opposed to nest excavation which involves only the rear flippers).

Initially, contact with the turtle should occur only after the female has finished camouflaging the nest. Once a general level of tolerance on the part of females utilizing a particular study site is well known by patrol crews, certain aspects of data collection, such as measuring and egg counting, might be carried out safely once the egg chamber is complete (that is, prior to covering the egg chamber and camouflaging the nest). The general consensus is that tagging should not be done until the eggs have been laid (see Balazs, this volume). Prior to departure, the turtle should be examined with a soft light to confirm species (see Pritchard, this volume), identify potentially obscure tags (such as in the groin area), and document injury. Lastly, pertinent information is registered, such as nest location and distance from the sea, number of eggs, date, time and name of observer.

Looking for tags and potential tag scars is important even if the local project does not carry on a tagging program because nesting females may have been encountered in other regions and tagged by other researchers. If tags are found, the date and locality where the turtle was found, together with the names and institutions participating, should be sent to the return address engraved on the tag or to a central tagging database depending on the procedure operating in the region.

Internesting Behavior

While the focus of research and management on gravid females has been on terrestrial activities, there is a need to understand the at-sea activities of these females as it relates to interpretation of beach data and protection of nesting turtles in the marine environment during the nesting season. Documentation of internesting movements (the at-sea movements of females between nesting emergences within the same season) aids in identifying the areas and habitats used most frequently by gravid females. These should be key areas for protection from threats such as trawling, gill nets, dredging, and oil and mineral exploration during the nesting season.

Monitoring turtles in the marine environment usually requires the use of remote sensing techniques. This can involve balloons or floats for daytime monitoring, the use of "chem-lights" or battery powered light sources for nighttime monitoring, or the use of electronic telemetry techniques (see S. Eckert, this volume). The activities of gravid sea turtles which are monitored using remote sensing techniques may be used to 1) document habitat protection needs; 2) better understand behaviors associated with nesting; and 3) validate data collected on the nesting beach. The specific management needs of a rookery, combined with the personnel and funding available and local characteristics of the study area, will dictate the methods employed.

With the exception of leatherback turtles, which typically venture into deep offshore waters between nestings, the most direct approach to *short-term* monitoring of gravid females is the use of floats attached to the turtle by a leader or lanyard. The length of the lanyard used will depend on the water depths around the study area. In most cases the lanyard should be longer than the normal maximum depth. In addition to the tracking float at the end, there should also be floats placed at intervals (3 m) along the lanyard to prevent entanglement when the turtle is in shallow water. A length of PVC tubing may also be mounted at the point of attachment to the turtle to deflect the lanyard away from the turtle's flippers. The tensile strength of the lanyard or the point of attachment should be such that if the float or lanyard becomes snagged, the turtle could easily break free. In addition, the point of attachment should have a corrodible link which will detach all equipment through the action of salt water.

The float should be large enough to be visible at a distance of at least 1 km, under the conditions of a particular study area. The use of a mast to elevate a flag above the float will enhance visibility. A mast will usually require a counter weight to keep it vertical. The float can be attached during oviposition or after the female has completed the nesting process. The use of elevated observation areas, such as rocky headlands or towers, can enhance tracking. The position of the tracked turtle can be determined by recording two simultaneous compass bearings of the floats. The main limitation in the utilization of floats is that the tracking distance is restricted to the limits of visual observation. Also, the size and colors of the float can attract predators and people not involved in the project.

Monitoring the nocturnal activities of turtles will generally require the addition of lights to the float package. These lights should facilitate tracking but should not influence the turtle's activities. While chemical light sticks must be replaced daily, they do provide a highly visible but diffuse light source. Battery powered lights should be sealed from salt water to avoid the corrosive effects of the sea.

The use of electronic tags such as sonic or radio transmitters enhances the distance from which a turtle may be located. This advantage comes with the associated costs of transmitters and receiving equipment. Adult hard-shelled sea turtles are well suited to electronic monitoring because they can carry relatively large transmitters and have a convenient point of attachment in the form of a bony shell. Typically, sonic transmitters have a more limited range than radio transmitters but have the advantage of transmitting precise signals while the turtle is submerged. Shell-mounted sonic and radio transmitters are frequently used in combination, with the radio used to locate the general vicinity of the turtle during brief surface activity and the sonic to acquire continuous data once contact is established.

Radios are either attached directly to the carapace or incorporated into a float at the end of a lanyard. Direct attachment by gluing or fiberglassing to the dome of the shell provides a signal only when the turtle is at the surface. For most species this is only a small fraction of time while at sea and during terrestrial nesting emergences. The use of a float package enables continuous contact in shallow water but has the disadvantage of frequent detachment, especially in rocky or coral habitats. Float transmitters can be used in combination with carapace mounted transmitters. This double configuration allows continuous contact as long as the float remains attached but has the added advantage of reestablishing contact during terrestrial emergences if the float becomes detached. The float transmitter can usually be recovered and reused if it breaks free from the turtle. The float/lanyard configuration can also be used to recapture the turtle by "fishing" the turtle to the surface using the lanyard.

The use of satellite telemetry has the advantage of nearly unlimited range. Satellite transmitters (PTT's) also have the greatest initial cost as well as significant costs associated with monitoring and access. The accuracy and frequency of locations will vary with latitude, duty cycle, surface time of the turtle, sea state, attachment method, pass duration and satellite position. While internesting movements can be monitored via satellite or ground based VHF telemetry, it is the extensive post-nesting movements that can only be monitored with satellite technology (see S. Eckert, this volume).

Research and Management Techniques for the Conservation of Sea Turtles
K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, M. Donnelly (Editors)
IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Reproductive Cycles and Endocrinology

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Introduction: Why and When to Study Reproductive Systems?

The study of sea turtle reproductive cycles and their endocrine control falls primarily into areas often thought of as basic research. Should this type of research be done at all on threatened or endangered species? This is an important concern that must be considered very carefully before starting any such project. The answer must be "sometimes yes and sometimes no." Four questions should be answered in the affirmative before initiating any study of reproductive physiology of marine turtles:

(1) Has the investigator received scientific peer approval to do the research through proper institutional, state, national, and international permitting agencies? In other words, scientifically speaking, is the project a high priority?

(2) Does the investigator have the technical skills to safely undertake the project from the standpoint of both the animal's and the human investigator's welfare?

(3) Is the proper equipment on hand or available to safely handle the turtles and complete the protocols and analyses?

(4) Since it is expensive, are the financial resources available to do this type of research?

If the proposed project can meet these standards, then the research should go forward. Are these standards too high? They do not appear to be, since at least five separate labs in Australia and the USA have clearly met these standards and made significant contributions to sea turtle endocrine/reproductive science without harm to study animals.

Identifying projects which might be appropriate for sea turtles is critical since one does not want to attempt a protocol which could further damage an already depleted population. For this reason it is useful for the physiologist to collaborate with a conservation biologist (they could be the same person) whenever possible. A set of guidelines were suggested several years ago which still have some application in terms of justifying new research projects in this area (Owens, 1995). They are: (1) identifying critical and possibly unique reproductive processes of major concern to species survival; (2) developing improved techniques for accomplishing high priority applied and basic research; and (3) moving vigorously ahead in basic reproductive physiology research, especially where critical areas have been identified.

Research Potential: What Uses Are There for this Type of Study?

Sea turtles have been surprisingly useful models for reptilian reproductive biology because, despite their awkwardly large size, blood is easily obtained for hormone studies and sea turtles are good surgical patients. The first relatively complete reptilian hormone cycles were documented in green sea turtles from the Grand Cayman Turtle Farm (reviewed in Owens, 1997). Where solid ecological data are also gathered on free-living populations, important life history questions can be addressed, such as, sex ratio of immature populations, chronology of ovulation for the female, fecundity within a season, percent of a population that is reproductively active at one time, and questions of time and age at reproductive maturity. Because of several unusual life cycle traits seen in sea turtles *(e.g.,* late maturity, long life, variable breeding cycles, temperature dependent sex determination), population modelers need the kind of precise reproductive information that can result from combining detailed ecological (field) studies with carefully designed physiological investigations. The important studies at Heron Island, Australia, show the full potential of such hybrid studies *(e.g.,* Limpus, 1985; Wibbels *et al.*, 1990).

With increased capabilities for capturing and tracking individuals (see S. Eckert, this volume), it should be possible in the near future to vastly improve our understanding of migration controllers and mating systems, as well as nutritional dynamics and stress during reproduction. While an improved understanding of captive breeding potential was an initial motivation as well as a clear product of this research field, the successes of these programs (Wood and Wood, 1980) have actually reduced the need for intensive research in captivity except as it can relate to gaining a better understanding of basic physiology.

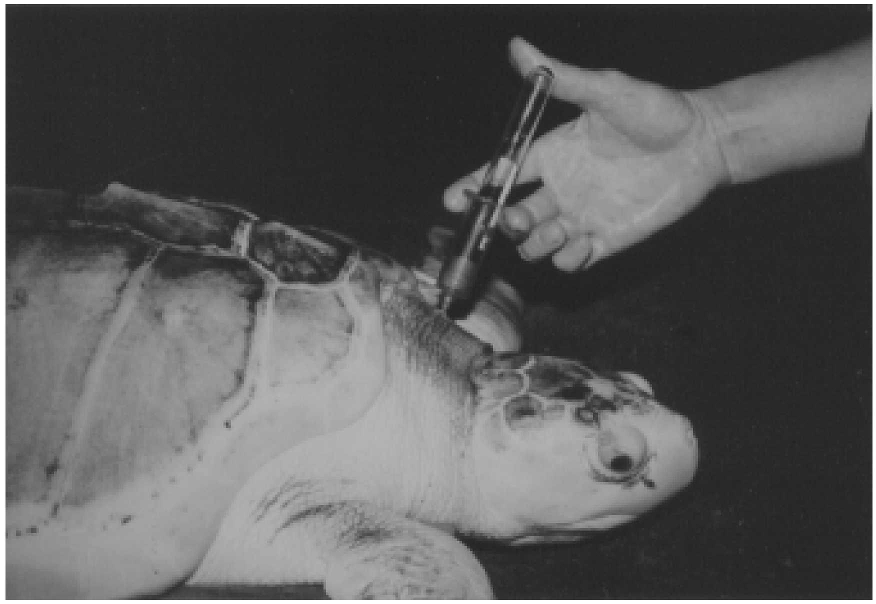

Figure 1. Blood sampling from the dorsal cervical sinus in a Kemp's ridley using a vacutainer system vacuum collection tube. Careful cleaning of the neck region is required prior to sampling.

Specific Techniques

There are four techniques that are often used in endocrine studies: blood sampling, hormone radioimmunoassay, laparoscopic surgery, and ultrasonography. The applications, strengths, risks, and analysis of each will be discussed.

Blood Sampling

Blood is considered a body tissue, as are muscle and bone. The advantage of blood is that it is easy to sample and can provide, through its subcomponents, excellent indicators of many aspects of an individual's health and reproductive status. Taking a blood sample from the sinuses in the dorsal side of the neck is now routine (Owens and Ruiz, 1980). After a modest amount of practice it is possible to obtain a blood sample at least 95% of the time (Figure 1).

Either a syringe and needle or a vacuum tube, needle, and holder system work well for drawing blood. With practice the sample can be taken within 30 seconds. For turtles less than 0.5 kg, a 23 gauge 1/2 inch needle works best. For turtles from 0.5-5 kg, a 1 inch 21 gauge needle is satisfactory, while a 1.5 inch 21 gauge needle works well in most larger animals except leatherbacks (*Dermochelys coriacea*; see below). Lithium or sodium heparin is best for an anticoagulant. EDTA (also an anticoagulant) should be avoided since it causes hemolysis in sea turtle blood. It is important to position the turtle so that the sinus fills with blood. For this reason, consistent results have been obtained when the turtle's head is lower than the body. An angled restraining rack, a slanted table or bench, or an inclined nesting beach (with assistants doing the restraining) all work well. Always carefully clean the neck with alcohol (containing at least 70% concentration of ethanol), or other antiseptic prior to sampling.

The sinus is on either side of the midline of the neck about 1/3 to 1/2 way toward the back of the head from the anterior edge of the carapace. Depending on the size of the turtle, the sinus is from 0.5-3 cm lateral to the midline. There is some variation in individuals, thus is it not unusual to have to insert the needle three to five times in order to locate the sinus. If one side of the neck does not produce blood, try the opposite side. Always insert the needle vertically (90 degrees to the plane of the neck) into the neck and *do not* move the needle laterally to locate the sinus. This will cause unnecessary tissue damage. Once the needle is inserted, apply suction and move the needle slowly up and back down until the sinus is located. *Do not* remove the needle from the neck while still applying suction as this can damage your sample.

Centrifuge the blood immediately or keep it on ice until centrifuging. Separate the plasma from the red cells and save both fractions, preferably at ultracold temperatures, for research and archiving. Several brands of small, portable electric centrifuges are available from biomedical and scientific supply companies. Ultracold temperatures (< -50°C) are preferred for storing blood products because these cold temperatures reduce protein changes. For the short term, ultracold temperatures may be achieved by dry ice or liquid nitrogen. Long term storage in an electric ultra cold freezer or liquid nitrogen freezer are recommended.

Because their neck sinuses are larger, both species of ridley (*Lepidochelys* spp.) and loggerhead (*Caretta caretta*) turtles (particularly immature individuals) are more easily sampled than are green (*Chelonia mydas)* and hawksbill (*Eretmochelys imbricata*) turtles. Adult nesting females of all species can be problematic to sample after they have been crawling on a beach. The sinus appears to be more constricted than in an animal taken freshly from the water. Sampling leatherbacks is more difficult because their necks are very large and require a long (3 – 3.5 in) spinal tap needle. The leathery skin is exceptionally difficult to penetrate and there are several procedural challenges, including clotting in the long needle (the inside of the needle should be coated with anticoagulant before sampling). A promising alternative technique for sampling leatherbacks from their rear flippers has been described (Dutton, 1996). As a general rule, extracting blood from a female in the process of covering her eggs can be difficult because the sinus is reduced in size and hard to locate. Sampling is easier if one can anticipate the end of oviposition and obtain the sample before covering begins; however, a drawback of this technique is that the turtle may abort the end of her clutch.

Hormone Radioimmunoassay

Just as blood chemistry work for ions or sugars can give an indication of an animal's health status, the specific levels of certain hormones in the circulation can also provide clues as to the precise reproductive or behavioral status of the individual. Many hormone (endocrine) assays are now available in kits from several companies such as ICN Pharmaceuticals (Costa Mesa, California USA) or Diagnostic Products Corporation (DPC) (Tarzana, California USA). Many steroids can be analyzed at veterinary teaching hospitals or specialized labs. Each assay should be validated (proven to work) for the species and hormone being studied. Several U.S. labs currently have good experience with sea turtle assays (see Guillette *et al.*, 1991; Owens, 1997; Wibbels *et al,* 1990). The testosterone-based sex determination technique (Owens *et al.*, 1978) requires a special assay for testosterone which is set sensitive enough to detect hormone at the very low levels seen in young animals (see Wibbels *et al.*, 1993 and Wibbels, this volume). This sensitive assay is not a routine procedure.

Laparoscopy

This form of surgery uses a miniature telescope to directly view inside the peritoneal cavity. It is a potentially dangerous procedure and should not be attempted until proper veterinary training has been obtained (Wood *et al.*, 1983). Laparoscopy can be used to determine the sex of immature turtles or the reproductive status of adults (see also Wibbels, this volume). It can also have value in diagnosing liver, lung, bladder, and intestinal tract problems; however, these types of evaluations require considerable veterinary experience and should not be undertaken by the novice. The minimum equipment necessary is a laparoscope, trocar, sleeve, fiber optics projector, and standard surgical instruments (Figure 2). The estimated minimal cost for this equipment is now about US$ 4000, depending on the size and options of the equipment purchased.

Complete familiarization with sea turtle anatomy is essential prior to doing surgery. In addition, the surgery should be performed in collaboration with a veterinarian until proficiency is developed. It is important to use aseptic techniques at all times to prevent infections. Following a surgical scrub (three alternating applications of 70% ethanol and a surgical iodine soap), the animal is restrained in an inverted position and a local anaesthetic injected into the muscle and dermis of the peritoneal wall of the inguinal area. A 1-2 cm incision is then made just through the skin and the trocar and sleeve used to push through the muscles and peritoneal wall into the body cavity. Particular caution is necessary to avoid an entry that is too far posterior (where the trocar might strike the kidney) or an entry that goes too deep (where the trocar might strike the lung or gut). After entry into the peritoneal cavity is achieved, it should be verified with the laparoscope prior to inflating the body cavity with filtered air. Inflation (known as insufflation) is necessary to visualize the internal organs. When the exami-

Figure 2. After local anaesthesia the turtle is restrained for laparoscopy in the inverted position. Sterile surgical techniques are required in this procedure to prevent infection. The laparoscope is being inserted into the sleeve which has been introduced into the peritoneum.

nation is complete, all air must be removed prior to suturing the wound. A single deep suture and two superficial sutures are usually adequate to seal the wound.

It is currently common practice to avoid the use of general anesthetics (with veterinary approval) for this particular surgery since a local anaesthetic incurs less risk of mortality, is adequate for reducing apparent pain, and allows a much shorter post-operative observation period (Wood *et al.,* 1982; Wibbels *et al.,* 1990).

Striking vital organs during trocar entry has the potential of inducing severe bleeding and mortality. In sea turtles, even experienced laparoscopists can expect a mortality rate in the order of 1-2%, under good conditions. The two most common causes of mortality include excessive bleeding due to poor trocar placement and death due to non-specific symptoms in a turtle that has already been compromised due to other conditions. For example, an overheated turtle may have a gas-distended lung or gut which can easily be perforated even with the best of technique. In addition, sea turtles with a heavy parasite load, a severe bacterial infection or acute obesity may succumb very easily during surgery. Captive animals are particularly susceptible to infection in the area of the sutured wound. Animals with any of the above-mentioned compromising symptoms must not be subjected to this type of surgery. If a turtle does die during the operation, it is essential to have an independent veterinarian conduct a necropsy to determine the cause of death.

Ultrasonography

The use of ultrasound imaging (Rostal *et al.,* 1990) has proven ideal in the rapid evaluation of an adult female's ovarian condition (Plotkin *et al.*, 1995). While additional research is needed to realize the full potential of this technique, it has some clear advantages over laparoscopic surgery in some situations. Most importantly, it does not require the aseptic technique, incisions, and sutures of surgery. Thus ultrasonography is generally fast, very safe, and non-invasive. An additional advantage is that the reflected images (sonograms) can be stored as video or still frames and exact measurements of structures such as follicles or eggs can be made from the real-time image or from the saved video. The disadvantages compared to laparoscopy are that one does not see the real tissue color or the smaller figures where the structures naturally lack heterogenous densities. For example, it has been difficult to distinguish immature ovaries from immature testes, a task that is easily done using the surgical approach. Other disadvantages are that the instrument, which is essentially a microcomputer with sensing probe, costs several thousand US$ when purchased new and requires a safe and dependable power supply. The ideal field system links a generator to an Uninterrupted Power Supply (UPS) and then to the instrument.

In ultrasounding the ovary, the turtle is placed on its carapace in a comfortable position for restraint (Figure 3). An assistant can easily restrain one of the smaller species (such as a ridley) in an automobile tire while the ultrasound is done. Larger turtles require more assistants to ensure the safety of the turtle, researchers, and machine. Ultrasound has not been very useful in males, but one unusual circumstance should be noted in case it might be of use in sea turtle anatomy and physiology studies. Adult males show a softening of the medial plastron (Wibbels *et al.,* 1991). With ultrasound, one can directly visualize the heart beating as well as blood flow in the major vessels. This is not possible in immatures or females due to their dense plastron shell.

Figure 3. An ultrasound evaluation of the ovary is possible using the ultrasonic probe placed on the inguinal area just posterior to the plastron. No anaesthetic is used as three assistants restrain the turtle on an automobile tire.

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Research and Management Techniques for the Conservation of Sea Turtles
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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Determining Clutch Size and Hatching Success

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Introduction

Determining clutch size and hatching success provides data fundamental to the conservation and management of sea turtles. These data are essential because they assist in understanding the suitability of the beach (or hatchery) to act as an incubation system and the general health of the nesting population.

To understand the success of the reproductive effort of sea turtles, it is necessary to determine the number of eggs laid, the diameter and weight of eggs, the number that incubate successfully, and the number of hatchlings that emerge from nests as well as the number of hatchlings that cross the beach into the water. Any significant change (based on means and standard deviations derived from the studied population) in these numbers through time indicates that problems may be occurring. For example, a significant change in the number of eggs that incubate to produce hatchlings indicates that some factors influencing incubation *(e.g.,* gas, moisture, temperature, and biotic factors) have changed. It is not necessary to count the eggs in every clutch that is laid; a random sample obtained from clutches counted throughout the nesting period will suffice. The same logic applies to the other quantifiable data collected from the eggs, embryos and hatchlings.

When based on a long term monitoring program, quantification of change provides the foundation for management decisions. If changes are small, no action may be required and conservation effort may be placed on other issues; if changes are large, management action should be tuned to address the specific threats first. Critical decisions concerning the management of *in situ* or hatchery habitats must be based on accurate data (see Boulon, this volume; Mortimer, this volume). Such data can be obtained by counting, measuring and weighing eggs as they are laid and by counting (and categorizing) the contents of nests after the hatchlings have emerged. This effort must continue through several years. In support of critical decision making, it is essential that definitions are clear and that data collection is standardized.

Definitions

Definition of an Egg

Sea turtles lay two categories of eggs: normal and odd-shaped. Normal eggs are spherical, white and comprised of (1) a pliable shell (ca. 3% of total weight), (2) a capsule of albumen (ca. 48.5% of total weight) and (3) a yolk (ca. 48.5% of total weight) (Miller, 1985). The vitelline membrane that supports the embryonic disc (see Miller, 1985 for detailed descriptions of sea turtle embryonic development) encases the yolk. The mean diameter of normal eggs varies among the species (Miller, 1985, 1997; Van Buskirk and Crowder, 1994).

Odd-shaped eggs may be extra large, multi-yolked (double or chain-form) or very small when compared to the other eggs in the clutch. Extra large diameter eggs are usually 1/4 (or more) larger in diameter than normal eggs of that species. Extra large diameter eggs typically contain two yolks surrounded by a single envelope of albumen and the shell; these seldom produce hatchlings, although one of the two embryos may develop for a while. Multi-yolked eggs are made up of several units of yolk and albumen contained within a continuous shell. The shell may be more or less constricted between the units; some may be connected by a small tube of shell, whereas others may show little constriction between the units. As a general rule, the greater the separation between the units, the greater the chance of producing hatchlings.

Very small eggs (usually smaller than 1/2 the diameter of normal eggs) are commonly termed 'yolkless' eggs. They contain mostly albumen and a few granules, or more, of yolk encapsulated by a shell. The yolk material is not encased by a vitelline membrane and, because there is no embryonic disc present, no development can occur. When a bright light is shown through a 'yolkless' egg, the image is white in contrast to a normal egg and other odd-shaped eggs, which show a yellowish hue.

Definition of a Clutch

A clutch is defined as the number of eggs laid into the nest, excluding yolkless eggs (as defined above). Yolkless eggs should be counted and reported separately. Because extra large and multi-yolked (double or chain-form) eggs actually contain viable embryos, they should be counted as part of the clutch. Multi-yolked eggs should be counted as a single egg because they contain viable embryos; however, because they are encased in a single shell, they should be counted as one egg, *i.e.,* do not count the number of contained yolks as eggs. The mean number of eggs in a clutch varies among the species (Miller, 1997; Van Buskirk and Crowder, 1994).

Clutch size can be determined by counting eggs at oviposition (the time of laying) or, if the clutch is to be moved, counting is more easily accomplished at reburial. To facilitate egg measurement and weighing in an *in situ* clutch, the eggs should be gently excavated once the turtle has finished filling in and moved away from the nest. Any eggs that are broken during excavation and handling must be counted as part of the clutch; a note of the number of broken eggs should be recorded on the data sheet. Although it is not necessary to count the eggs in every clutch laid on the beach, it is a good idea to count the eggs in some *in situ* nests as well as counting the eggs in all clutches that are moved. This allows comparison of the number of eggs and an initial assessment of the relocation activity. The number of clutches processed must be in balance with the other priorities of the total work program. If possible, successive clutches laid by several turtles throughout the season should be processed.

Laying of a partial clutch occurs when a turtle abandons a nesting effort after she has started to lay eggs. Any turtle that is found attempting to nest a second time within six days after laying some eggs has been disturbed (Miller, 1997). The partial clutches should be added together to obtain the actual clutch count; unfortunately, if the turtle has not been tagged, identification of the individual (hence linking of the partial clutches) is not possible.

Methods

Monitoring Incubation Temperature

Because the temperature of the sand during incubation (1) varies through daily and seasonal cycles, (2) influences embryonic survival, (3) determines hatchling sex and (4) the duration of incubation, temperature data are extremely important to understanding the incubation environment, including if conservation options include egg reburial (see also Merchant, this volume; Godfrey and Mrosovsky, this volume).

Temperatures should be taken as a routine part of working with nests throughout the nesting period. Because the mean nest depth varies among species, two approaches may be used to obtain the necessary data. First, a standard depth of 50 cm below the beach surface can be used over a wide range of beaches for comparison within a region and/or between regions. The second approach is to use an average nest depth for a species at a particular beach. A combination of these approaches allows for an integrated approach to understanding the variation in temperatures within the nesting habitat. The depth at which the temperature is measured must be standardized to allow comparison of temperatures within and between habitats on the beach, among nesting sites and among species. The methods used should be clearly stated in all reports.

Temperatures should be obtained in habitats that represent the range of nesting sites and positions on the beach. The date, time, depth, location and weather at the time of oviposition or emergence should be recorded for each sand temperature. Godfrey and Mrosovsky (1994) provide a useful overview of field methodology for measuring temperature on nesting beaches.

Sand temperatures may be obtained using a thermometer that displays a 0.2° C accuracy. Field thermometers should be calibrated against a certified thermometer before use. Calibration should be checked at six different temperatures (15°, 20°, 25°, 30°, 35°, 40° C) to establish any error in the device. Miniature temperature data logging devices are available from several companies that advertise in, for example, herpetological newsletters. They vary in price and features; selection should be based on the specific requirements of the study. The primary advantage of the data logging devices is that they can provide daily and seasonal profiles of sand temperatures when buried in the beach for the entire season at different depths *(e.g.,* 25 cm, 50 cm, 75 cm).

Handling Eggs

Eggs should be handled carefully. Hands should be clean of all chemical residues *(e.g.,* sunscreen, insect repellent, etc.) prior to handling eggs. All handling (excavating, measuring, weighing, transporting, reburial) of eggs should be completed within 2 hr of oviposition or the eggs should be allowed to remain *in situ* for at least 25 days to reduce the impact of movement induced mortality (Limpus *et al.,* 1979; Parmenter, 1980). Although freshly laid eggs are not as susceptible to movement induced mortality, it is good practice to dig out the clutch without rotating the eggs, in case a more advanced clutch must be moved some time in the future. The new location for the eggs must provide adequate conditions of moisture, temperature and gas exchange to support the developing embryos and be secure from predators and poachers (see Boulon; Mortimer, this volume).

Measuring and Weighing Eggs

Different species of sea turtle lay eggs of different diameters and weights (Miller, 1985, 1997; Van Buskirk and Crowder, 1994). Within a species, the eggs tend to be similar in size, although some variation may exist between populations.

To establish the size of eggs, ten eggs chosen at random from each clutch should be measured for the greatest and least diameters and individually weighed. The use of ten eggs provides an adequate statistical basis for assessing within and between clutch variation. Using fewer than ten eggs does not provide an adequate basis and using more than ten eggs does not improve the statistical basis for comparison. Each egg should be cleaned of adhering sand. Sand may be wiped off using a cloth (or brush) or by hand. When being measured the egg should be held so that the shell is tight by gently pressing a finger against the shell to form a dimple. Calipers should be used to locate the greatest diameter; the least diameter is usually located 90 degrees to the axis of the greatest diameter, but may be located anywhere around the egg. Both values should be recorded. The greatest and the least diameters should be added together and divided by two to obtain an average.

Similarly, to establish the weight of eggs, ten eggs from each clutch should be weighed using a spring or electric balance capable of being read to a minimum accuracy of 0.5 g. Ideally, the measured eggs (as above) should be the same used for weighing. Eggs may be identified by marking them with a soft, blunt pencil or an ink based marking pen.

A standard statistical textbook will explain how to calculate a mean and standard deviation. Briefly, the average diameter and weight for each of the 10 eggs are used to calculate the mean and standard deviation (SD) for the clutch. Once a clutch has had a mean egg diameter and weight calculated, an overall beach mean and standard deviation may be calculated. Results (diameter, weight) should be reported as the mean, standard deviation, maximum, minimum, and number of clutches for the beach.

Marking Nests

Relocating a nest following oviposition can be very difficult, unless its position has been marked. During oviposition a nest that is to be counted or moved can be marked be inserting a small rope (or colored tape) into the egg chamber so that it extends onto the surface of the beach. Once the turtle has finished filling in the egg chamber and moved ahead, the clutch can be located by following the cord to the eggs.

Individual clutches of eggs left *in situ* on the beach or relocated to a hatchery, can be identified later by inserting a nest tag among the eggs at oviposition. A nest tag may be a brightly colored piece of plastic tape (ca. 20 x 3 cm, surveyor's tape) or some other marker that contains a unique code by which to identify that clutch with associated data. This can be accomplished while the turtle is laying or when the eggs are counted immediately after oviposition. The nest tag should be written in permanent ink; the label should contain the tag number of the female and nesting date. When the nest is excavated following emergence of the hatchlings, the recovery of the nest tag allows data on the hatching success and emergence success to be linked to data on the female as well as the eggs (counts, measurements).

The nest tag should not be visible from the surface of the beach, especially in areas where poachers threaten nests. Another advantage of using a nest tag located among the eggs is that other nesting turtles will not disturb the marker (as they sometimes do with stakes on the beach surface) unless they dig into the clutch; if this happens the nest is still identifiable. The use of stakes with above ground clutch identification to indicate nests is very useful in protected hatcheries.

Table 1. Minimum data set for each clutch examined.

Tag Number	Tag number of female turtle
Date and Time Laid	Date laid; time based on 24-hr clock
Nest Depth – Top	Depth from beach surface to the top of the first egg in the chamber
Nest Depth – Bottom	Depth from beach surface to bottom of egg chamber
Nest Location Along Beach	Sector code (if beach length is divided into sectors), or triangulation coordinates from known points along the beach
Nest Location Across Beach	Position on beach (e.g., on slope or dune, above/below high water, etc.)
Nest Location Habitat	Habitat surrounding nest (e.g., bare sand, grass, in/under vegetation)
Sand Temperature	Temperature at a standard depth using a calibrated thermometer
Clutch Count	Count of normal eggs, plus count of yolkless eggs
Egg Diameter	Diameter of 10 normal eggs/clutch, greatest and least (same eggs that were weighed)
Egg Weight	Individual weight of 10 eggs from a clutch (same eggs that were measured)

Recording Data

There is a minimum set of information that should be collected from each clutch examined (Table 1). Data sheets should be designed to include the minimum data set. A set of headings with blank boxes on the same data sheet used to record information about the nesting female is the optimal place to record clutch count, egg measurements and other data *(e.g.,* sand temperature) recorded at the time of oviposition.

Field Equipment

Basic field equipment should include: data sheets, clipboard, pencils, calipers, balance (metric scale), thermometer, 2 m tape measure (flexible, non metal), 25-100 m flexible tape measure (for nest location), headlight (for hands free recording of data), a bag for field equipment, and a suitable bag for routine or emergency transport of eggs. All measuring equipment should be routinely calibrated.

Locating a Nest after Emergence of the Hatchlings

When hatchling tracks are encountered on the beach, the emergence crater can usually be located by turning away from the water and following the hatchling tracks back up the beach to the vicinity of the emergence crater. The tracks should form a wide "V" with the point at the emergence crater (when the sand is damp, the crater is obvious). Rubber gloves should be worn when excavating nests because nests regularly contain rotten eggs and petrified, dead hatchlings. Sand in the neck of the nest (the channel through which the hatchlings traveled to the surface) will be loose and soft compared to the surrounding beach. Care should be taken not to disturb adjoining clutches that are still incubating.

After the hatchlings have emerged from a nest and the nest has been excavated, the data contained on the nest tag, if present, should be recorded (Table 2).

Categorizing Nest Contents

Nest contents should be examined and divided into categories (Table 3). These categories may be further subdivided to provide finer definition of the nest contents; however, the extra work involved is probably not worth the result, unless specific questions are being addressed. For example, a skilled observer may require several hours to find evidence to distinguish early embryonic death from infertility or intra-oviducal death, all of which are contained in the undeveloped category (see Miller, 1985, for detailed descriptions of sea turtle embryonic development).

Table 2. Recommended data entries after the hatchlings have emerged from the nest.

Tag Number	Tag number of nesting female (from the nest tag)
Date Laid	Date (from the nest tag)
Date Emerged	Date when hatchlings emerged
Time of Emergence	Time hatchlings were observed
Incubation Length	Date emerged – Date laid

Table 3. Categories and definitions of nest contents to be recorded on data sheets.

E = Emerged	Hatchlings leaving or departed from nest
S = Shells	Number of empty shells counted (>50% complete)
L = Live in nest	Live hatchlings left among shells (not those in neck of nest)
D = Dead in nest	Dead hatchlings that have left their shells
UD = Undeveloped	Unhatched eggs with no obvious embryo
UH = Unhatched	Unhatched eggs with obvious embryo (excluding UHT)
UHT = Unhatched term	Unhatched apparently full term embryo in egg shell or pipped (with a small amount of external yolk material)
P = Depredated	Open, nearly complete shells containing egg residue

The other categories assist with identifying potential problems that might have occurred during incubation. For example, if a high proportion of the eggs contained UHT embryos, sub-sand flooding of the nest from a recent high tide (that cut off the oxygen prior to pipping) might be the problem. Infertility is difficult to demonstrate; even a skilled observer may miss a fragment of the embryonic disk (the presence of which indicates fertility) in an unhatched egg exhumed from a nest at the end of incubation.

Counting empty egg shells is difficult and contains counting error depending on the skill of the person counting. Only shells that make up more than 50% of the egg size should be counted; shell fragments should not be counted. An estimate of the counting error can be made by counting the shells in clutches in which the number of eggs was counted at oviposition. The error is the percentage of the difference between the two counts.

A researcher may choose to have the category UH include UHT embryos; if so, field notes should be used to identify dead, pipped embryos to aid the identification of the cause(s) of death. The shells of eggs depredated in the nest (P) seldom resemble the torn shells from which hatchlings have emerged; the shells of depredated eggs (P) usually have holes or small torn areas and contain a quantity of egg material. When eggs have been exhumed by predators and scattered on the beach, counting is difficult and obtaining an accurate count may be impossible.

After categorising and counting the contents of the nest, the number of eggs in the clutch may be calculated using one of the following formula (see symbols above):

if all hatchlings were intercepted:
Clutch = E + L + D + UD + UH + UHT + P;

or, if all hatchlings were not captured, estimate E for use in the equation above by: E = (S - (L + D))

Determining Hatching Success and Emergence Success

Assessing incubation success is a two stage process consisting of determining hatching and emergence success. Hatching success refers to the number of hatchlings that hatch out of their egg shell (equals the number of empty egg shells in the nest); emergence success refers to the number of hatchlings that reach the beach surface (equals the number of empty egg shells minus the number of live and dead hatchlings remaining in the nest chamber). Hatching success is often 1% or more higher than emergence success. Both hatching and emergence success should be reported when presenting data on incubation success.

$$\text{Hatching Success (\%)} = \frac{\#\text{shells}}{\#\text{shells} + \#\text{UD}+\#\text{UH}+\#\text{UHT}+\#\text{P}} \times 100$$

$$\text{Emergence Success (\%)} = \frac{\#\text{shells} - (\#\text{L} + \#\text{D})}{\#\text{shells} + \#\text{UD}+\#\text{UH}+\#\text{UHT}+\#\text{P}} \times 100$$

Simply counting hatchlings on the beach is not accurate enough to assess emergence success because some hatchlings may escape before being counted or may be eaten by predators or some hatchlings may be slow in emerging from the nest. When excavating nests, live hatchlings just below the beach surface *(i.e.,* not trapped by vegetation or debris) should be included in the count of hatchlings that successfully reached the beach surface.

Measuring and Weighing Hatchlings

The mean length and weight of hatchling sea turtles varies among the species (Miller, 1997; Van Buskirk and Crowder, 1994). Ten hatchlings from each of several clutches should be measured and weighed to establish hatchling size. Straight carapace length (SCL) should be measured using calipers from the nuchal scute to the division between the post central scales. SCL measurements obtained for 10 hatchlings should be added together and divided by 10 to obtain an average SCL for hatchlings in the clutch. The same 10 hatchlings from each clutch should be weighed using a spring or electric balance capable of being read to an accuracy of 0.5 g. Weighing should be done out of the wind. The weights obtained for the ten hatchlings should be added together and divided by 10 to obtain the average weight for the hatchlings in the clutch. Results (SCL, weight) should be reported as the mean, standard deviation, minimum-maximum, and number of hatchlings. Significant changes from these annual means may indicate a problem during incubation *(e.g.*, change in the moisture in the sand around the nest).

