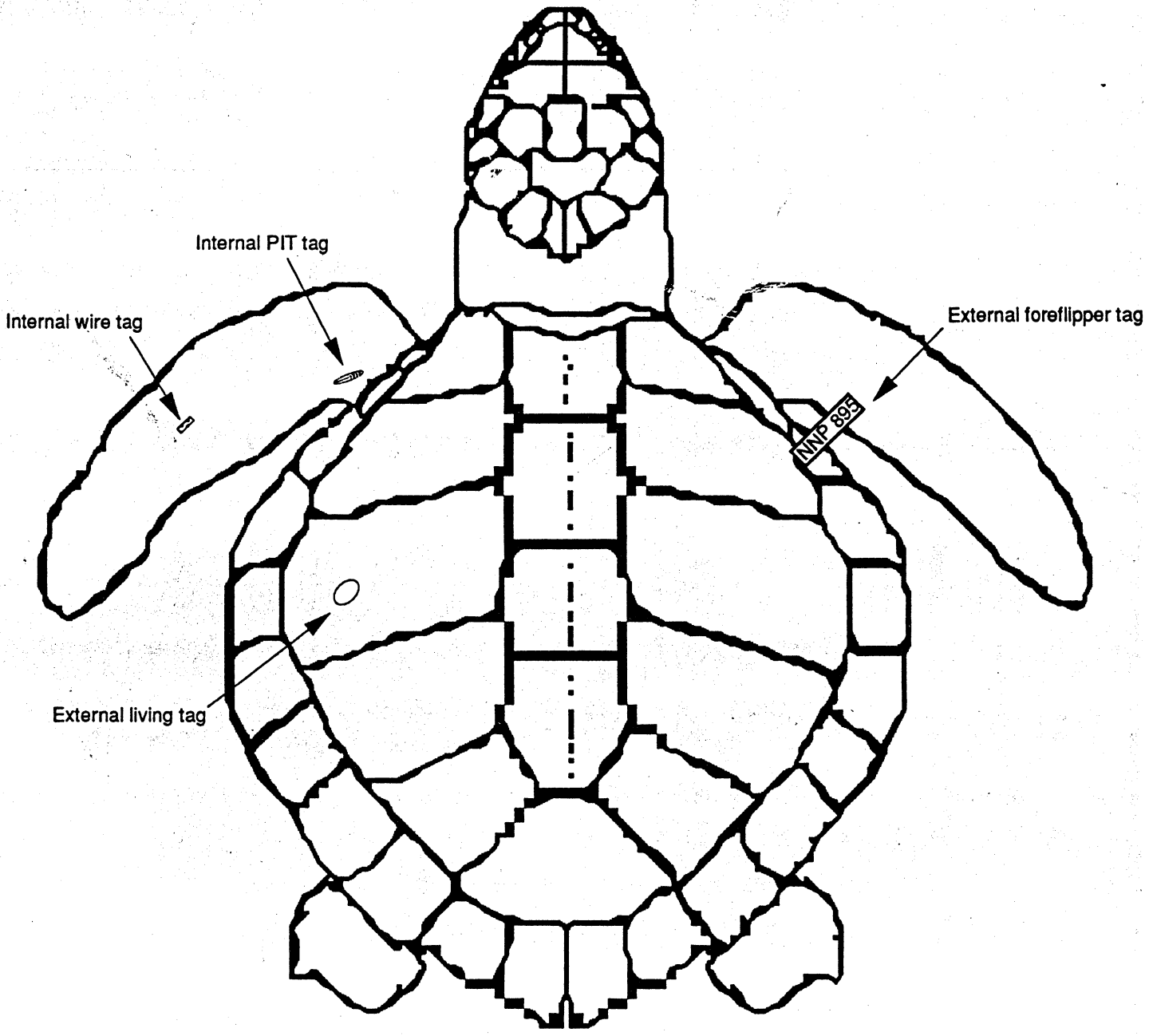
NOAA Technical Memorandum NMFS-SEFC-334

Detection, Verification and Decoding of Tags and Marks in Head Started Kemp's Ridley Sea Turtles, *Lepidochelys kempii*



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DETECTION, VERIFICATION AND DECODING OF TAGS AND MARKS IN HEAD STARTED KEMP'S RIDLEY SEA TURTLES, *LEPIDOCHELYS KEMPII*

BY

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INTRODUCTION

Preparation of this manual was motivated by recommendations made by a peer review panel of experts who evaluated the Kemp's ridley sea turtle (Lepidochelys kempii) head start experiment at the National Marine Fisheries Service (NMFS), Southeast Fisheries Science Center's (SEFSC) Galveston Laboratory on September 22-23, 1992 (Eckert et al. 1992). After examining the head start experimental design, data and analytical results, the panel expressed the goal of the experiment in the following hypotheses (modified slightly for clarity by the authors of this paper; see also Wibbels et al. 1989):

(1) Head starting can provide Kemp's ridley juveniles which are able to join the natural population, mature, find their way to nesting beaches and procreate (produce viable progeny), and

(2) head started turtles demonstrate equivalent or superior biological fitness, defined as equal or better survival rates from egg to reproductive adult, and equivalent fecundity as compared to wild Kemp's ridleys.

Testing these hypotheses requires adequate samples of tagged or marked Kemp's ridleys, both head started (the experimental group) and wild (the control group) (Eckert et al. 1992). The panel recommended large scale mark-recapture programs to determine, for all life stages:

1. Survival rates
2. Growth rates
3. Behavior (habitat selection, movement and migration patterns)
4. Physical fitness
5. Sex ratios
6. Size frequency
7. Age at maturity.

The panel applied this recommendation to wild as well as head started Kemp's ridleys, emphasizing that such mark-recapture programs are essential for comparisons between head started and wild Kemp's ridleys in any evaluation of head starting. The numbers of tagged wild juvenile Kemp's ridleys (Carr and Caldwell 1956; Marquez 1984; Henwood and Ogren 1987; Byles 1989; Ogren 1989; Schmidt and Ogren 1991; Rudloe and Rudloe 1991; Rudloe, Rudloe and Ogren 1991) are small compared to the 22,596 head started Kemp's ridleys of the 1978-1992 year classes that have been tagged and released.

Documentation of nestings of head started Kemp's ridleys will require greater efforts in surveying nesting beaches and examining

turtles for internal tags and external tags or marks (Caillouet et al. in press, a and b). Most, if not all, tagged wild nesters encountered at the primary nesting beach at Rancho Nuevo, Tamaulipas, Mexico have been tagged (and many retagged) there as adults. However, most head started Kemp's ridleys have been tagged and released off Texas when less than 1 yr old (Manzella et al. 1988; Fontaine et al. 1985, 1989a, 1989b). Therefore, to be documented as nesters, head started Kemp's ridleys must survive to maturity, copulate, find their way to a nesting beach and be inspected by someone qualified and equipped to detect, verify and decode the tags or marks used on head started Kemp's ridleys. Documentation of nesting wild Kemp's ridleys is much easier, if one assumes that every nester is wild whether tagged or not.

The metal foreflipper tag, the most widely used sea turtle tag, probably is not retained to adulthood (Henwood 1986). External living tags, internal wire tags and internal PIT (passive integrated transponder) tags show promise as lifetime tags, but require special expertise, equipment or both for detection (Fontaine et al. 1989a).

Although 22,596 head started Kemp's ridleys of the 1978-1992 year-classes have been tagged and released, this number has decreased through mortality and tag loss. In contrast, many wild nesters are retagged when encountered more than once at Rancho Nuevo. The smaller the number of tagged, head started individuals remaining in the population, the higher is the level of effort required to detect them (Eckert et al. 1992). Present efforts are insufficient to observe all wild Kemp's ridley nesters at the Rancho Nuevo beach (Pritchard 1990), so such efforts would be even less likely to detect head started nesters. With current levels and methods of beach coverage and tag detection, the likelihood of detecting head started Kemp's ridleys at Rancho Nuevo or any other nesting site is probably very low (Caillouet et al. in press, a and b).

This manual establishes protocols for examining Kemp's ridleys observed nesting, found stranded live or dead, or captured by various means, to determine if they are head started.

