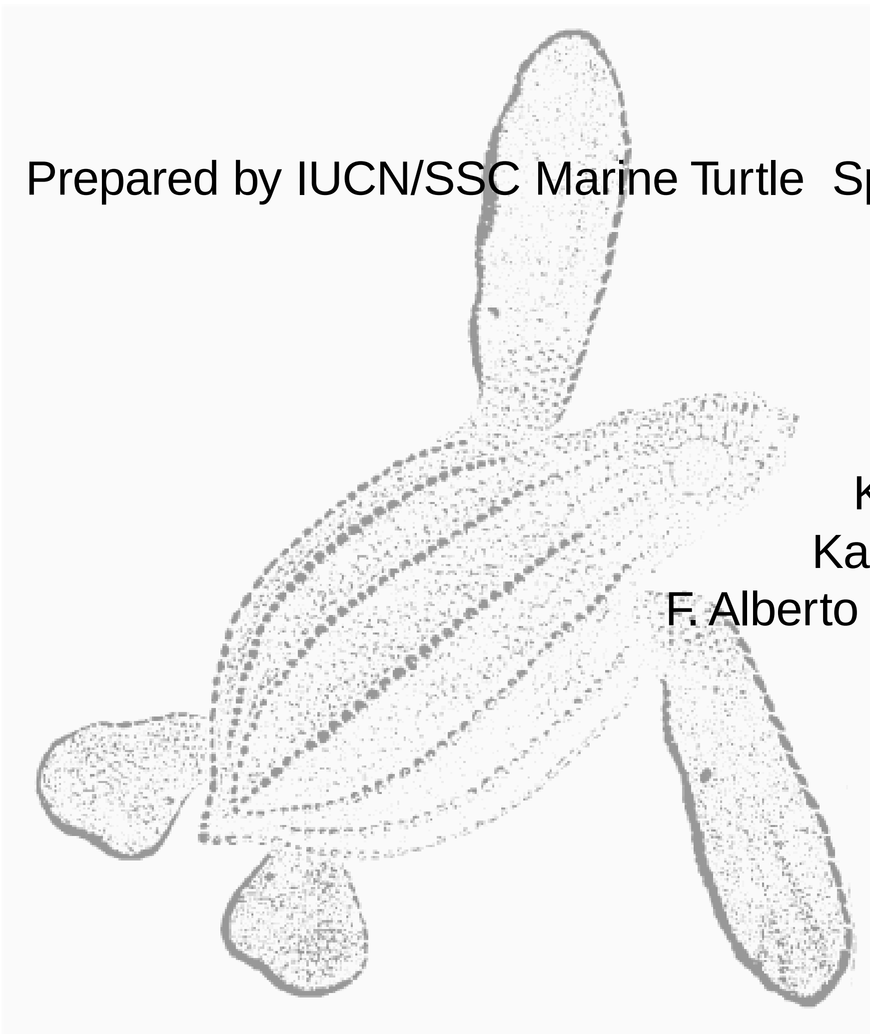
Research and Management Techniques for the Conservation of Sea Turtles



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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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Table of Contents

1. Overview

2. Taxonomy and Species Identification

3. Population and Habitat Assessment

4. Data Collection and Methods

5. Reducing Threats

6. Husbandry, Veterinary Care, and Necropsy

7. Legislation and Enforcement

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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.

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