Hatchlings that have been captured after entering the sea or that have remained in the nest (they are often misshapen) should not be weighed or measured as being representative of normal hatchlings in the clutch. Because hatchlings lose water (weight) quickly after emerging, they should be processed and released as soon as possible after emerging from the sand. They should not be kept throughout the next day.

Summary

The careful recording of data on the number of eggs laid and the results of incubation, hatching and emergence can assist in identifying the reproductive characteristics of the nesting population. Methods should be clearly stated and data should be reported in the form of the mean, standard deviation, minimum-maximum, and number. These data assist in the management of the nesting site by providing a basis for comparison among nesting seasons and sites as well as among species.

Acknowledgments

I wish to thank Kirstin Dobbs for her valuable assistance in preparing this contribution.

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Determining Hatchling Sex

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Introduction

Sexual differentiation in mammals depends on the transformation of the undifferentiated gonad into a testicle. The gene that controls this initial event is SRY and is localized in the short arm of the Y chromosome (Koopman *et al.,* 1990). In non-mammalian vertebrates, SRY related genes of the SOX family are found in both sexes and are independent of the presence of sex chromosomes (Tiersh *et al.,* 1991). The identity of the gene or genes that control sexual differentiation in non-mammalian vertebrates is still unknown. However, as in placental mammals, morphological sexual differentiation in the embryo seems to initiate in the embryo's gonad. It is reasonable to postulate that the factor or factors required for sexual differentiation act primarily at the level of this organ, controlling its transformation into an ovary or a testicle.

Although the physiological mechanism by which temperature or other environmental factors influence sexual differentiation is unknown, vertebrates have been divided into two groups: (1) Organisms in which environmental factors have no influence over their sexual differentiation are classified as having Genotypic Sex Determination (GSD); (2) Organisms whose sex determination is influenced by the environment, undergo an Environmental Sex Determination (ESD) (Bull, 1983). In sea turtles, determination of sex by temperature has been found in *Caretta caretta* (Yntema and Mrosovsky, 1980), *Chelonia mydas* (Miller and Limpus, 1981), *Dermochelys coriacea* (Rimblot *et al.,* 1985), *Lepidochelys olivacea* (Morreale *et al.,* 1982), *Lepidochelys kempii* (Shaver *et al.,* 1988), and *Eretmochelys imbricata* (Dalrymple *et al.,* 1985).

Identifying Sex in Hatchlings

There is a thermosensitive period (TSP) for sex determination during development placed around the second third of total time of incubation. TSP is defined as "that time span or developmental-stage span outside of which temperature manipulations do not exert any influence on sexual phenotype" (Mrosovsky and Pieau, 1991). Furthermore, in all species of marine turtles, there are no external morphological characters which may be used to determine the sex of organisms at hatching stage, and only through dissection and direct observation of the gonads is this possible.

Invasive Methods (Dissection)

Three procedures, based on morphological observations, are available: (1) direct observations of the gonads in situ; (2) clearing technique of gonads in toto; (3) histological study of the gonads.

Criteria based on (1) are concentrated on gonadal morphological details observable immediately after the viscera that cover them *(e.g.,* intestines, liver, stomach) are removed. Gonads appear as two clear bands that extend along the length of the kidneys (mesonephros). McCoy *et al.* (1983) attempted to sex *L. olivacea* gonads based on the fact that ovaries tend to have a wrinkled surface and are larger in size than the testicles. As this criteria is questionable, van der Heiden *et al.* (1985) proposed method (2) which requires the dissection of the urogenital complex (gonad and kidneys) and fixation in 10% formalin. Afterward, the gonad is separated from the kidney and submerged in 100 ml of 4% formalin solution and 5 ml glycerol (a few drops of copper sulphate should be added to avoid fungal contamination). Using a dissection microscope, these authors sexed *L. olivacea* and *C. mydas* hatchlings. Besides the gross external morphology, from which the sexes can be clearly distinguished when the material is processed while still fresh (ovaries have a wrinkled surface and are larger than testicles), gonads in their interior also show clear differences. This is particularly true in the anterior and posterior ends (since they are narrower there) where more detailed observation is possible. According to the authors, testicles are distinguishable by a granular appearance that possibly corresponds to the presence of seminiferous tubules.

In spite of the ease with which the previous procedures can be performed to determine the sex of hatchlings, some authors have expressed serious concerns of their value, proposing that the only reliable criterion is provided by a histological study of the gonad (Mrosovsky and Benabib, 1990; Mrosovsky and Godfrey, 1995). In this case, gonads need to be fixed, dehydrated, embedded in wax or plastic from which stained sections can be obtained and observed under the microscope. An adequately equipped laboratory is required for this procedure.

For the purpose of exemplifying key features, a detailed interpretation of hatchling gonad histology, based on a embryological study of *L. olivacea* hatchling gonads (see Merchant-Larios *et al.*, 1989), is presented below:

In males (Figure 1), the surface epithelium is flat, monostratified and frequently contains various germinal cells. The medullary cords appear separated from the surface epithelium although some remain attached to it. The medullary cords, surrounded by a basal membrane, are formed by epithelial type cells with abundant lipid droplets. Germ cells are scarce and there is no lumen within the medullary cords that would justify the name of "seminiferous tubules", the correct term would be "seminiferous cords". Among these formations and bordering the surface epithelium there is a basal membrane and abundant stromal tissue, formed largely by extracellular matrix, fibroblasts cells and blood vessels. Ovaries are distinguished by a conspicuous thickening of the surface epithelium (Figure 2). It appears as a columnar epithelium, one or more cells in thickness. It contains a thick basal membrane that separates the surface epithelium from the medullary region of the gonad. The medullary cords are vestigial and appear as small groups of epithelial cells surrounded by a basal membrane. Stromatic tissue is abundant in the medullary region.

In our experience it is possible to combine procedures (2) and (3) sequentially, taking advantage of the practical advantages of the first and the precision of the second. In cleared gonads, the sex can be easily identified but only if they are well differentiated and the preservation is satisfactory. However, in difficult cases of inter-sexes (alternating regions along the gonad with well developed cortex and medullary cords) or indifferentiation of the gonads (when both medullary cords and surface epithelium remain poorly developed), the same cleared gonads can be dehydrated in ethanol and embedded in paraffin or plastic for a further histological analysis. Preservation is excellent as can be seen in Figure 3.

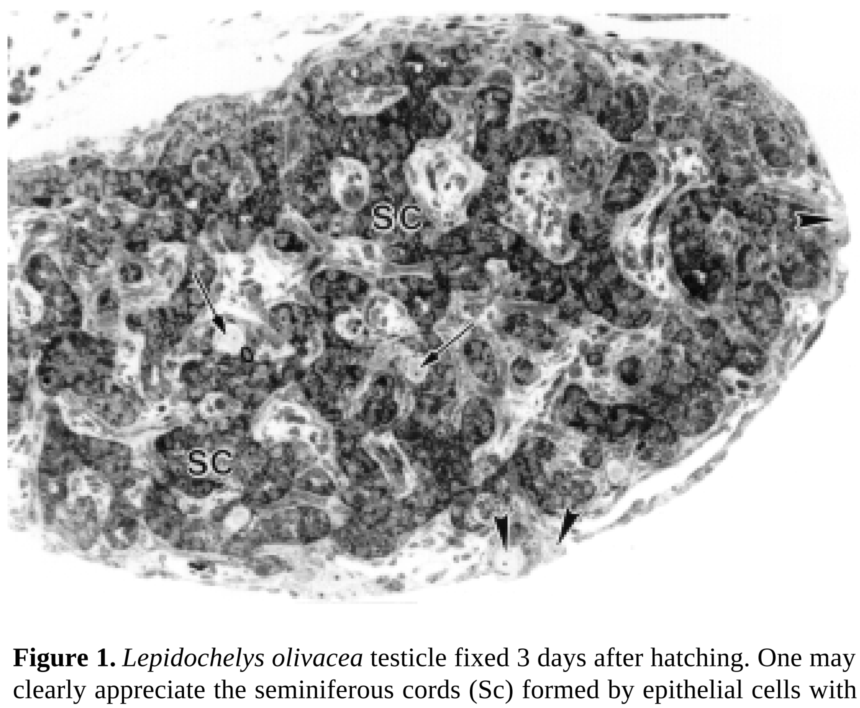

Figure 1. *Lepidochelys olivacea* testicle fixed 3 days after hatching. One may clearly appreciate the seminiferous cords (Sc) formed by epithelial cells with dense cytoplasm due to numerous lipid granules. Some germ cells are situated in the cords (arrows) and others in the epithelial surface (arrowheads). Semithin section (2mm) fixed with paraformaldeyde-glutaraldehyde (Karnovsky, 1965), post-fixed with OsO4 and embedded in Epon. 200X magnification.

Non-invasive Methods (Radioimmunoanalysis)

A non-invasive method for the diagnosis of sex in recently hatched organisms has been attempted. Gross *et al.* (1995) reported the possibility of sexing *C. caretta* hatchlings using radioimmunoanalysis (RIA) of the chorioallantoic and amniotic fluids (CAFs). They found that in males the ratio of estradiol (E) to testosterone (T) concentrations is significantly lower than in females, allowing them to predict sex with acceptable precision. The same authors found a similar E:T ratio in plasma from hatchlings of the same species. In olive ridley turtles we have carried out RIA of serum prior to and post-hatching of various steroid metabolites including E and T (Merchant-Larios and Salame-Méndez, unpubl. observ.). Unfortunately, no significant differences could be found in any of the metabolites which would permit a distinction of sex in this species. It is possible that in *C. caretta*, the endocrine activity of the gonads is more advanced than in *L. olivacea*. This is suggested by the presence of mullerian ducts at hatching in the latter species while in *C. caretta* they have disappeared almost totally (Yntema and Mrosovsky, 1980). Bearing in mind these significant differences in developmental timing between species, it is recommended that hatchling hormonal patterns for the species under study and its correlation with sex be established before RIA is used as a method for sex identification.

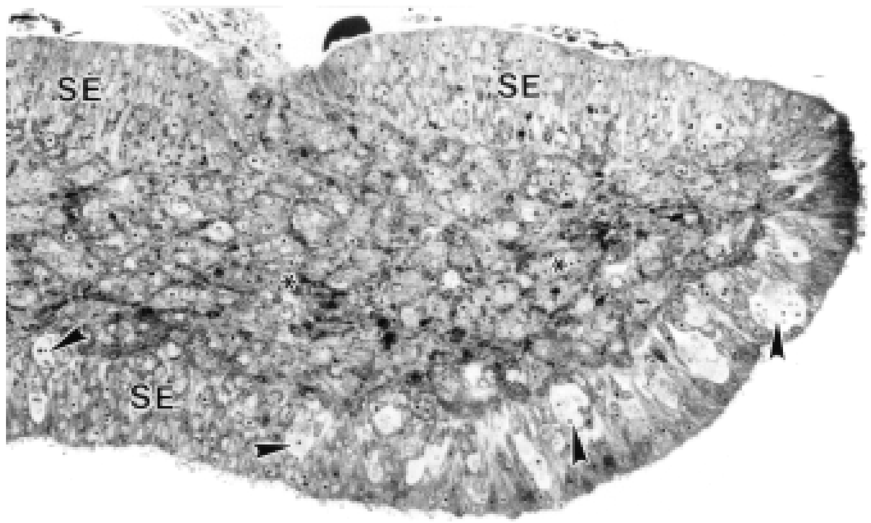

Figure 2. *Lepidochelys olivacea* ovary fixed two days after hatching. The surface epithelium (SE), formed by a columnar, multistratified epithelium, contains numerous germ cells (arrowheads). In the medullar region, there are remnants of fragmented medullary cords (*). Sample processed as in Figure 1 200x.

Estimating Sex Ratios in Hatchlings

In order to make an estimation of the proportion of the two sexes (sex ratio) present in a natural population in the field, it is convenient to know beforehand the “pivotal” or “threshold” temperature. Once this is known, *in situ* nest temperatures of nests chosen from representative zones in the beach can be used to extrapolate the overall temperature range and, from these, derive the sex ratio for the rookery during the particular season nesting season.

The pivotal temperature is defined as the incubation temperature at which the resulting sex ratio in the clutch is 1:1. Experimentally, this value is obtained from incubating groups of eggs at various constant temperatures and determining the resulting proportion of male and female hatchlings. At a range of temperatures still allowing normal development (around 24-34 C), one can determine the masculinizing and feminizing temperature range (which produce 100% males or females, respectively) and estimate the pivotal temperature (50% of each sex). Precise, constant values are difficult to determine because of genetic variations of the individual specimens in each experimental group (see Mrosovsky and Pieau, 1991, for a major discussion on this point). For sea turtles species which have been studied, the pivotal temperature reported is around 30°C. Studies of *C. caretta* (Mrosovsky, 1988) specimens have shown variations of pivotal temperature as high as one degree centigrade, depending on nest size and genetic factors.

It has been suggested that temperature possibly counteracts a genetic control of gender. If this is true, then the pivotal temperature could be taken as the condition under which the genetic sex is expressed without external alterations (Mrosovsky and Pieau, 1991). Considering that in a nest or in a population at a beach there is a variable ratio of genotypical males to females and that response to temperature varies according to genetic sex, the pivotal temperature may vary as much as one degree centigrade (Mrosovsky, 1988). Thus, an estimation of pivotal temperature among the different turtle populations that nest in different beaches is recommended. A minimum of 5-6 nests per beach per season, following up for at least three consecutive years, would render values in close approximation of the true range of the pivotal temperature for the population being studied.

Although pivotal temperature is a reliable indicator when estimating sex ratios under natural conditions, a knowledge of "transition ranges of temperature" (TRT) is also convenient. This parameter refers to the difference in values between the low temperature producing 100% males and the high that results in 100% females (Mrosovsky and Pieau, 1991). As with pivotal temperature, TRT does not have a fixed value. No doubt there will be some variations depending on the sample size. Hence, as with pivotal temperature, estimation of standard deviation in a particular population will be optimized by measuring temperatures in as many nests as possible in different places on the beach in question and repeating the study over several years.

The equipment necessary for measuring temperature on the beach has been fully discussed by Godfrey and Mrosovsky (1994). They designed a module that memorizes maximum and minimum temperatures. Apart from the equipment being economical and resistant, it withstands burial, reducing the possibility of theft. The core sensor is a commercial memory thermometer (Radio Shack 277-302 or 630/1020) protected inside a Plexiglas box.

Converting sand temperatures into hatchling sex ratio is not as straightforward as it may seem. Pivotal temperatures of turtles are generally derived from incubation of the eggs at constant temperatures in the laboratory, unlike field incubation conditions (Bull, 1985). Microenvironmental factors, such as metabolic warming of the eggs, can cause nest temperatures to differ from sand temperatures (Mrosovsky and Yntema, 1980; Godfrey *et al.*, 1997). This factor should be taken into account when measuring temperatures on the beach, and sensors should be positioned as close as possible to the nest or among the eggs in each nest in order to derive the sex ratio. Also, inter-clutch variation in pivotal temperatures can complicate conversions of beach temperature to sex ratio; hence, adequate sample sizes are important (see above). Nevertheless, sand temperature profiles are useful, particularly in assessing the impact of management techniques on sex ratio (see Godfrey and Mrosovsky, this volume).

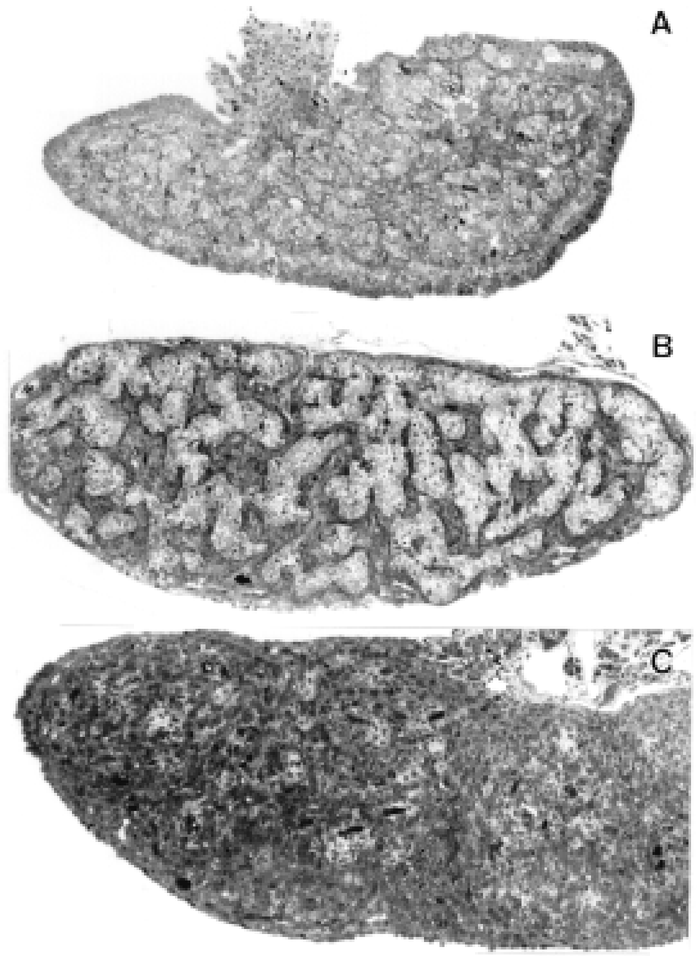

Figure 3. *Dermochelys coriacea* ovary (A), testicle (B) and undifferentiated gonad C previously treated with the clarifying technique (see text). Glycerol was eliminated with a phosphate buffer and the samples were treated as for Figure 1 and 2 material.

Final Considerations

The gonads of different species of sea turtle reveal variations in the degree of differentiation at hatching. The most and least differentiated gonads are those of *C. caretta* and *D. coriacea* respectively, while an intermediate differentiation may be observed in *L. olivacea* and *C. mydas.* However, the gonads may be considered morphologically and physiologically immature in all sea turtle species. In vertebrates, differentiated ovaries contain oocytes surrounded by follicular cells and differentiated testes have seminiferous tubules and Leydig cells. In sea turtle hatchlings, as in other species, the onset of meiosis is delayed and there is no follicle formation in the ovaries. In the testes, no differentiated seminiferous tubules are present and only medullary cords, with few germ cells, are found. In most species, some germ cells remain in the surface epithelium and no differentiated Leydig cells have been observed (Figure 1). Therefore, complete differentiation of gonads must occur sometime after hatching and when this happens still has to be established.

Genetic variations among different sea turtle populations and the varying environmental conditions of beaches located at different latitudes means that differences in pivotal temperature in turtles of the same species are expected. Therefore, estimations of the parameters for each beach is recommended and freely extrapolating from the results obtained in other beaches should be avoided.

Finally, it is important to consider the relative frequency of gonads referred to as "inter-sex". In these samples, the medullary cords are conserved in some regions, as in the testicles, and the surface epithelium appears enlarged, in other regions, as in the ovaries. In other cases, the gonad remains "undifferentiated" and there is no clear development towards either sex. Considering the gonad's vulnerability to temperature and its immaturity at the hatchling stage, it is not surprising to find variations in its development, probably in response to abrupt changes in temperature during the sensitive period.

Acknowledgments

I am indebted to M. Cristina Ordoñez for providing me with her samples of clarified *D. coriacea* gonads. The project had the support of CONACyT (National Council for Science and Technology), 400315-5-4037N. This chapter was improved by comments from M. Girondot, C. Pieau, and A. Abreu.

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Estimating Hatchling Sex Ratios

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Introduction

When confronted with the information that hatchling sex is affected by incubation temperature (see Merchant, this volume), one of the first questions to be asked is, "What is the natural sex ratio of sea turtles?" A second question that soon follows is, "What is the optimal sex ratio, for conservation?" These questions are closely related, and at the present time neither can be easily answered. No one has designed and completed a long-term study that shows whether the manipulation of sex ratio is beneficial or detrimental. Until more information is made available, and in the light of some possible negative consequences of interference (Lovich, 1996; Girondot *et al.*, 1998), for the time being it is assumed that the safest course of action is to maintain natural hatchling sex ratios.

Knowledge of the natural sex ratio of nesting sea turtle populations is an important component of any management plan. Such information provides a baseline against which the effects of certain conservation techniques can be evaluated. These techniques may include: (1) nest relocation elsewhere on the nesting beach or to a hatchery, either of which may be thermally different from the original site (see Boulon, this volume; Mortimer, this volume); (2) limited egg harvest, which may result in the disproportionate removal of one sex from the beach (for instance, by authorizing egg collection only during certain times of the year); and (3) beach renourishment, which may alter the thermal characteristics of a beach by introducing a different type of sand (see Witherington, this volume).

Methods

Estimating sex ratios requires the synthesis of two types of information. First, the sex of the hatchlings must be determined (see Merchant, this volume). Second, data on sex must be combined with information on the nesting patterns of a population. It is necessary to know where the turtles are nesting on the beach, and when they nest, as there is spatial and temporal variation in sand temperature.

Spatial Variation

On a sea turtle nesting beach there may be distinct zones that are thermally different. For example, some zones have vegetation, others do not. Nests laid under dense vegetation are likely to be cooler, and thus produce more males than those laid in the open zone, which are likely to be warmer (cf. Spotila *et al.*, 1987). The distance from the high tide line may affect the depth of the water table, and thus influence temperatures at nest depth. In addition, if a nesting beach is long, all subsections should (ideally) be sampled to account for any thermal variation along beach length. Finally, if the aim is to estimate the hatchling sex ratio of the entire breeding population, then information from all the nesting beaches in the range of the meta-population should be included. Genetic information (see FitzSimmons *et al.*, this volume) is likely to be required to ascertain the extent of the breeding population (note that despite the apparent segregation of females by distinct nesting beaches, these groups of females may still be part of a larger interbreeding population if the males move and mate freely among the different groups).

Temporal Variation

Over the course of a nesting season, which may last several months, there are likely to be changes in weather. For example, rainy seasons can affect sand temperatures at nest depth, which in turn can affect sex ratio. Therefore, a proper estimate of the sex ratio should be based on samples taken throughout the nesting season. In practice, it is often easier to divide up the season into discrete units of time, such as month or half-month periods, and estimate the mean sex ratio for each of those periods *(e.g.,* Godfrey *et al.*, 1996). Also, it is important to remember that individual sea turtles tend to nest every second or third year (or more). Therefore, an estimate of hatchling sex ratios from a single season may represent nest site selection by only a third or so of the total adult population. Ideally, estimates of hatchling sex ratio should be based on data from several consecutive years. Of course, some variation in sex ratios from year to year is to be expected, since weather and nesting frequency are variable. By collecting data on sex ratios from a number of years, it is possible to determine an average hatchling sex ratio. If it is possible to determine sex ratios in only a single year, then it is desirable to consider whether or not that year was thermally typical. Meteorological records can be used for this purpose.

Consider Nesting Frequency

In general, more turtles nest in the middle of the season than at its beginning or end. This change in nesting frequency must be integrated with the information on how sex ratio varies over the season. The aim is to combine sex ratio information for specific periods of the nesting season with data on the relative numbers of nests laid during that same time. For example, a sex ratio profile of a hypothetical nesting beach is shown in Figure 1. The nesting season spans three months, and marks the transition from the dry to the rainy season. The relative nesting frequency in each month is shown on the right, with the majority of the nests being laid in June. The mean sex ratio from several sampled nests laid in each month is also shown on the right, presented in % female. Combining the two sets of data from all three months produces an overall seasonal sex ratio of 57% female. However, if sampling is restricted to one month *(e.g.,* June), then the estimate of sex ratio *(e.g.,* 40% female) would be inaccurate. Also, data from one beach or one year may well not be representative of the average long term population sex ratio (Figure 2). Finally, it may be the case that clutch size or hatching success varies from beach to beach (or over time). If the variation is large, it would be important to take these factors into account when calculating the sex ratio.

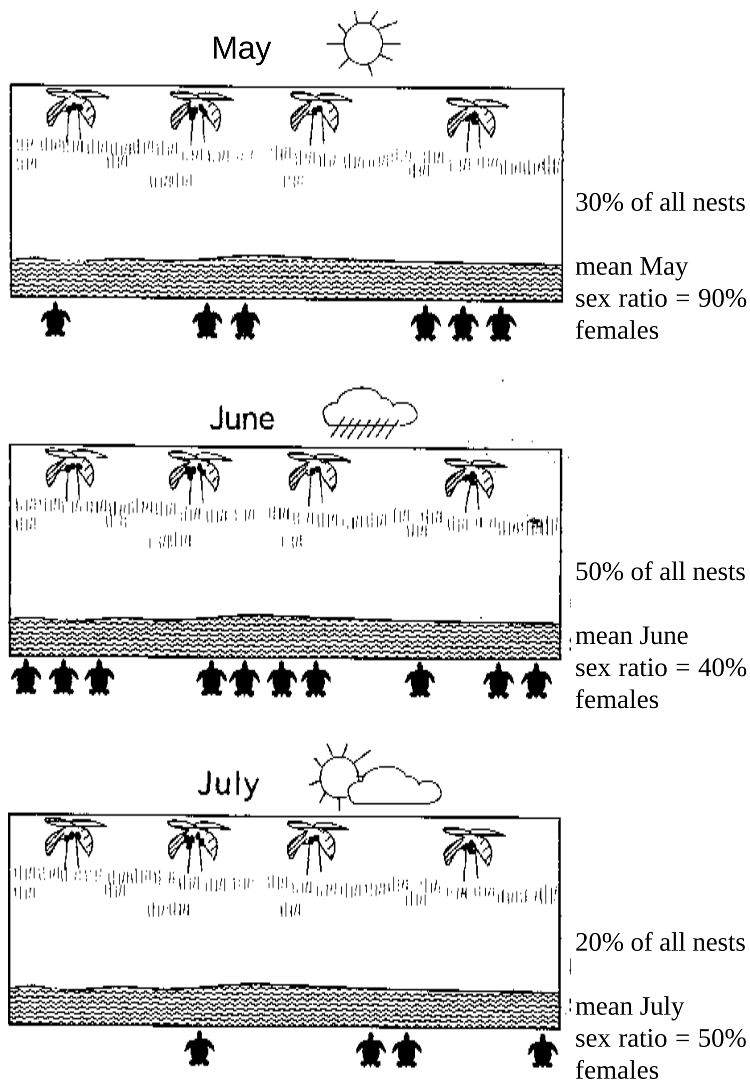

Figure 1. Example of turtle nesting frequency and sex ratio in a single season.

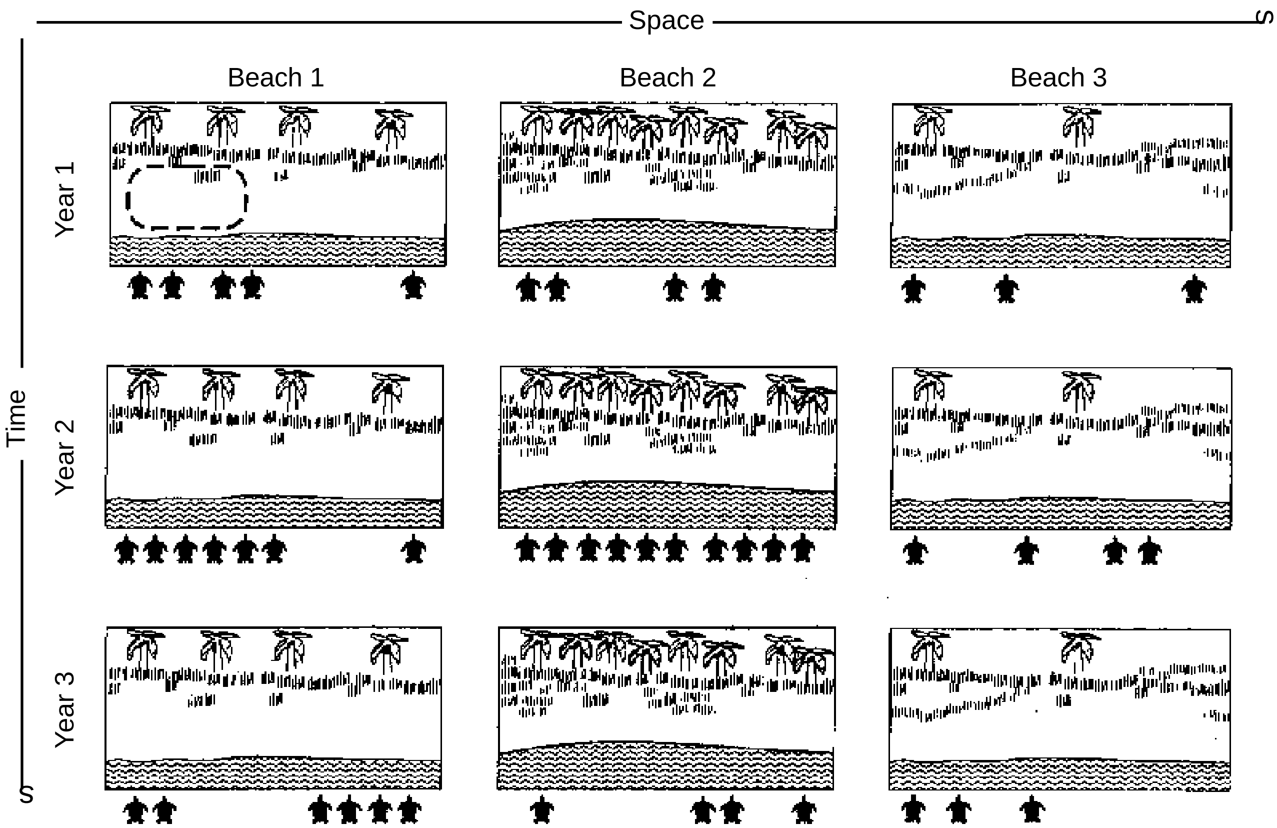

Figure 2. Requirements to constitute an adequate sample. If sampling is confined to one area only (dashed circle at top left), this does not represent the full spatio-temporal variation, or changes in nest density.

Determining Confidence Intervals for Overall Sex Ratio Estimate

In order to facilitate statistical comparison among different beaches or populations, it is necessary to calculate a confidence interval for the overall sex ratio estimate for the nesting population in question. In most cases, the overall estimate will not be based on data from all nests laid during the season(s); rather, it will be based on a sample of nests from a larger population. A good way of determining a confidence interval is to use the bootstrapping technique, which involves computing a large number of estimates by random sampling with replacement from the original set of data. For more detail, see Effron and Gong (1983).

Conclusion

In summary, understanding relationships between temperature and sex ratio on a beach enables protection to be organized in such a way that conserves both sexes. For instance, when relocation of eggs is necessary, it helps managers avoid an undesirable influence on sex ratios. However, measures taken in one place or time should be assessed in the context of the spatio-temporal variation of sex ratio (and nesting patterns) in the population as a whole.

Acknowledgments

Financial support for our research came from the Natural Sciences and Engineering Research Council of Canada.

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Diagnosing the Sex of Sea Turtles in Foraging Habitats

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Determining the sex of sea turtles on foraging grounds is of interest to biologists and conservationists for a variety of reasons. The fact that sex determination in sea turtles is strongly influenced by the temperature at which the eggs are incubated *(i.e.,* temperature-dependent sex determination or TSD) (see Merchant, this volume) raises numerous questions which are of ecological, evolutionary and/or conservational significance. For example: What are the natural sex ratios in sea turtle populations? Do sex ratios vary between and among populations? What effect does sex ratio have on the reproductive success of a population? Are certain sex ratios optimal for the survival of a population? These types of questions are of particular interest to conservationists, since information of this sort is essential for understanding the reproductive dynamics of a population and thus for generating optimal management strategies for endangered populations. Natural sex ratios produced by TSD can vary widely (reviewed by Wibbels *et al.,* 1991, 1993; Mrosovsky, 1994). Thus, the above questions may not be easily answered, and a comprehensive database of sex ratio information may be required to produce reliable answers.

The concept of examining sex ratios in sea turtle populations seems straightforward. However, to successfully complete such studies, one must make several decisions regarding experimental design and then overcome a number of logistical difficulties presented by sea turtle biology and life history. First, one must decide which portion of the population to examine *(e.g.,* embryonic, hatchling, immature, adult). Differential survival relative to sex could occur in sea turtles, and thus sex ratios could vary between various age classes within a population. For example, hatchlings emerging early in a nesting season could experience different water conditions and food availability in comparison to hatchlings emerging late in a nesting season, and hatchling sex ratios can change significantly during a nesting season (Mrosovsky *et al.,* 1984). Therefore, optimal sex ratio studies would include the various age classes within a population. This chapter reviews nonlethal methods for identifying the sex of sea turtles from foraging grounds *(i.e.,* immature and adult turtles) and analysis of sex ratio data.

Identifying the Sex of Adult Sea Turtles

One of the fundamental necessities in sea turtle sex ratio studies is a valid means of identifying the sex of individual turtles. This is normally not a problem with adult sea turtles since males develop secondary sexual characteristics *(e.g.,* tail length, carapace morphology, morphology of the nails on the front flippers) during puberty. The most obvious secondary sexual characteristic is the large and muscular prehensile tail which extends well beyond the carapace in an adult male (Figure 1). While actual tail lengths will vary with species and possibly populations, the tail of female sea turtles is short and, at most, will project only slightly beyond the edge of the marginal scutes. However, one should be cautious when using tail length to indicate the sex of sea turtles that are near the minimum adult size for a given population; some large immature or pubescent males may not have yet developed long tails and could therefore be mistaken as small adult females (Limpus, 1985; Limpus and Reed, 1985).

Sexing Techniques for Immature Turtles

In contrast to adults, the sexing of immature and hatchling turtles represents a significant logistical hurdle. Tail length is not an accurate sexing technique for immature sea turtles (Limpus, 1985; Wibbels, 1988). However, tail length may be indicative of sex in some males as they near sexual maturity (Limpus, 1985).

A variety of nonlethal methods has been proposed and/or developed for determining the sex of immature sea turtles. The most definitive method is the direct observation of the gonads by laparoscopic examination. In addition, several techniques have been evaluated as physiological or molecular markers of sex. These include karyotyping (Bickham *et al.*, 1980), H-Y antigen assay of blood cells (Wellins, 1987), Bkm DNA fingerprinting (Demas *et al.*, 1990), and assay of blood testosterone levels (Owens *et al.*, 1978; Wibbels *et al.*, 1987; Wibbels, 1988). Karyotyping has yet to reveal sex specific differences, whereas H-Y antigen assay of blood cells and Bkm DNA fingerprinting have been proposed as potential sexing techniques but have not been well validated. Further, the logistics and costs of these three methods would hinder their widespread use for examining large numbers of turtles; therefore, they will not be discussed in detail in this chapter. Laparoscopy and testosterone RIA are also characterized by logistical difficulties and expensive equipment, but they have proven to be practical in successfully sexing large numbers of immature turtles. For a sexing technique to be useful it should be accurate, logistically practical, and cost effective.

Laparoscopy

Laparoscopic examination has been shown to be an effective method of sexing immature sea turtles (Wood *et al.*, 1983; Limpus and Reed, 1985; Limpus, 1985) since the gonads can be viewed directly through the laparoscope (Figure 2). A detailed description of immature and mature gonads is in Limpus and Reed (1985) and Wibbels (1988), and several photographs of immature gonads are shown by Rainey (1981). A detailed description of the laparoscopic procedures is provided by Wood *et al.* (1983). Owens (this volume) provides a technical overview. The main disadvantage is that the procedure is invasive and potentially hazardous to the turtle. Moreover, it is logistically difficult and should not be attempted without proper veterinary training. Despite the caveats, laparoscopy has been used successfully by a number of researchers and has been used to sex thousands of sea turtles (C. Limpus, Qld. Dept. Environment, pers. comm.). Further, the use of laparoscopy is currently a necessity for evaluating the effectiveness of other nonlethal sexing techniques for immature sea turtles.

Testosterone Radioimmunoassay (RIA)

Serum testosterone level can be used as an accurate indicator of sex of immature sea turtles (Owens *et al.*, 1978; Morris, 1982; Wibbels *et al.*, 1987; Wibbels, 1988). For example, in a study of sea turtles on Heron Atoll, serum testosterone was examined in immature green (n=197), loggerhead (n=61) and hawksbill (n=25) turtles in which the sex was verified through laparoscopy (Wibbels, 1988). In all three species, males exhibited significantly higher testosterone levels than females. In all hawksbill and loggerhead turtles, as well as 98% of green turtles, testosterone levels were an accurate indicator of sex *(i.e.,* the ranges of male and female testosterone levels did not overlap). A study of immature loggerheads captured in the Cape Canaveral channel (Florida, U.S.) provided similar results with no overlap of the ranges of male and female testosterone levels (Wibbels *et al.*, 1987).

More recently, testosterone levels have been used in studies to sex relatively large numbers of immature

Figure 1. Adult male green turtle showing muscular tail which extends well beyond the margin of the carapace.