IDENTIFICATION OF KEMP'S RIDLEY SEA TURTLE

Pritchard et al. (1983) presented the following identifying characteristics of Kemp's ridley sea turtle (Figures 1):

1. Hard shell with horny scutes
2. Two pairs of prefrontal scales
3. Five pairs of lateral scutes

4. Dorsal color grey in immatures, light olive-green in adults, white below in immatures, yellow below in adults
5. Plastron with four pairs of enlarged scutes connecting it to the carapace
6. Head to about 13 cm wide
7. Carapace to about 70 cm long (straight line measurement) and weight to about 45 kg
8. A pore near the rear of each inframarginal
9. Carapace scutes do not overlap

Photographs of head started Kemp's ridley sea turtles of varying ages are shown in Figures 2, 3, and 4, and a wild adult female nesting on the beach is shown in Figure 5. These photographs of Kemp's ridley are shown for use in identification.

TAGS AND MARKS

All head started Kemp's ridleys released into the wild have been marked or tagged in one or more ways:

1. external, metal, foreflipper tag
2. external, living tag (plastron to carapace tissue graft)
3. internal, magnetized, coded wire tag
4. internal, passive integrated transponder (PIT) tag

Numbers tagged and released with each type of tag or mark are presented in Table 1. Various tag codes and living tag locations are presented in Table 2.

Foreflipper Tag

All head started Kemp's ridleys were tagged with metal foreflipper tags before release (Fontaine et al. 1985, 1989a, b; Manzella et al. 1988). Metal foreflipper tags were usually placed on the trailing edge of the right foreflipper (Figure 6), but left foreflippers have been tagged occasionally, and in some cases both foreflippers have been tagged. Foreflipper tags were Hasco Style, Number 681, manufactured by National Band and Tag Company, Newport, Kentucky. Each tag was engraved on one side with the message "SEND NMFS LAB, VIRGINIA KEY, MIAMI, FL 33149" and on the other side with a five or six character identification code consisting of letters, numbers or both. When recording this code, pay particular attention to distinguishing between letters and numbers that might be confused

(e.g., zero and the letter 'O', letters 'Q' and 'O', letters 'M' and 'N', etc.).

In some cases, when metal foreflipper tags are lost they leave a residue of metal particles in the flipper tissue (Figure 7). If at all possible, the left or right foreflippers of Kemp's ridleys showing a suspected tag scar should be X-rayed and examined for this evidence.

Living Tag

Starting with the 1984 year-class, all head started Kemp's ridleys were living tagged before release (Tables 1 and 2). The living tag was formed by transplanting a piece of lighter colored plastron tissue into a scute on the darker carapace (Figure 8), to create a light mark on an otherwise dark background. Each year, the living tag was applied to a different carapace scute to distinguish year-classes (Caillouet et al. 1986; Fontaine, et al. 1988a).

Wire Tag

Beginning with the 1984 year-class, all head started Kemp's ridleys were tagged with magnetized wire tags in either the right or left foreflipper before release (Manzella et al. 1987). Magnetized wire tags were of the notched-code variety (Figure 9). All wire tags used on a given year-class usually had identical codes (Table 2). All were magnetized before being implanted, but unmagnetized wire tags can be used and magnetized later. Wire tag location on head started Kemp's ridleys is shown in Figure 10.

The magnetized wire tag can be detected either by magnetometer or by X-ray. Both types of detection require expensive equipment. X-ray is not readily available in the field, but portable magnetometers are available from the tag manufacturer. Care should be taken to avoid false positive responses due to extraneous sources of magnetism (i.e., magnetite in beach sand, steel reinforcement rod in concrete, electrical wiring, electronic equipment, etc).

We feel that the wire has value as a lifetime tag for sea turtles. The review panel (Eckert et al. 1992) considered the wire tag to be an archival tag for Kemp's ridleys. This small tag is relatively inexpensive and easy to implant. However, because it is internal, it is not recognizable by the general public.

Passive Integrated Transponder Tag

Beginning with the 1990 year-class, all head started Kemp's ridleys were tagged with PIT tags placed into the latero-ventral muscle of the left axillary (arm pit) before release. The PIT tag is a tiny microprocessor capable of transmitting a 10-character identification code (such as 7F7D23187E) when excited by the detector. PIT tags have high detection accuracy and retention rate in fish (Prentice, Sims and Park 1985). A PIT tag is shown on the X-ray in Figure 10.

A turtle can be scanned for the presence of a PIT tag by holding the portable detecting device as close to the skin as possible without touching it. Both dorsal and ventral surfaces should be scanned. Several passes should be made with the scanner over the area in question. A PIT tag sometimes will not respond to excitation by the reading device, so several scans may be necessary.

The PIT tag is not recognizable by the general public because it is internal. It costs considerably more than flipper tags and wire tags. The portable electronic detecting devices also are expensive. Nevertheless, PIT tags probably will prove to be excellent lifetime tags for sea turtles.

EXAMINING TURTLES FOR TAGS OR MARKS

When a Kemp's ridley is recovered alive, whether stranded or caught by nets, hook and line or other methods, the first consideration must be the well being of the animal. A turtle with open wounds or other evidence of physical trauma or disease should be treated immediately by a qualified veterinarian. Lethargic or comatose turtles should be brought immediately to a rehabilitation facility and placed in a tank containing only enough sea water to keep the turtle wet. Make sure the turtle's mouth is above water so the turtle can breath without taking in water. Seawater can damage lung tissue (Stabenau, Moon and Hemingway 1993). The turtle should be closely observed to assure it is able to breathe freely.

A qualified veterinarian should examine the turtle, recommend initial treatment and provide guidance for resuscitation and rehabilitation. Procedures for resuscitation of comatose sea turtles should be followed by qualified personnel (Balazs 1986; Stabenau et al. 1993). If strong enough, the turtle should be X-rayed by a veterinarian to determine if it contains hooks in the throat, esophagus or stomach (Figure 11) (Canon et al. in press). Embedded hooks should be removed by veterinary surgery if the turtle is strong

enough to withstand anaesthesia and surgery.

Procedures for maintaining and caring for captive sea turtles should be followed (Fontaine et al. 1988b). They are presented in Appendix I. Rehabilitated turtles should be released after examination for tags, but only on the recommendation of a qualified veterinarian.

Examination of a live sea turtle for the presence of tags may cause stress to the turtle, so it should be delayed until it can be done without harming the turtle. Examinations of live or dead Kemp's ridleys should proceed as follows:

1. Examine all four flippers for metal or plastic tags. Do not remove attached tags if the turtle is alive. When recording tag codes, pay particular attention to distinguishing between letters or numbers that might be confused (e.g., zero and the letter 'O', letters 'Q' and 'O', the letters 'M' and 'N', etc.).

2. All exterior surfaces of the carapace and plastron should be examined for the presence of external tag scars or marks (apparent living tags and notches or scars on the carapace or plastron could be marks or scars made by taggers, but they could also be from wounds acquired accidentally). If a scute contains a suspected living tag, identify and record the particular scute on which the mark was located. Describe the scar or mark and depict it with a drawing, photograph, or both. Examine all flippers for wounds or scars that could be signs of lost flipper tags. Whether or not it is suspected that an observed wound or scar has been caused by tagging, it should be depicted with a drawing, photograph, or both.

3. If electronic detecting devices are available, examine each turtle for PIT tags and magnetized wire tags as follows:

    a. Use a portable PIT tag detector to scan both fore-flippers and the adjacent shoulder and axillary (armpit) areas thoroughly. A positive response to a PIT tag will be a reading of a 10-character code (such as 7F7D23187E). When recording tag codes, pay particular attention to distinguishing between letters or numbers that might be confused (e.g., zero and the letter 'O', letters 'Q' and 'O', the letters 'M' and 'N', etc.).

    b. Use a portable magnetometer to scan both foreflippers for magnetic tags, avoiding extraneous sources of magnetism that might cause a false positive response

when no magnetic tag is present. The turtle should be held at least 1 m away from the ground, sand, metal equipment, vehicles, electronic circuits, walls containing pipes or reinforcement steel, etc. It may be possible to shield a turtle on a nesting beach from magnetic particles in the sand by placing a piece of plywood under the turtle before passing the detector over its flippers. Pass the detector over dorsal and ventral surfaces of both foreflippers, holding the wand as close as possible to the turtle without touching its shell or skin. A positive response to a magnetic tag is a loud beep from the scanning equipment. If no magnetic tag is detected, pass a magnet over surfaces of the fore-flippers to re-magnetize any wire tag that might be present. The magnet should be passed in only one direction. Again attempt to detect a magnetic tag. Record all information completely, noting whether or not a positive response was received from the magnetometer and other measures attempted (e.g., re-magnetizing, re-scanning, etc.).