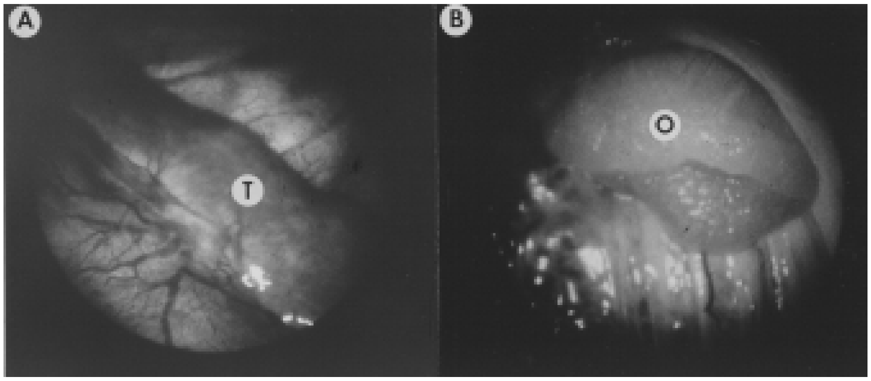

Figure 2. Appearance of immature testis and ovary through a laparoscope. 2A) Immature testis (T) is shown running diagonally through photograph. 2B) Immature ovary (O) is shown.

green and loggerhead sea turtles captured in the wild (Wibbels *et al.*, 1991, 1993; Bolten *et al.*, 1992). The minimum size limits of sea turtles that can be sexed by testosterone RIA has not been well documented. However, an unpublished study (A. Meylan, Florida Dept. Environ. Protection, pers. comm.) suggests that it could be used to sex green turtles with straight carapace lengths as short as approximately 25 cm.

There are several advantages in using a testosterone RIA to sex sea turtles. The RIA is conducted in the laboratory, so the field component is limited to the capture and blood sampling of turtles. Testosterone is a rather stable hormone, so serum samples from turtles can be stored for prolonged periods of time (at least several years) at -20 C or below with little or no degradation. A single testosterone RIA can easily include 50 to 100 samples or more, thus providing a practical and cost effective means of sexing relatively large numbers of sea turtles.

There are also limitations to using an RIA to sex sea turtles. First, as with any sexing technique for sea turtles, an RIA should be well-validated. For example, results from RIAs may vary within and between laboratories. Additionally, testosterone levels can vary slightly between sea turtle species, and possibly between populations (Wibbels, 1988). Therefore, whenever possible, a particular RIA should be validated using serum samples from turtles of known sex *(e.g.,* laparoscopically verified sex) from the species and/or population which is to be analyzed. In those analyses, various size classes of turtles should be examined to validate the size range of turtles that can be accurately sexed. Second, the results described above for green turtles (Wibbels, 1988) show that in some populations the testosterone levels of a small percentage of males and females can overlap. This again shows the necessity for accurate validation of the RIA. Female-only and male-only ranges must be determined. Only turtles falling within those ranges can be accurately sexed as male or female. Finally, once validated, the RIA should include interassay controls to verify assay reliability over time.

Blood Sampling

Blood samples for RIA analysis (or for other sex determination techniques) can be obtained from blood vessels located parallel to the spinal cord on the dorsal portion of the neck of sea turtles (Owens and Ruiz, 1980). The turtle can be placed in a slanted orientation with head down for optimal results, but in many cases blood samples can be readily obtained from turtles that are in a horizontal position. The optimal length and size of the needle required for blood sampling may vary depending of size of the turtle and the species, but a 3.8 cm, 21 gauge needle works well for most immature sea turtles. To obtain a sample, the needle is attached to vacutainer or syringe and then inserted into the neck at a steep angle at the approximate locations shown in Figure 3. Blood should be collected in sterile vacutainers if serum will be used in the assay or in treated, sterile tubes *(e.g.,* heparin or sodium citrate tubes) if plasma will be used in the assay. A minimum of several milliliters of blood should be taken, so that enough serum can be obtained for running samples in duplicate. Sample tubes should be placed on ice until

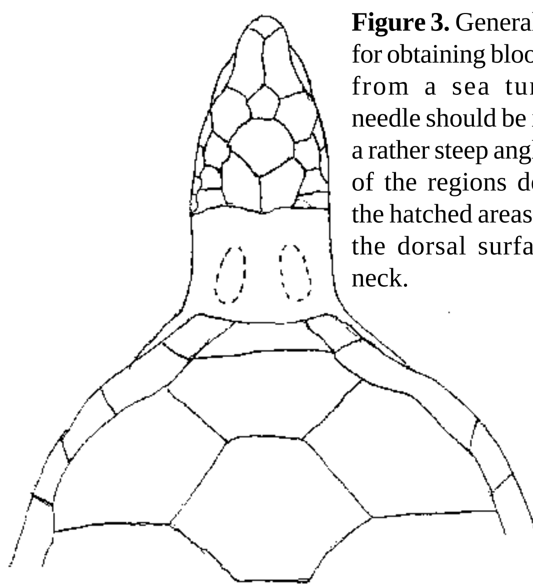

Figure 3. General locations for obtaining blood samples from a sea turtle. The needle should be inserted at a rather steep angle into one of the regions denoted by the hatched areas shown on the dorsal surface of the neck.

they can be centrifuged. The serum or plasma is separated from the blood cells by centrifugation and then transferred to a separate container and frozen.

Analysis of Sex Ratio Data

Once sex ratio data have been collected from a foraging ground, appropriate methods must be chosen for analysis. Specific questions relating to the sex ratio data should be formulated so that meaningful analyses can be conducted. For example, it may be insightful to examine whether a sex ratio differs from a 1:1 ratio, or whether sex ratios from different feeding grounds vary. Further, in addition to examining pooled data from a population, it may be advantageous to subdivide the data based on such factors as size classes of turtles, time of year when sampled, and sampling location.

Once specific questions have been generated, appropriate statistical analyses can be conducted. The sex of a sea turtle represents a qualitative rather than a quantitative variable, and a sex ratio is a derived variable. Therefore, when examining a single sex ratio from a population, many of the familiar statistics and their descriptive parameters *(e.g.,* mean, variation, confidence limits) do not apply (Siegel, 1956; Sokal and Rohlf, 1969; Zolman, 1993). However, there are statistical tests that are appropriate for sex ratio data. To compare observed frequencies of males and females in a population to a predicted value (1:1 for example), the chi-square goodness of fit test is appropriate when working with moderate to large sample sizes (all expected cell frequencies should be 5 or greater). Additionally, a Fisher's exact test can be used for these analyses and should be used instead of a chi-square test when working with small sample sizes (Siegel, 1956; Sokal and Rohlf, 1969; Zolman, 1993). These goodness of fit tests allow researchers to examine whether the observed sex ratio in a given population differs significantly from a 1:1 ratio. These tests can also be used to compare sex ratio data. For example, it may be useful to compare sex ratio data collected at different times of the year from a particular feeding ground, to compare sex ratios from different feeding grounds, or to compare sex ratios of different size classes of sea turtles within a population. Such analyses can also be accomplished with a replicated goodness of fit test (Sokal and Rohlf, 1969). This test generates "G" statistics which will indicate if the sex ratios are homogeneous and if the pooled male and female frequencies significantly differ from predicted values.

Acknowledgments

The completion of this manuscript was made possible through funding by the National Oceanic and Atmospheric Administration, U.S. Department of Commerce under Grant #NA56RG0129, through the Mississippi-Alabama Sea Grant Consortium.

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K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, M. Donnelly (Editors)
IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Diet Sampling and Diet Component Analysis

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The ability to sample the diet of sea turtles allows studies of the feeding ecology and physiology of these animals. Data from such studies can provide insight into questions relating to habitat utilization, digestive physiology, energetics, diet contaminants, trophic ecology, endoparasites, and the relative health of an individual turtle. Additionally, knowledge of the breadth of the diet of a turtle population allows conservation efforts to be directed to protect areas that provide such foods.

The feeding habits of wild turtles can be determined by a variety of methods, but the preferred technique is gastric lavage or stomach flushing. This comparatively simple and reliable technique has been used successfully to sample the gut contents of various vertebrate groups without harm to the animal. A system of stomach flushing of sea turtles has been developed (Forbes and Limpus, 1993) that allows rapid retrieval of large volumes of undigested food from the esophagus and anterior stomach regions of sea turtles. The technique described below has been widely and successfully used on green turtles, hawksbills, flatbacks, olive ridleys, and loggerheads ranging in size from approximately 25 to 115 cm curved carapace length (CCL). The technique should be equally successful on leatherbacks, if they could be lifted and moved as required throughout the procedure.

It is useful to note that other procedures (other than gastric lavage) also offer research potential, but they are not without their shortcomings. In analyzing samples from dead or moribund turtles, care should be exercised in the interpretation of results as the diets of these animals may not reflect the diets of healthy individuals. Diet may also be inferred from observations of turtles feeding in the wild. However, the difficulties of approaching and observing free-ranging sea turtles underwater preclude such studies under most circumstances. Collection of food fragments from the mouths of captured wild turtles can provide insight into diet, but the sample may represent only those dietary items that are hard to swallow *(e.g.,* the tentacled hydrozoan *Physalia* spp.) or are caught on various mouth structures such as the nasal choanae. The sampling bias inherent in this technique would be difficult to overcome.

Data on the food habits of wild sea turtles can also be obtained from direct underwater surveys, or from the examination of feces. Underwater surveys aimed at finding and evaluating evidence of turtle feeding activity require that an investigator locate physical evidence of turtle cropping, such as seagrass grazing plots or bite marks in sponges and gorgonians. The reliability of this technique depends on the ability of the observer to locate and accurately identify turtle cropping marks on sessile benthic organisms. Collecting fecal samples is problematic and time consuming. Additionally, the quantitative data available from fecal analyses are limited by the differential digestibilities of various dietary components which affect their representation in the feces when measured by either volume or weight.

The examination of digestive tract contents from healthy turtles captured in the wild and then sacrificed is one of the best determinations of diet. However, the ecological and moral implications of sacrificing sea turtles generally preclude this technique unless the turtles are taken in fisheries activities.

Gastric Lavage Technique

Turtles are placed on their carapace at a height which allows the head to be positioned lower than the dome of the carapace while allowing unencumbered access to the animal's head. The carapace should be supported to prevent the animal from rocking. Placing the turtle on a small automobile tire laid flat (wheel removed) in a wheelbarrow provides an excellent surface for support, restraint, and subsequent transport of the animal. For optimal drainage, the posterior end of the turtle should be elevated slightly higher than the head. It is rare for turtles to struggle once secured as described. Small turtles can be hand-held in the lap. Gyuris and Limpus (1986) have described a method for restraining the front flippers of large turtles.

The mouth is opened by holding the head securely and gently inserting a thin stainless steel pry bar between the maxilla and mandible. Pry bars can be easily made from flat steel stock but care should be taken to round and smooth all surfaces to reduce the risk of damage to the mouth cavity (Table 1). Although pry bars are the most effective and safe instruments, other common items such as wide blade screwdrivers and steel scalpel handles can be modified as a temporary pry bar although care must be used to prevent harm to the turtle.

The pry bar is inserted vertically between the maxilla and mandible and a gentle downward pressure is applied until the pry bar can be felt butting against the palate. At this point, the free end of the bar should be rotated downward (towards the cranium). This motion should be made gently as the intent is not to force the mouth open but to provide an irritating pressure which will cause the turtle to open its mouth. Attempting to force the jaws open will result in damage to the jaws and may hinder the animal's ability to feed. As the turtle opens its mouth, the bar is slid rapidly across the mouth cavity and out the other side at which time it is held in place at both ends until a mouth gag can be placed into position (Figure 1). Caution must be exercised to avoid striking the internal nares while passing the pry bar through the mouth.

A standard veterinary canine mouth gag is inserted into the mouth while the pry bar is held in place by an assistant (Figure 1). The gag should be inserted at the anterior end of the mouth and then expanded. The gag should be checked for stability before removing the pry bar. The gag should be expanded only to the point at which it is secure and not as far as the mouth will open as this will tear the soft dermal tissues at the junction of the mandible and maxilla. Should the turtle open its mouth further, the gag's tension spring will automatically expand the gag.

If veterinary gags are not available, polyvinylchloride (PVC) water piping can be used as a tubular gag for small to medium sized turtles. Thick-walled (4.0 mm) PVC pipe is cut into lengths of 1.5 cm. The inside diameter (ID) of the PVC pipe will be determined by the size of the turtle. Turtles >65 cm CCL require an ID of at least 4.5 cm, turtles 40-65 cm CCL an ID of 3.5 cm, and turtles <40 cm CCL an ID of 2.0 cm. Extremely large animals and loggerheads may require a tubular gag made from steel piping rather than PVC. Steel gags should have a soft coating, such as tire inner tubes, bonded to their surface to prevent slipping and damage to the mouth. The PVC or steel tubular gag should be positioned so that its opening is in line with the esophagus. It is more difficult to open the mouth wide enough to secure the tubular gag than with the adjustable veterinary gag.

Following the insertion of the gag, two flexible clear plastic tubes are inserted into the esophagus, one on each side of the gag. The first tube inserted is the retrieval tube that carries the displaced stomach contents into a mesh collection bag. The second tube is the water injection tube that carries the lavage water into the turtle. The retrieval tubing should have a wall thickness of 2.0 mm. A thinner wall may allow the tubing to collapse while a thicker wall will not provide enough flexibility. The largest diameter of tube possible should be used as large pieces of food may clog the retrieval tube (Table 1). The water injection tube should be 5.0 mm ID with a wall thickness of 1.0-1.5 mm and 3 m in length. Turtles <40 cm CCL require a tube of 3.5-4.0 mm ID. The ends of all tubes should be sanded or melted with a flame to provide smooth, rounded ends.

A mesh collection bag is fitted at one end of the retrieval tube. This bag can be made from fiberglass window screen netting or similar small mesh material. The top of the collection bag is equipped with purse draw strings that allow the bag to be drawn tightly against the tube. To prevent the bag

Table 1. Recommended dimensions of pry bars and retrieval tubes for three size classes of sea turtles. CCL is curved carapace length; ID is inside diameter.

CCL (cm)	Pry Bar	Retrieval Tube
25-50	2.0 mm x 12 mm x 15 cm	12 mm ID x 1.0 m
50-60	2.5 mm x 20 mm x 20 cm	16 mm ID x 1.5 m
>60	2.5 mm x 25 mm x 20 cm	20 mm ID x 1.5 m

from slipping off the tube, several cable ties or automotive hose clamps should be permanently placed on the outside of the tube 2-4 cm from the end. Markings are made on both tubes at 10 cm intervals from the insertion end to monitor the length of tubing inserted into the esophagus.

Before inserting the retrieval tube, one person must firmly grasp the head and extend the neck fully while keeping the head in line with the mid-line of the plastron and level with the plane of the plastron. This position must be maintained throughout the flushing procedure to prevent harm to the animal.

The tip of the retrieval tube should be dipped in a lubricant such as vegetable oil and then gently placed into the anterior end of the esophagus. If the glottis hinders the entrance of the tube, it can be depressed with the pry bar. Resistance from a muscle group near the anterior of the esophagus is frequently felt once the tube passes the glottis. If careful manipulation of the tube into the esophagus is not made at this point, delicate dermal tissues could be damaged and slight hemorrhaging could occur as evidenced by drainage of blood into the tube. As adult turtles may have large and partially convoluted trachea that hamper the insertion of the tube, they may require external manipulation of their trachea to facilitate passage of the tube.

Once the retrieval tube has passed the esophageal muscle group, the lubricated injection tube is slid in laterally along the retrieval tube (Figure 2). Lateral positioning of this tube will reduce the risk of entering the trachea which should already be sealed by the retrieval tube. Both tubes are now passed down the esophagus simultaneously until resistance is felt from either the food bolus or the junction of the esophagus and the stomach. This junction occurs ventral to the heart. In feeding turtles, a food bolus will normally be encountered before the junction. The distance to this junction can be determined prior to tube insertion by laying the tube along the midline of the plastron and measuring from the junction of the humeral and pectoral scutes to the tip of the mouth. The stomach flushing procedure should not begin at a depth greater than this measured distance.

Fresh or saltwater is now delivered through the injection tube. The flow valve to the water delivery system must be close by so that it can be turned off rapidly. If water delivery is through a pressurized domestic system, an optimal delivery pressure to the injection tube is 10-25 psi (9 liters/min). Delivery pressures for turtles <40 cm CCL should fall in the low end of this range. Delivery pressures can be de-

Figure 1. Positioning of head, pry bar and gag in a green turtle.

Figure 2. Lateral positioning of injection tube (left) and rerieval tube in a green turtle, *Chelonia mydas.* Note alignment of head with plastron.

termined easily with the installation of an inexpensive in-line pressure gauge placed just upstream from the flow valve. In lieu of a pressurized system, hand operated bilge pumps have been used quite successfully. Water must not be delivered at pressures or volumes greater than what can be expelled easily through the retrieval tube as the accumulation of excess water pressure within the turtle could cause it serious injury or death.

As water enters the turtle, return flow should begin within seconds through the retrieval tube. The exit flow volume should equal the delivery flow. If this is not the case, the retrieval tube should be withdrawn slightly to allow free entry of water into the tube as the tube may be obstructed. If water does not exit or the flow rate is low for more than 15-20 sec at any time during the lavage, stop the entry of water and reinsert both tubes. Once proper return water flow is achieved, food particles should be seen traveling within the tube. If particles are not present or to increase the quantity, while holding the injection tube in place, move the retrieval tube firmly against the bolus and then withdraw several centimeters to allow the dislodged particles to enter the tube. If food is not entering the tube, do not increase the force of the forward movement of the tube as the tube most likely is against soft tissue rather than the bolus. Instead, the tube should be withdrawn several centimeters, rotated slightly and reinserted until food particles begin to exit.

Although the entrance to the trachea should be sealed by the retrieval tube, the actual lavage should not exceed 3 minutes to reduce the chance of the turtle inhaling. Once the desired quantity of sample has been collected, the water to the injection tube is turned off and water and food are allowed to drain until all flow has stopped. The posterior of the turtle can be elevated slightly at this point to assist in drainage. Complete drainage is important prior to removing the retrieval tube as the turtle may breathe as the tube is removed and the airway must be free of standing water to prevent aspiration. The injection tube should be removed first and then the retrieval tube. Immediately after removing the tubes, the gag should be removed rapidly and the head elevated slightly to drain any remaining water clear of the glottis and back into the esophagus. The head should be held in this position until the first breath is taken which should be almost immediately. At this point the procedure is complete.

Proper lavage technique may yield up to 1 liter of food from healthy and actively feeding adult green turtles and 500 ml from subadults. Subadult hawksbills may yield up to 200 ml. Lavage samples should be preserved in a 6.5% buffered formalin/seawater solution. Stronger formalin solutions will discolor most plant matter as well as some animal matter making identification more difficult.

Many individual turtles have been lavaged more than three times without any known detrimental effect. Individuals have been recaptured from the day after the procedure up to three years later and appear to be quite healthy and feeding. Laparoscopic examination of the intestines following the procedure has not detected any swelling or damage to the intestines. The entire technique can be performed in less than 10 minutes and is rarely unsuccessful.

This system has proven to be a quick, safe, and inexpensive method by which sea turtles' stomach samples can be obtained in the field without injury to the animal. The technique is readily learned and proficiency can be achieved in a short time. However, care should be taken in the interpretation of the significance of the sample retrieved. The sample contents are a function of the size of the retrieval tube used, the size of the diet components in the anterior digestive tract, the duration of the lavage, the distance or depth at which the digestive tract was sampled, and the experience of the person performing the lavage.

Diet Component Analysis

Once a diet sample has been collected by gastric lavage or any other technique, the next step is to analyze the contents. A simple qualitative list of the components present in the diet sample may be all that is desired, or a detailed quantitative analysis of diet composition and the relative contribution of each diet component may be required. A reference collection of potential diet items should be established by preserving the diet items in 6.5% buffered formalin/ seawater solution in clear plastic vials stored in darkness to reduce color fading.

The two most common methods of quantifying a diet component's contribution to the diet is to determine either its weight or volume relative to the total diet sample. Attempting to quantify a component's importance to the diet by its gravimetric or weight contribution has several drawbacks. The importance of diet items with a high ash content and therefore high relative weight *(e.g.,* calcareous algae, sponge spicules, exoskeletons) will be overestimated in a gravimetric analysis while low ash content items will be underestimated.

If a gravimetric procedure is used, diet components can be freeze dried or oven dried until a constant weight is obtained. Freeze drying is the preferred method if biochemical analyses are to be performed on the components as heating may damage heat labile compounds. If freeze drying is not possible, samples should be dried at 60°C to avoid heat damage. After drying, the diet components should be maintained in a desiccating chamber with silica gel to prevent rehydrating prior to weighing.

The relative volume of each dietary component can be determined with two techniques. One technique uses water displacement. Each diet component is placed in a graduated cylinder containing water, and the increase in volume recorded in the graduated cylinder is the volume of the diet component. For reasonable accuracy, the size of the graduated cylinder should be appropriate for the volume of the sample; that is, displacement of a 1 ml sample should not be measured in a 100 ml graduated cylinder.

The second technique uses the principles of microstereology (Weibel *et al.*, 1966; Schaefer, 1970) and a quantification technique (Forbes, 1996). For this approach, each lavage sample is emptied into a large tray and mixed until visually homogeneous. A subsample sufficient to cover the bottom of a Petri dish is removed and spread across the dish to a depth at which substage light can still be transmitted through the sample in amounts sufficient to illuminate the sample. The sample is then viewed under a dissecting microscope with wide-field ocular lenses fitted with a Weibel graticule consisting of 21 straight lines arranged in 3 rows of 7 lines. Although the Weibel pattern is the most efficient sampling graticule (from Bunton Instrument Company, 9607 Doctor Perry Road., Suite 99, Ijamsville, Maryland 21754 USA), a variety of grid patterns can be used. Filamentous species of algae can be viewed with substage lighting transmitted through a blue filter to enhance cellular definition.

Sampling field locations should be marked and numbered sequentially every 4 cm along the circumference of the Petri dish with a permanent marker. The Petri dish is rotated within a stage mounted template until the sampling field lines up with an indicator line on the stage template. The template is made by cutting a hole (equal to the diameter of the Petri dish) out of cardboard or plastic. Each diet component's contribution to the volume of a sample is determined by counting the number of graticule line endpoints that it intercepts relative to the total number of intercepts counted for all components combined.

The power of magnification will be determined by the resolution required to identify the specimen. However, all intercepts should be counted at the same magnification. If higher magnification is required, the diet item can be removed carefully from the Petri dish and viewed under a compound microscope. The number of fields required to ensure an adequate analysis of the lavage sample is determined by sampling a series of the most diverse lavage samples. The results are plotted to determine (1) the point at which there is no significant increase in the number of components added with the addition of another sampling field and (2) the point at which the cumulative percent contribution of each component levels off without significant change with the addition of another sampling field.

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Measuring Sea Turtle Growth

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Studies of growth have played an important part in defining critical aspects of sea turtle life histories, in particular for assessing age to maturity. Growth rates measured in several populations have been used to demonstrate that sea turtles are very slow-maturing and potentially long-lived animals. Turtles are especially suitable subjects for growth research as their size can be determined precisely by measuring the carapace or plastron. However, the elusive nature of free-living sea turtles, especially juvenile and subadult animals, has limited the extent of mark and recapture studies yielding growth data to those species and populations most accessible for research. For example, a considerable amount of growth data has been collected for green turtles (*Chelonia mydas*) in Australia (Chaloupka and Limpus, 1996) and the Bahamas (Bjorndal and Bolten, 1988), but only limited information is available for other species of sea turtles.

Two types of techniques for determining growth in sea turtles can be recognized: direct and indirect. Direct growth measurement consists of determining the size increase of individual animals over time. Because of the long time periods involved with this method, several alternative approaches have been explored that promise to produce results over shorter time periods. These indirect methods yield estimates of past growth rates and include skeletochronology (the examination of periosteal layers in the humerus of individual turtles see Zug, 1990) and length-frequency analysis of sample populations (Bjorndal and Bolten, 1995). Validation of the growth estimates obtained with these indirect methods, however, requires comparison with direct growth measurements from the population studied. In this chapter we will restrict our discussions to direct techniques of growth measurement and methods of data analysis.

The measurement of growth in sea turtles through mark and recapture of juvenile and adult animals is in principle a simple matter (see Ehrhart and Ogren, this volume, for capture methodology), and much can be achieved with a tape measure or calipers. It is important to recognize that the quality of the growth data to be collected is greatly enhanced by developing and following an appropriate research protocol. Collecting direct growth data requires: (1) the unequivocal identification (tagging/marking) of individual turtles (see Balazs, this volume), (2) the measurement of well defined body structures (see Bolten, this volume), and (3) the re-encounter and re-measurement of marked turtles.

Measuring growth over time consists of determining the difference between two or more measurements. This calculation yields size increment data that are highly sensitive to measurement error. Fortunately, sea turtle carapaces are generally rigid body structures that allow precise measurements to be made. It is important to select unambiguous reference points on the turtle carapace so that measurements can be taken consistently; these points may vary among species.

Most growth studies have used carapace length as the principal measure for assessing turtle body size changes. Straight-line carapace length (SCL) measurements taken with calipers have been shown to be preferable to over-the-curve tape measurements because of their greater precision (Bjorndal and Bolten, 1989). Errors associated with tape measurements may be generated by tape stretch or shrinkage over time, variable tape tension, and interference by barnacles or other epibiota along the measurement path. Inter-observer measurement errors are discrepancies caused by differences in measurement technique between observers; these errors are eliminated when a turtle is always measured by the same person.

An assessment of overall measurement error is easily performed, and the results can be of great value to the interpretation of growth data (see Bolten, this volume). For growth increments to be reliably measured, they should be at least one order of magnitude greater than the measurement error. Given a turtle population for which a rough estimate of growth rate already exists, the *(measurement error)/(growth rate)* quotient can be used to determine an appropriate minimum time interval between captures. Intervals of close to one year (or multiples thereof) are ideal, as this minimizes the possible distortion of growth data by seasonal effects. Because of the generally slow growing nature of marine turtles, growth increment data collected over periods of only a few months should be avoided, unless measurements can be performed under controlled, laboratory conditions.

In practice, measured SCL increments occasionally yield negative values, reflecting actual decrease in measurable turtle length or measurement error. Such negative values should be included in any subsequent analysis, unless the measured size decrease is attributable to physical damage *(e.g.,* scute breakage). In damaged individuals, a size increase can sometimes still be inferred from measurements of unaffected body parts.

Turtle size and growth can also be expressed in terms of body mass. For understanding certain physiological processes, a knowledge of body mass growth rates may be more important than information on linear growth. Body mass of turtles is typically measured by weighing with a spring or platform scale. It should be noted, however, that variation in nutritional status and reproductive condition of turtles generally introduces greater variability in body mass data than in linear size measures.

Once growth increment information has been collected, a variety of analytical methods may be employed to interpret the data. Perhaps the simplest but most insightful of all is the conversion of individual growth increments to growth rates using the formula:

mean annual growth rate = (measurement 2 measurement 1) ÷ interval in years

Likewise, the measurement error associated with each calculated growth rate can be obtained using the formula:

mean growth rate error = measurement error ÷ interval in years

Growth rate information is usually presented for individual turtles (in scatterplots of individual growth rates vs. carapace length) or in tabular form, by individual or grouped by size class.

Model-based approaches assume that the examined turtles all follow similar growth trajectories. Turtle growth patterns can be expressed concisely once the critical parameters of an appropriate model *(e.g.,* von Bertalanffy, logistic, Gompertz) have been determined. The use of models facilitates comparisons of growth patterns between populations by allowing differences between parameters to be detected with standard statistical tests. A technical review of growth rate analyses is presented in Chaloupka and Musick (1997).

Sea turtle growth rates have often been found to be highly variable, even within a single population *(e.g.,* Bjorndal and Bolten, 1988). Growth rates are thought to be controlled by a variety of factors that can be divided in two categories: factors intrinsic to an individual, and environmental factors. Intrinsic factors besides size that are likely to affect growth include the sex, genotype, and health status of individual turtles. Environmental factors include water temperature, food quality and availability, and foraging opportunity. Effects of intrinsic factors can be examined by partitioning the collected growth data into groups of interest and testing for differences between groups. Using this method, Bolten *et al.* (1992) found no significant differences between growth rates of male and female juvenile green turtles in the Bahamas. Determining the environmental factors that influence growth is likely to be a much more complicated process requiring extensive ecological knowledge.

Studies of growth in free-living sea turtles have the potential for yielding valuable insight into the time-scale of developmental processes in these animals, such as time to maturation. Whereas growth rates have now been determined for several green turtle populations, the growth rates in other sea turtle species (and in additional green turtle populations) remains a fertile ground for research. Due to the nature of their subjects, growth studies are necessarily very labor intensive and long-term endeavors. Many different methods for turtle capture, tagging, and measurement are available and determining the most appropriate for a given turtle population at the onset of the study will greatly increase the potential for success.

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Stranding and Salvage Networks

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Overview: The Importance of a Network

Stranded sea turtles are defined as those that wash ashore dead or alive or are found floating dead or alive (generally in a weakened condition). Sea turtles strand in the vicinity of migratory routes, foraging habitats, developmental habitats, and nesting beaches. The numbers that strand are typically influenced by a variety of factors and vary at different geographical locations and during different years and seasons.

Systematic data gathering for stranded sea turtles can provide resource managers and scientists with biological information useful in improving the conservation and management of these species. Data gathering is best accomplished through a formal stranding and salvage network that can document stranded sea turtles, salvage dead individuals for necropsy, and transport live individuals to rehabilitation facilities. Data collected through the network can be used to identify sources of mortality, document locations of negative human/sea turtle interactions, evaluate the effectiveness of various regulations, and serve as a basis for management decisions. Documenting stranded sea turtles and associated tag returns can enhance an understanding of species composition, distribution, seasonality, sizes, migratory patterns, and habitat use. Through salvage, necropsy, and specimen collection from dead stranded turtles, information is obtained on sex ratios, diseases, foraging ecology, and other topics. Live stranded sea turtles that are located and taken to appropriate rehabilitation facilities can often be successfully rehabilitated and released back into the wild.

A national Sea Turtle Stranding and Salvage Network (STSSN) has operated in the USA since 1980. It serves as a basis for many of the protocols and recommendations offered in this chapter.

Network Components

Network Participants and Coordinators

Funding is typically limited; thus, it is recommended that most participants and coordinators volunteer to provide data without compensation. Whenever possible, these volunteers should be trained biologists who understand the importance of accurate data collection, and who will be able to participate in the network for several years. Among those that should be considered to participate are employees of natural resource agencies, zoos and aquaria, as well as park managers, educators and dedicated local residents. Once informed about the importance of the stranding network, employers may allow participation in network activities during normal work hours. In order to facilitate timely data collection, participants should be distributed throughout the geographical area where the network will operate, and they should receive training in standardized data collection protocols.

A Network Coordinator, as well as several Regional Coordinators, should be designated. Each Regional Coordinator should be located within a specific geographical area and oversee network activities conducted there. The Network Coordinator should be an employee of the agency willing to commit to the long-term maintenance of a central computerized database that will contain all stranding records.

Detection of Stranded Turtles

Network participants document turtles stranded within their geographical area. Stranded turtles are detected either by network participants or by other individuals who report the turtles. Participants should immediately attempt to find turtles reported alive, so that they do not succumb prior to transfer to rehabilitation facilities, and promptly attempt to locate those reported dead, so that they do not deteriorate appreciably before data collection.

Turtles may be detected opportunistically, or during surveys designed specifically to identify stranded turtles. Depending on funding and time availability, surveys can be undertaken intermittently or systematically. If systematic monitoring is undertaken, surveys should be made from 1-3 times per week so that turtles can be located before they deteriorate or are taken by people or predators. Index areas for systematic monitoring can be established if those areas are surveyed consistently and effort expended is recorded. Regardless of the method used to detect stranded turtles, the numbers documented should be considered minimum stranding figures since they represent only reported strandings and not all stranding events.

Documentation of Stranded Turtles

Each stranded turtle located should be documented by a network participant on a standardized form. The form used by the STSSN is included as an example (Figure 1). Managers and researchers establishing networks in other areas will likely need to modify the STSSN form to meet their specific needs. Only one standardized form should be developed and used for a particular network. The form should include the data parameters and notation codes listed below, but contain only those species occurring within the geographical area covered by the network. It should be as short, concise, self-contained, and easy to complete as possible. The data to be collected for each stranded turtle should be printed on the front; a species guide and the Regional Coordinator s address should be printed on the back.

All data parameters listed on the standardized form should be recorded for each turtle. Straight and curved carapace length and width should be measured using standard methodology (see Bolten, this volume). Straight line measurements made with calipers are more accurate than curved measurements made with a flexible tape. Attempts to determine sex using blood serum testosterone assays, laparoscopy, and examination of gonads during necropsy should be noted. It is not recommended to use tail length to identify sex since this method is unreliable for decomposed carcasses and immature turtles.

If possible, each stranded turtle should be photographed at the stranding site, necropsy location, or rehabilitation facility. Photographs provide additional documentation of the stranding authenticity and characteristics. Network participants should immediately submit each completed original stranding form to the appropriate Regional Coordinator, who should immediately review it for accuracy and submit it to the Network Coordinator. Both the network participant and Regional Coordinator should retain a copy of each form for archival and reference purposes.

Other Associated Activities for Stranded Turtles

Once an animal has been documented, it should be marked or removed from the stranding site (to prevent it being counted again). Live stranded turtles should be transported to rehabilitation facilities (the facility should be noted on the data form). Dead stranded turtles (fresh or moderately decomposed) and live stranded turtles that succumb during rehabilitation efforts can be salvaged for necropsy and specimen removal and are an important resource for obtaining additional information. Necropsies should be conducted using standardized protocol (see Jacobson, this volume). Dead turtles not salvaged for necropsy should be buried high on the beach or pulled behind the dunes. It is not recommended to mark them with paint or other materials since these markings usually disappear over time.

Acknowledgments

We would like to thank participants of the USA s Sea Turtle Stranding and Salvage Network, including those early participants who developed the standardized stranding form and protocols used by the network today, and those participants who have continued the network s activities.

Record Keeping: Standard Information for a Data Form

1. Observer s name, address, telephone number
2. Turtle number by day (enter a consecutive number for the individual observer for that day)
3. Stranding date (enter yr / month / day)
4. Stranding location in reference to the closest town or landmark. Include county, state, or other relevant geographical breakdown, as well as latitude and longitude. Note whether stranding was located inshore (bays, estuaries, or passes and their beaches) or offshore (oceans and their beaches).
5. Species code: CC = Loggerhead; CM = Green/Black; DC = Leatherback; EI = Hawksbill; LK = Kemp s ridley; LO = Olive ridley; ND = Flatback; UN = Unknown
6. Reliability of species identification (indicate unsure , probable , or positive).
7. Species verified by Regional Coordinator (yes or no)
8. Sex of turtle (female , male , or undetermined)
9. How sex was determined (enter the method used)
10. Condition of turtle, coded as follows: 0 = Alive; 1 = Fresh dead; 2 = Moderately decomposed; 3 = Severely decomposed; 4 = Dried carcass; 5 = Skeleton, bones only.
11. Final disposition of turtle, coded as follows: 1 = Painted, left on beach; 2 = Buried, on beach/off beach; 3 = Salvaged specimen, all or part; 4 = Pulled up on beach or dune; 5 = Unpainted, left on beach; 6 = Alive, released; 7 = Alive, taken to holding facility.
12. Tag number(s). Enter type of tag (metal, plastic, PIT, living, etc.), tag numbers, tag position, tag return address, and disposition of tag. Draw located tags on the diagram.
13. Remarks. Enter information on tar or oiling, gear or debris entanglement, wounds or mutilation, propeller damage, papillomas, epizoa, etc. Draw noted items on the diagram.
14. Measurements (straight length / width; curved length / width). Circle measurement units.

SEA TURTLE STRANDING AND SALVAGE NETWORK   STRANDING REPORT

PLEASE PRINT CLEARLY AND FILL IN ALL APPLICABLE BLANKS. Use codes below. Measurements may be straight line (caliper) and/or over the curve (tape measure). Measure length from the center of the nuchol notch to the tip of the most posterior marginol. Measure width at the widest point of carapace. **CIRCLE THE UNITS USED.** See diagram below. Please give a specific location description. **INCLUDE LATITUDE AND LONGITUDE.**

Observer s Full Name ______________________________ Stranding Date ______ — ______ — ______ (year / month / day)

Address / Affiliation ______________________________

Area Code / Phone Number ______________________________

Species ______________________________ Turtle Number By Day ______________

Reliabiity of I.D.: (CIRCLE) Unsure Probably Positive   Species Verified by State Coordinator? Yes h No h

Sex: (CIRCLE) Female Male Undetermined   How was sex determined? ______________

State ______________________________ County ______________

Location (be specific and include closest town) ______________________________

____

Lattitude ______________________________ Longitude ______________________________

Condition of the Turtle (use codes) ______________ Final Disposition of Turtle (use codes) ______________

Tag Number(s) (include tag return address and disposition of tag) ______________________________

____

Remarks (note if turtle was involved with tar or oil, gear of debris entanglement, wounds or mutilations, propeller damage, papillomas, epizoa, etc.) continue on back if necessary.

____

____

MEASUREMENTS: CIRCLE UNITS

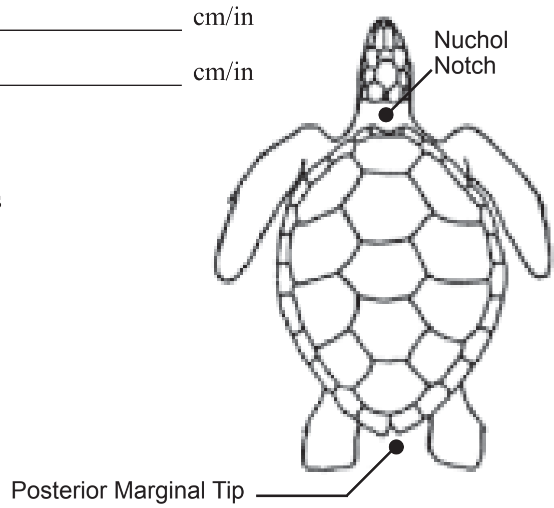
Straight Length ______________ cm/in

Straight Width ______________ cm/in

Curved Length ______________ cm/in

Curved Width ______________ cm/in

Mark wounds,
abnormalities
and tag locations



CODES

SPECIES:
- CC = Loggerhead
- CM = Green
- DC = Leatherback
- EI = Hawksbill
- LK = Kemp s Ridley
- UN = Unidentified

CONDITION OF TURTLE:
- 0 = Alive
- 1 = Fresh dead
- 2 = Moderately decomposed
- 3 = Severely decomposed
- 4 = Dried carcass
- 5 = Skeleton, bones only

FINAL DISPOSITION OF TURTLE:
- 1 = Painted, left on beach
- 2 = Buried: on beach / off beach
- 3 = Salvaged specimen: all / part
- 4 = Pulled up on beach or dune
- 5 = Unpainted, left on beach
- 6 = Alive, released
- 7 = Alive, taken to a holding facility

Figure 1. STSSN standardized stranding form

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Interviews and Market Surveys

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Interviews

Interviewing is the process of compiling information and viewpoints by verbal questions, discussions or meetings. Interviews provide opportunity for: (1) obtaining information in an inexpensive and time-saving manner; (2) summarizing the experience of knowledgeable people; (3) compiling information that has been maintained only in an oral tradition or when written information is scarce; (4) supplementing data collected by direct observation; (5) sharing of information; and (6) collaboration.

There are different types of interviews, recording methods, approaches to conducting interviews *(e.g.,* question structure), and interpreting the information compiled. The recommended method could be one of those described below, or a combination thereof. Effort should be made to tailor methods to the situation with careful understanding of the politics surrounding the information to be compiled, the people to be interviewed (= interviewees), and the interviewer's own position. Preliminary visits to a location and discussions with residents provide insight into the best approach. Critical information can be lost if the interviewer does not understand the vocabulary, interpretations, and politics of the persons being interviewed, and likewise, if the person being interviewed does not understand the interviewer in a similar way.

Interviewing requires a few basic requirements of the interviewer: be prepared, listen carefully, be clear and concise, be courteous and appreciative, be respectful, be patient, and be perceptive. Know the language, or work closely with an interpreter accepted politically and culturally by the interviewee and whose personal opinion does not affect the translation.

Designing Questions for an Interview

A good interview depends on carefully designed questions (see Appendix I for guidance) which incorporate local terms, names, and phrases to facilitate interaction and familiarize the subject matter. Critical information is lost when the interviewee does not understand the vocabulary used in the questions.

To identify misunderstandings and/or discrepancies in the information compiled, a number of different questions aimed at obtaining the same information should be posed. If the answers to these related questions are similar, the quality and accuracy of the information are acceptable. Answers to similar questions can be used to assess how the interviewee is reacting to the interviewer's presence or questions *(e.g.,* openness vs. caution), and how honest and knowledgeable the interviewee is about the topic.

Questions should be phrased so as not to indicate the interviewer's own interpretation of a situation, or give the interviewee insight into what the interviewer would like to hear. If the questions are slanted to a certain position, the interviewee can often identify this bias, and may, sometimes out of courtesy, provide answers to the questions in support of the interviewer's viewpoint.

Depending on the question, the interviewer may need to understand how the person being interviewed arrived at a conclusion. The interviewer should not hesitate to inquire how such information was determined. However, such an inquiry should not be done in a manner that questions the knowledge, experience, or authority of the interviewee.

Types of Interviews

a) Questionnaire: A questionnaire is a printed list of questions with space to record answers. Questionnaires help to standardize information, which can be helpful when compiling or comparing information across multiple sources. However, questionnaires limit opportunities for expanding discussion of a topic based on new knowledge generated during an oral interview. Such limitations can be overcome to some extent by designing questions that are broad, and based on preliminary tests of the questionnaire.

When time is limited, quantitative information is sought, and a large sample size is required, questionnaires can provide the most productive results. Questionnaires also allow easier recording of information and simplified comparative and statistical analyses. Questionnaires can be completed by the interviewer, by the interviewee in the presence of the interviewer, or completed and sent (by mail or other means) to collection centers, such as a conservation agency or organization.

b) Using Lead Questions: Essentially a question and answer format, this method uses lead questions to solicit specific information (as opposed to promoting an open-ended discussion) about an issue and compile the larger knowledge and insight of the interviewee without limiting the number or the scope of the questions. This method is better than the questionnaire method when the interviewer has little knowledge about a situation. This method can also be used effectively to compile basic terms, names, phrases, and gain an understanding about the politics of a situation that could later be used to develop an excellent questionnaire. Due to the less structured nature of this method in comparison to questionnaires, a good balanced interview can emerge, yielding both qualitative and quantitative information.

c) Open (Open-Ended) Discussions: This is the least structured format and requires a greater degree of language skill and socio-political sensitivity on the part of the interviewer in order to accomplish a successful interview. In this method the interviewer may provide the lead questions or statements, and the interviewee may carry the discussion in a variety of directions and depths. Sometimes the only cue that the person being interviewed may need is the topic of interest. This method is excellent for understanding larger, more complex issues, such as attitudes and conflicts. It often brings out information, connections, and interactions that were previously unknown to the interviewer.

This method offers the best situation for dialogue and sharing between interviewer and interviewee. It is also an effective method when interviewing a group, where people will prompt each other into discussing the issue at hand while asking questions of each other and of the interviewer. It is most effective when time is not a limiting factor. This method has been found to be most compatible with the cultures of most rural communities in developing countries, where a questionnaire may be viewed as an interrogation. However, open-ended interviews can be more difficult and time-consuming to record, and the more qualitative nature of the process makes statistical analyses difficult.

Methods for Recording Information

The method used to record an interview is as important as the interview itself. The recording method depends primarily on what is comfortable for the person being interviewed. People are often uncomfortable with having their thoughts recorded. The situation can be improved through prior collaboration, understanding, or agreement. Recording methods are:

a) Handwriting: Using this method, blanks are filled on a printed questionnaire or abbreviated notes (or detailed answers) are recorded in a notebook.

b) Tape Recording: Although using a tape recorder documents an interview most accurately, it can be intimidating to the interviewee. Seek permission before beginning an interview; if the interviewee is hesitant, agree on a less intimidating method. Use of a hidden recorder is unethical and can have negative, sometimes unsafe, repercussions, which can cause distrust and hinder continued work in the area.

c) Photographic Memory: In this method, the interviewer memorizes a series of topics for which information is sought, remembers the interviewee's answers, and later records the answers. This method relies heavily on the interviewer's memory to recall the conversation accurately, and therefore runs the risk of being misrecorded, especially with long interviews. From the viewpoint of the interviewee, this method resembles a conversation and is the least intimidating.

d) Photography and Videography: Audio-visual records can be used to document and/ or supplement information provided by the interviewee. Visual imagery allows the interview to be revisited or reinterpreted at a later time, or to identify locations, distances and associations. It can also be used to assist in identification and illustration during interviews.

e) Map Supplements: Maps can be used to compile information on locations and distributions referred to by the interviewee, and/or to present historical associations and trends.

Interview Process

The interviewer must establish him/herself and his/her methodology carefully, as the interviewer and the methodology can influence the interview process and the information compiled. If the interviewer is not careful in setting the "stage" for the interview, people interviewed will interact with or answer the interviewer in a "safe" manner (so as to protect themselves). The following process, although not exhaustive and not always applicable, may assist in conducting a productive interview:

First, make preliminary visits to become familiar with the location and the people. Seek collaboration with an organization or people from the area. Compile locally used terms, names, and phrases *(e.g.,* sea turtle species, nesting/foraging sites, turtle products/uses). Determine locally conducive conditions for an effective interview *(e.g.,* times which do not obstruct livelihoods or lifestyles; appropriate locations). Determine the best interview method (or combination) from those described above. Develop questions and test them informally. Identify persons to be interviewed.

Introductions are very important. The interviewer should ensure appropriate and unbiased introductions, as interviews can be greatly enhanced or hurt by the political affiliation of the introducer and of the supportive organization(s). Explain the purpose of the interview and of the information that will be compiled. Encourage the interviewee to ask questions of the interviewer, so as to prevent the feeling of an interrogation. Agree on place and time of interview, method of interviewing and recording, amount of time available, how information will be used and conditions for such use *(e.g.,* compensation, credit, sharing of information summaries). Record the identity of the interviewee (if the person is willing to offer that information), and pertinent background information, such as livelihood and experience.

Finally, conduct the interview(s). Record and cross-check information through subsequent visits to the same locality and follow-up meetings with interviewees and others. Provide compensation (monetary or material) if that was part of the interview agreement. Share with the person being interviewed local and worldwide information on turtles and other issues discussed, including materials which can be retained by the interviewee. Analyze data and prepare summaries and reports. Circulate reports that include interview information among organizations, collaborators and, where appropriate, those who participated in the interviews, and update the information obtained through periodic communication.

Interpreting Interview Information

The quality of the information compiled and the strength of its interpretation depend on many issues: methodology, understanding local vocabulary and interpretations, biases, expectations, and the political nature of the issue. The types and limitations of the information must be known before any form of interpretation or analysis is conducted.

a) Quantitative Information: Statistical analysis is appropriate when quantitative information has been compiled in a standardized manner across an adequate sample size. Subjective answers such as "many turtles" cannot be statistically analyzed, although "about 100-150 turtles" can provide a workable range. Obtain numerical values (or ranges) or yes/no answers whenever appropriate.

b) Qualitative and Anecdotal Information: Such information can include opinions, ideas, reactions, and general observations, even information that is considered unimportant in the daily activities of those interviewed. Analysis here is more difficult, as there are more variables involved. Distilled information can reveal the magnitude of a problem, diversity of opinion, and degrees of complexity surrounding solutions. To attempt to quantify all aspects of conservation, especially when conservation is such a political issue, would mean losing valuable information about the diversity of people interacting with sea turtles and their conservation.

Some information may seem false or ridiculous to the interviewer. However, discarding this information without verification can bias the information compiled in favor of the interviewer's own expectations. Testing such "questionable" information through direct observation or further inquiry could indicate that the information is true, reveal an innovative explanation for an observation, or reveal attitudes or biases towards a certain issue.

c) Interpreting Sensitive Information: Since sea turtles are protected in most locations, and yet continue to be utilized by many coastal and island communities in these locations, compiling information about turtles from these same people can be challenging. How interviewees respond to being questioned on controversial issues will influence the accuracy (and therefore should influence the interpretation) of the interview information.

Interview Ethics

Interviewing is often practiced as the collection of information. However, ethical research calls for an exchange or sharing of information in interviews, so that information does not serve only the objectives of the interviewer. With the greater recognition and enforcement of intellectual property rights, interviewers must be aware and acknowledge the value of knowledge imparted during interviews. Mutual gain and equitable exchange must be the expected outcomes of an interview.

Information compiled has often been used against the very people who provided that information, especially on controversial issues such as sea turtle utilization. In such situations the result is often greater distrust of interviewers and researchers who may follow, as well as opposition to programs implemented using such information. An interviewer is responsible for ensuring ethical outcomes from information compiled through an interview.

The identity of people who are interviewed based on an agreement of anonymity should not be revealed. Further, if the interviewer has received information confidentially such information should not be made public unless agreed upon by the source person, and the person's anonymity has been ensured. Depending on the sensitivity of the information, the promise of anonymity can be fulfilled by erasing a person's name, address, photograph and video recording, and the date and location of the interview.

Market Surveys

Market surveys use interviews and observation to compile and assess information on: (1) levels and types of sea turtle utilization and commercialization; (2) structure and organization of local, national or international markets; (3) increasing/decreasing product availability; (4) role and importance of turtles in the diet and income of the people in the area; (5) cultural connections to turtles; (6) attitudes to turtles as a commodity; (7) conservation programs; and (8) ecological information (such as seasonality, distribution, and numbers of individuals of different species and size classes in the area frequented by turtlers).

Items for sale at markets include whole turtles, meat, eggs, shell products *(e.g.*, jewelry, trinkets, souvenir shells), stuffed turtles, mounted parts, extracted products *(e.g.*, soaps, lotions, oils) and prepared foods *(e.g.*, turtle soup, grilled steak, drinks with raw eggs). Where the sale of sea turtle products is illegal, obtaining accurate information from market surveys is difficult. This limitation has to be considered carefully when planning, conducting, and analyzing a market survey. Because market surveys are largely based on interviews, methodology explained above will be useful.

Market Survey Process

a) Locate Markets: Determine where fish and meat markets are; explore and inquire for turtle products. Visit beaches and inquire from people collecting turtles and turtle products where their products are sold. Check garbage piles and other disposal sites associated with markets for turtle remnants, especially shells.

b) Locate Vendors: Visit and converse with vendors, or be introduced by a respected individual. Be aware of politics between vendors and your introducer or those accompanying you. A controversial accomplice could jeopardize the representation and accuracy of your survey.

c) Explain Survey Objectives and Use of Information: Solicit participation in the survey through collaboration and support from a vendors' organization or other form of respected leadership.

d) Extend Courtesy: Respect the wishes of individuals who do not want to participate in the survey, and understand their concerns. Their concerns may provide insight into other more appropriate survey techniques and the complicated and sensitive nature of turtle sales.

e) Solicit Information: Interview vendors, consumers, and middle-merchants (see Appendix II for questions).

f) Observe Market Activities: Spend a day at the market, with a vendor, or follow a group of captured turtles from the collection point to the final consumer.

g) Verify Information: Cross-check and ground-truth information compiled by observation and supplementary interviews of the same vendors or other persons in the area. If possible, visit the same market or location over a period of time to compare information compiled at different times of the day, on different days of the week, and between seasons.

h) Follow-up: Provide feedback to interviewees on survey results, discuss relevance of information, and discuss potential problems and solutions. Share information on sea turtles in the area and worldwide; if appropriate, offer materials to the interviewee to keep.

A similar process is recommended when surveying for worked products; that is, the retail sale (often but not always to foreign tourists) of tortoiseshell items, painted whole shells, and restaurant fare.

Appendix I

General Questions for Compiling Information on the Ecology and Conservation of Sea Turtles in a Locality

Biology, Status, and Distribution

1) How many turtles are seen in this area (abundance; number seen per day per distance or area unit, how many nests per distance or area unit)?

    What types or species? How do you identify species (descriptions or diagnostic characteristics)? What names are used locally? Which turtles are the most common? Can you rank them in abundance?

[Note: Later, use color photographs to obtain further identification and verification.]

2) Where are turtles found (habitats)? What are they doing there?

    What times of the year (seasonality) are the turtles encountered? When is the peak period? Are turtles seen moving through the area? Where do you think they come from and where do you think they are going?

    What sizes and sexes are seen? How do you tell the difference? Where is each group found? During what times of the year?

3) How many turtles were found in the area (specify nesting, foraging or captured) 10 / 20 / 50 years ago? Why has a change occurred?

    What are some of the ways turtles die or are killed or are lost in the area *(e.g.,* human utilization, habitat destruction, incidental catch)? How many? Where? When?

Utilization and Commercialization

4) Does turtling occur in the area? Are turtles and turtle products sold locally, nationally or internationally?

    How many turtles are caught (species, sizes, sexes)? How, where, when, and how often are they caught?

5) What are they used for (products: meat, eggs, etc. and purpose: daily food/subsistence, for commercial sale, ceremony/cultural use, etc.)?

    How many people are involved with catching and distributing turtles/products? Has this number changed in recent years/decades?

    How much of food and/or income is obtained from turtles in relation to daily diet and sources of income?

    What percentage of people in community regularly utilize turtles?

6) What are the selling prices of turtles and their parts?

    Since when have sea turtles been utilized as a source of food/income?

[Note: See Appendix II for more questions relating to Market Surveys.]

Laws and Conservation Programs, and Attitudes towards Them

7) Are there local agreements or laws that control the collecting of sea turtles? Are they working? Why? Who enforces them? How are people's lives and livelihoods touched by them? How have they responded? Are they necessary/unnecessary? Are they fair/unfair?

    Are there any conservation programs in the area? Do they include sea turtles? What turtle conservation activities are undertaken? Who is in charge of these programs? Are people from the area involved with these programs? What do the people in the area think about these programs? Where do the funds come from for these programs?

8) Are there any government agencies or other organizations managing sea turtle projects in the area? Who are they? What do they do? What do the people in the area think of them and what they do?

    Has any information been shared with the people in the area about the turtles? By whom? What kind? When? How was it received?

9) Have flipper (or other) tags been seen on any turtles? Are these tags collected? What is done with collected tags? What do you think these tags are (purpose, origin)?

Information on the Locality

What is the spoken language?

10) How many people live here? How many communities? What are the names of the communities?

How long have the people been in the area? How do they identify themselves, by what name?

11) What are their common livelihoods? How long have they been in these livelihoods?

    What facilities are available in the area (school, hospital, hotel, industry, port, etc)?

12) What is the total area of coastline/ reefs/ seagrass in the area? What are the names of the locations (waters, beaches)?

Information Source (Interviewee)

13) Name

    Address

14) Occupation / numbers of years in occupation

    Sex / age

15) Date and location of interview

Appendix II

General Questions for Compiling Information on Market Aspects

Market Survey Information

16) Name and location of market, retail outlet, or restaurant.

    Date, day of week, and time visited.

17) Numbers of vendors offering sea turtles, turtle parts, and turtle products.

    Numbers of turtles, species, sizes, and sexes.

18) List of items, frequency/seasonality of availability, and popularity/demand of selected items.

    Prices of items for sale by item, size, or weight for whole turtles, parts, and products, including cost to vendor to acquire items offered for sale. Prices may vary by demand and supply of turtles, as well as time of day *(e.g.* vendors in localities without cold storage may try to dispose of remaining meats and eggs towards the end of the day at relatively low prices).

19) Sources of turtles, turtle parts, and products, including locations of collection from the ocean/ beach, seasonality, locations and livelihoods of collectors, presence of middle-buyers/sellers.

    Intended consumers: purpose of and reason for purchase *(e.g.* food, decoration, ceremony/ festivity, belief, resale, home use, restaurant/bar).

20) Organization of market and vendors. This information may provide insight into price determination and price fluctuations, competition, control of the number of vendors selling turtle items, and the presence/absence of a participatory body in turtle conservation activities.

21) Record the number of vendors surveyed (also record this as a percentage of the total number of vendors at that location) to determine sample size and statistical reliability.

5

Reducing Threats

Research and Management Techniques for the Conservation of Sea Turtles
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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Reducing Threats to Turtles

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Around the world, the survival of seven species of sea turtle is threatened by a variety of man-induced factors, including the direct and indirect harvest of adults and juveniles (see Oravetz, this volume), threats to eggs and hatchlings (see Boulon, this volume; Mortimer, this volume), the degradation or loss of nesting habitat (see Witherington, this volume), and pollution of the seas (see Gibson and Smith, this volume). Perhaps no threat is as pervasive and devastating to declining populations as the persistent take of adult and juvenile turtles. The take continues, often in contravention of existing national and international legislation, largely because of familiar and ineffective "top-down" approaches to conservation, and a lack of grassroots support for or understanding of conservation initiatives. In response, conservation organizations and regulatory agencies alike are investing heavily in community-based conservation (see Frazier, this volume). Community-based conservation involves changing habits and outlooks, neither of which happens easily.

Changing Habits

One of the greatest and most complex challenges to the long term conservation of sea turtles is changing the habits of coastal communities in which natural resource use is a vital source of income and essential to survival. Conservation of endangered species has traditionally implied interfering with human survival in such communities. It is necessary to change the paradigm that conservation is a barrier to human survival, or to socio-economic development. Local people who depend on these creatures for their livelihood must be drawn into conservation and research programs which generate direct and indirect benefits to their communities. This is the only way to immediately address the challenge without negatively affecting the socio-economic structure (or potential) of those involved in natural resource use.

Assess and Understand Primary Community Needs, As Well as Potential Program Benefits

In establishing a conservation program, it is essential to evaluate all pertinent socio-cultural issues. To propose viable alternatives, it is necessary to learn about and understand the most important needs of each community, respect local culture, and analyze the role of sea turtles in generating family income. Creating jobs and new environmentally friendly sources of income tailored to each individual community are realistic ways of promoting the conservation not only of sea turtles, but of the ecosystem as a whole.

Alternative ways of life can only be identified and understood when program managers live in local communities. By participating in local celebrations and meetings, witnessing day-to-day problems, identifying natural leadership and organized groups, and helping whenever possible, communication with residents is increased. The information obtained from these interactions is valuable in evaluating practical measures intended to compensate for previous harvesting activities. Community involvement also allows the program to represent or aid the communities in gaining support from government and non-government organizations involved with sustainable development, health, education, and, consequently, conservation.

Program managers and participants should support existing community organizations, such as residential associations, groups of fishermen, schools, cooperatives, and regional producers; to encourage the formation of such groups where they do not already exist so that collectively beneficial activities are carried out; and to actively participate in community and environmental councils at local, state, and federal levels, as a means of sharing responsibility and obtaining more support for and knowledge of measures required for implementing conservation programs.

Develop Alternative Programs and New Sources of Income

Program activities can increase community involvement if local circumstances (training, available materials) are considered. Such activities, ranging from production to education, can provide income and disseminate information and culture, heightening environmental consciousness and preparing new generations for the future. Production and marketing of conservation oriented products based on species conservation programs has provided an alternative for financing such activities through a direct relationship with the communities, where profit is reinvested in education, health, jobs, and training. With these objectives in mind, small companies producing clothing (T-shirts, hats, beach wear) or artisan groups may be organized by the conservation program or encouraged to work as cooperatives or individually, always aiming to include as many people as possible.

Before initiating such activities, a budget must be projected which will support them until they become self-sufficient. There are many ways of financing specific social programs, including various inter-governmental development banks, non-government organizations, and government sources. Integrated activities, such as the production of T-shirts in cooperation with a paper recycling group whose product is used as packaging, make more efficient use of local talent, increase profitability and broaden the educational scope of the program. Selective garbage collection is required for paper recycling, and thus those responsible for one project must become involved in related projects, enabling the system to function as a whole.

Many lucrative activities can be carried out by children and young people, as long as these jobs do not replace school (which often happens in developing countries). The formation of links between sea turtle conservation and tourism in suitable communities (that is, in communities where essential infrastructure, including access, is available) can involve a significant percentage of the population and fuel local economies. These endeavors *(e.g.,* small permanent visitor's centers, museums, bars, bed and breakfast facilities, restaurants, craft outlets) should aim to provide direct benefits to each community. Economic exploitation "from the outside to the inside", where only a small part of the profits effectively reach the communities, is counterproductive and should be avoided.

Visitor's centers in areas of program activity provide opportunities for direct contact between residents, visitors, and sea turtles. Such centers, which may include a small museum, retail store(s), display tanks containing local species in various life cycle stages, and signs explaining species biology and status, as well as program activities, are important tools for education and fund-raising campaigns. They must be adapted to local characteristics, ranging accordingly from small structures tailored to local demands, to more sophisticated projects capable of accommodating large numbers of tourists. The museum may serve multiple purposes, sponsoring activities such as video clubs, art centers, and school group presentations.

Hiring fishermen to carry out sea turtle conservation and management activities not only provides an alternative source of income, it also makes future resource administration by the community possible. Providing information about more efficient and responsible fishing methods may improve local living conditions and avert stock depletion. Other ecologically viable solutions include changing habits and implementing nontraditional activities, such as managed fisheries, thus exposing young fishermen to conservationist views.

Changing Outlooks

In establishing conservation programs, it is important to identify where gaps exist in the knowledge of the constituencies being addressed. Local communities are indispensable to conservation programs, as are other sectors of society, including politicians, corporate interests, the scientific community, foundations, donors, sponsors, and opinion-makers in general. Public support perpetuates the conservation program, and, consequently, enhances the survival of sea turtles and other target resources.

Those responsible for technical and legal aspects of conservation (legislation in priority feeding and breeding areas, creation of national parks and biological reserves), are often physically distant from the problem, or may not possess basic information on the subject. This is also the case with foundations and sponsors (government, non-government), as well as coastal landowners and developers. As a deeper understanding of (and public approval of) the pertinent issues increases, leverage is gained for achieving advances in all aspects, including improving legislation, securing financial resources, and garnering further private and public sector support.

It is increasingly important for conservation program managers to understand that technical work plans and personal dedication are insufficient for success. It is essential that the process of securing financial resources for conservation activities be professional and viable. Involvement of executive directors must be complete, integrated to all aspects required for successful project completion, and not only those linked to academia, administration, or conservation. There are many formal and informal ways of promoting environmental consciousness and consequent increases in favorable public opinion.

Environmental Communication and Education

Promotion and development of educational campaigns can be undertaken using various communication tools, such as marketing (publicity, public relations, events, merchandising), mass media (radio, television, newspapers, magazines) and others, including multi-media, flyers, posters, exhibits, oral presentations, debates, and publications regarding program initiatives. Many politicians, businessmen, and institutional leaders, among others, possess neither the opportunity nor the will to learn about conservation program initiatives, including field activities. These are usually carried out in remote areas which are difficult to reach and offer limited accommodations. Therefore, it is important to "bring" these programs to all relevant sectors, using the means described above.

Media interest in environmental issues is on the rise, and can be taken advantage of by providing information regularly to media channels. This presents a difficult task for program directors, whose professional education typically has not included this process. Nevertheless it is as important as field work, for distribution and granting of financial resources, as well as public approval, are often heavily influenced by media coverage. Conservation programs must absorb and use all of the modern communications tools available, just as other public and private institutions do.

The relative ease with which sea turtles can be photographed or filmed, as compared to other wild animals, is a positive aspect of using visual images in publicity campaigns. Images of females nesting, juveniles diving and foraging, and hatchlings crawling to the sea have great visual appeal, and are thus capable of positively influencing public opinion. Incorporating these images in public events, campaigns, T-shirts, festivals, and handicrafts, as well as publicizing the idea of conservation and encouraging various sectors of society to support it, also benefits local community members who emphasize the familiar images in their own marketing initiatives.

Sponsors are more interested in financing programs that provide potential market advantages, where results and achievements can be publicized. Publicity increases institutional, financial, and political credibility. Thus, the "image" of the program must be valued as much as possible. By creating an exclusive logo that identifies the program, it is even feasible to partially finance field work by charging royalties and licensing the logo's use. Programs with well established images are also more easily publicized.

Short institutional videos (12-20 minutes) focusing on project aspects and sea turtle biology are an effective means of presenting a conservation program. Technical films, often too long and too detailed, are not as effective for most viewers. To most efficiently counter the persistent lack of funding besieging conservation, promotional videos and films should be generic and simple, so as to be useful in various situations (communities, sponsors, schools and universities, government and non-government institutions; the videos can also be sold).

A high quality photographic collection is essential for organizing exhibits and talks. It is also useful for compiling teaching materials *(e.g.*, pamphlets, posters) and providing images to newspapers and magazines. Those working in the field are most likely to document natural phenomena; thus, it is always a worthwhile investment to include high quality photographic and video equipment in the project budget. Presentations which include photographs, videos, multi-media and other resources in well visited areas *(e.g.*, aquaria, museums, schools and universities, retail malls) also increase public awareness of sea turtle conservation.

Support for the conservation program in terms of the legal aspects of protection is secured through a steady and constant relationship with Government. Sea turtle conservation is also promoted by employing lobbying techniques that seek to educate government sectors and demonstrate that cooperation is possible. It is also important to involve renowned politicians in environmental issues, to participate in decision making, and to share positive results. A priority in meeting program goals should be to undertake an educational campaign that makes use of media channels targeting particular constituencies, such as the policy-making community.

At the grassroots level, integrating the program into daily life ensures that new generations are raised with a more conservationist outlook. Applied methods of environmental education include specific courses and activities *(e.g.,* paper recycling, selective garbage collection, junior ecological tour guiding, community gardens) that involve youth groups. It is also useful to include local inhabitants in enjoyable program aspects, such as the release of hatchlings. In this way, sea turtles act as "flagship species", encouraging a general ecological sensitivity and concern. Isolated conservation programs which do not have public support become fragile and vulnerable. Chances for long-term success are increased by support on all levels, from ministers to fisherman.

Secondary and University Student Training Programs

Training programs and internships for secondary, university, or post-graduate students provide practical experience and are vital in educating future conservationists and natural resource managers. Interns should be exposed not only to sea turtle biology, but also to the realities and difficulties of conservation programs. Courses taught in school do not often include community interaction, fund-raising, and institutional representation in varying real life situations. At the same time, programs must also make research that complements conservation activities a priority. Technical co-operation and partnerships with local and international universities are indispensable in this respect. Universities, besides being focused research institutions, are endowed with financial and human resources usually unavailable to conservation programs.

Evaluating Success

The following milestones should be taken into consideration when evaluating program success: (1) the number of community members involved in the program's conservation, production, and marketing efforts and other related services, or receiving indirect benefits from the program; (2) improvements in quality of life at the community level *(e.g.,* education, per capita income, access to consumer goods, health); (3) a decline in the number of nests poached, nesting females killed, and animals captured accidentally or intentionally during fishing; (4) the implementation of specific, effective legislation for the protection of sea turtles; (5) creation of and support for protected areas benefitting sea turtles; (6) profit generated by program products, and the percentage invested in the protection of sea turtles and in local community programs; and (7) an increasing number of community members and others familiar with the sea turtle conservation program.

Acknowledgments

The authors wish to thank Otto Freitas for his help in the final text (Portuguese version) and Eugenia Naro, Alexandre Silveira and Manjula Tiwari for the translation to English.

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Reducing Threats to Eggs and Hatchlings: *In Situ* Protection

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Once a clutch of sea turtle eggs has been laid, the female leaves the beach, offering no protection to eggs or emergent hatchlings. From this point forward, eggs and hatchlings are subject to a number of natural threats *(e.g.,* beach erosion, storm and tidal inundation, native predators) and other hazards *(e.g.,* poaching, non-native predators and livestock, coastal development). A variety of *in situ* methods have been developed to reduce the effects of these threats. This chapter will describe some of these techniques, and offer examples of their application and success.

It should be noted from the outset that the preferred option is always the least manipulative intervention that will yield the desired result. Collecting and reburying eggs should be considered only as a last resort (see also Mortimer, this volume). Against some threats, including depredation, beach surveillance and nest caging are likely to be more effective and result in higher rates of hatching success than could be expected from *in situ* egg reburial programs. Of all the options discussed in this chapter, aversive conditioning and predator control are least likely to produce desirable results.

The reader is referred to Witherington, this volume, for solutions to specific threats *(e.g.,* armoring, artificial lighting, and recreational activities) posed by coastal development.

Beach Patrols and Disguising Nests

The presence of researchers or surveillance personnel *(e.g.,* law enforcement officers, voluntary game wardens, community activists) on the nesting beach can reduce or even eliminate a variety of threats, including egg poaching, depredation, and, in the case of hatchlings, entrapment in beach debris or disorientation inland toward artificial light sources. Some predators, such as wild hogs (*Sus* sp.) or chronically undernourished dogs, may not be dissuaded, but most small mammals and predatory birds (and poachers) are reticent to act in the presence of humans.

To reduce the likelihood that poachers will determine the pattern of surveillance (enabling them to focus their activities during non-surveillance periods), beach patrols should either be continuous (all-night) or should occur at random intervals, often enough to act as a deterrent. In either case, nests should be disguised by effacing the physical evidence with a palm frond, rake, or by walking back and forth repeatedly over the site. The objective is to smooth out the site to match the surrounding beach, reducing the likelihood that a poacher will expend energy probing the area. This method should not be used on beaches where ongoing management efforts depend on daily or weekly crawl counts to assess the status of the turtle population.

When “disguising” a nest from predators, masking odors *(e.g.,* urine, pepper sauce) are sometimes applied to the immediate vicinity of the nest with an aim to confuse or repel non-human predators. There are no data available with which to evaluate the success of these actions. Care should be taken not to introduce noxious chemicals to the beach environment that may be harmful to the developing embryos, emergent hatchlings, gravid females, or non-target wildlife.

Buried Mesh and Caging

Depredation of turtle eggs generally involves digging into a freshly laid or newly hatched nest. The placement of treated *(e.g.,* galvanized or plastic coated) wire or rigid plastic mesh just below (and parallel to) the sand surface or, alternatively, formed as a cage over the nest can deter nest excavation. It is important to use mesh small enough to prevent access by the predator, yet large enough to allow the passage of hatchlings to the surface.

For medium-sized mammals *(e.g.,* dogs; raccoons, *Procyon lotor;* hogs; coati mundis, *Nasua nasua, N. narica),* a 1 m square section of 5 x 10 cm mesh galvanized welded wire, anchored with corner stakes, should be placed over the nest as soon as possible (see Jordan, 1994). In Jordan's study, the stakes were fashioned from 60-90 cm steel reinforcing bar, bent to form a hook at the top which secured the corners of the screen. For smaller mammals, such as mongoose *(Herpestes auropunctatus),* a smaller mesh can be used but must be removed prior to hatching. In any case, the mesh should be buried 8-10 cm below the surface to conceal it from predators and curious pedestrians on the one hand, while precluding any interference with the incubating eggs on the other hand.

Galvanized wire mesh cages can be formed in a ring or in a square. The square shape is often advocated because it allows the wire constituting the four faces to be bent outward at the bottom, discouraging digging by small mammals. Addison (1997) illustrated the construction of a 90 x 90 x 75 cm cage from 5 x 10 cm mesh screening, with the bottom 15 cm bent horizontally outwards. Optimally, cages are buried to a depth of 30 cm over the nest. This is accomplished by centering the cage over the nest, setting it aside, and then excavating a 90 x 90 cm trench 30 cm deep around the nest. Dry, surface sand should be swept aside prior to digging. Once the trench is in place, the cage is placed in the trench and backfilled, leaving approximately 45 cm of the cage above the sand, thus preventing predators from digging into the nest. Addison and Henricy (1994) determined that cages were more effective than just using flat mesh, although they are more visible than the buried mesh.

Ratnaswamy (1995) used predator removal, nest screening and conditioned taste aversion at Canaveral National Seashore, Florida USA. She found nest screening to be the most effective, though the most costly, method in reducing nest depredation. In addition, this method reduces any direct impact to local raccoon populations, and therefore presumably reduces potentially adverse ecological effects of predator removal.

Translocating Eggs

While the first and best management choice should always be to protect eggs *in situ*, there are circumstances under which the movement of eggs is a viable conservation option. The removal of eggs from a natural nest (typically at the time of deposition) and their reburial elsewhere on the beach can be effective in mitigating for a variety of threats that reduce hatching success or result in high levels of nest loss. The technique is most useful under the following circumstances:

Severe and Predictable Erosion

Many sandy beaches are subject to seasonal or storm-related erosion and deposition (accretion) cycles which can lead to nest loss when portions of the beach succumb to changes in current direction or velocity. By carefully relocating nests laid in known high risk areas (areas with serious and predictable erosion) to more stable beach zones, seasonal reproductive output can be significantly enhanced.

Inundation

Low profile areas where the subterranean water table comes within 50 cm of the beach surface can result in standing water in the nest cavity; the usual result is high embryo mortality. Relocation of these nests to higher profile areas can significantly increase hatch success. Similarly, nests laid very near the sea may be lost prior to term. Relocation of these eggs can result in at least moderately good hatch success.

Poaching

Field signs, including beach crawls and nesting pits, can be rendered ineffective by removing eggs from their natural nest and relocating them to another site, even one very close to the original nest but outside of the crawl and body pit area. Excavation of the original site may ensue, but to no avail, making it less likely that the egg collector will return.

In situ

(non-hatchery) relocation is best accomplished using regular beach patrols, enabling the collection of eggs at deposition. Eggs are gently gathered as they drop and placed immediately in a clean bag, bucket or basket. Alternatively, a plastic bag can be positioned in the hole to receive the eggs. In either case, the bag or other container must be strong enough to reliably carry up to 12 kg of eggs. If a bag is placed in the hole, the opening should be clasped shut (to exclude falling sand) and the bag swiftly dug out from behind as soon as egg-laying is complete. Assistance may be needed during this process to hold flippers out of the way, provide light or to receive eggs. Efforts should be made to minimize the amount of sand gathered with the eggs. Sand will score (abrade) the egg shells and can reduce hatch success. If the eggs are to be transported a long distance, they should be covered to reduce moisture loss.

Care must be taken to record nest depth so the original dimensions can be replicated. Suspend a stiff tape measure or weighted string (scored in metric units) in the hole until it reaches the bottom and read the depth at the sand surface. Since the original sand surface is often effaced by the nesting process, it is suitably accurate for most species to record the depth at the bottom edge of the carapace just behind a rear flipper. It is important not only that the measurement be as accurate as possible, but that the technique be consistent. A measurement should also be taken of the diameter of the neck of the nest.

Eggs should be transported immediately to the relocation site (if transport occurs by vehicle, the egg bag/bucket should be secured and cushioned). Reburial should occur within 1-6 hr to minimize movement-induced injury to embryos, and the negative effects of changes in the temperature and moisture content of the eggs. To simplify project logistics, minimize transport trauma, and promote the perpetuation of the population at its chosen nesting beach, every effort should be made to translocate eggs elsewhere on the same nesting beach. The new nest site should be sufficiently above the high tide line and conform with species-specific parameters; *e.g.,* leatherback nests on the open beach, hawksbill nests in the beach forest (if appropriate to the site). Care should be taken not to locate the nest too near (< 1.0 m) other translocated clutches, or natural nests.