4. If a sea turtle has suffered no catastrophic trauma resulting in its death, but otherwise looks dead and shows no signs of decomposition, then it should be treated as if comatose. Comatose turtles appear dead. An ultrasonic Doppler flow meter can be used to detect a heart beat in comatose sea turtles (Stabenau et al. 1993). Ventilation of lungs should be administered to all comatose turtles, otherwise they most likely will die. If, after appropriate resuscitation procedures (Stabenau et al. 1993) have been attempted, the turtle has not recovered, then it may be considered dead. Fresh Kemp's ridley carcasses should be packed in ice and immediately transported to a qualified veterinary pathologist for necropsy in an attempt to determine the cause of death. Also, such specimens are valuable sources of tissues for physiological, genetic and forensic research.

5. If, during examinations of carcasses or parts of dead turtles, no responses were obtained from internal tag detection devices (magnetometer and PIT tag detector), foreflippers should be X-rayed. Carcasses or foreflippers can be taken to a veterinarian for X-ray. Both PIT and wire tags are detectable by X-rays (Figure 10). If internal tags are detected in dead turtles or foreflippers by X-ray, the frozen foreflippers containing the PIT or wire tags should be shipped to the NMFS Galveston Laboratory where the magnetic and PIT tags can be excised and decoded. Any unusual objects (hooks, GI tract obstructions, etc.) detected by X-ray should be noted. Whenever possible, photographs should be taken of all carcasses and turtle parts X-rayed. Photographs, X-rays or reproductions of same, should be included with STSSN reports and proper labels and other information (tag codes

and location or marks) in the records for each turtle. After all examinations are completed, any remaining turtle carcasses or parts not shipped to the Galveston Laboratory should be disposed of by incineration or burial.

FREEZING AND STORING CARCASSES AND PARTS

Examinations of frozen carcasses or parts can be done at the examiner's convenience. Entire carcasses of dead turtles weighing less than 6 kg should be stored frozen, along with proper labels and completed STSSN reports (stranding report forms). Carcasses should be placed in heavy plastic bags sealed prior to freezing. Completed STSSN reports should be placed inside a heavy-duty, zipper-sealed plastic bag and placed with the carcass inside the heavy plastic bag. Each bag should bear an external label. All records and labels placed in bags containing frozen turtles should be written in pencil or indelible ink.

For carcasses weighing 6 kg or more, both foreflippers should be removed (entire flippers including as much of the axillary and shoulder musculature and skin as possible) and stored frozen, along with proper labels and STSSN reports. Flippers should be placed in heavy plastic bags sealed prior to freezing. Completed STSSN reports should be placed inside a heavy-duty, zipper-sealed plastic bag and placed with the flippers inside the heavy plastic bag. Each bag should also bear an external label. All records and labels placed with the frozen turtle parts should be written in pencil or indelible ink.

NECROPSY

Complete and thorough necropsies of sea turtles are not possible in the field. Whenever feasible, a qualified veterinarian should be employed to conduct complete sea turtle necropsies in the laboratory (Appendix II). If this is not possible, try to complete an external examination in the field. Detailed necropsy procedures can be found in Wolke and George (1981), Ashley (1955), Jacobson (1978) and Van Kruningen (1971).

The following protocol was paraphrased from Wolke and George (1981). Large portions of their necropsy protocol are presented (with written permission of the senior author) in Appendix II.

Carapace length, carapace width, plastron length and total weight should be taken and recorded along with the type of

measure (straight or over the curve). Straight line measurements are preferred (Pritchard et al. 1983). Carefully examine skin and shell for color, texture and lesions. All lacerations, masses, discolorations and scars, including rope burns, tag scars, propeller cuts, bullet holes, puncture wounds and entangling materials should be described accurately. General condition of the body should be noted, because it reflects the state of decomposition and nutrition.

Stage 1 (rigor mortis): Within a short time after death, the ventral skin becomes pink to purple and as postmortem changes progress, explicit lines of purple-green will be seen encircling the neck and limbs.

Often, nutritional state of the turtle may be concluded from an external examination. A starving or chronically ill turtle has markedly sunken eyes and plastron. Its skin, carapace and plastron may be covered by an abnormal number of barnacles. Muscle masses of the neck and the extremities are reduced. This may be so marked that the supraoccipital crest at the back of the head will stand out sharply and the shell appears too large for the animal. These signs are valid only if the turtle is relatively fresh and has not undergone advanced decomposition.

Carefully inspect the head for presence of trauma and hemorrhage. Examine the eyes for abnormalities, with special reference to the cornea and sclera. The oral cavity and the mucus membranes should be examined for erosions, trauma and barnacles.

Examine the extremities for lesions and palpate them for fractures. The anal area should be examined for protrusions, exudates, and consistency of fecal matter. A fecal sample should be placed in a small vial containing 10% buffered formalin.

Stage 2 (putrefaction): Decomposition or postmortem autolysis is recognized by color changes of the skin, collection of blood in contingent areas, and by the production of gas with a foul odor. If the carcass is bloated so that gas escapes when a knife is inserted, advanced decomposition has occurred. In such cases and in those instances in which the flesh is falling from the skeletal system, necropsy methods are of little value, because postmortem changes will mask antemortem events. Determination of the degree of postmortem decomposition, and therefore the values of the necropsy, is subjective and learned by experience.

SHIPPING CARCASSES, PARTS OR TISSUES

All shipments of sea turtle specimens (carcasses, parts or tissues) within the Continental U.S. must be accompanied by proper Endangered and Threatened Species Permits from the U.S. Fish and Wildlife Service or the NMFS. Transfers must be documented with chain-of-custody papers. Certain states also may require permits or letters of authorization for such transfers.

Frozen sea turtle specimens should be wrapped or contained within heavy duty plastic bags. Plastic bags should be placed within heavy burlap or canvas sacks (to keep the plastic bags from becoming damaged during shipment) and securely tied or sewed shut. The shipping container should be a heavy duty polystyrene foam box or plastic ice chest (insulated). When bagged specimens have been placed within the shipping container, a double layer of cardboard or one layer of 1/4 in.(0.6 cm) plywood should be securely placed on top of the specimens. On top of this layer, 1 to 3 kg of "dry ice" should be placed, wrapped in newspaper or other suitable paper. Specimens should be shipped via express delivery.

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Table 1. Number of head started Kemp's ridley sea turtles tagged with various tags or marks and number released, by year-class.

Year-class	Flipper tag[a] Single[b]	Double[c]	Carapace tag[d]	Magnetized wire tag[e]	Living tag[f]	PIT tag[g]	Number released
1978	2061				1		2019
1979	1511						1370
1980	1723		180		180		1723
1981	1639						1639
1982	1353	436		25	436	4	1329
1983	192						190
1984	1078			1017	1017	22	1040
1985	1546			1534	1534	8	1534
1986	1730			1730	1730	96	1727
1987	1282			1282	1282	5	1280
1988	899			899	899		899
1989	1962			1962	1962		1962
1990	1979			1979	1979	1979	1979
1991	1942			1942	1942	1942	1942
1992	1964			1964	1964	1964	1963
Total	22861	436	180	14334	14926	6020	22596

[a] Hasco Style, Number 681, monel or inconel flipper tags manufactured by National Band and Tag Company, Newport, KY. Plastic flipper tags also were placed on 20 turtles of the 1984 year-class.

[b] Usually attached to the trailing edge of the right foreflipper, but occasionally to the left foreflipper.

[c] One attached to the trailing edge of the right foreflipper and the other to the trailing edge of the left foreflipper.

[d] Small monel tags placed on the carapace. This tagging was unsuccessful, for within a very short time the tags had been expelled from the carapace tissue.

[e] Manufactured by Northwest Marine Technology of Shaw Island, Washington. Non-magnetized, non-coded wire tags were used to tag 79 released turtles of the 1992 year-class, and 80 more released turtles of this year-class were tagged with non-coded but magnetized wire tags.

[f] Tissue transplant of a small piece of light colored plastron into the darker carapace. To distinguish year-classes, the living tag is placed on a different carapacial scute each year.

[g] The passive integrated transponder (PIT) tag is a glass-encapsulated microchip manufactured by Destron/IDI, Westminster, CO.

Table 2. Various tag codes and living tag locations for head started Kemp's ridley sea turtles[a].