To begin the reburial process, dry surface sand is swept aside (to a depth of 5-10 cm, depending on local conditions) to prevent it from sifting into the excavation. Once the damp subsurface is exposed, a narrow shaft to the desired depth is excavated using one hand. The weight of the person excavating the nest should rest heavily on the other hand, and as far from the rim of the hole as possible. When proper nest depth has been confirmed using a tape measure or weighted line, the neck of the nest is widened, again using one hand, to the desired diameter. Finally, the egg chamber is widened at the bottom so the finished nest resembles a flask or inverted light bulb.

The eggs should be placed carefully, not dropped, in groups of 2-5 (a comfortable handful) and counted. In the case of leatherback turtles, the yolkless eggs should be placed last *(i.e.,* on top). Burying a short length of colorful surveyor's tape with the eggs (see Miller, this volume) is useful if nest contents will ultimately be examined. Using a permanent marker, record the tag number of the female (if tagged), the date the nest was laid, and, if different, the date the nest was reburied. Cover the nest by replacing the damp subsurface sand removed from the hole (do not place hot surface sand on the eggs), firmly tamping it in place in layers of 8-12 cm.

Once the hole is completely filled, it is difficult to locate the nest with accuracy. Therefore, if the nest will be monitored through time or excavated at hatching, coordinates should be recorded (measurements to the nest from numbered stakes or natural landmarks on either side of the nest) or the nest otherwise marked at this time. Once the hole is filled, disguise the nest by smoothing over the disturbed sand surface, sweeping dry surface sand evenly all about.

In assessing this technique it should be noted that average hatch success will likely be measurably lower than that of undisturbed natural nests. But, when the process is undertaken with care, the technique is effective at reducing nest loss to threatening agents listed at the beginning of this section. More than a decade of experience with the leatherback population nesting at Sandy Point National Wildlife Refuge, U. S. Virgin Islands, clearly shows that annual reproductive success can be doubled or better using this technique in the context of regular all-night patrols of the nesting beach, collection of eggs at deposition (if laid in documented high risk zones), and immediate reburial (Boulon *et al.*, 1996).

The technique enjoys several advantages over moving eggs to an enclosed hatchery. Maintenance and personnel (surveillance) costs are high in properly maintained hatcheries; in addition, losses due to depredation, storms, and various other factors can be severe because eggs (and hatchlings) are artificially concentrated. Other factors also favor *in situ* reburial, including the fact that nest sites are unmarked, temperature and moisture profiles are likely to be closer to the norm since nests can be individually placed in appropriate habitat, and hatchlings emerge naturally.

Notwithstanding, it cannot be overemphasized that eggs should never be collected and reburied unless there is compelling evidence that significant losses will accrue which cannot be countered using non-manipulative strategies. Moreover, in choosing this technique, managers must be willing to commit the resources requisite to ensure that eggs are properly collected either at deposition or at first light the following morning (before the heat of the day). Under no circumstances (barring the emergency rescue of eggs found washing out to sea) should eggs be collected more than 12 hr after deposition. Once the embryo has settled on the wall of the egg shell, movement can be fatal. Indeed, some investigators have recommended that eggs not be moved at all between 3 hr and 21 days after oviposition (Harry and Limpus, 1989).

Aversive Conditioning

With this technique, predators are conditioned to avoid prey items by the selective use of chemicals which cause an unpleasant (sometimes very unpleasant) reaction when consumed. Researchers have used lithium chloride and various hormones in and on eggs in hopes that predators, especially small mammals, will lose their desire to consume turtle eggs. The author has been unable to find any researchers who can report the successful accomplishment of this technique for turtle eggs. Hopkins and Murphy (1982) found that lithium chloride did not work with raccoons in the lab or in the field. They determined that it might work on individuals who had never before eaten sea turtle eggs, but individuals characterized by a prior positive experience with eggs would not become aversively conditioned using this technique.

Ratnaswamy (1995) used oral estrogen (17-alpha-ethinyl-estradiol) to treat chicken eggs in an attempt to produce conditioned taste aversion (CTA) in raccoons. The hope was that any CTA developed towards chicken eggs would be transferred to turtle eggs. She found no significant difference in turtle egg depredation before and after treatment. She concluded, given the relatively large size of the raccoon population, that it may be impossible to develop a level of CTA adequate to protect turtle nests at the study site. It is not known whether raccoons can detect taste differences between chicken and turtle eggs which may complicate effective application of the CTA treatment.

One study in the U. S. Virgin Islands successfully conditioned mongooses to avoid chicken eggs using the hormone estradiol (D. W. Nellis, USVI, Div. Fish Wildl., pers. comm.). The process involved familiarizing mongooses with several sources of eggs over a period of time. The eggs were then injected with estradiol for two days, before returning to untreated eggs. After their experience with the treated eggs, the mongooses refused subsequent offers of chicken eggs. The test was never performed on sea turtle eggs, as the sacrifice of eggs from Endangered sea turtles did not seem prudent, but the technique shows promise.

Predator Control

While some nest depredation is certainly opportunistic, the habit of raiding turtle nests is, for at least some predator species, clearly a learned behavior. Predator control, broadly speaking, encompasses a variety of techniques, all of which are time consuming, some of which are very expensive, and few of which have shown consistently favorable results. Nevertheless, some methodologies may be worth pursuing if depredation constitutes a serious threat; that is, a threat well beyond the natural cycles of the food web.

Perhaps the least complicated method is to shoot the offending animals. This method has been used to cull feral dogs at some Central American nesting beaches, raccoons and hogs in the southeastern U.S., and a variety of “pest” species elsewhere in the world. Public hunts work moderately well, depending on the circumstances; relatively unpopulated areas are most conducive. If planning such a course of action, bear in mind the possibility of outcry from animal-rights groups or individuals. Some success may also be achieved with inexpensive poisoning campaigns, but such initiatives are almost certain to bring unwanted consequences in the form of death to non-target (and often beneficial) coastal species, as well as to children and/or domesticated animals.

Trapping programs are more expensive, but can yield satisfactory results. In the Caribbean region, where the mongoose is a significant predator on both eggs and hatchlings, conventional live traps (15 x 15 x 45 cm) baited with chicken or fish are sometimes set at 30 m intervals in shaded areas on the upper beach. Traps are checked at least daily. Captured individuals are either relocated to distant locales or euthanized. Five days of trapping can remove over 80% of the mongooses in a localized area (Coblentz and Coblentz, 1985). In the Coblentz and Coblentz study, hawksbill turtle nests suffered no depredation during or immediately following the trapping interval at the nesting beach. By trapping just prior to the nesting season, nest depredation may be significantly lowered during the season, depending on the number of immigrants and young-of-the-year, both of which may be less apt to depredate turtle nests until they have learned this behavior.

Similar success is reported by George *et al.* (1994) in the trapping and relocation of raccoons from the nesting beaches of the southeastern U.S. They concluded that the removal of large numbers of individuals from the predator population at the nesting beach clearly reduces the incidence of nest depredation. The real success of relocation programs ultimately depends on the propensity of the offending animal(s) to return to the territory from which they were removed. When possible, traps should be set along game trails leading to the beach. Again, the highest degree of effectiveness is obtained by campaigns just prior to the nesting season. If the problem persists into the nesting season, traps can be set near known nests. For humane reasons, live traps are preferred. Barring government restrictions, trapped animals may be transported inland and released; alternatively, humane disposition *(e.g.,* lethal injection, shooting) should be considered.

In New Zealand, Hawaii (U.S.), the Philippines, and the Galapagos Islands (Ecuador), trained "pig dogs" have been used to control feral hogs which threaten turtle nests. The use of these dogs was originally to harvest pigs, but their use has recently been considered for both sea turtle and tortoise protection (Clarke and Brisbin, 1994). This technique may be beyond the undertaking of most sea turtle management authorities, but is worth considering if feral (unclaimed) hogs are a serious problem.

Among insect predators, fire ants, which have been known to burrow into nests and attack hatchlings as they emerge from their eggs, may present the most serious threat. The use of chemicals around nests to deter ants is unacceptable, due to the potential for secondary toxicity and harm to hatchlings. On Ocean Isle Beach, North Carolina, U.S., dry grits (coarse ground corn) are sprinkled around nests twice a week and after any rain. Fire ants eat the grits, which supposedly swell and kill the ants; none of the treated nests were infested with fire ants (J. Simmons, pers. comm.).

Before contemplating any eradication program, the following caveats must be kept uppermost in mind. The systematic control of non-native species *(e.g.,* hogs, rats, dogs, mongooses), or widespread species of small mammals or birds whose population sizes are clearly exaggerated due to the presence of human settlements, is unlikely to have a detrimental effect on the larger coastal ecology. However, the removal of native predators *(e.g.,* vultures and other birds of prey, crabs, shore birds, snakes) from a nesting beach and its environs may be ecologically devastating. Poisons and indiscriminate traps may extract a heavy toll on non-target species, as well as on children. Their use should be carefully controlled.

Public Education

While not generally considered an *in situ* protection measure, education can play a significant role in protecting sea turtle nests and hatchlings. For example, in lieu of undertaking a predator control program, managers should consider whether changing the behavior of people might achieve a similar result. Establishing public dump sites well away from nesting beaches and controlling beach litter may reduce the number of scavengers *(e.g.,* dogs, rats, mongoose, vultures) visiting the area. Promoting license and leash laws for dogs and reasonable controls on the ranging of livestock *(e.g.,* hogs) may also curb rates of depredation. Where nests are exhumed (after hatchling emergence) for study purposes, nest contents should be completely reburied in the nest cavity. Indiscriminate disposal attracts the attention of predators.

Public education campaigns can enhance the success of virtually any nest protection program. The public should be made aware of the importance of stakes and other research landmarks, as well as nest cages and other equipment left on the beach. Residents should be encouraged to support nest protection efforts by volunteering to participate in beach surveillance programs, disguising nest crawls (unless this compromises ongoing research and monitoring programs), and reporting illegal activities. Education programs should be designed for a variety of audiences, including fishermen, school children, and coastal landowners (or land controllers), both residential and commercial. By involving all parties in a conservation program, it is possible to create an attitude of stewardship that will foster compliance with conservation and management strategies.

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K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, M. Donnelly (Editors)
IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Reducing Threats to Eggs and Hatchlings: Hatcheries

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When to Build a Hatchery

Ideally, sea turtle eggs should incubate in the natural nest. Relocation of eggs to a protected hatchery site should be undertaken only as a last resort and only in cases where *in situ* protection is impossible. At most rookeries, egg relocation programs only benefit clutches deposited in dangerous circumstances—for example, those laid too near the sea, too near artificial sources of light, in armored or erosion-prone areas, or in the path of vehicle or concentrated foot traffic. But even in such cases, *in situ* protection is often sufficient (see Boulon, this volume). In many parts of the world, however, egg depredation by people or by animals associated with people is so intense that mortality approaches 100% in any clutch not relocated to an enclosed hatchery. At sites where the dominant threat is human exploitation, the hatchery must be guarded at all times.

Since the negative effects of hatcheries (see below) are often greater than the risks posed by non-human predators, managers must quantitatively assess rates of depredation before adopting a hatchery program. At sites where depredation is high enough to warrant a hatchery program, the predators involved are usually species introduced by people *(e.g.,* feral dogs, cats, pigs) or species whose populations are unnaturally high as a result of conditions created by people. Such conditions can occur where human refuse provides a supplementary food source for the predator *(e.g.,* raccoons, rats, vultures) or where people have eliminated a predator's natural enemies. Under these circumstances, predator eradication or aversion methods might be considered as complementary to or as an alternative to a hatchery (but see Boulon, this volume).

The Limitations of Hatcheries

Because hatchery programs have the following serious limitations, *they can produce a net negative impact on turtle populations.* Preliminary assessment must conclude that less manipulative options are impractical or ineffective, that hatchery sites are available, that a sufficient proportion of eggs can be collected and suitably transported to the hatchery, that personnel are available to guard the facility, and that financial resources are sufficient for maintenance. The following caveats should be considered:

1. Hatcheries are very expensive in terms of the financial and human resources required to collect and maintain each clutch;
2. The effective operation of a hatchery depends on well-trained, reliable staff, but budgetary constraints usually provide only minimum wage salaries (or force the operation to rely on volunteers);
3. Hatching success in hatcheries is usually lower than in natural nests even when hatcheries are constructed and supervised by conscientious staff;
4. Hatchling sex ratios are often skewed towards one sex or the other, depending on conditions in the hatchery (see Merchant, this volume; Godfrey and Mrosovsky, this volume, for a discussion of temperature dependent sex determination in sea turtles);
5. Improper methods of hatchling release produce high rates of mortality. When hatchlings are released at the same time and place each day, fish feeding stations are created. Moreover, by the time of their release (usually morning), the hatchlings are exhausted from a night of fruitless struggle in the hatchery, most of them having emerged from

the nest within a few hours of sunset the night before. During the night, many succumb to predators (ranging from ants and crabs to birds and small mammals);

6. The establishment of hatcheries as a compromise measure to mitigate the destruction of nesting habitat (such as that caused by artificial lighting) creates a dangerous dependence on human intervention which may be impossible to maintain over time; and
7. Hatcheries have a harmful psychological effect on people. Because they are so labor intensive, they promote or endorse a belief that participants and supporters are doing more good for turtles than they actually are. As a result, programs that are more effective but politically less attractive may be ignored.

Recommended Hatchery Methodology

Hatchery Siting and Construction

Hatcheries should be located as close as possible to the nesting beach to minimize physical trauma to eggs during transportation, to reduce the time interval between when the eggs are laid and when they are planted in the hatchery, to provide the opportunity for embryos and hatchlings to imprint on the nesting beach, and to facilitate hatchling release. To maximize the diversity of conditions in which eggs are incubated and hatchlings released, several hatcheries should be established if possible. The hatcheries should be positioned to include the range of microhabitats utilized by nesting turtles, keeping in mind the need to include representative temperature regimes. Beach surveys can provide information on nest site selection (see Schroeder and Murphy, this volume).

All sea turtle species nest above the high tide line. The surface of the hatchery site should be located at least 1 m vertical distance above the level of the highest spring tides to prevent underground flooding of the eggs. Avoid placing the hatchery where it might be inundated by tidal streams that form behind the beach during very high tides, or near the mouths of rivers or streams where routine or unpredicted flooding could destroy the hatchery. Chain link fence, wire mesh or barbed wire should enclose the hatchery. To discourage crabs and other small burrowing predators from entering the enclosure, a 1-2 m wide strip of netlon mesh (1 cm mesh) should be buried to a depth of at least 0.5 m along the inside of the fence. To prevent infestation from fungus and bacteria, the same hatchery site should not be used during two consecutive nesting seasons.

At beaches where individual nests are at risk from localized threats such as erosion, inundation, or unintentional human disturbance, threatened egg clutches can be selectively relocated to safer points along the beach (see Boulon, this volume). In such cases, where human or animal predation is not a serious problem, it is best not to construct an enclosed hatchery or to employ cylindrical mesh enclosures (see below). Ideally, hatchlings should not be dependent on people for their release.

How Many Eggs?

Many resource managers believe that to maintain a healthy nesting population, at least 70% of the eggs laid should be protected. In cases where the population has already suffered a history of over-exploitation, that figure needs to approach 100%. At some sites, predictable weather patterns produce primarily male offspring during some months and primarily female offspring during other months. To help ensure a natural sex ratio, eggs destined for the hatchery need to be obtained throughout the nesting season in numbers proportional to the amount of nesting that occurs each month for each species.

Collection and Transport

To minimize embryonic mortality due to handling, all eggs should be planted in the hatchery within 2 hr of being laid (no clutch should remain unburied for periods exceeding 5 hr). Where there is an opportunity to collect eggs as they are laid, some workers catch eggs by hand as they drop from the cloaca and place them gently in a bag or bucket. Others position a large clean plastic bag in the nest beneath the cloaca, taking care not to collapse the egg chamber or disturb the turtle (the eggs drop directly into the bag without being handled or coated by sand). The sack of eggs is removed quickly before nest covering commences, or exhumed after the turtle has finished covering her nest. In other circumstances, clutches must be excavated after the nesting turtle has returned to the sea. Eggs should always be handled with care; when transported by vehicle they should be cushioned from vibrations.

Special care is needed when handling eggs that are more than 2 hr old (for example, when translocating eggs the following morning or when salvaging mid-term clutches that become threatened by erosion). The delicate embryonic membranes of older eggs are easily torn if the eggs are rotated or jarred. Dislodgement of the embryo results in death. Precautions should include marking the top of the egg with a soft grease pencil and transfering the eggs to a bucket or other inflexible container (not a sack) to ensure that they are not rotated either during transport or during reburial.

Reburial

Insofar as possible, each egg clutch should be planted within the hatchery enclosure in microhabitat approximating its natural nest. Hatchery nests should be situated at least 1 m apart to minimize their impact upon one another and to allow room for hatchery caretakers to move about. Nests should be constructed in the shape of a flask or urn, with a rounded bottom and a straight narrow opening leading from the egg chamber to the surface. Natural nest depth should be measured and duplicated in the hatchery. If nest excavation is hampered by cave-ins during periods of very dry weather, pour a bucket of fresh water into the unfinished nest, and then continue nest construction. Place eggs into the hatchery nests a few at a time if eggs are less than 2 hr old (one at a time if eggs are holder than 2 hr); under no circumstances should the eggs be “poured” into the nest. The damp sand removed during excavation of the artificial nest should be used to cover the eggs, firmly tamping it in place in layers of 8-12 cm. Dry sand should not contact the eggs, and should be used only during the final stages of covering the nest. Each nest should be numbered and associated with a standard data record form (see also Miller, this volume).

Cylindrical Mesh Enclosures

Most managers recommend placement of a cylindrical mesh enclosure over the top of each nest. These should be constructed from plastic netlon mesh (<1 cm mesh). “Chicken wire” should not be used; the mesh is too large and hatchlings get injured when their heads and flippers protrude through the openings. Netlon mesh should be cut into pieces approximately 40 cm in height and 195 cm in length, to form a cylinder 60 cm in diameter. A metal stake 0.25 cm in diameter can be used to join the ends of the mesh to form the cylinder and to secure it into the substrate. The mesh should be buried about 10 cm into the sand to reduce entry by burrowers, such as crabs. Depending on local rates and sources of depredation, the top of the cylinder can be fitted with a netlon cover, mosquito netting, or other appropriate mesh. By restraining the hatchlings that emerge, data recording *(e.g.*, number, size, weight of hatchlings) is facilitated. The disadvantage is that unless the hatchlings are released within a short time after they emerge from the nest, they are likely to suffer exhaustion, desiccation, loss of vigor, and possibly injury or death from predators.

Hatchling Release

Under natural conditions, groups of hatchlings enter the sea at random points along the nesting beach and at unpredictable times. Ideally, hatchery turtles should be released in groups as soon as possible after emerging from their nests, but early emergents should not be held back in order to create a larger group. To randomize release sites (reducing the prospect of creating fish “feeding stations”), each release should occur at a point hundreds of meters from previous release points. Hatchery personnel should anticipate hatchling emergence (noting that hatchlings usually emerge about 45-55 days after eggs are laid) and check mesh enclosures at frequent intervals (at least every 30-60 min) during periods of anticipated emergence. To promote natural imprinting, hatchlings should be allowed to crawl across the beach and enter the sea unassisted. When immediate release is impossible, hatchlings should be placed in a soft, damp cloth sack and kept in a cool, dark, quiet place. They should not be kept in water prior to release. Hatchlings kept in a container of water will engage in “swim frenzy” behavior and are likely to exhaust energy reserves stored in the yolk sac; they may even imprint to conditions in the container rather than to those at sea.

Special Techniques

Special incubating techniques have been used with varying degrees of success depending on local conditions. In the Philippines, the Sabah Turtle Islands, the Pacific coast of Guatemala and elsewhere, excessively warm hatcheries suspected of producing only female offspring have been cooled by placing coconut thatch as shade over a portion of the nests. In Malaysia, splitting egg clutches into complements of 40-60 eggs, each buried in a separate nest, has improved rates of hatching success (Mortimer *et al.*, 1994). In Natal, South Africa, hatch success improved in clutches placed inside cylindrical mesh baskets constructed of plastic netlon, and then planted in the hatchery inside the basket (G. Hughes, Natal Parks Board, *in litt* 14 September 1988). However, the same technique when employed in Malaysia produced nearly 100% mortality among late stage embryos and hatchlings (Mortimer and Aikanathan, unpubl. data). In Australia, cooling of newly laid egg clutches to a temperature of 7-10°C within a few hours of oviposition was found to delay formation of embryonic membranes long enough to allow long distance (>1000 km) translocation of egg clutches without reducing viability (Harry and Limpus, 1989).

Egg clutches incubated in Styrofoam boxes enjoy particularly high rates of hatching success, but require careful manipulation of temperature and moisture conditions. Cool temperatures characterize the boxes and favor male offspring. Because warming the boxes in the sun or with artificial heat sources causes moisture loss, careful application of fresh water is needed to keep conditions humid, but not water-logged. To prepare a Styrofoam box nest, pierce the entire bottom of the box with 0.5 cm diameter holes spaced at intervals of about 5 cm to facilitate drainage. Cut three pieces of nylon fabric, each to a size slightly larger than the surface area of the bottom of the box. Place the following materials into the pierced box, in order, starting on the bottom: one piece of nylon fabric, one 10 cm layer of moist beach sand, one piece of nylon fabric, 3-4 layers of freshly laid turtle eggs, one piece of nylon fabric, one 10 cm layer of moist beach sand. When the eggs begin to hatch, the top layer of sand and fabric need to be removed and only then should the Styrofoam lid be put in place to maintain high humidity.

Newly emerged hatchlings should be left inside the closed Styrofoam box for several days prior to their release until they have absorbed their external yolk sac and their plastron has flattened. No one knows with certainty whether Styrofoam box hatcheries interfere with the yet poorly understood mechanisms by which hatchlings imprint to their natal nesting beaches. What is known is that, without careful monitoring and intervention, hatchling sex ratios are strongly skewed and this point can invalidate nearly all other conservation measures. In Malaysia, decades of production of 100% female offspring in hatcheries has been implicated in high rates of infertility among nesting leatherback sea turtles (Chan and Liew, 1996).

Monitoring and Evaluation

Ideally, a statistically viable sample of nests should be monitored for incubation temperature in every hatchery, and certainly this is true in programs which rely on Styrofoam boxes, to ensure that both male and female offspring are produced in approximately equal proportions (see Godfrey and Mrosovsky, 1994, for a review of methodology). To determine hatch success within the hatchery, excavate a sample of clutches at the end of their incubation period (see Miller, this volume).

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K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, M. Donnelly (Editors)
IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Reducing Threats To Nesting Habitat

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Overview

Favorable nesting habitat is critical for sea turtle reproduction and is central to the survival of sea turtle populations. Threats to nesting habitat are defined as any action or process that can alter the sand substrate of the nesting beach, injure or kill sea turtles or their eggs, and/or cause the disruption of normal behavior patterns. The purpose of this section is to describe several such threats and to propose responses that promote sea turtle conservation.

There are at least four response categories in mitigating agents that threaten nesting beach habitat. The first and best response is to eliminate the threat. For example, restricting sand mining to inland deposits, prohibiting beach driving, and turning off beachfront lighting that would otherwise misdirect hatchlings. In some cases, it may be sufficient to restrict harmful activities to periods outside the nesting and hatching seasons, which extend from the date of first nesting to approximately two months after the last nest is laid.

A second response is risk reduction, or managed risk. The goal of managed risk is to reduce the probability of a threat occurring and to reduce the negative effects of a threat when is does occur. Managed risk is an important proactive response to oil spills, for example. Other applications of managed risk include the use of “turtle friendly” beachfront lighting in development plans in order to reduce the probability of hatchling misdirection and mortality, and the establishment of setback requirements for beach developments so that the need for coastal armoring is reduced.

A third response is to move eggs from high-risk areas to safer natural beach areas (see Boulon , this volume) or enclosed hatcheries (see Mortimer, this volume). Although moving eggs can sometimes be the only way to save them, this response can have many negative effects. Even careful excavation, movement, and reburial of sea turtle eggs can reduce hatching and emergence success, alter hatchling sex ratios, and reduce hatchling fitness. Moreover, egg translocation does not protect nesting females from the same effects threatening their eggs and can eliminate incentives to remove threats on the nesting beach. For these reasons, the translocation of eggs should be considered *only* as a last resort and only when high egg mortality has been demonstrated and is certain.

A fourth response is to do nothing. Some threats *(e.g.,* chronic erosion) either cannot be eliminated or threaten too few nests to justify costly mitigation. The cost of mitigation may be a financial loss, the loss of conservation opportunities elsewhere, or a biological loss *(e.g.,* harming when one intends to help). Care should be taken not to overestimate the consequences of natural threats. It is reasonable to assume that the selective pressures of these threats on sea turtles have shaped biological mechanisms to mitigate them and that nesting in locations that seem risk-prone may actually provide a fitness advantage to developing hatchlings. For instance, some nests deposited low on the beach may be successful despite moderate erosion and overwash. On some beaches, the reduction of pathogens by overwash can make these nests among the most productive nests on the beach.

Erosion and Accretion

It is in the nature of beaches to erode and accrete. When these processes become extreme during the nesting-hatching season, females can experience difficulty in nesting and eggs can be uncovered, inundated, or swept away. Extreme erosion and accretion can occur during storm events, during periods of high wind, or when the placement of man-made structures modifies the natural movement of sand along the coastline. Nesting access is reduced by eroded escarpments and by uprooted, woody dune vegetation that may subsequently accumulate on the beach. Severe accretion can deposit sand over existing nests so that developing eggs suffocate and hatchlings are prevented from escaping.

Although the natural events that cause erosion and accretion cannot be stopped, their consequences can sometimes be lessened. Fallen trees and debris can be removed (but should not be excavated) from the beach, escarpments can be leveled, and the profile of the beach can be restored by artificially "nourishing" the beach with sand (see below). No action that requires heavy machinery should be conducted during nesting and hatching seasons. Even a beach that appears devastated by erosion may have surviving nests that would be damaged by work vehicles and the movement of sand. Sometimes doing nothing is the best strategy; beaches that are unaffected by man-made stabilization structures often recover fully over the course of a few months.

Tropical storm forecasting seldom gives accurate predictions of landfall more than 24 hr in advance. Although damage from these storms can be severe, it is often localized in an area that cannot be predicted. Given the negative effects of translocating eggs and the unpredictability of storms, moving large numbers of nests prior to a forecasted storm is not recommended.

Chronic erosion, as opposed to acute storm-generated erosion, may destroy some nests placed low on the beach, but these losses are frequently overestimated. As a general rule, nests should only be translocated if they are low enough on the beach to be washed daily by tides or if they are situated in well documented high-risk areas that routinely experience serious erosion and egg loss *(e.g.,* nests laid near river mouths or beneath eroding sea walls)

Beach Armoring

Beaches are sometimes armored to protect coastal property from erosion. Armoring can include sea walls, rock revetments, sandbag structures, sand fencing, gabions, and other rigid structures. Beach armoring can eliminate nesting habitat, exacerbate erosion, block access by nesting turtles, and fatally entrap turtles. Structures built perpendicular to the coast and intended to control long-shore sand movement *(e.g.,* groins and jetties) present similar threats to nesting habitat. Such structures typically exacerbate erosion on down-current sand beaches.

The best way to reduce the threat of armoring is to eliminate the necessity for it. Any permanent structure built immediately adjacent to the beach or on the primary dune is likely to become threatened by erosion; thus, development near sea turtle nesting beaches should adhere to conservative setback requirements. On relatively stable beaches construction should not take place within approximately 50 m of the zone of mean high water. This setback distance should be greater for shorelines with more dynamic cycles of erosion and accretion. If structures do become threatened by erosion, they should be moved away from the sea if at all possible; armoring (which is expensive and very often ineffective) should be the last resort. Replacement of beach sands by beach nourishment is a preferred alternative to armoring, but presents its own suite of adverse consequences (see below).

Artificial Beach Nourishment

Artificial beach-nourishment (sometimes referred to as beach renourishment or rebuilding) is the artificial replacement of sand that has been lost to erosion. Like beach armoring, artificial nourishment only becomes necessary when valuable man-made structures are threatened by erosion (although there may be ancillary incentives, such as the desirability of a wide beach for tourism). Methods for beach nourishment include mechanically dumping or pumping sand from outside sources onto the beach or scraping sand from the lower beach to deposit it onto the upper beach.

Although beach nourishment is a preferred alternative to armoring, it is not without negative effects. The suitability of a nourished beach as nesting habitat depends on the quality of sand used and the method(s) of deposition. Some nourished beaches have an excessive clay, silt, and shell content, and may have a spatial distribution of sand grains that is poorly sorted. These conditions may leave the nourished beach prone to the formation of escarpments and may produce sand that is too compact for nest excavation by sea turtles. Sand on a nourished beach also may vary greatly in moisture content, solar reflection, and thermal conduction, which can affect nesting, hatching success, and hatchling fitness (reviewed by Crain *et al.*, 1995).

If artificial nourishment is selected as a management response, it should only be undertaken outside of the nesting-hatching season. Nourishment during the nesting-hatching season will bury nests and destroy eggs. Translocation of nests prior to nourishment projects is an incomplete way to protect nests from burial. Movement-induced mortality (to embryos) is likely and surveyors will be unable to locate some nests. Data from Florida (USA) indicate that approximately 8% of freshly deposited sea turtle nests are incorrectly identified as abandoned attempts even by trained surveyors. Nourishment activities commonly take place continuously, day and night, and require lighting, activity, and equipment that can be disruptive to nesting and fatal to hatchlings.

No nourished beach will perfectly match the sand that has eroded away. It is not clear how well quality-control of sand and choice of spreading methods can limit the differences between nourished and natural sands. A principal criterion by which nourished beaches are judged is the similarity of sand compaction to the original beach sand. Crain *et al.* (1995) offer a range of compaction values and methods by which compaction can be measured. Nourished beaches that are too compact are often tilled, however, it has not been determined whether this will significantly soften beach sands.

Sand Mining

Sand mining operations remove large quantities of sand from beaches to be used as fill, in the making of concrete, and for other construction activities. Sand mining diminishes the profile of the beach and promotes instability. The persistent removal of beach sand disrupts stabilizing vegetation, exacerbates erosion, and can eliminate nesting habitat. Mining should not be allowed to occur on sea turtle nesting beaches.

Commercial sand mining extracts sand at a faster rate than it is replenished by natural coastal processes; thus, it is a serious threat whether conducted during or outside the nesting-hatching season. Translocation of nests away from a beach to be mined is a poor solution to this threat. It is noteworthy that mining sand on beaches up- or down-current from nesting habitat also degrades nesting habitat, since large scale sand extraction disrupts the complex interchange of sediments along the coast. Similarly, mining sediments from the water near beaches should be carefully evaluated for potential effects on beach erosion, since offshore material is essential for natural beach maintenance. It is recommended that sand extraction sites be confined to inland quarries or properly evaluated offshore sites.

Beach Lighting

Artificial lighting near nesting beaches deters sea turtles from nesting and interferes with the ability of hatchlings to move from their nest to the sea. In part, hatchlings reach the sea by orienting toward the brightest horizon (see Witherington and Martin, 1996, for a review). The brightness of artificial lighting can misdirect hatchlings away from the sea and leave them vulnerable to dehydration, exhaustion, and predation. As a consequence, any artificial lighting visible from a nesting beach can cause high hatchling mortality.

Nighttime beach surveys should be conducted so that specific problem light sources can be identified. A surveyor should walk the entire length of the beach at the tide line looking for artificial light sources. Any source visible from the beach should be noted by describing its location, appearance, and methods by which it can be corrected (see methods below). Because artificial lighting problems may develop during the nesting-hatching season, multiple surveys should be conducted. A survey conducted before the nesting season begins will allow managers time to correct potential lighting problems and follow-up surveys during the season will reveal what corrections have yet to be made.

There are many ways to alter light sources so that their effect on sea turtles is reduced (Witherington and Martin, 1996). Although permanent alterations are best, temporary alterations made during the nesting-hatching season can be sufficient to protect sea turtles. The most widely applicable solutions include the following:

1. Turn lights off during the nesting-hatching season. This is the simplest, most effective, and least expensive solution, but it may not be accepted by property owners in cases where lighting is deemed essential for security or other reasons.
2. Lower, shield, recess and/or redirect lights. These actions are effective to the extent that they reduce the amount of light reaching the beach. Dune vegetation, existing buildings, and opaque shields can be used to hide light sources from the beach. Fixtures that are designed to control light well and that are directed down and away from the beach are among the best types of lighting to use near sea turtle nesting beaches (Figure 1).
3. Close curtains or blinds after dark and apply a dark tint or film to windows that face the beach. Light from the interior of buildings can also be

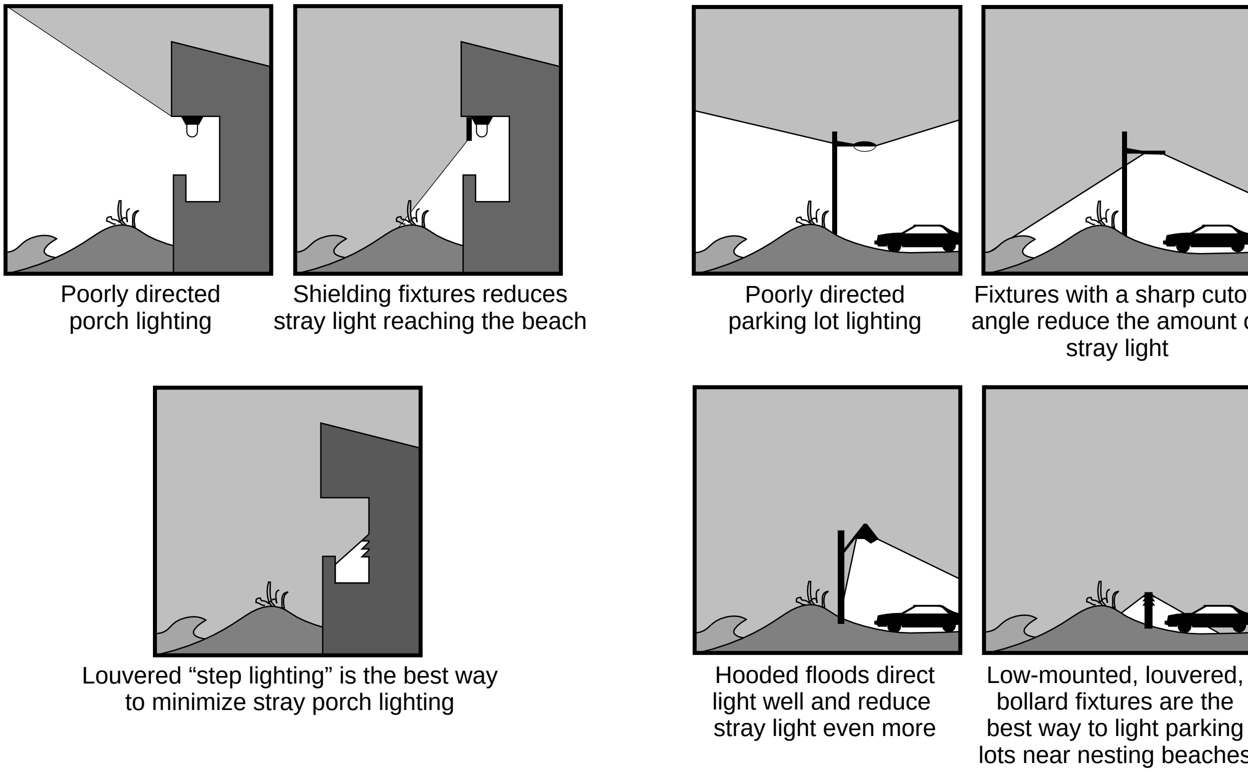

Figure 1. Light management techniques for building and pole-mounted lighting near sea turtle nesting beaches.

reduced by moving lamps away from beach-side windows.

4. Use light sources that sea turtles see poorly. Sources that emit very little short wavelength light (*e.g.,* pure yellow and red sources) are less disruptive to nesting and hatchling sea turtles than are sources that emit a substantial amount of short-wavelength light (*e.g.,* violet, blue, and green sources, or any source that appears whitish or golden). Low-pressure sodium vapor sources (not to be confused with high-pressure sodium vapor sources) are the purest yellow light sources and may be the best commercially available light sources for applications near nesting beaches. Yellow incandescent light bulbs, commonly called "bug lights," can be acceptable if used sparingly. Neither low-pressure sodium nor bug lights are completely harmless and they can affect some species more than others (Witherington and Martin, 1996); therefore, they should be shielded or directed so that they are minimally visible from the beach.

Light management rather than light prohibition is the most realistic conservation policy for developed sea turtle nesting beaches. To gain cooperation from property owners, they should be reassured that light management will allow them to direct light onto their property where it is needed as long as that light does not "leak" out onto the beach. For a detailed presentation of light management techniques for sea turtle nesting beaches, see Witherington and Martin (1996).

Vehicles, Foot Traffic, and Livestock

Vehicular activity (including beach cleaning equipment), foot traffic, and livestock on the beach all have the potential to expose or crush eggs, and to interfere with the ability of hatchlings to reach the sea. Hatchlings awaiting emergence within nests are particularly vulnerable to crushing and to entrapment resulting from a collapse of the airspace within the nest.

Heavy vehicles such as automobiles, trucks, earth-moving equipment, and beach-cleaning tractors can cause much greater disturbances than foot traffic. Wheeled and tracked vehicles that deeply penetrate soft sand leave ruts that can entrap hatchlings. Although hatchlings can escape from most footprints, they often choose to crawl for great distances within tire ruts, thereby decreasing the chances that they will enter the sea. Hatchlings may stay crawling within ruts due to their tendency for orientation toward open areas.

Mechanized beach cleaning involves the raking of flotsam and litter from the beach. Mechanical raking can penetrate nests, expose eggs, and destroy them.

Other effects of beach cleaning include effects that are common to other vehicular activities.

During the nesting-hatching season, vehicular traffic and livestock should be kept off nesting beaches and the dune, especially at night when most hatchlings emerge from nests and when females of most species attempt nesting. Due to the effects of tide on beach width, it is seldom practicable to simply restrict vehicles or livestock to the lower beach where nesting is infrequent. Because it is not yet known how extensive mortality from these sources can be, translocating nests to mitigate the damage may not be justifiable; efforts to remove the threat(s) should take precedence.

Where vehicular use is required for emergency access, law enforcement, research, or management activities, only vehicles with low-pressure tires (< 35 kPa or 5.0 psi, as with most “balloon-wheeled” all-terrain motorcycles) should be used. Vehicular activity should be restricted to below the high tide mark. Where human foot traffic is extensive, as is the case for urban bathing beaches, or where mechanized beach cleaning is conducted, nests can be cordoned off to protect them from disturbance. Raking by hand is preferred over the use of beach-cleaning machines.

Obstacles

Debris *(e.g.,* rope, fishing line, glass, metal, plastic, and Styrofoam), recreational and work equipment *(e.g.,* chairs, chaise lounges, watercraft, umbrellas, parked vehicles, pipes, refuse cans, tarpaulins), structures *(e.g.,* cabanas, shanties, animal pens, boardwalks, fencing), and other obstacles have the potential to entrap, entangle, and impede nesting turtles and their hatchlings. Potentially harmful debris should be removed from the beach at regular intervals. Complete cleaning of the beach (from the extraction of large stumps to the removal of low density accumulations of beached seaweed) is seldom necessary and may be detrimental. Seaweed and other debris should never be buried on the beach during the nesting-hatching season.

Most of the threat from recreational equipment can be eliminated by pulling equipment and watercraft off the beach at the end of the day. Cabanas and shanties should be positioned away from areas where turtles nest. Structures on the beach should be supported by a single pole rather than multiple poles which can entrap turtles. Ideally, specific areas with no nesting should be designated for watercraft launching.

Oil Spills

Oil spills frequently occur in catastrophic proportions and can pose grave threats to marine and coastal ecosystems. Sea turtles are one group among many groups of organisms affected by spills. Spills that take place during the nesting-hatching season can be lethal to all life stages on or near the beach: mating pairs, nesting females, eggs, hatchlings, and young post-hatchlings at sea. Oil cleanup activities can also be harmful. Earth-moving equipment can dissuade females from nesting and destroy nests, containment booms can entrap hatchlings, and lighting from nighttime activities can misdirect them.

The difficulty of mitigating the effects of oil spills on sea turtle nesting beaches should provide an incentive to locate oil transport activities away from important nesting areas. Nonetheless, oil spills have some potential to occur on almost any beach. Because of this threat, many areas have government or contract teams prepared to respond to spills with extensive equipment and personnel.

The best strategy for lessening threats to sea turtles is for local sea turtle conservation biologists to coordinate with these spill response teams before spills occur. Response teams or the government entities that oversee them should be given summary information on nesting and hatching seasons, density of nesting, species occurrence, and whom to contact about specific nest information. Where possible, sea turtle workers should keep in summary form, specific information on where nests are and when they were deposited. Sea turtle workers can assist in reducing the harm from oil cleanup activities by clearly marking nest areas (if known) and examining containment booms for trapped hatchlings.

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Reducing Threats to Foraging Habitat

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It is intuitive that protecting sea turtles, eggs and hatchlings from harm is only the first step to ensuring the survival of threatened and endangered populations. Strategies to reduce or eliminate threats to foraging and nesting habitats must be an important part of any management plan (see also Witherington, this volume). Foraging habitat is, to a large extent, species specific; collectively, however, most species rely heavily on coastal marine ecosystems for food. There are some life-stage exceptions, including epipelagic post-hatchling dispersal *(e.g.,* see Carr, 1987).

Since most marine resource managers are concerned with waters under national or provincial jurisdiction, coastal waters receive the most management attention. This is befitting, since most threats to the marine environment emanate from land and thus the coastal zone is disproportionately affected. This section reviews major threats to sea turtle foraging habitats in coastal waters, especially coral reefs and seagrass. Integrated Coastal Zone Management (ICZM) is proposed as the most effective long term response. Specific remedies to persistent threats *(e.g.,* anchoring) are also described. To maximize the effectiveness of specific remedies, they should be imposed as part of a holistic coastal zone protection strategy.

Threats to Foraging Habitat

Declining Water Quality

An overall general decline in water quality, particularly in relation to activities which increase turbidity, is perhaps the most important factor affecting coastal habitats. Seagrasses require a greater percentage of incident light than most other marine aquatic plants. Their distribution is restricted by depth, temperature and salinity, but depth as a function of light availability is the main limiting factor. Hence, the first areas to be affected by a decline in water clarity are the deeper seagrass beds where light attenuation is more severe (Kenworthy *et al.,* 1988, 1991).

Turbidity can be increased by sediment runoff from land-based sources as a result of poor land clearing practices for agriculture, forest products, road construction and other development. Similarly, dredging for navigational purposes or shoreline reclamation can significantly increase nearshore turbidity in localized areas, thus affecting proximal seagrasses. Physical alteration of the sea bed, such as occurs during dredging, blasting and anchoring, can also be an important contributing factor in reducing the area of seagrass cover.

Increased levels of nutrients *(e.g.,* sewage, agrochemicals) discharged from land-based sources can also lead to higher turbidity because the nutrients result in higher levels of phytoplankton in the water column. In addition, increased nutrient levels generally enhance the growth of epiphytes on the blades of seagrasses, causing a shading effect which can lead to grass mortality. If water quality is improved, seagrass productivity increases and with deeper light penetration, seagrass distribution also increases. As a direct result, fish and other wildlife dependent on seagrasses also benefit, including both herbivorous and omnivorous sea turtles (the latter preying upon crustaceans and other invertebrates inhabiting seagrass ecosystems).

As in the case of seagrass, water quality is a limiting factor for coral reefs. Sedimentation and eutrophication are major factors in the worldwide decline of coral reefs (Ginsburg, 1994). Increased levels of sediment smother reef organisms and reduce the light available for photosynthesis. Heavy sedimentation is also associated with lower coral growth and diversity, less live coral cover, reduced coral recruitment, and decreased calcification and coral productivity (Rogers, 1990). Consequently, sedimentation of the coastal zone from dredging and land-based runoff is one of the biggest potential sources of reef degradation from human activities. The effects can be long-lasting, with resuspension and transport of dredged sediment occurring years after dredging has stopped.

Similarly, increased levels of nutrients, such as from under-treated sewage, can cause significant changes in reef communities. Studies in Kaneohe Bay, Hawaii (U.S.) demonstrate that sewage effluent enhances benthic algal biomass and phytoplankton in the water column. The latter led to an increase in benthic filter feeding invertebrates which, together with the benthic algae, competitively excluded corals (Pastorak and Bilyard, 1985).

The many inter-linkages between these dominant tropical marine ecosystems (coral reefs and seagrasses) amplify the negative effects of anthropomorphic threats acting on either one of them. Seagrasses trap and stabilize sediments, preventing sediment from settling on the reef (Ogden, 1983); simultaneously, coral reefs provide a natural breakwater, reducing wave energy and thus creating ideal conditions for the growth of seagrass. A change in one ecosystem as a result of man's activities often has repercussions in an adjacent ecosystem, illustrating their ecological interdependence and emphasizing the need for a holistic approach to their management and conservation.

Anchoring

Indiscriminate anchoring can result in significant scarring to both coral reefs and seagrass, and this problem is increasing as tourism and pleasure boating intensifies around the world. Anchors uproot seagrasses and break the rhizome system; once the roots are disturbed, recovery is slow. Repetitive anchoring in many coastal bays of the U.S. Virgin Islands has so reduced seagrass cover that pastures once extending to 18.5 m now rarely persist below 4 m. With disturbance rates higher than recovery rates in many areas, the ecosystems' capacity to support foraging green turtles is declining (Williams, 1988).

Anchors and anchor chains cause significant localized destruction to corals and other reef organisms, including in protected areas; this and other consequences of multiple use present formidable challenges to coastal zone management *(e.g.,* Rogers *et al.*, 1988). In addition to coral breakage and direct mortality, holes and channels in the reef structure created by repetitive anchoring can alter current patterns and result in the erosion of sediments, thus causing further damage. In his review of the impacts of recreational activities on coral reefs, Tilmant (1987) noted three major concerns for reefs experiencing intensive recreational use: boating, diver and fishing impacts. He noted that physical damage to corals by anchors can be extensive; for example, an estimated 20% of staghorn coral *(Acropora cervicornis)* was destroyed at a popular anchorage area in Florida.

Oil Pollution and Marine Debris

Oil pollution and tar fouling are potential (or actual) hazards in many coastal areas. The Wider Caribbean hosts several large refineries and is characterized by active shipping lanes; more than 700,000 tons of oil are transported through the region each day. Following a spill in Caribbean Panama in 1986, seagrasses declined in biomass and infauna was severely affected, intertidal reefs declined, and sub-tidal reefs suffered significant mortality and sublethal effects (Keller and Jackson, 1993). In addition to damage effected by high profile spills, bilge washing by tankers results in chronic pollution which can stress seagrasses and coral reefs (such as by reducing rates of reproduction).

Garbage disposed at sea, or finding its way to the sea from land-based sources, is a serious global threat to the coastal zone. Death to marine organisms as a result of ingestion or entanglement in marine debris is widespread and well publicized *(e.g.,* Balazs, 1985; Laist, 1987), but perhaps less widely known is the threat that debris poses to the environment. For example, plastic bags can wrap around corals and suffocate underlying tissues (Rogers *et. al.,* 1988). Debris also smothers seagrass, and can leak noxious elements and pose other threats to important foraging habitats.

Dynamite and Chemical Fishing

The use of dynamite, chemicals and coral smashing techniques to capture fish cause irreparable harm to the sea bed, and especially to coral reefs. In the case of dynamite, many non-target fish are killed; others do not float to the surface and therefore are not collected. The physical damage effected by methods such as these destroys the very foundation of the reef, reducing or eliminating its capacity to support commercial fishes and invertebrates, as well as sea turtles. Chlorine and a wide variety of other chemicals are extremely toxic to corals. The application of chlorine bleach or other noxious substances to a reef for the purpose of snaring lobster or obtaining fish (including tropical specimens for the pet trade) kills corals, poisons important nursery areas for commercial fishes, and degrades sea turtle foraging habitat.

Other Threats

Other threats to turtle foraging areas include vessel groundings, certain fishing techniques *(e.g.,* bottom trawling, dropping traps or anchoring blocks indiscriminately on living reef), near shore construction *(e.g.,* piers, marinas), shoreline armoring *(e.g.,* jetties, seawalls), careless snorkeling and diving *(e.g.,* touching, collecting, trampling), reef walking (subsistence gleaning of shallow reef organisms, common throughout the insular Pacific), and other activities which directly or indirectly affect the health or physical integrity of seagrasses, coral reefs, mangroves, estuaries and related coastal ecosystems.

Integrated Coastal Zone Management (ICZM)

A holistic approach to the sustainable management of coastal resources is requisite to the survival of sea turtles and the foraging habitats upon which they depend. The diversity of threats acting upon these habitats requires an integrated management strategy which can coordinate the activities of many sectors, and involve their input in planning exercises. ICZM meets this need by offering a framework within which concerned agencies can work together toward their common objective of sustainable use of coastal resources (Clark, 1992). The components of a national strategy might include planning and zoning for multiple use of coastal areas, developing a marine protected areas network, conducting research and monitoring programs, identifying and fulfilling needs for legislation, policies and special guidelines, and promoting environmental education. The following components are important for sea turtle habitat.

Planning and Zoning

Effective planning must be closely coordinated at (or between) the appropriate government level(s). ICZM components with direct consequences to sea turtle foraging habitat should focus on improving water quality. For instance, replacing septic tanks and primary/secondary waste water discharges with tertiary waste water treatment; improving watershed management to reduce erosion, thus lowering the sediment load in coastal waters; reducing the use of agrochemicals, thus lowering the runoff of fertilizers and pesticides; developing guidelines with respect to dredging, blasting, and construction in nearshore waters; etc.

Through the process of zoning for multiple use or the designation of Special Management Areas, particular regulations can be introduced which afford protection to, or require mitigating measures for the conservation of, sea turtle foraging areas. For example, prohibiting shrimp trawling in certain areas (such as offshore Playa Rancho Nuevo, Mexico; National Research Council, 1990) or introducing no anchoring zones (Rogers *et. al.*, 1988). One of the simplest methods for preventing damage to seagrasses and coral reefs by anchors is to install mooring buoys at popular anchorage sites; benign and inexpensive technology is readily available *(e.g.*, Halas, 1985).

Network of Marine Protected Areas

As part of the process for planning a system of marine protected areas, sea turtle foraging areas should be identified and mapped. The most important areas should be included within the boundaries of marine parks, thus providing a measure of regulatory protection.

Oil Spill Contingency Plans

In areas subject to oil spill risk, an Oil Spill Contingency Plan should be prepared to ensure that an effective emergency strategy is readily implemented in the event of a spill. An Ecosystem Vulnerability Index should be developed; maps should highlight ecosystems and natural resources most vulnerable to oil pollution (Price and Heinanen, 1992). Emergency response workers should be fully appraised of appropriate protocol in the event that oiled turtles are rescued.

Monitoring

Coral reef and water quality monitoring programs are an essential component of ICZM. Routine monitoring of reefs will indicate changes (positive or negative) over time. The following parameters should be included in the monitoring protocol: species diversity, percentage live coral cover, sedimentation rates, and fish censuses. Several methodologies have been described *(e.g.* UNEP, 1984; Rogers *et al.*, 1994). Appropriate management measures, including zoning for multiple use or closing areas to promote recovery, can be introduced if significant harmful changes are demonstrated.

Water quality management should include establishing criteria or standards which not only avoid further deterioration but also promote improvement. In the case of seagrasses, distribution is tightly controlled by the depth of light penetration. Therefore, the following parameters should be included in the monitoring protocol: total suspended solids, chlorophyll a, dissolved inorganic nitrogen and phosphorus, secchi depth and water color. The light attenuation coefficient can be measured by irradiance meters which detect the wavelengths of light utilized by seagrasses (Kenworthy *et al.*, 1991).

Monitoring programs often require coordination between agencies. When this is the case, data collection techniques should ensure compatibility for the purposes of data analysis.

Education

To ensure that the value of coastal resources and the survival of endangered species, including sea turtles, is appreciated, that the process of integrated coastal management is accepted, and that a participatory approach to management is encouraged, an education program should be introduced at all levels, ranging from policy-makers to school children. Special programs should be prepared to target specific groups such as fishermen and recreational boaters, coastal landowners, and tourists.

Concluding Remarks

The goal of the ICZM process is "to ensure optimum sustainable use of coastal natural resources, maintenance of biodiversity, and conservation of critical habitats, thus providing the basis for long-term economic development" (Clark, 1992). To conserve migratory species, such as sea turtles, the national planning process should complement a broader international perspective. Issues such as pollution, watershed management, and the designation of protected areas often require a multinational approach (see Trono and Salm, this volume).

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Reducing Incidental Catch in Fisheries

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Incidental catch in fisheries is widely recognized as a major mortality factor for sea turtles. Several gear types, including shrimp trawl nets and fish seines, are known sources of injury and mortality. Pritchard *et al.* (1983) offered three solutions for reducing mortality: restricting fishing activity in areas and during seasons when sea turtles concentrate, pulling trawls and other fishing gear to the surface more frequently, and using excluder devices to release sea turtles from trawls. The description and analysis of the incidental catch problem offered by Pritchard *et al.* (1983) more than a decade and a half ago constituted the best available information at that time. While there is still much to learn about the extent of and solutions to the incidental capture of sea turtles in fishing gear, new information is now available.

In an extensive national review of the threats to sea turtles, shrimp trawling was singled out as the most important human-associated source of sea turtle mortality to "juveniles, subadults, and breeders in U.S. coastal waters" by the National Research Council (1990). The report estimated the annual mortality of loggerhead *(Caretta caretta)* and Kemp's ridley *(Lepidochelys kempii)* turtles associated with shrimping at between 5,500-55,000. Other trawling, traps, gill nets, longlines and entanglement in discarded fishing gear were also cited as major sources of mortality. Mortality from discarded fishing line was mentioned under the general area of entanglement, but there was little discussion of the capture of sea turtles by hook and line recreational fishing.

Current information indicates that the major sources of sea turtle mortality by fishing gear worldwide are: (1) trawling, (2) pelagic and bottom longlines, (3) gill/entanglement nets or entrapment gear *(e.g.*, seines, pound nets), (4) entanglements in buoy or trap lines, and (5) hooks and lines from recreational and commercial fishing. The purpose of this chapter is to summarize information available on gear that impacts sea turtles, the known or possible magnitude of takes by gear type, and potential solutions to reduce the take. Whenever practicable, comatose sea turtles recovered from fishing gear should be resuscitated (described below).

Trawling

Trawls are highly efficient gear for catching a variety of marine crustaceans and fish around the world. A variety of types are in use, with sizes ranging from 10 ft (3 m) head rope length (used by artisinal and recreational fisherman) to massive commercial trawls up to 200 ft (61.5 m) head rope length. Fortunately for sea turtles, massive trawls typically target cold water fish species where sea turtles are unlikely to occur. However, in the U.S. Gulf of Mexico shrimp fishery, the simultaneous use of four 100 ft (30.75 m) trawls by large shrimp vessels is not uncommon.

Problem Quantification

There are no reliable estimates of the global extent of trawl fishing in areas where sea turtles occur, but the incidental take of sea turtles in shrimp trawls is widely cited as very significant. Based on worldwide shrimp trawling effort, and making assumptions about the rate of capture (based on documented U.S. takes), a reasonable estimate of annual mortality of sea turtles in shrimp trawls worldwide is 150,000.

Worldwide, the principal marine species targeted in warm waters by trawling is shrimp. Fish are also harvested extensively, not always as a directed fishery with species specific trawls, but incidentally in shrimp trawls. Regardless of the target species, if bottom trawls occur in habitats frequented by sea turtles, turtles will be taken as bycatch. Unable to surface to breathe, many of those taken will drown.

Mitigation

The use of excluder devices, reduced tow times, and/or time and area closures are among the options touted to prevent or reduce turtle mortality. The Turtle Excluder Device (TED) has become the standard for reduction of sea turtle mortality from shrimping and, to a lesser extent, from fish trawling. The principle of the TED is simple: a barrier with an opening through which sea turtles voluntarily or involuntarily escape is installed into the trawl. Small openings in the TED, either spacing between the metal bars of a grid or large mesh size (8 in / 20 cm) webbing panels, allow most of the target species to pass through the openings into the rear or cod end of the net.

Research by the U.S. National Marine Fisheries Service (NMFS), fishermen and universities, has demonstrated that some types of TEDs work more efficiently at both target species retention and sea turtle release. All TEDs likely lose some target species, either because shrimp, which are weak swimmers, escape out the turtle release opening, or large fish do not pass through the openings in the TED and also escape out the turtle release opening. Despite some shortcomings, to date the TED is the best technical solution to allow turtles to escape from trawls with minimal effect on the target catch. Research and experience confirm that grid-type ("hard") TEDs seem to be the best for both purposes. Mesh webbing ("soft") TEDs divert a greater proportion of shrimp through the exit openings and, due to net stretching, create pockets in which turtles can become entrapped.

An oft overlooked part of the shrimp trawl fishery is the use of try nets or sample trawls. Because these trawls are pulled frequently to provide fishermen with an indication of what the large nets are catching, it was believed that they had little impact on sea turtle mortality. However, in almost 20,000 hr of tows conducted between 1992-1995 in U.S. waters, 41 turtles captured in try nets were recorded by NMFS observers for a calculated catch rate of about 0.002 turtles/net hr/try net (average try net size is 15 ft, or 4.6 m). By comparison, Henwood and Stuntz (1987) reported a catch rate of 0.0031 turtles/net hr/100 ft (30.75 m) net, for observer data collected from commercial trawls between 1973-1984. While most of these turtles observed captured in try nets were alive when brought aboard, their ultimate fate is unknown.

Reducing tow times can improve sea turtle survival under certain conditions. However, recent research and review of physiological data suggest that forced submergence of turtles for even a few minutes causes changes in their blood chemistry. Recovery to normal levels is dependent on the length of time submergence is forced, as well as turtle size. For small turtles, recovery from even a few minutes of forced submergence can require as long as 24 hr. Thus, reduced tow times may not be a viable alternative to TEDs where the conservation of sea turtles is the goal.

Pelagic Longlines

Longlines, used for the capture of pelagic species such as swordfish and other billfish, tunas, and sharks, consist of a surface line buoyed at each end, with lines of smaller diameter (sometimes called gangions) spaced uniformly from the main line. Baited hooks are attached to the smaller lines which hang vertically in the water column. Longlines can be several miles long, and are deployed from vessels and allowed to soak, usually overnight. The lines are retrieved after the specified soak time, and the catch brought aboard. There is increasing evidence that sea turtles both bite the baited hooks and become entangled in the lines. Swordfish, a major target species, tend to concentrate along frontal zones with high topographic relief and high biological productivity. These are often the same areas where sea turtles concentrate, creating a scenario for incidental take.

Problem Quantification

There are no worldwide estimates of sea turtle bycatch in pelagic longline gear. It is estimated by the NMFS Northeast Fisheries Science Center that in the U.S. Atlantic Ocean swordfish fishery, 1218 sea turtles were taken in 1992. More than 20,000 subadult loggerhead turtles are hooked annually by the Spanish longline fleet (in the eastern Atlantic and in the Mediterranean Sea) (Aguilar *et al.*, 1995). Additional longline fleets operate in the Mediterranean Sea and eastern Atlantic waters, so this number represents only part of the total take by longlines.

Mitigation

Mitigating measures to reduce sea turtle take should include additional research on the distribution and abundance of sea turtles, as well as a reduction of fishing effort when sea turtles occur in concentrations. Alternatives include limiting entrants to these fisheries, modifying fishing quotas, setting seasonal limits based on sea turtle distribution and abundance, and pulling lines more frequently.

Research on gear types can also be undertaken to reduce potential interactions with sea turtles. Alternative gear placement, bait, and hook types and materials can be developed to reduce interaction with turtles. The Japanese are reportedly conducting research on a rubber or plastic iridescent material that turtles supposedly bite in preference to the baited hooks; however, such a solution would not address the twin threat of line entanglement.

Research on reducing sea turtle take by longlines is in its infancy compared to technical solutions in the shrimp trawl fishery because the incidental take of sea turtles by longline gear is a problem documented only fairly recently. However, long line fisheries are expanding rapidly throughout the world, and this problem needs to be addressed.

Bottom Longlines

Bottom longlines differ from pelagic longlines in that they are set on the sea bottom, usually over a reef or other hard bottom. Bottom longlines use the principle of a main or mother line from which smaller diameter lines with baited hooks are evenly spaced. Principal species targeted are reef fish *(e.g.*, snappers, groupers). Evidence on the incidental take of sea turtles on bottom longlines is sparse, but they have the potential to take reef dwelling turtles such as loggerheads and hawksbills *(Eretmochelys imbricata).*

Problem Quantification

There are no national or regional data from which to estimate the global extent of sea turtle mortality due to bottom longline fishing effort.

Mitigation

Possible measures to reduce sea turtle takes include pulling lines more frequently, setting gear in areas where turtles are not in abundance, and using degradable hooks that would not cause long-term problems for turtles. More research is needed to define the extent of the problem, and to devise potential solutions.

Gill/Entanglement Nets

There are generally two types of gill nets used in fisheries around the world. Pelagic (deep ocean) drift nets target species such as swordfish and other billfish, sharks, mackerels, and dolphinfish. These large drift nets are an indiscriminate fishing technique that, in addition to the target catch, take various non-target species of sea turtles, marine mammals, seabirds, and other marine life. On the other hand, coastal gill nets are used around the world to capture coastal fishes. Mesh sizes vary depending on the target species, mainly between 2-3 in (5-7.6 cm) stretch mesh up to the 12-16 in (30.5-40.6 cm) mesh used in shark gill nets.

Problem Quantification

Because of the indiscriminate nature of gill nets, sea turtles are likely to be captured in both the pelagic and coastal habitats where they occur. As an example, incidental capture of leatherback turtles *(Dermochelys coriacea)* in the swordfish gillnet fisheries of Chile and Peru has been implicated in the recent collapse of the breeding colony on the Pacific coast of Mexico (Eckert and Sarti, 1997). Until recently, Mexico supported the largest nesting assemblage of leatherback sea turtles in the world (Sarti *et al.,* 1996). Mortality of sea turtles entangled in Chilean gillnets is estimated to be 80% (Frazier and Montero, 1990).

In some parts of the world, such as in Brazil, coastal gill nets represent a larger mortality problemfor turtles than trawling (Maria Marcovaldi, Projeto TAMAR, pers. comm.). Projeto TAMAR (the national sea turtle research and conservation program in Brazil) is working with fishermen to tag and release turtles caught in nets, but this project needs to be expanded.

Mitigation

Measures to reduce the incidental take of sea turtles in gill nets include setting nets in areas where turtles are unlikely to be present, limiting the length or depth of the nets, reducing the soak time of nets and requiring nets to be attended, establishing quotas or restrictions for target species, and using mesh sizes that are less likely to take turtles.

To reduce the incidental catch problem on Florida's east coast (USA), the State of Florida has limited the size of gill nets to no more than 600 yd (554 m), established a green turtle conservation zone in the area of greatest take, limited the number of gill nets allowed to one per fisher, prohibited use of trammel nets (which are actually a double gill net of varying mesh sizes), and established a zero soak time (that is, fishermen were required to begin retrieving their nets as soon as the set was complete). Shortly before these measures were instituted, the citizens of Florida, through constitutional amendment, banned the use of all gill nets in state waters in November 1996. Fishery managers around the world may take note of the Florida situation, which illustrates that a public outcry can force stringent management measures when less stringent measures are too little or too late.

Seines, Purse Seines, and Pound Nets

Seine nets are gear types that can be considered small mesh gill nets that are pulled through the water to capture a target species of fish both for food and bait. Usually one end of the net is anchored in shallow water or on shore and the other end carried by boat or wade fishermen out to sea; then brought back to shallow water or shore, entrapping the target species. Purse seines are deployed from vessels or boats. The target species is encircled by the net and the bottom of the net pursed or closed to entrap the target species. Pound nets employ the entrapment principle, and are generally anchored with stakes forming a pound or net corral. A single length of netting called a lead line stretches perpendicular from the middle of the pound and is used to guide the target species into the pocket of the net. Pound nets are used in coastal bays and sounds where the water is generally calm.

Problem Quantification

All three gear types (haul seines, purse seines, pound nets) have been implicated in the capture and mortality of sea turtles (NRC, 1990). However, mortality of sea turtles in these gears is probably not significant because turtles are usually not forced to be submerged and the mesh sizes used are usually small enough that turtles are not entangled. However, pound nets with more slack have more potential for accumulation of debris and marine organisms.

Significant mortality of sea turtles captured in seines is likely to be the direct result of fishermen who kill them for meat.

Mitigation

Measures to reduce the incidental take of sea turtles in pound nets would include setting the nets in areas where sea turtles are unlikely to occur. However, based on available evidence, few sea turtles are likely killed in pound nets, as long as due care is employed in releasing the animal. The type of lead lines used in some pound net fisheries can be modified, sometimes simply by stretching it tighter to avoid the potential for capture. In the case of haul seines and purse seines, since this gear is continuously tended by fishermen any turtles incidentally captured can be released from the net in a timely fashion.

Buoy and Trap Lines

Entanglement of sea turtles in buoy lines from anchor markers, crab pots, lobster pots, and fish traps has been documented in the U. S. and elsewhere. Loggerhead turtles feed on spiny lobsters and crabs and have been known to break into traps to reach the crustaceans. Kemp’s ridleys also feed on crabs and have been known to destroy traps in search of prey. In addition to the possible entanglement in buoy lines, some turtles are likely killed by fisherman because of gear damage.

Problem Quantification

There are no national or regional data from which to estimate the global extent of sea turtle mortality due to accidental entanglement in buoy and trap lines.

Mitigation

Obvious alternatives to mitigate the potential for sea turtle entanglement in buoy/trap lines are reduction of fishing effort, establishment of restricted fishing zones, and requirements to tend fishing gear more frequently. Management actions to conserve spiny lobster and stone crab stocks, instituted at the state and federal levels in the U.S., have included seasonal fishing restrictions, limits on the number and sizes of traps, and the installation of biodegradable panels in traps to limit their fishing life. Some of these measures will reduce the chances of entanglement of sea turtles.

Hook and Line Gear

The abundance of fishing gear using hooks and line around the world is unquantifiable. Hooks, and especially discarded fishing line, have the potential to adversely impact all species of sea turtles. Foul hooking and ingestion of hooks are additional problems.

Problem Quantification

There are no national or regional data from which to estimate the global extent of sea turtle mortality due to accidental catch by hook and line gear.

Mitigation

There are no obvious or reasonable mitigation measures to reduce this take, other than a general educational effort. Fishermen should be continually reminded not to discard their fishing gear in the marine environment, and should be encouraged to use hooks of degradable material. Educational efforts should include information on the proper release of turtles. Where feasible, programs should be established to notify marine resource or protection agencies of turtle takes by hook and line gear. This would at least help ensure proper release of turtles, recording of the incidents, and provide opportunities for tagging and other research.

Resuscitation and Release

Sea turtles that are dead or actively moving should be released over the stern of the boat. In addition, they should be released only when trawls (or other offending gear) are not in use, when the engine gears are in neutral position, and in areas where they are unlikely to be recaptured or injured by vessels. Resuscitation should be attempted on sea turtles that are comatose or inactive but not dead by: (1) placing the turtle on its carapace (back) and pumping its plastron (breastplate) with hand or foot, or (2) placing the turtle on its plastron and elevating its hindquarter several inches for a period of 1-24 hr. The amount of elevation depends on the size of the turtle; greater elevations are required for larger turtles. Sea turtles being resuscitated must be shaded and kept wet or moist.

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Husbandry, Veterinary Care, and Necropsy

Research and Management Techniques for the Conservation of Sea Turtles
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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Ranching and Captive Breeding Sea Turtles: Evaluation as a Conservation Strategy

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The controversy over sea turtle "farming" has proceeded for 30 years with little change in polarized positions and little objective analysis. Proponents promote farming as a method to save turtles, while opponents claim that farms actively contribute to sea turtle declines. This chapter discusses the general implications of sea turtle farming from a conservation perspective and provides readers with a basis for their own opinion. Discussion is restricted to sea turtles raised primarily for commercial purposes, and whether such activities might have conservation benefits. The technical aspects of turtle farming are beyond the scope of this chapter. Wood and Wood (1980) and Jacobson (1996) provide an entry to this material.

There are two ways to "farm" sea turtles: (1) maintaining captive adults who breed in captivity and whose offspring are raised for use ("captive breeding," often termed "farming") and (2) collecting turtles from wild populations (usually as eggs) which are then raised in captivity for use ("ranching"). These definitions are derived from the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which regulates international commercial trade from captive breeding and ranching in different ways. In this chapter, the term "farm" is used interchangeably to describe any facility holding captive turtles from either wild or captive bred sources, and sometimes both, for commercial production.

Constraints on Farms

Three factors affect the practicality and economic viability of sea turtle ranching and captive breeding: their marine habitat, their slow growth rates (measured in decades in most wild populations), and our relative ignorance of their diseases and parasites. Sea turtles must be maintained in sea water and require locations near the sea and expensive systems to supply flowing salt water. Attempts to maintain sea turtles commercially in natural or artificial enclosures in the sea have been uniformly unsuccessful. Sea turtles have reptilian physiology and the species of most commercial interest (green turtles *(Chelonia mydas)* and hawksbills *(Eretmochelys imbricata))* have a natural diet of very low nutrient and protein content. These two factors cause natural growth rates to be slow, increasing the expense of growing animals to economically marketable size. This can be offset by improving diet quality and protein content and providing warmer water, but again these necessitate increased expenses. Sea turtles are also subject to a wide variety of pathogens and parasites. In natural situations and at wild population densities these may have imperceptible effects, but in crowded and often unhygienic conditions of captivity, epizootic diseases cause catastrophic mortality *(e.g.,* Jacobson, 1996).

These factors create constraints to sea turtle farms which must be developed on a capital-intensive and technical basis. Specialized technical expertise, veterinary supervision and intervention, water quality control systems, carefully balanced high protein diets, and water temperature control all enhance production and economic success but at high costs of production requiring that products be sold at high prices. Obtaining farm stock from wild sources is relatively easy, but this can create unrealistically low expectations about the amount of capital, time, and skill that farm development will need. There is no currently operating, economically successful sea turtle ranch and only one captive breeding farm (Cayman Island Turtle Farm, Grand Cayman Island).

For purely conservation purposes, funding to farm an organism is justifiable for species that are imminently in danger of extinction and for which *in situ* conservation mechanisms have been proven ineffective. Only one sea turtle, the Kemp's ridley *(Lepidochelys kempii)*, approaches this situation. It is instructive that a captive ranching and release ("head-starting") program for this species instituted by the United States government was discontinued after 15 years and many millions of $US, due to uncertainty about the results and other concerns (Byles, 1993; Williams, 1993; Eckert *et al.*, 1994). Long term head-starting programs, including those focusing on green turtles (Florida, USA; Huff, 1989) and hawksbills (Republic of Palau; Sato and Madriasau, 1991) have also been discontinued in recent years based on insufficient evidence of success.

History of Farming

Three attempts have been made to develop facilities for turtle farming, at Grand Cayman Island (U.K.) in the Caribbean Sea, Reunion Island (France) in the Indian Ocean, and in the Torres Straits islands (Australia), all with green turtles. Facilities were also started or planned in Suriname (Reichart, 1982) and Indonesia and are currently under development in Cuba.

Cayman Turtle Farm

Cayman Turtle Farm (CTF) was started under the name Mariculture Inc. in 1969 using green turtle eggs obtained from Costa Rica. The farm initially attempted to raise turtles in semi-natural surroundings, but quickly converted to closed tank systems located on Grand Cayman Island. Adult breeding stock was obtained from Mexico, Suriname, Costa Rica, and Ascension Island and these began laying eggs in captivity in 1973. Most of the farm's production was from wild eggs collected under license from Ascension Island, Suriname, and Costa Rica (constituting "ranching" in the present sense). The farm conducted intensive studies of reproductive biology of captive sea turtles and successfully bred captive raised sea turtles in 1975, and by 1978 discontinued importation of wild eggs, relying entirely on production from both wild-caught and captive-raised stock. Deep controversy ensued over whether CTF had legitimately achieved adequate captive breeding, and concerns were raised about the effects of re-opening the quiescent international trade in sea turtle products.

As a result of international opposition from the scientific community, CTF did not receive CITES approval to trade internationally. In 1979, CITES adopted a captive breeding definition requiring production of second generation offspring which CTF had difficulty meeting. Lacking CITES approval, the farm could not sell its products anywhere except the United Kingdom (being a U.K. dependency, such trade was considered domestic). The U.S. Endangered Species Act of 1973 prevented import or transshipment through the United States, greatly restricting CTF's marketing and sales. The farm went through a number of changes in ownership and serious economic difficulties. The addition of a component of tourism and diversification of products including shell, oil, and local sales of turtle meat in Grand Cayman failed to provide sufficient revenue. CTF entered bankruptcy in 1975 and was taken over from the second owners by the Cayman Islands government in 1983. Since then, CTF has continued to operate at a reduced scale largely as a tourist facility and to provide employment and turtle meat for the local market. The farm also releases immature green turtles into the waters around Grand Cayman Island (Wood and Wood, 1993). The farm returned its first operating profit in 1988, 19 years after establishment.

Farm Corail, Reunion Island

Sea turtle farming began on an experimental basis in 1972 under the direction of the Institutes de Peches on Reunion Island, a French overseas Department located in the southwestern Indian Ocean (Lebrun, 1975). The farm was stocked with hatchlings collected annually from green turtle nesting beaches on Tromelin and Europa islands located 600 km and 2,000 km distant. The farm has been producing meat and shell for sale to tourists and the French domestic market since about 1980. Several attempts to apply for international trading privileges under CITES were unsuccessful, and the farm remains oriented toward its local and domestic market and with a reduced scope of operations. The facility has had consistent problems with slow growth and disease, which are attributed to the artificial pelletized diet and the seasonally low water temperatures in the area. In 1996-1997, Farm Corail negotiated a transition to fish aquaculture, research, and education. No new turtle stock has been introduced, and the release of captives is proposed. Turtle tracks at the two nesting islands of Tromelin and Europa have been regularly counted to support the premise that the annual hatchling collection does not threaten the nesting colony. The data indicate normal fluctuations, but no decline in either population over the period of hatchling exploitation (Le Gall *et al.,* 1986).