Year-class	Foreflipper tag series	Magnetized wire tag	Living tag
1978	G----, F---- NNA260, G0985 13582		
1979	NNN---, NNA--- K----, J0096		
1980	NNB---, K---- 8001 - 8100LP,		LA[c], LC2, LC3, LH, N2, N3, RC2, RC3, RC4,
1981	NNG---, NNH---		
1982	NNL---, NNM--- NNZ---, AAK--- BBB---	D_1-2; D_2-[b]	LC3
1983	NNQ---		LC4
1984	NNT---, NNV--- NNZ---, AAL--- BBB---	D_1-2; D_2-72	LC5
1985	NNX---, NNY---	D_1-2; D_2-73	RC5
1986	PPK---, PPL--- BBB---	D_1-2; D_2-74	N4
1987	PPR---, PPS--- BBB911	D_1-2; D_2-80	RC1
1988	QQA---, NNZ701 BBB999	D_1-2; D_2-81	LC1
1989	QQF---, QQG---	D_1-2; D_2-82	RC4
1990	QQK---, QQL---	D_1-2; D_2-84	RC2
1991	QQW---, QQX---	D_1-2; D_2-85	LC2
1992	SSC---, SSD---	D_1-2; D_2-68[d]	RC3

[a]Each PIT tag has a unique 10-character code (e.g., 7F7F0C2263). The dashes following letters in the foreflipper tag codes represent digits 1-9.

[b]Only 25 turtles of the 1984 year-class were tagged with magnetic tags and released as an initial experiment with this tag. Each tag had and individual code. One of these turtles died while in captivity. The remaining tags were: D_1-2, D_2-1, -2, -4, -8, -9, -10, -16, -17, -18, -20, -21, -32, -33, -34, -36, -37, -40, -41, -42, -64, -65, -66, -68, and -69.

[c]Some turtles of the 1990 year-class were experimentally tagged between scutes RC2 and RC3, RC3 and RC4 on the carapace, and LP and RP or LH and RH on the plastron (RC=right costal, LC=left costal, N=neural, LH=left humeral, RH=right humeral, LP=left pectoral, RP=right pectoral, LA=left abdominal).

[d]159 released turtles of the 1992 year-class were tagged with non-coded wire tags, 80 of them magnetized and 79 non-magnetized.

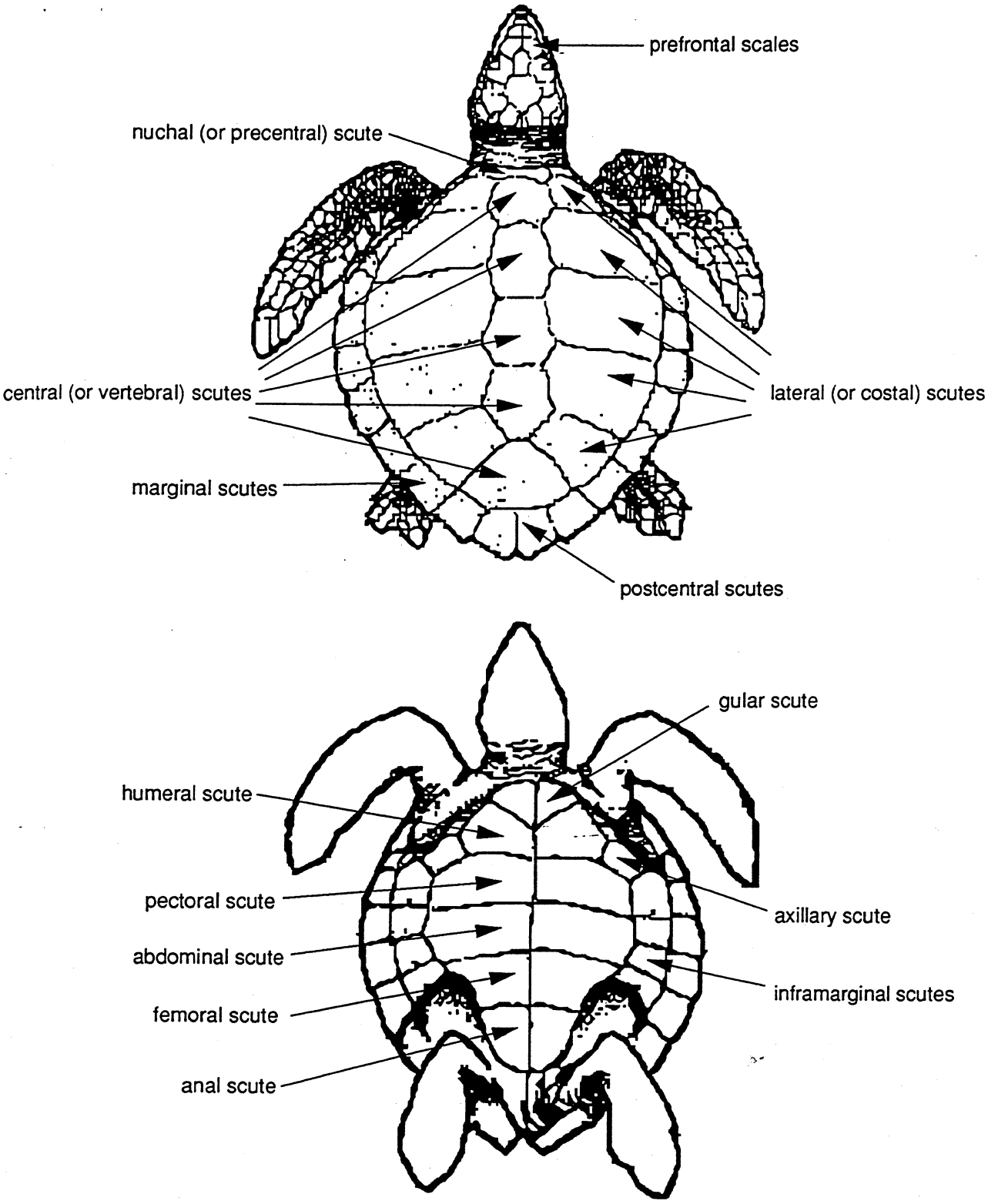

Figure 1. Morphological terms used in sea turtle identification (adapted from Pritchard et al. 1983).

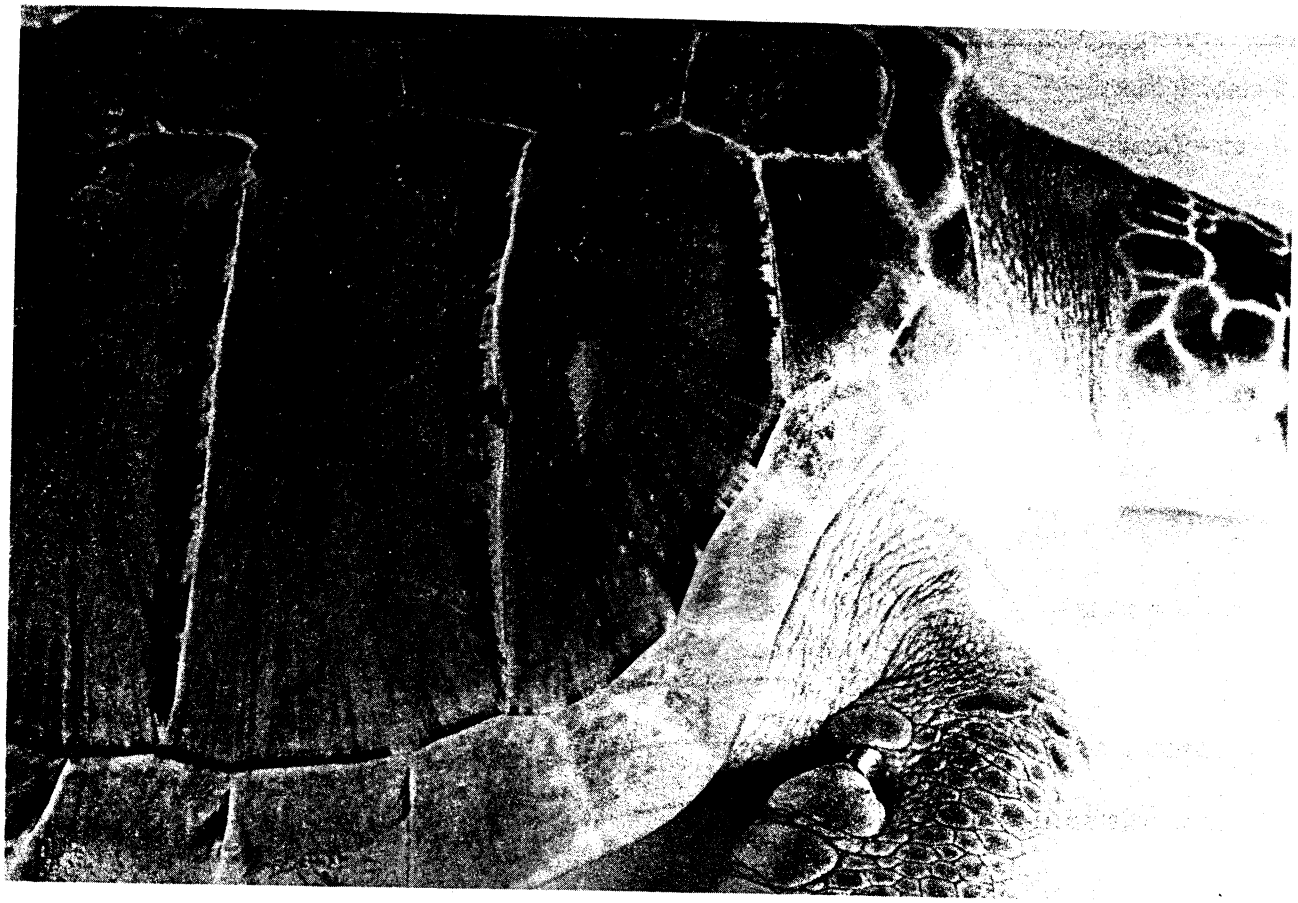

Figure 2. Carapace view of a head started Kemp's ridley sea turtle. Note the living tag in right coastal scute 2 and the metal tag attached to the right foreflipper. In the absence of a foreflipper tag, scarring in this area of a foreflipper might indicate a lost tag. X-ray of the scar might detect residual metal particles suggesting that the turtle had been tagged.

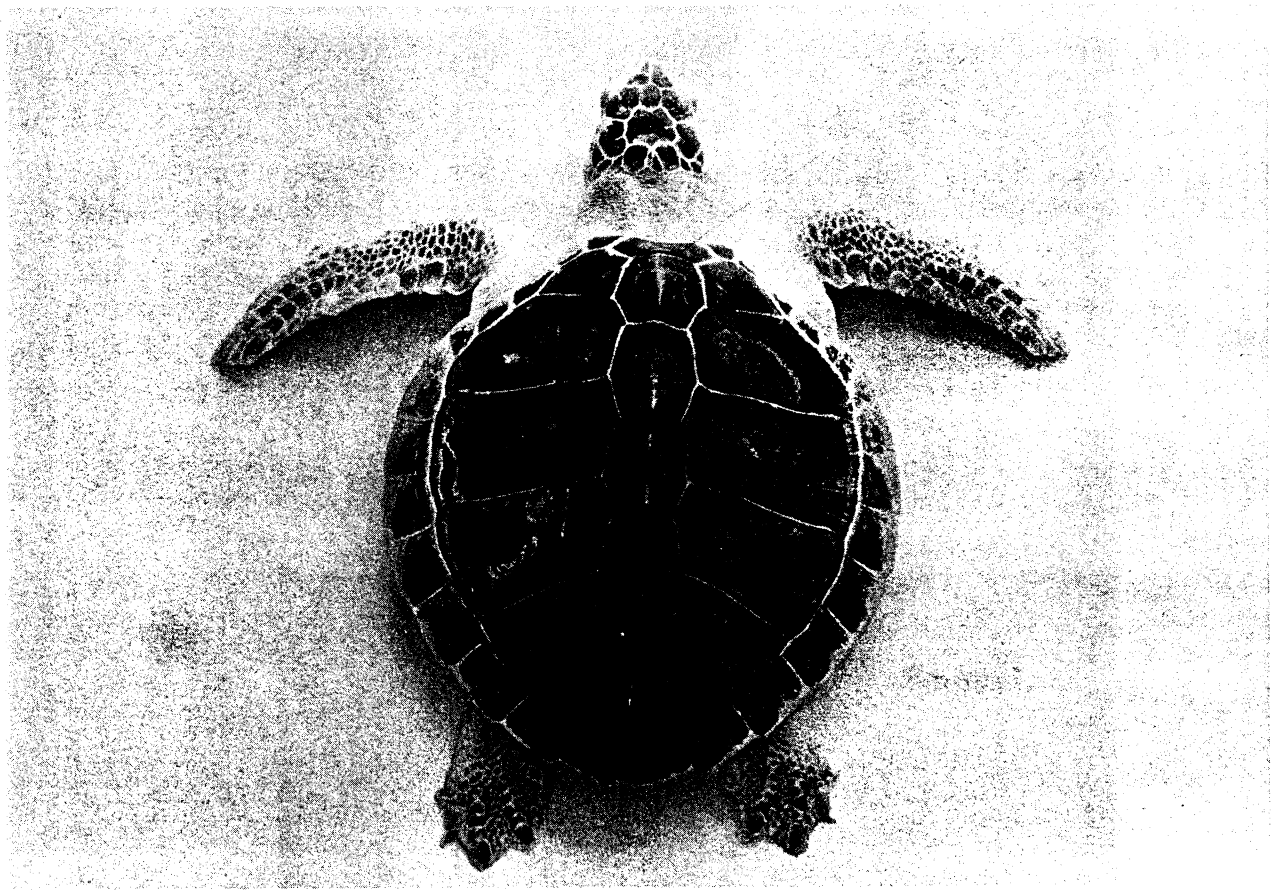

Figure 3. Photograph of a 2-yr-old head started Kemp's ridley sea turtle.

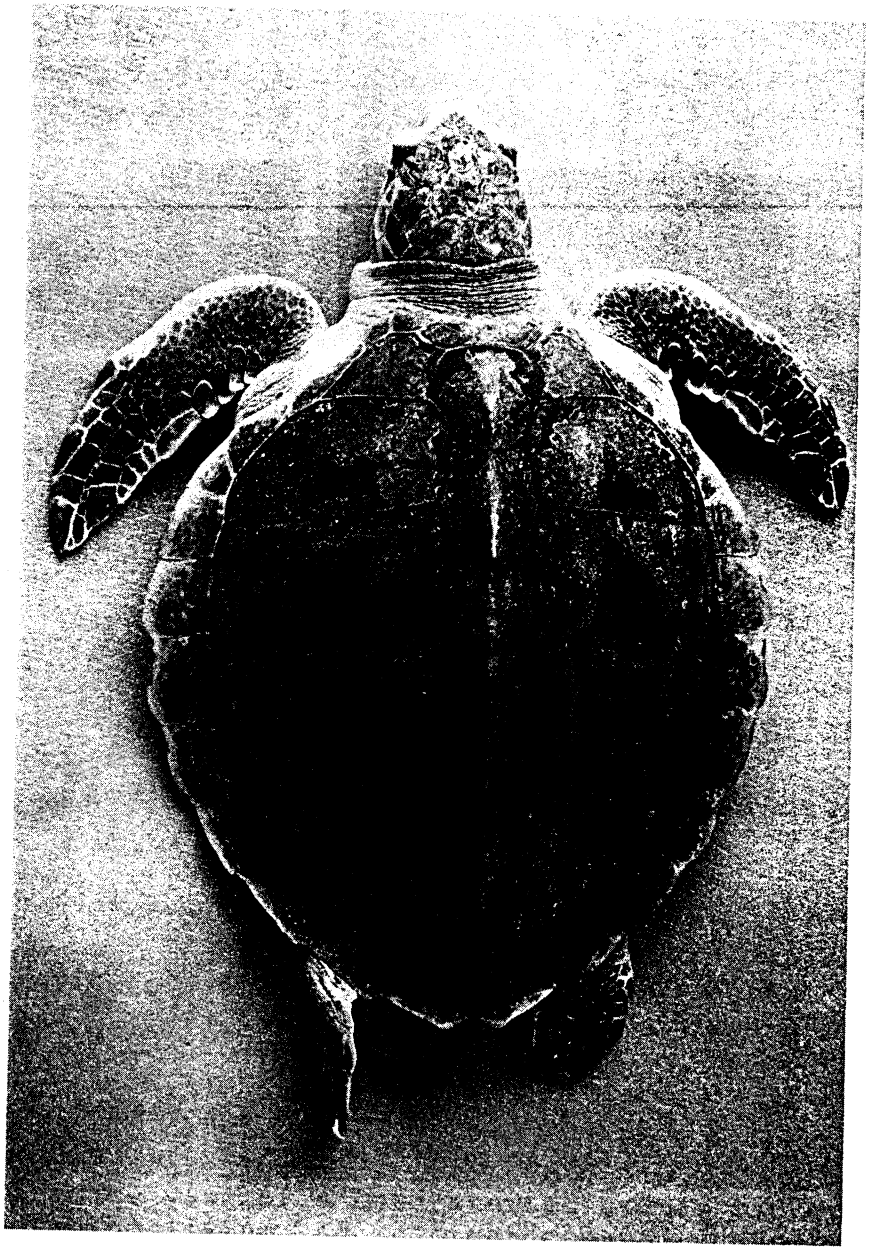

Figure 4. Photograph of a 3-year-old head started Kemp's ridley sea turtle.