Torres Strait

Following initial studies by the National University of Australia, an organization created by the Australian government to assist development in aboriginal communities established a network of village-level sea turtle farms on the islands of Torres Strait, Australia, in 1970. Green turtle eggs collected from the large nesting aggregations at Bramble Cay and Rayne Island were transported to about 150 villagers located on islands in the Torres Strait. Difficulties with low hatch rates and high mortality were experienced at an early stage. The project was critically evaluated in 1972 (Carr and Main, 1973) and reorganized to concentrate turtle raising on nine islands with more intensive technical support, each with a capacity for 100-500 small turtles. During the period 1974-1978, the project undertook research on husbandry and disease, as well as general studies of sea turtle biology in the region, but was unable to overcome the basic problems of limited food supplies for young turtles and disease and parasites. In 1980, after government expenditure of $AU 6 million, the project was terminated.

Benefits and Disadvantages

A variety of conservation advantages and detriments have been claimed for turtle farms. These all lack objective or quantifiable information to evaluate them, which has led to a highly polarized and emotional discussion of these factors with little resolution. Ehrenfeld (1974) and Hendrickson (1974) provide two contrasting views.

Production of a Food Source for Tropical Coastal People

The prospect of using sea turtles to produce high quality protein from unproductive tropical marine systems and provide food for residents of tropical countries was initially supported by Carr (1967) and later strongly self-criticized (Carr, 1984). The high cost of growing turtles to edible size ensures that the price of farmed turtle meat is higher than wild-caught turtle. To recover costs, turtle farm products must be sold to overseas markets or tourists (Ehrenfeld, 1982; Dodd, 1982). The flavor of captive turtles fed non-natural diets is alleged to be inferior to that of the wild product, causing low acceptability among coastal people used to the real thing. Farmed turtle has therefore not proven to be the low-cost protein source originally envisaged.

Substitute for Wild Products

Production of turtle products in large quantities from farmed animals has been claimed to reduce demand for products from wild-caught turtles in both local and international markets, extending protection to wild turtle populations. Their high price may exclude farmed products from most local markets. Critics of farms, and of commercial use and international trade in turtles in general, argue that any increase in the availability of products on the international market will stimulate demand, which existing farms will be unable to satisfy, increasing pressure on wild populations and trade through illegal channels. Objective evidence on the reality of this scenario is contradictory, and some economic theory would argue that such stimulation is illusory. Clearly, effective national regulations and strict control of trade to prevent illegal commerce is necessary to prevent or minimize any such effect.

Removal of Animals from Wild Populations to Stock Farms

In both captive breeding and ranching, stock must be removed from the wild. For captive breeding, a relatively small number of adult breeders of both sexes is required. The very high reproductive value of such adults to the population, as determined by modeling studies *(e.g.,* Crouse *et al.,* 1987), may make the ecological effects of such removal significant, although data are lacking. For ranches, a continuing supply of eggs from breeding beaches is required. Some schemes for egg removal have used spurious models of sea turtle biology to support unreasonably high levels of collection (see Heppell *et al.,* 1995). Continued removal of a majority of the eggs must eventually cause population collapse. However, considering the life history strategy of sea turtles and the very high natural mortality of younger stages, it can be argued that removal of a small proportion of eggs is likely to have little effect on adult recruitment. More knowledge about juvenile survivorship and density dependent constraints on adult recruitment are needed to evaluate this factor and estimate what proportion of eggs may be safely harvested.

Animals for Release/Restocking

A proportion of the turtles raised on farms can be released back to the wild. Because of the presumed high mortality of sea turtles in the smaller size classes, largely from predation, it is argued that recruitment to wild breeding populations can be augmented by releasing larger sized turtles that are less subject to predation in a process termed "head-starting." Proponents point to documented cases of long term survival of released turtles, and growth and movements suggesting that they have successfully adapted to the wild (Wood and Wood, 1993). Critics point out that very few head-started turtles have joined a breeding population (Shaver, 1996; Shaver and Caillouet, 1998) and argue that the complex migratory movements of sea turtles in their subadult years are compromised and that behavior is unlikely to be normal (Dodd, 1982). The aberrant behavior and movements of some newly released turtles are widely documented. The potential introduction of disease and parasites from released captives into wild populations is also a serious concern (Jacobson, 1996), and there are further concerns about releasing turtles from different genetic stocks into wild populations (Dodd, 1982). Criteria for evaluating the success of head-starting are described in Eckert *et al.* (1994).

Research

Farms provide a unique opportunity to study some aspects of the biology of sea turtles. Holding turtles in captivity allows manipulation and experimentation that is not possible in the wild. CTF made major contributions to the understanding of the physiology of sea turtles, supporting research by visiting scientists and making its facilities and animals available for studies (Owens, 1995). The farm undertook to hold and breed the highly endangered Kemp's ridley turtle starting in 1980, and by 1984 was successfully breeding and raising this species. The farm successfully solved numerous husbandry problems involving nutrition, disease, and reproductive physiology. Farm research is often directed toward questions of maintenance and husbandry that have only indirect application to conservation and wild populations. However, most commentators concede that the research activities, particularly those at CTF, have been broadly beneficial to our general understanding of sea turtle biology.

CITES Guidelines

A new perspective was introduced between 1992 and 1994 when a task force of the Animals Committee of CITES was assembled to draft guidelines for the evaluation of proposals to CITES for ranching sea turtles under Resolution 3.15 of the Convention. Attempting to move beyond the unproductive arguments of the past, the task force proceeded under two broad assumptions: (1) the conservation benefits required by Res. Conf. 3.15 (and also needed to satisfy a very skeptical conservation community) must be made explicit in any ranching proposal, and (2) the solution to all the issues raised about effects of increasing international commerce in turtle products must be met by a very strict control of international trade.

Returning to the fundamentals of sea turtle biology, the task force recognized that because of their migratory habit, sea turtles were rarely or never solely the jurisdiction or "property" of a single nation, and therefore represented a special case for CITES which justified some extraordinary solutions. Responding to the most recent results on the genetic composition of sea turtle populations, and on a long recognized need for international cooperation in sea turtle conservation, the task force proposed that genetic population units be defined and all the nations in which a population spent time be identified. Communication, cooperation, and a regional approach to conservation of the population was then proposed as a necessary component of any ranching proposal for that population.

To address the need for effective trade controls, the task force proposed measures that would prevent sea turtle products from entering trade from any source except legal, approved ranches, and again called for international and bilateral cooperation between producing nations and consuming nations to achieve this.

These two new approaches to ranching allowed a prospective scenario where a sea turtle ranching project would become the vehicle for regionally coordinated conservation programs. The application of similar guidelines for all commercial sea turtle use is similarly feasible. Lack of funds to develop research, conservation, and enforcement is the major impediment to all sea turtle conservation. By linking the development of commercial sea turtle farms to required conservation activities, a source of funding, an incentive, and political support to meet the CITES guidelines could be encouraged.

The proposals were accepted by the parties to the CITES convention in 1994 (CITES Res. Conf. 9.20). The requirements for regional cooperation and scientific and biological knowledge remain difficult obstacles to meeting these new guidelines. It remains to be seen, on one hand, whether the new guidelines can indeed be applied as they were conceived (that is, to be a very positive factor for sea turtle conservation) and on the other, whether the requirements for international cooperation and coordination are too complex to be feasible.

The first proposal to change the CITES listing for a sea turtle was submitted by Cuba to the 10th Meeting of the Conference of CITES Parties in 1997, and it failed to receive the two-thirds majority required for approval. Additional proposals from Cuba and elsewhere are anticipated.

Conclusion

Sea turtle farms, whether for captive breeding or ranching, cannot be shown to be directly beneficial or proven to be fatally detrimental to the conservation of wild populations. What can be demonstrated is that they are very expensive, require advanced technical knowledge, and are, to date, of unproved economic viability. The linkage of farms to direct conservation activities and strict trade control, through international cooperation, provides the potential that farms could contribute to the conservation of sea turtles, but this potential remains unrealized.

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Rehabilitation of Sea Turtles

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Transport to Facility

Once rescued, ill or injured sea turtles should be transported to a primary care facility as soon as possible. Turtles should not be flipper tagged prior to evaluation because tagging can cause blood loss in severely anemic animals. Other procedures, such as gastric lavage (see Forbes, this volume), should also be avoided. Transport personnel are an integral part of the rescue effort and should always have essential information about the specifics of rescue and the condition and behavior of the turtle. If the medical facility is distant, individuals responsible for pickup and delivery should be educated in basic problems and complications of inappropriate care. Transport facilities should have containers of varying sizes that can comfortably hold different turtles. Fiberglass or plastic boxes are easily cleaned and can be reused. Containers with rounded corners and walls that slope slightly outward prevent a weakened turtle from crawling into a corner (or a straight wall) and obstructing breathing.

Historically turtles have often been transported on their backs to decrease movement, but this position can be very compromising to many individuals, and so all turtles should be moved in a plastron-down position. Because weak individuals can drown, turtles should not be transported in water, especially cool water during times of cold stress. The bottom of the carrier can be canvas-covered foam kept slightly moist. Moist towels can be placed over the carapace but must avoid obstructing breathing. The carapace and skin can be coated with lanolin or petroleum jelly (such as Vaseline) to avoid drying. Do not transport in open vehicles during excessive heat or cold; the best range of temperature for transport is 20-25° C. Turtles should not be picked up by their flippers. The turtle may be lifted by grasping both sides of the carapace (which can better support its weight) or by using a stretcher that provides adequate support and attachments for carry straps or poles.

Initial Presentation and Evaluation

A delay in therapeutic onset can be fatal to some individuals. At presentation the turtle is first visually evaluated. Visual inspection should categorize overall gross body condition as normal, underweight, or emaciated. While these are subjective categories, anatomic changes become obvious with experience. Turtles with severe weight loss have decreased muscle and fat tissue. In the neck area, the back of the skull has a prominent occipital process that becomes very obvious in thin turtles. In addition, bilateral dorsal and lateral neck muscle groups, which are often hidden among other tissues, become more obvious with weight loss. The soft tissue of the foreflipper and the shoulder area is decreased in thin animals. In very thin animals, the plastron may be sunken or appear indented centrally. Ulcerations of the skin of the plastron are also more common in chronically debilitated animals, and bony spicules of the plastron may perforate the skin and become evident. The eyes may appear sunken, especially when the head is elevated. Chronically ill turtles may be covered with barnacles, worms, and crabs. Leeches on the skin, eyes, mouth, and cloaca are sure signs of chronic illness.

Initial treatment will depend on the turtle's behavior. Turtles out of water may appear more inactive and non-responsive than they actually are. The clinical responses of a normal turtle when dry should be differentiated from sicker animals. While dry, observe respirations. A stronger animal usually picks up its head during breathing. It may also try to move away from activity. When placed into a pool of water, flipper movement is coordinated, and the head is raised to a 45° angle with each breath. A short, monitored swim test, if the turtle is strong enough, will help in the evaluation if the clinician is not sure of the behavior. Individuals who are thin, do not pick up their head in water to breathe, are uncoordinated, and/or float with their flippers dangling in the water, should be maintained out of water initially. When out of water, it is best if they are kept in a walled container which is padded with foam and covered with canvas as described above. Wet towels may be used over the shell except when temperatures are below 20°C and there are no heating blankets. A light coating of lanolin, vitamin A and D ointment, or petroleum jelly can be used to avoid drying of the shell and skin.

Diagnostic Techniques

After initial evaluation, a blood sample is taken (see Owens, this volume) for a complete blood count and serum chemistries. Until these values are available, a rapid glucose determination is made with a glucose strip, Chemstrip bG (Boehringer Mannheim Corp., 9115 Hague Road, Indianapolis, Indiana 46256 USA). Additionally, a packed red cell volume and total protein can be important indices with which to begin treatment. Complete blood counts (CBC) and serum chemistries, although expensive, should be done on each ill animal because they lead to more efficient diagnosis and treatment. Anemia is common in sick turtles and hampers therapy. Local hospitals may donate some of their services although they will not have the background for initially interpreting the CBC. Radiographs (X-rays) are also essential. Bacterial cultures of wounds and feces may provide some insight into the types of organisms present. Feces should be examined for the presence of parasitic organisms. Cytologic evaluation of the colon may also be helpful in determining the presence of infection and inflammation.

Treatment Techniques

Hypoglycemia

A weak turtle may be hypoglycemic which may be determined by Chemstrips and verified by serum chemistry. Normal serum glucose levels range between 70-120 mg/dl, but can vary in healthy individuals (such as females that are laying and are thin - Brenda Lee Philips, pers. comm., 1996). Individuals that are thin or emaciated and have glucose levels <60 mg/dl should be considered candidates for treatment with glucose supplements by one of the following methods:

1. Oral supplementation depends partially on the ability of the turtle to transfer glucose-containing material (liquid or gruel) to the intestinal tract for absorption. The patient must be somewhat stable, allowing time for complications, such as regurgitation or constipation, to become evident. If the intestinal tract is functional, the turtle may be given up to 1 ml of 50% dextrose per kilogram of body weight 3-6 times per day. This solution should be diluted with ringers, saline or gruel to make the solution less hypertonic. Unfortunately this volume may be difficult to administer in recently hospitalized turtles, and care must be taken to balance the oral approach with serum glucose levels. Esophageal tubing can result in upper intestinal food buildup, regurgitation and aspiration of food, especially in turtles kept out of water. Personnel involved in these procedures should be experienced because of the potential for treatment related complications. During the procedure, the turtle is placed in a vertical heads up position either in a support or held by personnel as discussed in a later section. If possible, the turtle should first be tubed with plain water marked with food coloring to see if it can hold down fluids. If it must be placed dry, it should be maintained at a 45 degree angle to avoid aspiration.

2. Intravenous glucose administration is often the method of choice for severe hypoglycemia in other animal species . Though it can be used, this procedure has numerous disadvantages, including loss of the catheter, difficulty in placement and maintaining integrity when the animal becomes active, and the need for increased manpower and trained personnel. Interosseous administration is another possible method, but requires experienced medical personnel and constant monitoring (Howard Krum, pers. comm., 1996). If not carefully monitored, parenteral fluids can result in dilution of the blood in severely anemic patients.

3. Intracoelomic cavity glucose has been used to treat moderate to severe hypoglycemia. The turtle is placed on its back with its caudal shell elevated to allow the intestinal tract to slide forward. A 20 gauge needle, angled at 30 degrees anterior dorsal, is inserted slowly in the

anterior inguinal region. A 5% glucose solution has been administered to approximately 40 individuals at 11-17 ml/kg body weight depending on the level of serum glucose. This method is used to buy time to allow oral supplementation to be effective. As in every fluid supplementation procedure, veterinary supervision is needed to prevent overhydration and electrolyte problems. Blood samples for glucose determination should be taken at least every 12-24 hr to ascertain treatment response. This is our preferred method for initial stabilization. If the individual does not initially absorb the glucose as evidenced by no response and a continued low serum glucose, then a bolus should be given intravenously. This has been seen most commonly (about 10%) in young emaciated greens. As soon as possible, oral supplementation should piggyback and then replace injectable methods.

Nutritional Supplementation

Tube feeding is a common treatment in nutritionally debilitated individuals, but it has limitations. The basic technique involves placement of a flexible tube into the distal esophagus which connects via a left turn with the stomach. As a result of the turtle's anatomy, the material is delivered to the esophagus in a smaller volume than expected. The turtle is placed at a 45-90 angle with its head up and extended, which straightens the esophagus allowing easier passage. The tube is lubricated, and the mouth is maintained in an open position with a bite block which is covered with rubber to avoid damaging the beak and oral area. Care must be taken not to hyperextend the temporal mandibular joint. The amount of liquid or gruel given will depend on the turtle's size and coordination. As a guide, a 3-4 kg turtle may only be able to keep down 10 ml of gruel initially. If weak, the turtle should be maintained at a 45 angle for 5 min to facilitate movement of the material.

To avoid regurgitation and aspiration, the turtle should be placed back in the water as soon as possible; avoid tipping the head lower than the body. Very weak animals may fare better with thicker materials which are less likely to reflux over the glottis. Turtles that are too weak to stay in the water continually can sometimes be placed in water 1-5 min after eating to allow them to safely reflux any excess materials. Some material may be expelled in the water through the nose which does not indicate aspiration. Individuals that are too weak to place in water can be maintained with the head and body slightly elevated.

While initial feedings may seem discouraging, generally the amount can be increased over a few days. The number of feedings per day are determined by the condition and blood glucose status of the animal. A low residue elemental diet called Vital (Vital HN, Ross Products Division, Abbott Laboratories, Columbus, Ohio 43215-1724 USA) is often substituted for glucose solutions. This diet provides energy with carbohydrates and protein which can be absorbed without having to be processed by the liver. Turtles that are not thin and have normal glucose levels may be fed 3 times per day and should be weighed twice a week until stable or gaining weight. Others may require 3-4 feedings per day, and turtles with low glucose may need up to 6 low volume feedings per day when requiring chronic glucose supplementation. These animals should be weighed every day or at least every two days to keep therapy on a responsive time schedule.

Treatment Considerations

Treatment of ill or injured turtles may require additional therapy beyond food, shelter and antibiotics. A debilitated individual is not only deficient in protein and fat but may have inadequate tissue stores of minerals and vitamins. Young green turtles *(Chelonia mydas)* commonly have extremely low calcium levels coinciding with soft shells. These individuals respond well to calcium supplementation, initially by giving calcium gluconate subcutaneously until the animal can be supplemented orally. The need for this supplement may also be suggested by high levels of muscle enzymes on the serum panel. These animals often have a severe myositis that may be related to a number of factors that may include nutritional debilitation and secondary muscle fatigue. In addition, vitamin E is supplemented orally at 20-30 I.U.'s of dl-alpha tocopheryl acetate per kilogram of body weight once a day for one week and then every other day for one to two weeks. Additional research is being conducted to further clarify the levels needed.

If the turtle is maintained on a predominately fish diet, a multi-vitamin (Mazuri Vitamins, Purina Mills, Box 66812, St. Louis, Missouri 63166-6812 USA) is given per instructions, based on the amount of fish being ingested. A B-complex injection is also given which includes thiamine at a dose of 0.6-1.0 ml per 22 kilograms of body weight once. This is also followed up with oral multi-vitamin supplements as mentioned above.

Severely anemic animals may benefit from vitamin K supplementation. Initially 0.5mg/kg body weight may be administered usually once. These individuals may also be iron deficient when compared to other normal individuals. A safe dose of iron has not yet been determined but 0.5 mg/kg of elemental iron per day split for 10 to 14 days has not resulted in excessive serum levels. Iron should be used with caution and under veterinary supervision.

Constipation has been found as a common sequelae to emaciation, dehydration, and debilitation in young green turtles. Loggerhead turtles *(Caretta caretta)*, usually adults, often have large amounts of shell debris in the lower intestinal tract. Initially loggerheads were treated with surgery, but survival was poor since they tended to have severe anemia and emaciation. They were found to be poor surgical candidates unless they were also given blood transfusions.

An alternative medical solution (for constipation) is intestinal stimulants such as metachlopromide (Danbury Pharmaceutical Inc., Danbury, Connecticut 06810 USA) at 0.5 mg/kg orally every 48 hr, or, if the animal cannot feed, by injection at 0.3 mg/kg once per day. This schedule works best when combined with mineral oil on alternate days. The oil can be used at a rate of 2.2 to3 ml/kg body weight in small individuals. Oil should be used only after it is shown that the turtle will be able to keep it down by first giving the turtle water orally. It can also be administered in gelatin capsules. Turtles should be placed in water after being given oil to avoid aspiration. Larger turtles may not need as much oil on a per kilo of body weight basis. A 45 kg turtle may only require 1.0 ml/kg. Caretakers should note on a daily basis if the animals are defecating. The life support systems for sea turtles are heavily impacted by oil, and a foam fractionator can help to mitigate the problems. When feces are not observed and therapeutic success is in doubt for re-establishing normal gut motility, barium can be administered orally at a dose ranging from 5 to 15 ml / kg of a 30 % solution to evaluate intestinal movement. Individuals that can not handle this volume may require several smaller doses.

Many turtles tend to have noticeable parasite loads, such as young green turtles with tissue trematode infestation and loggerheads with trematode and nematode involvement. Ill turtles may not be able to deal with the addition of large numbers of parasites so all individuals should be treated for trematodes and nematodes. We currently use fenbendazole for nematodes at dose of 50-100 mg/kg once and repeated in 2 weeks, and praziquantel at 16 mg/kg once then repeated in two weeks for trematodes.

Basic Facilities

The main focus for most rehabilitation programs is to return animals to the environment where they originated. In general the more successful rehabilitation facilities combine basic husbandry methods, hardworking employees and experienced veterinary personnel. Most facilities can easily be compromised if there are too many animals being retained longer than necessary. Turtles should be returned after attaining adequate health that will maximize survival or when traumatic problems have been successfully treated.

Facilities should include pools, with filter systems if not oceanside, and systems to cool or heat the water. Pools should be low maintenance and easy to clean, adapt and repair. Most facilities use above ground fiberglass pools because of lower expense. With age, seam areas may begin to peel, and turtles can ingest pieces of fiberglass, so caretakers must regularly inspect pools. Filtration systems may be sand and/or cartridge based when required. While flow through systems near shore have many advantages, they are prone to complications from the source including temperature, surrounding water quality, and biohazards such as red tide or pollution. Water temperatures should range between 22 and 26 C. Temperatures above 28 C may lead to lethargy and loss of appetite. Roofing or shade-screening over pools can minimize excessive heat and sunlight and protect from temperature extremes. Young turtles may also benefit from having 50% of their pool covered to allow them to hide. This seems to decrease stress levels, especially in young greens.

Salinity levels are usually maintained at 32-36 ppt. Lower salinity levels can be used to influence hydration and removal of leeches and barnacles, but this is usually recommended where serum sodium levels are elevated above normal. This may indicate a hydration imbalance or intake of saltwater. Excessive salt intake is normally handled physiologically by excretion in healthy turtles, but their capability may be compromised during illness. Lower salinity may be used to aid turtles with excessive buoyancy but can force others to work harder to stay at the surface. Changing salinity levels for short periods may also help control bacteria that are used to high salinity. Turtles should not be left in fresh or brackish water for extended periods of time without checking serum electrolyte levels.

Chlorine has been used in closed systems (those not constantly adding new water) for short periods to aid in control of severe skin and shell infection. Levels of chlorine up to 1 ppm appear to be beneficial.

Trauma

Watercraft Injuries

Propeller injuries may range from mild to severe and include head lacerations, eye injury, injury to limbs, and carapace lacerations and fractures. The wounds are initially examined for depth and extent of damage. Debris is often present, so the wounds may need to be flushed with sterile saline. Chronic, partially healed propeller wounds may be associated with secondary problems such as emaciation and increased buoyancy. These wounds may have retained dead bone and pockets of debris trapped deep in the tissue connected to the surface by fistulous tracts. Externally the wounds may appear healed, but any small openings should be examined for possible connection to debris channels.

Rapidly moving boats may strike the head or carapace resulting in fractures. Injury to the carapace can also involve fractures to the spinal column which is often complicated with buoyancy problems. Clinically these turtles may do well for extended periods or may have recurrent problems with granulomatous disease of deep tissues. If the spinal cord is damaged, there can be an increased incidence of constipation and colitis. Shell injuries historically have been treated by a variety of methods. The use of acrylic, fiberglass or other hard patch techniques for shell repair has decreased. While these methods may stabilize the wound, a sealed shell defect may also trap debris and inhibit healing. Most shell fractures require 2-6 weeks for damage tissue to be delineated. Hard patch material must be removed to maximize healing and promote normal shell calcification and repair. If a method of shell stabilization is required it should allow regular debridement of the wound.

Most traumatic wounds of the carapace respond well to the Tegaderm® technique. The wound is cleaned, debrided if necessary, and flushed with 5% betadyne solution (if not penetrating the lungs). The shell surrounding the wound is cleaned and dried. The exposed soft tissues are covered with a Vaseline-based triple antibiotic ointment avoiding the wound edges. A sheet of Tegaderm® (3M Health Care, St. Paul, Minnesota 55144 USA) is applied over the wound with 1.5 cm overlap onto dry shell. Multiple pieces of Tegaderm® can be used by overlapping portions on the wound center. All edges of the Tegaderm® that are exposed are then glued to each other and to the shell with cyanoacrylate glue *(e.g.,* Superglue - Loctite Corp., Cleveland, Ohio 44128 USA). The glue is allowed to dry for a few minutes, and then the turtle can be returned to the water. The bandage can be removed at regular intervals, usually once a week, for cleaning and then reapplied.

Wounds will not heal if the turtle's body condition is not adequate to support tissue replacement or, if it is continually losing weight. Proper shell repair may require many weeks of therapy, and turtles should not be turned loose with hard patches such as fiberglass.

Cold-stunning

Increased numbers of turtles may be brought in for rehabilitation when water temperatures fall below seasonal norms for extended periods. These individuals may range from thin to visually in good flesh, are often lethargic, hypothermic, in some cases hypoglycemic, and may have other problems such as septicemia. Initial evaluation should include body temperature. The goal is to elevate the body temperature to a preferred physiologic level. For turtles with short-term exposure that are still coordinated and able to pick up their heads in water, being placed in warmer salt water is often adequate. Water at 26 C is often adequate and can be slowly increased if needed. Animals that are chronically affected, thin, and nonresponsive may require heat supplementation with water heating pads. Treatment techniques used in other species include warm water enemas (difficult in turtles) and IV fluids (also difficult). Another technique that can be used is warm intracoelomic fluid, although this should be combined with an exogenous source of heat and continued body temperature evaluation. Cold stunning effects seen in one area may differ from those seen in another.

Blood samples should be taken from nonresponsive turtles for complete blood count, chemistries, and a rapid determination of glucose level. Care must be taken not to overheat the turtle by providing a cloth barrier between the patient and heating pad. Water based pads are preferred. Regularly check the pad and body temperature. Protect the carapace and skin from drying out by using lanolin or vaseline. Avoid water soaked cloth material unless it can be kept warm. Antibiotics may not be needed for acutely affected animals although use in nonresponsive turtles should be addressed with the veterinarian involved.

Fishing Hooks and Debris Ingestion

Fishing hooks may cause severe injury with the most damage done in the upper intestinal track, especially the esophagus. This may include retained hooks, perforation, and laceration. Diagnostic techniques may include visual inspection radiology (X-rays) and endoscopy. Removal may be accomplished by hand, endoscope, or surgery. Esophageal surgery is often difficult because of post-surgical complications, but it may be required. Every turtle that is rescued should be considered a possible victim of debris ingestion, including plastic material and monofilament line. Plastic bags and debris may cause intestinal blockage, and monofilament line or rope may result in blockage or perforation. Radiography may be a useful diagnostic technique although plastic material may not show on X-rays.

Exposure to Oil

Turtles exposed to oil-based compounds may suffer from external contamination and/or ingestion. External oils and tar can be removed by washing with dish detergent *(e.g.,* "Dawn") or with vegetable oils. Oral residue can be broken down by the use of organic fats such as mayonnaise. If ingestion is suspected, charcoal-containing compounds may decrease absorption of hydrocarbons which can cause organ damage. Additional supportive therapy such as fluids may also be helpful. Serial blood samples can help to direct therapy.

Acknowledgments

The author would like to thank John Kerivan, Ray Davis, Tom McHenry, Brenda Lee Phillips, Maggie Murphy, Kelly Pace, Ryan Lindell, Andy Cronkhite, and the rest of the Aquarium Staff at Sea World of Florida who donate so much time and energy to helping sea turtles.

Research and Management Techniques for the Conservation of Sea Turtles
K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, M. Donnelly (Editors)
IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

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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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

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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7

Legislation and Enforcement

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IUCN/SSC Marine Turtle Specialist Group Publication No. 4, 1999

Grassroots Stakeholders and National Legislation

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Prior to the 1960s, there was little concern on the part of national governments for the welfare of marine turtle populations. Although at that time most countries already had national conservation legislation, specific regulations for the protection and management of marine turtles were incidental, perfunctory, or non-existent. With the growing realization during the past few decades that virtually all marine turtle species were threatened with extinction, and that a renewable natural resource with economic, cultural, and ecological value may be lost, international agreements and national legislative measures gradually emerged in order to mitigate, or even call a halt to, human exploitation or marine turtles. While the aim of such legislation has been to rebuild depleted populations, and thus save these species from extinction, the results have been mixed.

In most countries, new regulations to protect marine turtles were integrated into an already existing framework of general conservation laws. This was not always an ideal codification for the intended protection. For example, in many countries sea turtles were (and still are) considered to be both fisheries (while at sea) and wildlife (while nesting on land) resources, and as such are often placed under the separate jurisdictions of both Fisheries and Wildlife departments. This has caused confusion and controversy, and in some cases clearly contradictory legislation. Moreover, rather than enjoy the attention and resources of two regulatory agencies, marine turtles have often been neglected, with each agency considering them to be the other's obligation. It must, therefore, be strongly recommended that clear lines of jurisdiction be established in conservation legislation for marine turtles, and that overlapping departmental responsibilities (and other excuses for neglect) be avoided.

Nearly universal principles (*e.g.,* sustainability, stewardship, accountability) are evident in conservation law at the national level. Nevertheless, there is an inevitable variation in detail because, aside from an obvious emphasis on the protection on natural resources, a nation's laws are also based on economic priorities and domestic culture. The cultural aspect is uniquely important. If stakeholders feel the law is unjust and contrary to what they deem to be their traditional right(s), it will be violated, defeating the original intent of the legislation. Therefore, an important consideration in the development of new conservation legislation, or in making adjustments to existing legislation, is an attempt at consensus, most of all among local and indigenous people because these are the people that are often affected the most but consulted the least.

Involving stakeholders, especially those who may be geographically or politically isolated, is never easy (see Frazier, this volume). To promote dialogue and encourage consensus, public hearings should be conducted before any political decision is made. These hearings should be in the form of “town hall” meetings or debates at the grassroots level. It would be a mistake to discuss the proposed legislation solely with local politicians or community leaders. Experience has shown that, while these leaders may endorse or agree with certain regulations, they do not always have the mandate of the entire village or region. Ensuing increased poaching by disappointed malcontents then often becomes an outlet of their disagreement with the law. Development of conservation legislation would therefore not be the exclusive realm of politicians, nor should a single special interest group be allowed to unduly influence the legislative process. Decision makers should also have the wisdom to consult specialists on the ramifications of the proposed legislation. The primary purpose must be to look at all facets of the issues at hand, and this can best be accomplished by a multidisciplinary approach.

While keeping the above points in mind, the conservation advocate must also ensure the integrity of the legislative process. For example, if grassroots dialogue has clearly established a firm recalcitrance on the part of hunters to curb the level of exploitation, or the "compromise" put forward is to protect eggs and hatchlings but not nesting females, then the next step is not to codify the compromise in legislation (for this will only ensure extinction), but to delve more deeply into the issues involved. What is the basis of the harvest? Is it protein? Income? Trade? Status? The root must be found and agreed upon, and then the dialogue renewed with an aim to meet the need by means other than killing sea turtles. Solutions might include making investments in alternative sources of income *(e.g.*, under-exploited fisheries, cottage industries, resource protection/conservation, eco-tourism) and/or protein *(e.g.*, goats, chickens, pigs, iguana, small-scale mariculture). Creative financing will almost certainly be needed.

Not only must the ultimate solution take into account the survival of both the hunter and the hunted, it must go beyond legislation or social programming designed to enhance the survival of indigenous people and/or depleted marine turtles. Since threats to marine turtles go far beyond the direct effects of human exploitation, protective legislation alone will not suffice to mitigate the problems or promote population recovery. Nesting beaches are being destroyed at unprecedented rates to accommodate the fastest growing industry in the world: tourism (see Witherington, this volume, for a discussion of threats to nesting habitat). Increasing rates of harvest of shrimp and fish cause death to numerous non-target species, including tens (if not hundreds) of thousands of marine turtles every year (see Oravetz, this volume). Supporting or complementary regulations and rules for other sectors within the legislative framework are often needed to encompass the entire spectrum of threats facing marine turtles in a particular country.

As in any advocacy process, the first step is to assemble the facts. If marine turtles are depleted, there must be a reason. The reason is likely to be some combination of local harvest, harvest elsewhere in the population's range, incidental catch in national or extraterritorial waters, and/or habitat degradation. The mechanisms to provide optimum protection to marine turtles will differ among nations and no unified set of laws will be appropriate. But there are general guidelines that can help conservationists to recommend legislation both to stakeholders and to lawmakers that will conform closely to international standards. With an aim to promote sustainable populations, the following points should be kept in mind: (1) all locally occurring populations should be afforded the protection necessary to promote healthy population levels; (2) restrictions should be placed, as necessary, on both direct and incidental take; (3) penalties and fines should be commensurate with product value and there should be provision for the confiscation of equipment (including boats and vehicles); (4) conflicting or contradictory legislation should be identified and repealed; (5) legislation should also be enacted or strengthened to protect critical habitat; and (6) relevant international agreements should be supported by national law.

If population recovery is the primary goal, some measures must, of necessity, be non-negotiable. Adult turtles, and especially females on the nesting beach, should be protected at all times and under all circumstances. Eggs should not be harvested unless it can be convincingly demonstrated that the collection quota consists of eggs which are otherwise doomed. It is an unfortunate reality that there are very few places in the world where this case can reasonably be made. At the present time, there are closely supervised and generally successful national egg collection initiatives in Costa Rica and Suriname. In the former case, successive *arribadas* of huge numbers of olive ridleys predictably exhume large numbers of incubating eggs laid early in the season. In the latter case, more than a million eggs are destroyed each season by cyclical erosion or other natural causes; a fraction of these doomed eggs are harvested and sold to the public. Only at isolated sites in Costa Rica, Mexico, and India do sizable olive ridley *arribadas* still occur, and few nations of the world have a coastline as dynamic as Suriname's.

The plight of marine turtles is so dire in most parts of the world that an indefinite moratorium on the harvest of turtles and eggs would be the only defensible option if biological considerations were all that had to be taken into account. In reality, time may be needed to involve stakeholders in the conservation agenda, and thus legislative advocates might consider codifying a future date certain, after which the harvest will cease to be legal. During this time, hunters, vendors, and consumers should be prepared for a moratorium—

nets should be purchased, alternatives offered, public education undertaken, and enforcement capacity strengthened. Once established, a moratorium on the capture and sale of sea turtles, their eggs, and products should not be lifted until such time as there is sufficient information to show that a regulated harvest will not compromise the full recovery of depleted populations.

If the obstacles to a full moratorium are insurmountable, advocates should emphasize to legislators the urgent need for national legislation which imposes a closed season that fully encompasses the nesting period for all species. If size limits are imposed to protect breeding age adults, such limits ought to be based on criteria that can be met at the point of capture; *e.g.,* shell length, as opposed to weight. If criteria cannot be assured at the point of capture, turtles should be landed alive (potentially lethal capture methods, such as spearing, should be banned). Logic should be used at every step. For instance, in addition to establishing a closed season on capture, the possession and sale of turtle products should also be prohibited during the annual closed season. In this way, possession is not easily excused by protesting that the turtle had been landed during the open season. If hawksbill turtles *(Eretmochelys imbricata)* are protected, the domestic sale, import, and export of tortoiseshell should be explicitly banned and export legislation should be modified to reinforce conservation statues.

Throughout the advocacy process, conservationists should make every attempt to ensure the accuracy of their data. For example, trends might be emphasized as opposed to precise population estimates if trend data are available and precise population estimates are not available (see Gerrodette and Taylor, this volume, for a discussion of estimating population size). Many politicians are well-informed and for proponents to promote passage of conservation legislation they must present well-formulated and well-documented arguments. To base petitions on superficial data or to advocate decisions based on emotional rhetoric would be foolhardy, especially in view of compelling arguments for the *status quo* likely to be presented by special interest lobbies. Proponents should also seek to express the value of the marine turtle resource in economic terms *(e.g.,* sustainable income from tourism) or functional value. Ethical or aesthetic considerations should never be compromised, of course, but most politicians are swayed primarily by economic arguments.

Finally, in establishing national conservation legislation for sea turtles, one should strive to make it compatible with, and complementary to, international agreements (see Hykle, this volume). It is one thing to ratify the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) or other international treaties, but quite another to ensure that national laws do not negate the goals of these various treaties.

In summary, national legislation should be clear in its intent, equitable in its objectives, uncompromising with regard to the basic biology of sea turtles *(e.g.,* recognizing slow growth, delayed maturity, and the unique importance of gravid females), adequate in the areas of enforcement and penalty, holistic *(e.g.,* include habitat protection), and harmonized with relevant international obligations. The involvement of stakeholders in the design and enforcement of conservation law should always be honestly and openly sought. Marginalizing the participation of grassroots shakeholders nearly always ensures the failure of national conservation law.

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Regional Collaboration

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The sea is a special environment where linkages established by currents, species, migrations, and passive larval dispersal can extend thousands of kilometers. Consequently, general marine conservation issues, and especially those relating to far-ranging species such as turtles and cetaceans, need to be addressed at a regional (multinational) level. There is consensus that conservation measures implemented independently or in isolation at a national level are inadequate to effectively arrest persistent declines in marine turtle populations. Moreover, conservation effort in one country may be negated by unsustainable activities directed toward the same turtle population in another range country. Truly successful conservation programs transcend geographical and political boundaries. When states share a common marine resource, they also share the common challenge of effective conservation.