Figure 5. Photograph of a wild adult female Kemp's ridley sea turtle on the nesting beach at Rancho Nuevo, Mexico.

Figure 6. Metal foreflipper tag used on head started Kemp's ridley sea turtles.

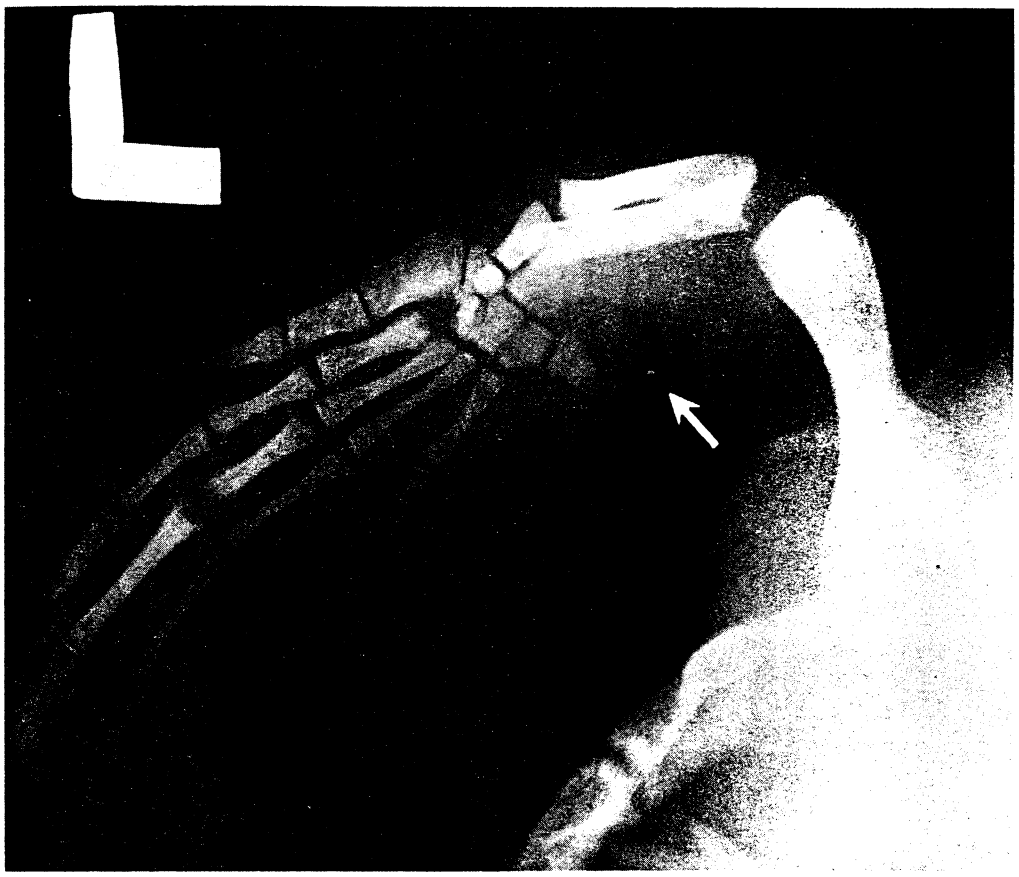

Figure 7. X-ray showing left foreflipper of a head started Kemp's ridley sea turtle that lost its metal foreflipper tag. Metal particles left behind upon loss of the tag remained within the tissue and were detectable by X-ray (arrow).

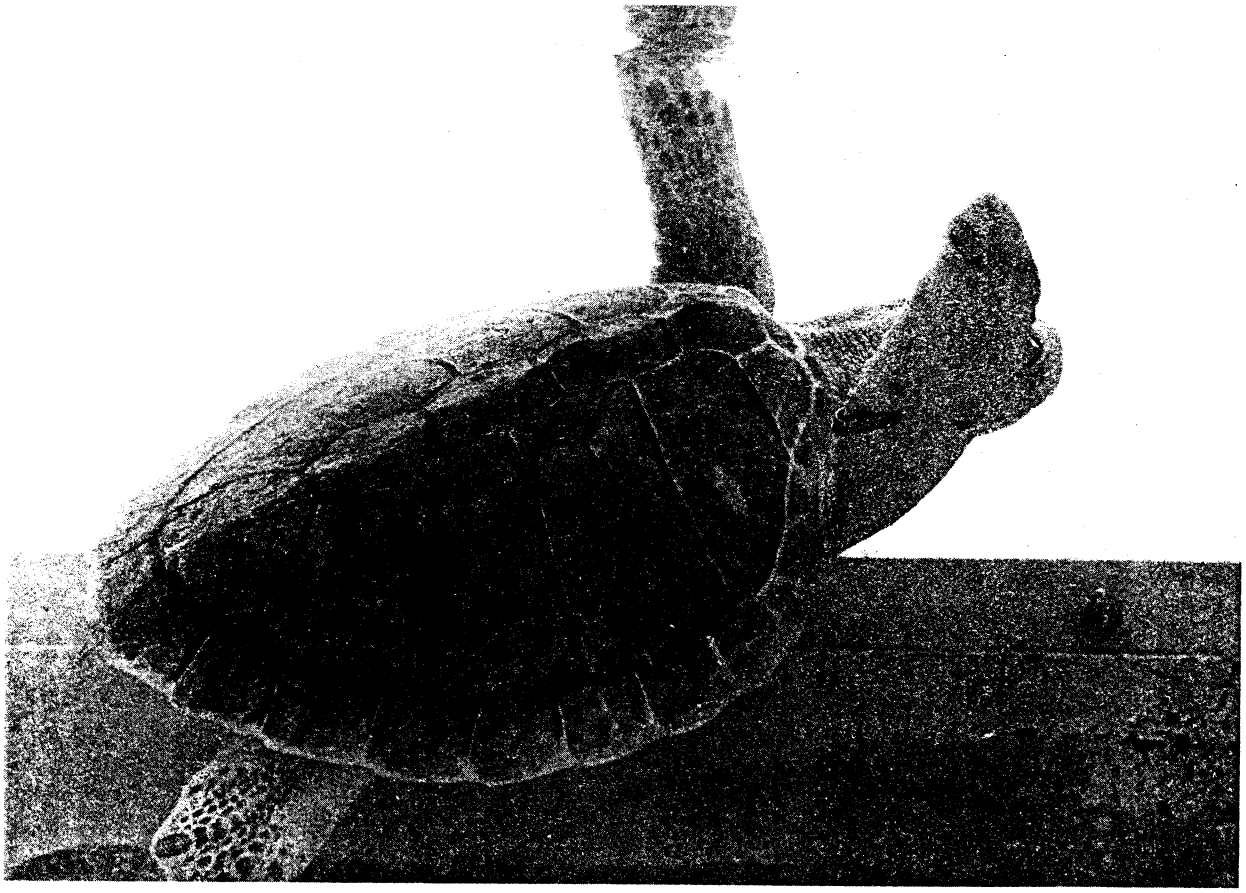

Figure 8. Example of a living tag on right costal scute 2 of a head started Kemp's ridley sea turtle.

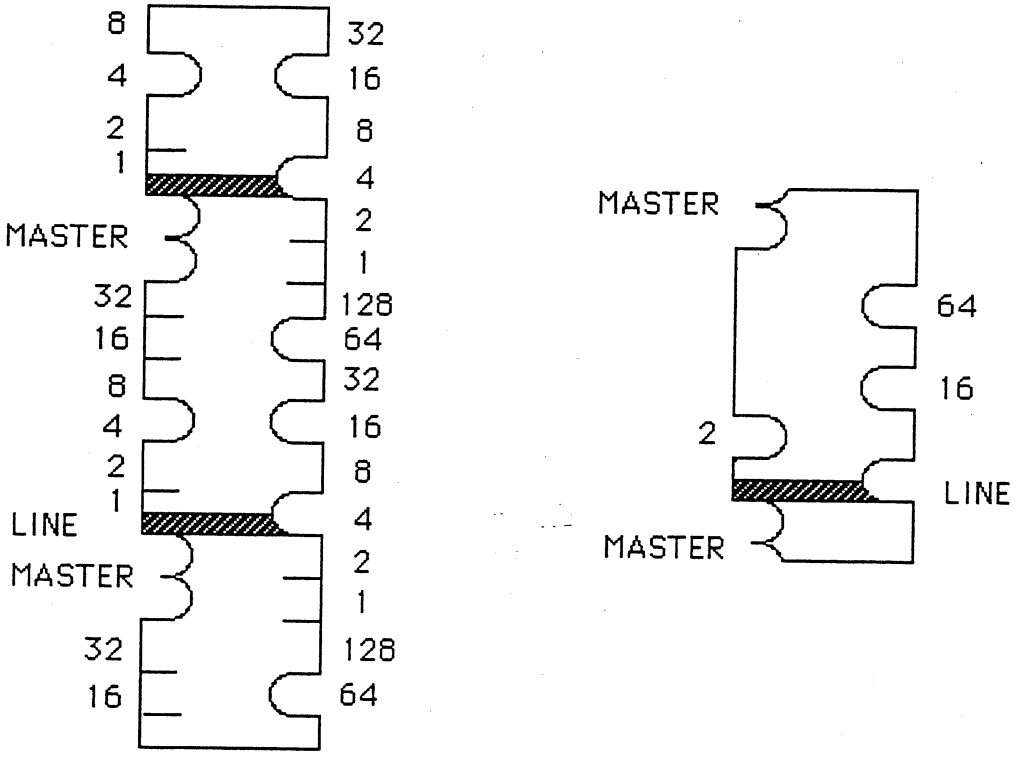

Figure 9. Schematic drawing of the coded, wire tag. The notch-coded tag on the right is encoded D_1:2 (on its left side), and D_2:80 (i.e., 64 & 16, on its right side). The diagram on the left shows the master code for the notched wire tags.

Figure 10. X-ray of a head started Kemp's ridley sea turtle showing location of coded wire tag (left) and PIT tag (right).

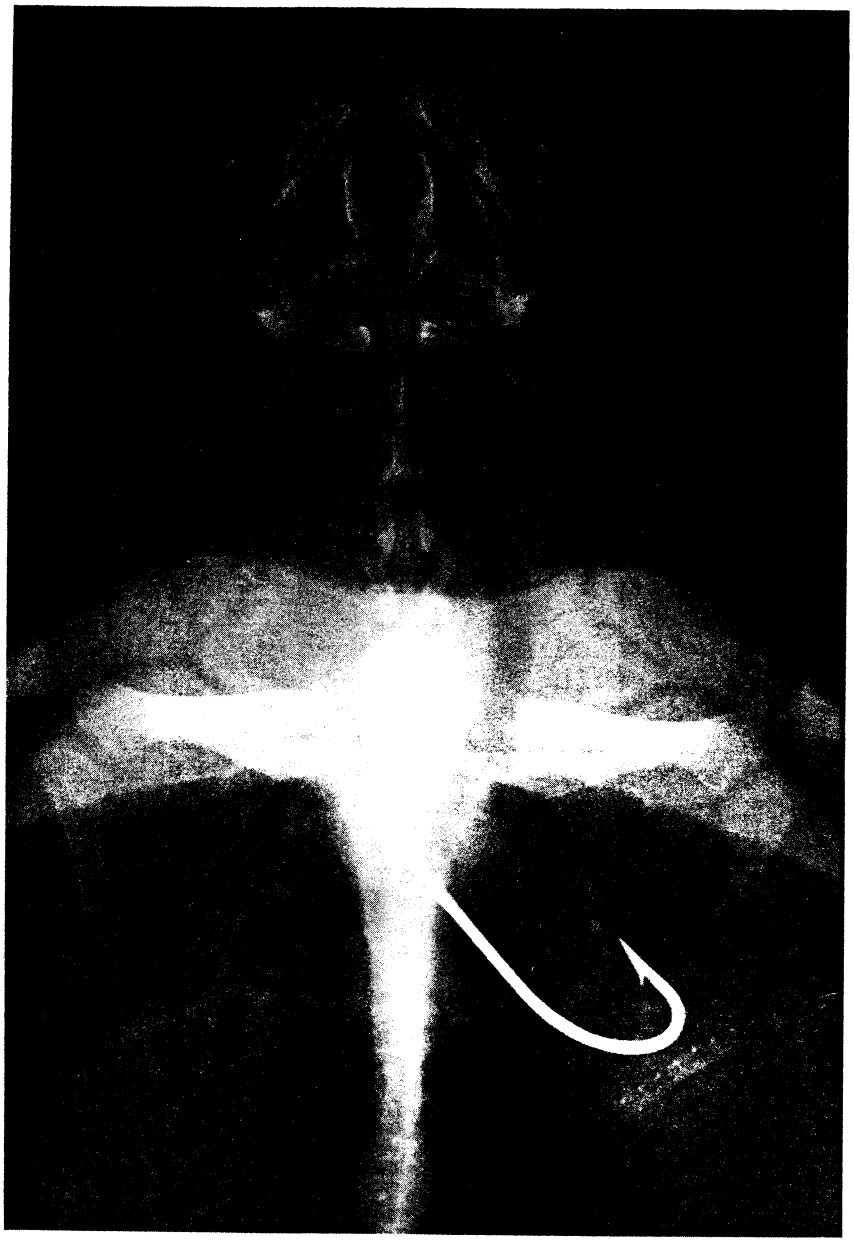

Figure 11. X-ray of a head started Kemp's ridley caught on hook and line. This demonstrates the importance of close examination of live stranded or captured sea turtles for internal problems (see Cannon et al. in press).

APPENDIX I

CARE AND MAINTENANCE STANDARDS FOR KEMP'S RIDLEY SEA TURTLES (Lepidochelys kempii) HELD IN CAPTIVITY

(adapted from Fontaine et al. 1988b)

HEALTH CARE

A qualified veterinarian should examine each Kemp's ridley held in captivity, diagnose its condition, and recommend any remedial treatments necessary. Before the turtle is examined for the presence or absence of identifying tags or marks the veterinarian must certify that it is safe to do so. Before a rehabilitated Kemp's ridley is released to the wild, the veterinarian must certify that it can be safely released.

When a Kemp's ridley sea turtle is recovered alive whether stranded on a beach, caught by hook and line, captured in a shrimp trawl or commercial fishing net, etc. the first consideration is for the well being of the animal. If the turtle appears to be healthy and in good shape, it should be returned to the wild as soon as possible. Before releasing the turtle, check for tags and make measurements, according to the protocol above. If the turtle is alive, no external lesions or evidence of physical trauma are apparent but the animal appears lethargic, it should be held for rehabilitation. A lethargic turtle should be taken to a holding facility or laboratory quickly and placed in an area where it can remain quite and undisturbed for at least 24 hours. One with open wounds or other evidence of physical trauma or disease should be taken to a qualified veterinarian for treatment immediately.

FACILITIES

Holding Tanks - The tanks in which Kemp's ridley sea turtles are held must be deep enough to hold sufficient water to allow complete submersion of the turtle, large enough to permit unimpeded turning and swimming, and of a configuration and surface texture that will not lead to injury. A rule of thumb is to provide no less than 38 liters (10 gal) per kg (2.2 lb) of sea turtle. The inside surfaces of holding containers must be non-abrasive, free of burrs or projections and free of toxic heavy metals or organics. Use of non-finished concrete tanks should be avoided as we have found that rough surfaces lead eventually to epidermal abrasions and severe lesions in the turtles, particularly in the plastron area. Further, the tanks must have adequate lighting (sunlight and/or artificial lighting) on a normal or simulated diurnal photoperiod.

SEAWATER

Quantity - There must be sufficient volume (no less than 38 liters/kg of turtle) of seawater to promote growth and allow complete submersion and free movement. Kemp's ridley sea turtles must be kept out of seawater a cumulative maximum of 10 hr per week (as when draining water for cleaning tanks, or in an emergency), but they must be kept moist and protected from physical damage during such periods. Additionally, turtles kept out of seawater must be protected from extremes of temperature and never placed in direct sunlight while dry. The facility must have the ability to provide adequate quantities of seawater for normal and emergency conditions.