Marine turtles routinely journey hundreds and sometimes thousands of kilometers between specific habitats which, in some cases, are separated by entire ocean basins. Consequently, a coordinated management strategy is requisite among range states. Some existing international conventions and global conservation strategies include provisions relevant to endangered and highly migratory species, including marine turtles. These conventions (see Hykle, this volume) and strategies *(e.g.,* the "Global Biodiversity Strategy" developed under the aegis of the World Resources Institute, World Conservation Union (IUCN) and U.N. Environment Programme (UNEP), and the "Global Strategy for the Conservation of Marine Turtles" by the IUCN/SSC Marine Turtle Specialist Group (MTSG)) provide a framework for regional collaboration. In developing strategies for such collaboration, participants must first diagnose key conservation issues and focus on those areas where national issues and needs require or would benefit from being addressed at a transnational level.

Regional collaboration in the collecting and sharing of research data has the potential to guide meaningful management actions and to assist in the determination of conservation priorities. In addition, many of the issues relating to marine turtle conservation are usually common throughout a region, and individual countries have much to gain from sharing their experience. There is no rigid formula when encouraging regional collaboration for marine turtle conservation. Various approaches have been employed in different geographical arenas. In the South Pacific, the regional marine turtle program is a component of a broader regional agreement pertaining to the environment in general. In the Caribbean Sea, cooperative action is effectively being achieved through an active network of international marine turtle experts, local country coordinators, and hundreds of interested citizens throughout the Wider Caribbean region. In Southeast Asia, the regional marine turtle conservation program underwent a long and formal process under the aegis of ASEAN, a regional organization, that, as a group, operates by consensus.

Whatever process is employed in establishing multilateral collaboration, it is advantageous to use a functionalist approach; that is, the program should focus on its function, which is the conservation of marine turtles throughout a geographic region. Avoiding potential obstacles, such as geopolitics, tariffs, sovereignty, and national security, will hasten acceptance and generate support from participating states. In cases where states oppose cooperation, unofficial planning by conservation groups or the pursuing of partnerships at lower government levels (such as between state-supported projects dealing with marine turtles) can provide a vehicle for cooperation. Particularly where the states involved are developing nations, expecting large financial commitments from governments may create further resistance. One way to partially overcome the funding issue is to integrate a regional conservation initiative into an existing intergovernmental agenda.

The South Pacific Regional Environment Program (SPREP) is an intergovernmental program for the oceanic Pacific Island nations. The successful Regional Marine Turtle Management Program (RMTMP) in this area is a component of SPREP's larger biodiversity program. Representatives from participating countries meet annually to provide information on species distribution and abundance, nesting and foraging areas, cultural importance and traditional use, the status of legislation and law enforcement, tagging data (including long distance recoveries which help define range states), the impacts of natural coastal processes and calamities *(e.g.,* cyclones), and threats associated with international trade, incidental catch, and ill-conceived coastal development. Based on agreed priorities established at these annual meetings, project proposals are developed and submitted to the SPREP Secretariat for funding. A collective database is maintained. Using this approach, the RMTMP is able to effectively address a variety of conservation issues relating to marine turtles, which constitute a prominent shared resource.

In the Wider Caribbean, the Caribbean Environment Programme (CEP) was established 20 years ago by governments of the region under the aegis of UNEP to work under a framework of regional cooperation for the protection and management of the coastal and marine resources of the region. The Wider Caribbean Sea Turtle Conservation Network (WIDECAST), a partner organization to CEP, is comprised of national coordinators in more than 30 nations and a well developed grassroots network. Its primary objectives are to promote a regional capability to implement scientifically sound marine turtle conservation programs ("by developing a technical understanding of marine turtle biology and management in local individuals and institutions"), and to assist Wider Caribbean governments in fulfilling their obligations under the SPAW Protocol (see Hykle, this volume). With the assistance of local network participants and regional experts, national coordinators oversee the development of national Sea Turtle Recovery Action Plans which summarize available information and make conservation recommendations. WIDECAST also sponsors training opportunities, assists with the design and implementation of conservation and management programs, and produces and distributes educational materials. Based on the experiences of developing and implementing national recovery plans and at the request of governments under the framework of CEP, WIDECAST is currently finalizing regional guidelines for sea turtle management in the Wider Caribbean. As is the case with the RMTMP, representatives from participating countries meet annually to share information and discuss national and regional conservation strategy.

In 1975, the Mediterranean Action Plan (MAP) was established by governments in the region under UNEP's Regional Seas Programme. MAP, which has as its focus the protection of the Mediterranean Sea, consists of three components: scientific, socioeconomic, and institutional and legal (Barcelona Convention and Protocols). The Protocol on Specially Protected Areas was adopted in 1982 and came into force in 1986 (see Hykle, this volume). Parties to the Barcelona Convention included the protection of marine turtles among their priority targets for the period 1985-1995 (Genoa Declaration, September 1985). For this purpose they adopted in 1989 the "Action Plan for the Conservation of Mediterranean Marine Turtles." Issues of regional significance relating to Specially Protected Areas and endangered species (including sea turtles) are coordinated by the Regional Activity Centre for Specially Protected Areas (RAC/SPA), based in Tunisia. Parties to the Convention have nominated National Focal Points in their respective countries for liaising with this Centre on technical and scientific issues. The National Focal Points meet every two years. The Centre provides the Parties with assistance in a variety of fields, including the organization of seminars and training courses. It also provides financial assistance, for example to trainees for participation in courses in marine turtle conservation techniques (such as the one held every year at the Lara Marine Turtle Station in Cyprus) and for carrying out beach surveys. The European Union also provides possibilities of financing Mediterranean States in projects related to turtle conservation through its EC instruments, such as LIFE/Third Countries or MEDA. RAC/SPA maintains relations with several intergovernmental organizations and NGOs, and it *inter alia* maintains inventories and databases.

The ASEAN Regional Conservation Program (conceived in late 1993) provides a framework for regional collaboration on marine turtle research and conservation in Southeast Asia. The ASEAN region (Brunei, Indonesia, Malaysia, Philippines, Singapore, Thailand, Vietnam) supports six species of marine turtles and globally significant aggregations of green and leatherback turtles. The regional program calls for the development and implementation of six major components, namely: (1) Establishment of Information Systems, (2) Institution Building, (3) Management Oriented Research and Monitoring, (4) Information and Education Campaigns, (5) International Efforts and Linkages, and (6) Resource Management. Recognizing that the full implementation of broad regional programs such as this entails huge amounts of funding, efforts are being made to implement model conservation initiatives. One such initiative is the bilateral approach between the governments of the Philippines and Malaysia to jointly manage the Philippine Sabah Turtle Islands. The Philippine Sabah Turtle Islands support the only remaining major nesting colony in the ASEAN region.

Transborder protected areas have gained popularity during the past decade as a novel and pragmatic approach to conserving endangered species and habitats shared by neighboring countries. The Turtle Islands Heritage Protected Area (TIHPA) takes its name from the Turtle Islands, a group of nine islands situated along the International Treaty Limits between the Philippines and Malaysia. These nine islands have a total land area of only 336 hectares, but they harbor one of the largest aggregations of green *(Chelonia)* and hawksbill *(Eretmochelys)* turtles in the world. Documented movements by tagged adults demonstrate that the cluster of islands constitutes a single rookery. More than 17 million eggs were laid between 1984-1995, 72 percent on the six Philippine islands and 28 percent on the three Sabah (Malaysia) islands. The impact of mass harvesting of eggs, a traditional source of income to the islanders, is now evident. Between 1951-1980, egg production in the Turtle Islands plunged by more than 88 percent. While most of the eggs were taken from Philippine nesting beaches, the majority were sold in Malaysia.

In response to the crisis in the Turtle Islands, the World Wildlife Fund Philippine Program (WWF-PP) developed a project proposal to establish the Turtle Islands as a transborder management area for marine turtles. A Working Group composed of representatives from the Pawikan Conservation Project, (a project of the Philippines Department of Environment and Natural Resources), WWF-PP, and the Marine Turtle Foundation was organized to lay the groundwork for the TIHPA. The group, chaired by WWF-PP, undertook to draft a Memorandum of Agreement (MOA) as its first task. At the Second Meeting of the Philippines-Malaysia Joint Commission for Bilateral Cooperation (JCBC) in February 1995, a Joint Technical Working Group (JTWG) was created to build on the efforts of the Philippine consortium.

After much deliberation, the JTWG eventually agreed on a final draft MOA establishing the TIHPA. The MOA stipulated that contracting Parties endeavor to develop an integrated management program including, at a minimum, the following: (1) Implementation of an integrated and uniform approach to conservation and research that is oriented towards wise management of the TIHPA, (2) establishment of a centralized database and information network on marine turtles, (3) development of appropriate information awareness programs primarily targeting inhabitants of the Turtle Islands on the conservation of marine turtles and the protection of their habitats, (4) implementation of a joint marine turtle resource management program, (5) development and implementation of a training and development program for the staff of the TIHPA, and (6) development and undertaking of eco-tourism programs.

In May 1996, during the Third Meeting of the Philippine-Malaysia JCBC, a landmark agreement was forged between the two governments establishing the Turtle Islands Heritage Protected Areas (TIHPA). The TIHPA is considered the world's first transfrontier protected area for sea turtles. It should be noted that the Philippines has an unresolved territorial claim over Sabah. While the present government is not aggressively pursuing this claim, it remains an irritant in an otherwise healthy relationship between the two countries. It is heartening to see that even in the face of diplomatic sensitivities, significant progress can be made on behalf of urgent conservation issues.

Building on the success of programs in other regions, a Western Indian Ocean Training Workshop and Strategic Planning Session (jointly organized by the IUCN East African Regional Office and the MTSG) was hosted by the Natal Park Board in Sodwana Bay, South Africa, in November 1995. As a result of a series of national presentations and group discussions designed to determine key issues in sea turtle conservation at national and regional levels, the meeting participants drafted a Marine Turtle Strategy and Action Plan for the Western Indian Ocean. Modelled after the MTSG's Global Strategy for the Conservation of Marine Turtles, the strategy addresses the following needs: research and monitoring; integrated management for sustainable marine turtle populations; building capacity for conservation, research, and management; public awareness, information, and education; community participation in conservation; regional and international cooperation; and fund raising. Twelve priority actions were identified which need to be taken at the regional level in order to facilitate national programs. In January 1997, the MTSG organized a similar Workshop and Strategic Planning Session in Bhubaneswar, India and participants drafted a Marine Turtle Conservation Strategy and Action Plan for the Northern Indian Ocean. Building on its global and regional predecessors, the Northern Indian Ocean Strategy also identifies priority actions and programs that are needed to facilitate and promote marine turtle conservation at the national and regional level. Requisite to the achievement of both strategies is the development of specific agreements for collaborative management at the regional level to encourage full integration of all states into the program and facilitate formal interagency or bilateral partnerships.

While it is not possible to describe or give credit to all successful examples of international collaboration, we hope that the reader is heartened by the overview herein presented, and inspired to both participate in and pursue international opportunities. At the very least, we encourage all those laboring on behalf of marine turtle conservation to integrate their localized efforts with the efforts of colleagues working with the same populations of turtles in countries hundreds or even thousands of kilometers distant. By this we intend not only that our field methodologies be consistent and sound (as advocated by this manual), but that resources wisely expended at the local and national levels will contribute to the survival of sea turtles throughout their ranges.

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International Conservation Treaties

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A variety of legal instruments concluded among governments underpins much of the conservation work related to sea turtles. This section describes a number of conservation treaties operating on a global and regional level which are directly or indirectly relevant to the conservation of these animals. The coverage is necessarily incomplete: in the limited space available it is possible only to summarize the main features of the most prominent conventions. Each subsection includes information on the date of adoption of the treaty, an acronym or short form in common use, the date of entry into force, and the membership status at the time of writing. No attempt is made to present the many regional action plans that have been developed worldwide, some of them having at least tacit governmental endorsement. Some of the conventions adopt a species-oriented approach to conservation, others place more emphasis on habitat measures, while others are all-embracing. A feature common to all of the treaties is that their effectiveness and usefulness depends on the political motivation and availability of resources to implement them.

Global Conventions

1. Convention on International Trade in Endangered Species of Wild Fauna and Flora, 1973 (CITES): entered into force 1 July 1975; 145 member States

Arguably the most widely-known wildlife treaty, CITES strives to regulate international trade in a wide range of wild animals and plants through a system of export and import permits. The provisions governing trade from one member State to another of species listed in Appendix I of the Convention are particularly stringent: permits are required certifying that a specimen has been obtained legally, and that the import will not be detrimental to the survival of the species and is not for primarily commercial purposes. All sea turtles are listed in Appendix I: therefore, commercial trade in live or dead specimens, their parts and derivatives is effectively prohibited between CITES parties—except for those which formally enter a reservation exempting them from the Convention's provisions in relation to the species in question. In November 1994 the Conference of the Parties to CITES adopted guidelines for evaluating proposals that may be submitted by Parties in order to permit the ranching (rearing in a controlled environment of specimens taken from the wild) of sea turtles for the purpose of regulated international trade. At the time of writing, the guidelines had not been put into practice. CITES benefits from a broad membership and relatively well-established national implementation structures, and it attracts considerable attention from governmental and non-governmental bodies. CITES regulates only international trade and does not impose on its Parties legally-binding measures with respect to the domestic harvesting of sea turtles.

Secretariat: CITES Secretariat, 15, ch. des Anémones, C.P. 456, CH-1219 Châtelaine-Geneva, Switzerland; Tel: (+4122) 979-9139/40; Fax: (+4122) 797-3417; email: cites@unep.ch; Website: www.wcmc.org.uk/CITES

2. Convention on the Conservation of Migratory Species of Wild Animals, 1979 (CMS or Bonn Convention): entered into force 1 November 1983; 57 member States

The Convention on Migratory Species contains strict measures for the protection of sea turtles at the national level and encourages regional cooperation through specialized Agreements and joint research activities. Parties which are Range States for species listed in Appendix I (which includes all sea turtles except *Natator depressus)* are to endeavor to conserve their habitat, to counteract factors impeding their migration, and to control other factors that might endanger them. Above all, Parties are obliged to prohibit the taking of animals of these species with few exceptions. Appendix II lists migratory species that require or would benefit significantly from international cooperative Agreements—which may range from legally-binding treaties to less formal memoranda of understanding. The more formal Agreements should provide for coordinated species conservation and management plans; conservation and restoration of habitat; control of factors impeding migration; cooperative research and monitoring; and public education and exchange of information among Parties.

Sea turtles have been identified as a priority group for concerted action by the decision-making bodies of CMS. The Convention is sponsoring basic research *(e.g.,* surveys of critical nesting beaches, genetic studies to help elucidate migration patterns), information activities *(e.g.,* identification posters for Atlantic sea turtles, publications such as a review of the state of knowledge of sea turtles along the Atlantic coast of Africa, a prototype GIS map facility for nesting beaches of the Indian Ocean) and capacity building *(e.g.,* regional training/policy workshops, conservation techniques manual.) Starting at a regional level and focusing in particular on developing countries, CMS is working towards an interlinked, global framework for the conservation of sea turtles.

Secretariat: UNEP/CMS Secretariat, Martin-Luther-King-Str. 8, D-53175 Bonn, Germany; Tel: (49)(288) 815-2401/2; Fax: 815-2449; email: cms@cms.unep.de; Website: www.wcmc.org.uk/cms

3. Convention on Biological Diversity, 1992 (CBD): entered into force 29 December 1993; 174 member States

The objectives of CBD are "the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources…". Parties are obliged *inter alia* to develop (or adapt existing) national strategies, plans, or programs for the conservation and sustainable use of biological diversity, to carry out *in situ* conservation activities *(e.g.,* establishment of protected areas, rehabilitation and restoration of degraded ecosystems, regulation or management of activities affecting biological diversity), to undertake identification and monitoring activities, and to encourage customary use of biological resources compatible with conservation or sustainable use needs. The Convention does not explicitly address the conservation of sea turtles—indeed, the CBD contains no annexes of species to which its provisions are to apply. However, it does provide a framework within which broader conservation objectives are pursued. While the Convention has attracted wide political and financial support, implementation of specific components of the CBD is expected to be achieved through other instruments.

Secretariat: Secretariat of the Convention on Biological Diversity, World Trade Centre, 413 St. Jacques Street, Office 630, Montréal, Québec, Canada H2Y 1N9; Tel: (+1 514) 288-2220; Fax: (+1 514) 288-6588; Website: www.biodiv.org

Regional Conventions

1. Convention on Nature Protection and Wildlife Preservation in the Western Hemisphere, 1940 (Western Hemisphere Convention): entered into force 1 May 1942; 22 member States

The Western Hemisphere Convention's stated objectives are to protect and preserve all species and genera of native flora and fauna from extinction, to promote the establishment of protected areas, and to foster greater international cooperation. Strict protection is to be accorded to species listed in the annex to the Convention (actually, a compilation of national species lists), including five species of sea turtles. Innovative for its time, the Convention attracted a wide membership and it is said to have provided a conceptual foundation for the creation of protected areas and to have stimulated technical cooperation. For the most part, however, the Convention has not lived up to its potential—lacking a fully-fledged secretariat and without broad governmental support to revitalize it.

Depository: Organization of American States, Secretariat for Legal Affairs, 19th Street and Constitution Avenue NW, Washington, D.C. 20006; USA; Tel: (+1 202) 458-3395; Fax: (+1 202) 458-3250

2. Convention for the Protection of the Mediterranean Sea against Pollution, 1976 (Barcelona Convention): entered into force 12 February 1978; 20 member States and the European Union

Protocol concerning the Mediterranean Specially Protected Areas, 1982 (SPA Protocol): entered into force 23 March 1986; 20 member States and the European Union.

The Barcelona Convention has general provisions for the protection of the Mediterranean marine environment, while sectoral issues are covered by a series of protocols. The Convention and the protocols are the legal component of the Mediterranean Action Plan (MAP), which functions under UNEP's Regional Seas Programme. In 1995 the Barcelona Convention was amended (new title: Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean). The amendments, not yet in force, *inter alia* oblige Contracting parties to "take all appropriate measures to protect and preserve biological diversity, rare or fragile ecosystems, as well as species of wild fauna and flora which are rare, depleted, threatened, or endangered and their habitats…". In 1985 the Contracting Parties, in their Genoa Declaration, adopted ten priority targets to be achieved. Among these was the protection of marine turtles. In addition, a network or Regional Activity Centres deal with sectoral issues (see below).

Secretariat: Coordinating Unit for the Mediterranean Action Plan (UNEP), Cas. Konstantinou 48, P.O. Box 18019, 11610 Athens, Greece. Tel: (+301) 72 73 100 (switchboard); Fax: (+301) 72 53 196/7; e-mail: unepmedu@unepmap.gr

The SPA Protocol currently in force deals with issues related to the establishment of specially protected areas. In addition to its provisions related specifically to SPAs, an Action Plan for the conservation of marine turtles was adopted in 1989. In November 1998 this Action Plan was revised and is to be submitted to the next Contracting Party meeting (October 1999) for approval. In 1995 a new Protocol was adopted and will, in time, come into force and replace the current SPA Protocol (new title: Protocol concerning Specially protected Areas and Biological Diversity in the Mediterranean). it contains general obligations similar to those found in the Convention on Biological Diversity. In addition, the Protocol requires Contracting Parties to protect, preserve, and manage threatened or endangered species (including the prohibition of taking, possession, killing, commercial trade, disturbance, etc.), to establish protected areas, and to coordinate bilateral or multilateral conservation efforts. In addition to the declaration of Specially Protected Areas of Mediterranean Importance (SPAMIs), the new Protocol has an Annex listing endangered species for protection and conservation. Marine turtles are included in this list.

Secretariat: Regional Activity Centre for Specially Protected Areas, Centre International de l'Environment, 1080 La Charguia, Tunisia. Tel: (+216 1) 795 760; Fax: (+216 1) 797 349; e-mail: racspa@tunisia.eu.net

3. Convention on the Conservation of European Wildlife and Natural habitats, 1979 (Bern Convention): entered into force 1 June 1982; 36 European and African States and the European Union

The Contention's aims are to "conserve wild flora and fauna and their natural habitats, especially those species and habitats whose conservation requires the cooperation of several States and to promote such cooperation. Particular emphasis is given to endangered and vulnerable species including endangered and vulnerable migratory species." The Contracting Parties undertake *inter alia* to protect the species of fauna and flora listed in Appendices I and II, as well as their habitats. Five species of marine turtles are included in Appendix II, as strictly protected species. They are, *Chelonia mydas*, *Caretta caretta*, *Eretmochelys imbricata, Lepidochelys kempii,* and *Dermochelys coriacea*, with most attention focused on the first two. NGOs are actively participating in the work of the Convention and are often the prime movers in specific protection and monitoring operations. The Convention's Standing Committee can and does adopt generic and specific recommendations. Several files have been opened on marine turtle conservation issues and recommendations have been addressed to several States. The Convention is building a network of protected areas known as the Emerald Network of Areas of Special Conservation Interest, and is also responsible for coordinating a European Action Programme on Threatened Species within the framework of the Pan-European Biological and Landscape Diversity Strategy.

Secretariat: Council of Europe, Environment Conservation and Management and Regional Planning Division, F-67075 Strasbourg Cedex, France. Tel: (+33 88 412000); Fax: (+33 88 413751), e-mail: gianluca.silvestrini@coe.fr; Website: www.coe.fr

4. Convention for the Protection and Development of the Marine Environment of the Wider Caribbean Region, 1983 (Cartagena Convention): entered into force 11 October 1986; 21 member States

Protocol concerning Specially Protected Areas and Wildlife (SPAW Protocol): adopted 18 January 1990; annexes adopted 11 June 1991 (not yet in force: only 7 of the required 9 ratifications)

The Cartagena Convention urges Contracting Parties to "individually or jointly, take all appropriate measures to protect and preserve rare or fragile ecosystems, as well as the habitat of depleted, threatened, or endangered species, in the Convention area." Its SPAW Protocol provides for a comprehensive set of protection measures benefiting sea turtles, including the establishment of protected areas, regulation or prohibition of deleterious activities, development of management regimes, and international cooperation. All six species of sea turtles inhabiting the Wider Caribbean *(i.e., Caretta caretta, Chelonia mydas, Eretmochelys imbricata, Dermochelys coriacea, Lepidochelys kempii,* and *L. olivacea)* are listed in Annex II of the Protocol. Article II of the Protocol prohibits for species in Annex II: (a) the taking, possession, or killing (including, to the extent possible, the incidental taking, possession, or killing) or commercial trade in such species, their eggs, parts, or products, and (b) to the extent possible, the disturbance of such species, particularly during breeding, incubation, estimation, migration, and other periods of biological stress. Exceptions to the prohibitions—for scientific, educational, or management purposes—are not allowed. Although it is not yet in force, the adoption of the SPAW Protocol has already stimulated the development and implementation of the Regional Programme for Specially Protected Areas and Wildlife under the framework of the Caribbean Environment Programme. This includes sea turtle conservation activities implemented through the WIDECAST network (see Trono and Salm, this volume) as well as other relevant activities *(e.g.*, establishment and management of marine protected areas and MPA networking.

Secretariat: Caribbean Environment Programme Regional Co-ordinating Unit, 14-20 Port Royal Street, Kingston, Jamaica. Tel: (+1 876) 922 9267-9; Fax (+1 876) 922 9292; email: uneprcuja@toj.com; Website: www.cep.unep.org

5. Convention for the Protection of the Natural Resources and Environment of the South Pacific Region, 1986 (SPREP Convention): entered into force 22 August 1990; 11 member States

The South Pacific Regional Environment Programme (SPREP), formally established in 1980, gave its name to the convention that was adopted at one of its meetings in 1986. Parties to the SPREP Convention shall "take all appropriate measures to protect and preserve rare or fragile ecosystems and depleted, threatened, or endangered flora and fauna as well as their habitat" and "establish protected areas, such as parks and reserves, and prohibit or regulate any activity likely to have adverse effects on the species, ecosystems or biological processes that such areas are designed to protect." A Regional Marine Turtle Conservation Programme, developed under SPREP's Natural Resource Conservation Programme, promotes sea turtle conservation and monitoring work in the SPREP region, and helped to launch a Year of the Sea Turtle campaign in 1995.

Secretariat: South Pacific Regional Environment Programme, P.O. Box 240, Apia, Western Samoa; Tel: (+685) 21 929; Fax: (+685) 20 231; email: sprep@apctok.peg.apc.org

Other Instruments

Various other legal instruments are relevant, directly or indirectly, to the conservation of sea turtles, including the International Convention for the Prevention of Pollution from Ships, 1973 (MARPOL: entered in force 2 October 1983), the Convention on Conservation of Nature in the South Pacific, 1976 (Apia Convention: entered into force 28 June 1990), the United Nations Convention on the Law of the Sea, 1982 (UNCLOS: entered into force 16 November 1994), the ASEAN Agreement on the Conservation of Nature and Natural Resources, 1985, and the Convention for the conservation of biodiversity and protection of priority wild areas in Central America, 1992 (Conenio para la conservación de la biodiversidad y protección de áreas silvestres prioritarias en América Central). Other treaties are also relevant, but have yet to enter into force (as of the time of writing), such as the Convention for the Protection, Management and Development of the Marine and Coastal Environment of the Eastern African Region, 1985 (Nairobi Convention) and its related protocol on Protected Areas and on Wild Fauna and Flora in the Eastern African Region, 1985. The Inter-American Convention for the Protection and Conservation of Sea Turtles, the world's first treaty dedicated to sea turtles, was concluded in 1996; it requires eight ratifications before entry into force. The objective of the Convention is "to promote the protection, conservation, and recovery of sea turtle populations and of the habitats on which they depend…," The Convention addresses intentional harvest, accidental capture, and habitat destruction and encourages states outside the Americas to sign complementary protocols consistent with its goals.

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Forensic Aspects

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Forensic Science

Forensic science has been defined as the "application of the natural and physical sciences to the resolution of matters within a legal context" (Thornton, 1994). Therefore, forensic science is concerned with the crime and the suspects, followed by investigation and comparative/analytical analysis of the evidence, and can culminate in testimony regarding an interpretation of what the analyses mean in the context of the crime.

The informal use of the term "forensics" is popular among the wildlife research community today; much of this research is typically for information use only and never intended to reach a court of law. Because forensic tests can have significant impact on the outcome of a trial or public decision process, the informal use of the term forensics should be avoided. Attempting to infer support for the legal process while not instituting formal forensic investigative procedures is likely to compromise the admissibility of the analysis as evidence in a court of law and prevent successful prosecution of law enforcement cases. It is imperative that researchers who agree to conduct biochemical identifications of wildlife evidence for legal proceedings be fully aware of the unique responsibilities that forensic scientists have when supporting law enforcement and the law.

When is Forensic Science Used for Sea Turtles?

In the United States, forensic techniques for species identification have been used in conservation efforts concerning sea turtles since the late 1970s by the marine Forensics Program at the Charleston Laboratory of the National Marine Fisheries Service. In 1997, the laboratory, with its Marine Forensics Program, came under the jurisdiction of NOAA's National Ocean Service (NOS). Though the majority of species identification analyses have been conducted in support of law enforcement activities for the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) or other violations of the U.S. Endangered Species Act, management and research questions can be addressed using the same technology. An example of a legal case involved the seizure of a chunk of red meat claimed to be venison (deer, *Odocoileus* sp.) during the boarding of a shrimp trawler. The meat was forensically analyzed using isoelectric focusing and positively identified as loggerhead sea turtle, *Caretta caretta*, (Colbert, 1993).

What Kind of Samples Can Be Used as Evidence?

The most common types of evidence from suspected illegal take or trade in sea turtles or turtle products are meat, eggs, shells, or cosmetics containing turtle oils (see Table 1). Unusual or difficult circumstances should not rule out forensic analysis, and discussion with marine forensic scientists can often lead to new approaches in evidence analyses. New questions in sea turtle conservation continually arise. The Marine Forensics Program attempts to initiate methods development and collaborative research to meet new needs. Such activities frequently require collection of new standard samples from some or all sea turtle species for comparison to evidence and for database development. If capabilities are not currently available to address a sea turtle forensic issue, efforts are made to locate researchers that may be able to provide technical support. In such cases, the Marine Forensics Program may refer an inquiry to another researcher, or may offer to act as liaison for the issue so that the evi-

Table 1. Collection and Analysis Methods for Sea Turtle Body Parts

Evidence Type	Collection/Preservation	Analysis/ Methodology	Limitations of Methodology
Edible Quality Raw Meat	Chain of Custody and Documentation Place Tissue in plastic bag, ice immediately upon collection, then freeze as soon as possible *For DNA Analysis, meat may also be dried, salted or placed in Ethyl Alcohol, If refrigeration is not available	Isoelectric Focusing (IEF) DNA – RFLP or Sequencing	• Availability of standards from certain geographical locations • Availability of standards • Database incomplete
Eggs – Raw	Chain of Custody and Documentation Place Eggs in plastic bags, ice, refrigerate or freeze *If above not immediately available, eggs may be kept in damp sand for up to 48 hours	Lipid Analysis DNA RFLP or Sequencing	• Loggerhead, Kemp's and Olive Ridley are indistinquishable from one another • Standards lacking from certain geographical locations • Availability of standards • Database incomplete
Eggs – Cooked	Chain of Custody and Documentation Place Eggs in plastic container and refrigerate or freeze	Lipid Analysis DNA – RFLP or Sequencing	• Same as for raw eggs • Method under development • Availability of standards across geographic range • Characterization of additional markers needed
Blood (Taken from Animal)	Chain of Custody and Documentation Refrigerate up to 48 hours Freeze at -20°C – -80°C Dry on cotton cloth, cotton swab or filter paper Place in Lysis solution (provided by forensic lab)	DNA – RFLP or Sequencing	• Availability of standards across geographic range • Characteristics of additional markers needed
Blood Stains	Chain of Custody and Documentation Dry, bag material containing stain Scrape dried blood into plastic bag or tube, keep dry	DNA – RFLP or Sequencing	• Availability of standards across geographic range • Characteristics of additional markers needed
Other Internal Tissues	Chain of Custody and Documentation Only small piece of tissue required, ~.2-2cm^3 Refrigerate or freeze Place in salt to dry tissue Place in Ethyl Alcohol	DNA Analysis	• Availability of standards • Database incomplete
Carapace – Fresh with Tissue Attached	Chain of Custody and Documentation Refrigerate See above for additional tissue preservation for DNA analysis	Visual/Morphological Analysis IEF DNA Analysis	• Subjective, based on expert interpretation • Tissue must not be decomposed • Availability of standards • Database incomplete
Carapace – Dried, No Attached Flesh	Chain of Custody and Documentation Bag material and document with chain of custody	Visual/Morphological Analysis	• Subjective, based on expert interpretation
Skeletal Remains	Chain of Custody and Documentation Bag material and document with chain of custody Keep dry	Visual/Morphological Analysis DNA Analysis	• Subjective, based on expert interpretation • Availability of standards • Database incomplete
Dried Tissue	Chain of Custody and Documentation Bag material. Keep dry.	DNA Analysis	• Availability of standards • Database incomplete
Cooked Meats	Chain of Custody and Documentation Bag material. Refrigerate up to 1 week and/or freeze	DNA Analysis	• Method under development • Availability of standards • Database incomplete
Canned Meats	Chain of Custody and Documentation Bag material AS IS	DNA Analysis	• Method under development • Availability of standards • Database incomplete
Oils and Cosmetics	Chain of Custody and Documentation Bag material AS IS, Protect from air and sunlight	Lipid Analysis	• Results may be confounded by additives • Concentration of Turtle Oil in cosmetics may be below level of assay detectibility
Jewelry/Skin Products/Dried Turtle Penis	Chain of Custody and Documentation Bag material AS IS	Visual DNA	• Subjective, based on expert interpretation • Extent of processing tissue/skin has undergone

dence is handled in a manner that would most likely be acceptable in court and in the scientific community.

What Kind of Documentation is Needed for a Forensic Analysis?

Permits are the first requirement for handling or possessing sea turtles, or their parts or products. In the field of marine forensics, seized property (evidence) or morphologically unidentifiable samples are compared to special samples from an archive. These special samples, called standards, have been collected from carefully identified whole animals by authorized experts who also provide signed documents verifying the species. Standards and evidence alike are accompanied by a “chain of custody” or a traceable audit trail that originates with the collector and accompanies the sample at all times. Every person in possession of an evidence sample or a standard sample signs and dates the chain of custody when they receive and release the sample.

Chain of custody is maintained by keeping the sample under secure conditions with limited access, shipping or transferring the sample in a secure manner so that tampering is detectable, and using a laboratory facility where chain of custody procedures are followed during analysis. Any person who signs a chain of custody can potentially be called into court to testify about his/her possession of a sample, as can a person who verifies the species of a standard sample. The analyst is the most likely person to be asked to testify regarding forensic activities, and should be able to testify that the procedure was conducted accurately and that no individual could have tampered with the samples during analysis. In addition to chain of custody documentation, the analyst must produce a case report and be able to provide case related notes and other laboratory information, if requested.

How are the evidence samples identified? It is often difficult in the field to definitively determine the species of origin of sea turtle eggs or other tissues when the whole animal is not available. When the species of a sample cannot be conclusively determined by observation with the naked eye, chemical or biochemical analysis can often be used to reliably and definitively identify the species. For example, egg morphology, in conjunction with beach or origin, are sometimes useful in identifying eggs to species. Egg size ranges do, however, overlap between many species, and often more than one species of turtle nests on a particular beach. In such circumstances, chemical analyses such as lipid chemistry or DNA analyses are usually necessary.

Meat with attached skin or flippers may be identifiable from claw counts and/or morphology of skin and scales, if a sufficiently large piece is available. However, if only a small piece of meat or skin is available, forensic methods such as DNA and protein techniques will be needed to make identifications. The potential now exists, through the application of DNA technologies, to ascertain the parentage of eggs or hybrid individuals resulting from crosses between two species, and to determine the species identity of bone and scute fragments, as well as make identifications from trace evidence such as blood spatters. Details for all of the methods described in this section can be found in the “Charleston Laboratory Marine Forensics Manual,” an internal standard operating protocol. Additional information is available upon request from the NOAA/NOS Charleston Laboratory.

How are Forensic Samples Collected and Stored?

The effectiveness of forensic activities in law enforcement cases, management, and research issues is largely dependent upon the handling of samples prior to analysis. Errors can be made in sample documentation, collection, storage, packaging, and shipping that can diminish the value of or even exclude the samples for forensic analysis. Samples should be separated to prevent the contamination of one sample by another sample. Therefore, when multiple samples are collected, they should be individually bagged and labeled and a chain of custody started at the point the evidence is seized. It is recognized that some researchers and enforcement personnel working in remote areas may not have access to ideal sampling tools or shipping supplies such as dry ice, gel-packs, and Styrofoam containers. Should this situation arise, select an appropriate storage or preservation method from Table 1 until shipping or transport to the laboratory can be arranged. Though most types of samples can be safely frozen and then shipped by over night courier on dry ice or frozen gel-packs, it is preferred than anyone seeking forensic assistance call the NOAA/NOS Charleston Laboratory at (843) 762-8500; Fax: (843) 762-8700; email: marine.forensic @noaa.cov. Ask for Forensics Program personnel and discuss the issue prior to storing or shipping samples.

What is the Transport or Shipping Protocol?

Once notice has been given to the appropriate analyst at the laboratory that a shipment is being sent, the samples may be sent to the scientist at the NOAA/NOS Charleston Laboratory. The phone number of the laboratory, (843) 762-8500, and the correct zip code, 29412, must be used on the shipping label. Use of the wrong zip code can cause delays in delivery and may result in loss of the samples for forensic analysis. The services of the Marine Forensics Program are available to scientists and law enforcement personnel from around the world.

Who conducts forensic analyses? Again using the United States as an example, there are currently two federal wildlife and marine agencies with active forensic programs: the Fish and Wildlife Service (National Fish and Wildlife Forensics Laboratory in Ashland, Oregon) and the National Ocean Service (Marine Forensics Program, Charleston Laboratory in South Carolina). The goal of the U.S. Marine Forensics Program is to provide forensic support in matters of marine resources enforcement, management, utilization, safety, and conservation.

Federal U.S. forensic services have been made available to anyone without charge if the request falls generally within the realm of the agency mandates. However, if the requested sample analyses are not strictly for law enforcement purposes and result in detection of illegal activities, the information must be made available to the proper authorities. The program interacts freely with federal and state wildlife law enforcement agents, fisheries managers, state marine resource agencies and university personnel engaged in marine research. When a request exceeds the program's normal capabilities, arrangements can often be made to accommodate the need on a collaborative or contractual basis, or on the basis of some other mutually acceptable arrangement.

Who is Qualified to Conduct Forensic Analyses?

Forensic analyses should be conducted by scientists who are familiar with and practice forensic procedures, who are experienced with the methods used for species identification and understand issues of cross-contamination, and who are well aware that they may be subpoenaed to testify in support of the evidence or opinion they produce. Previous experience or training in expert witness testimony is very beneficial. Such criteria for the conduct of forensic analyses should increase the likelihood that a scientist will be qualified as an "expert" in a court of law.

When compiling a national database, researchers are advised to follow forensic guidelines and to identify the species from which their standard samples are collected. Interested researchers should be aware that species identification by DNA requires an extensive database from numerous turtle individuals within each species category. A sound database, with a thorough statistical examination of data derived from well-documented and securely maintained samples, is likely to withstand legal and scientific scrutiny in law enforcement action regarding the conservation of marine turtles.

Literature Cited

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