Quality - Seawater containing Kemp's ridleys should be maintained at salinities of 25 ppt or higher and temperatures of 20°C or higher, preferably near 25°C. Lower and upper extremes may induce disease or injury, or cause death and should be avoided. Salinities of less than 25 ppt and temperatures of less than 24°C may result in disease in Kemp's ridley sea turtles. In particular, fungus diseases are a problem when seawater temperature drops below 24°C. Seawater pH must be maintained between 7.5 and 9.0. Seawater must be kept free of contaminants such as toxic heavy metals and organics that may be deleterious to the health of the sea turtles.

General Condition - The seawater surface must be unencumbered to allow the Kemp's ridley sea turtles to surface to breathe and float. It must be free of non-food , floating objects or substances that may be ingested or otherwise harm the sea turtles. A flow-through seawater system that allows 3-6 total replacements per 24 hr is preferred. Re-cycled wastewater treatment systems may be used, provided that adequate filtration is applied to maintain total ammonia at <0.5 ppm and nitrite at <0.8 ppm. If a static seawater system is used, the tanks should be cleaned and the seawater replaced at least three times weekly.

The staff of the facility must have the instruments and ability to monitor seawater quality, to correct any situation in which the described limits are exceeded, and to properly care for the sea turtles while corrective measures are being taken.

BEHAVIOR

Kemp's ridleys sea turtles less than 2 yr old are very aggressive and will bite others of their kind and other species with very little provocation, particularly at feeding time. They also will aggravate large sea turtles to the point of retaliation, as has

been observed especially with larger loggerheads (Caretta caretta). For these reasons, hatchling and juvenile Kemp's ridley sea turtles should be kept isolated from one another in individual containers and from other sea turtle species. Experience has shown, however, that aggression in these turtles is not a hard and fast rule. It may be related to age or size, nutrition, size of the holding tank and environmental factors presently not understood. In any case, when the situation dictates that Kemp's ridleys be placed together, they must be closely observed until it is determined that they display no aggression that might cause serious injury or death. Small Kemp's ridleys should never be housed with larger turtles of another species.

Feeding a group of large Kemp's ridleys held in a common tank should be done by broadcasting or spreading the food around the tank to avoid congregating the turtles. They may injure one another while competing for food and it may stimulate terminal aggressive behavior. Any turtle that develops a light spot or pathological lesion on its skin or shell should be isolated from others to avoid "worrying" of the spot by other turtles. Juvenile Kemp's ridleys should be monitored closely for aggressive behavior and the establishment of a "peck order". When any antagonistic behavior is noted, the turtles should be separated immediately.

FEEDING

Kemp's ridleys being maintained in captivity for rehabilitation should be fed a diet of natural foods such as squid, crabs and shrimp. Oily or fatty fish must be avoided as foods because they cause fatty degeneration of the liver and steatitis leading to death.

APPENDIX II

SEA TURTLE NECROPSY

The general guidelines presented herein were taken directly from those published by Wolke and George (1981) under contract no. NA80FAC00016 to the National Marine Fisheries Service (with written permission from senior author Wolke). A qualified veterinarian should conduct complete sea turtle necropsies. Otherwise, the guidelines presented below should be followed.

" Necropsies should always be conducted so that a minimum of logistical problems will occur. The prosector (person conducting the necropsy) should dress in light, easily washable clothes and wear gloves. If possible, a shaded, well ventilated, and easily cleanable site should be used. The site should also be chosen in relation to carcass disposal. Large animals are very difficult to incinerate and must be buried. Disposal should be in compliance with authorization from proper law enforcement agents or with a valid state or federal sea turtle permit.

The skin and shell are carefully examined for color, texture and lesions. All lacerations, masses, discolorations and scars, including rope burns, tag scars, propeller cuts, and bullet holes are measured and described.

The head is carefully inspected for the presence of trauma and hemorrhage. The eyes are examined for abnormalities with special reference to the cornea and sclera. The oral cavity and the mucus membranes are examined for erosions, trauma and barnacles.

The extremities are then examined for lesions and palpated for fractures. The anal area is examined for protrusions, exudates, and consistency of fecal matter. A fecal sample should be placed in a small vial with 10% formalin. Necessary scrapings or impression smears of suspected lesions are made and air dried for submission to the laboratory. Parasites are removed and placed in AFA solution for later identification.

Dissection

Prior to dissection, the turtle is placed on its back. A short autopsy knife is inserted along the bridge between the carapace and the outermost plastron plates, and the cartilage is cut. The incisions are made through the skin along the anterior and posterior plastron edges. The plastron is removed from the underlying muscle masses. In larger turtles, this is most easily done if the plastron is pulled upward by an assistant with a hay or stevedore's hook. A mid-ventral incision is made between the coracoids and directed posteriorly to the pelvis. Incisions are then made along the lateral border of the muscle masses at the carapace edge and extending forward so that the muscles and pectoral girdle can be lifted upward and pushed toward the head, thereby exposing the underlying heart and other viscera. The muscles may then be removed by disarticulating the contained girdle at the shoulder (gleno-humoral joint). A similar procedure can be used to remove the muscle masses of the rear flippers. A mid-ventral incision is made separating the left and right pelvis (in larger turtles long-handled pruning shears or a saw will be necessary) followed by lifting the pelvis upward and backward with disarticulation from the femoral heads. In all of these manipulations, separate the muscle masses carefully from the underlying pericardium, peritoneum and viscera in a manner that does not disturb the relationship of organs to one another.

Check the position of organs. Frequently the pleuroperitoneal cavity will be filled with fluid. The fluid obscures the lungs and urogenital tracts and may collect in the lungs if they are accidently cut. It is, therefore, important that this fluid be removed frequently by tipping the animal on its side. If water is available, the cavity may be flushed and drained.

If decomposition has progressed, the fat over the heart and on the dorso-medial side of the scapula will be black-red and gelatinous. Further, major organs lose their natural color and appear as if parboiled. The pleuroperitoneal cavity may contain large amounts of a dark-red serosanguinous fluid. If these signs are sever, the soft tissues are unlikely to be suitable for histological examination and need not be collected. However, information can still be gathered from examination of gross anatomy and such related parameters as gut contents, parasite load, toxicology screening and skeletal features.

Throat

A mid-ventral incision is made posteriorly along the neck from the mandibular symphysis. The skin is reflected, exposing the underlying trachea and esophagus. The oral cavity is examined, as are the tongue and glottis. Two incisions are then made along the inner edges of each mandible through the muscle into the oral cavity. A finger can be inserted through the incisions encircling the tongue, and the tongue pulled upwards after its anterior attachment is severed. The tongue, larynx, trachea and esophagus are then dissected free of the neck to a point at which the trachea and esophagus disappear dorsal to the heart. The thymic tissue occurs on either side of the trachea in young animals. Due to the dorsal location of the turtle's lungs, it is easiest to examine the gastrointestinal system before the respiratory system.

Gastrointestinal Tract

A large, bilobed liver lies over the heart and occupies the anterior abdominal cavity. It is dark brown (mahogany), shiny and sharp-edged under normal conditions. The right lobe is largest and covers the anterior portion of the stomach, which occupies the left abdominal cavity. Grasping the esophagus and pushing the liver to one side, the stomach can be dissected free along its inner curvature from the mesentery and liver. Tie with twine the anterior end of the stomach at its attachment to the esophagus to prevent the stomach contents from spilling into the body cavity. Lift the stomach up and cut its esophageal attachment. At the pylorus, the stomach is cut free and removed after tying both ends of the transacted gut with twine. The stomach is then examined internally after an incision is made along the lesser curvature. Contents are recorded and preserved in alcohol (40% isopropyl or 70% ethyl).

Before removing the liver, the duodenum is incised mid ventrally to expose the bile duct opening. The duct should be checked for obstructions by applying pressure to the gall bladder, which lies beneath and within the back edge of the right liver lobe. The liver and gall bladder are removed and carefully examined. The gall bladder is approximately 2 X 3 cm in a 60 cm turtle and dark green. The saccular colon lies beneath the stomach. The pancreas lies along the duodenum. It is normally grey, irregular in outline and elongate. Cut the mesentery from the intestine and strip the intestine from the abdominal cavity.

One or two large mesenteric vessels to the lower intestine should be removed and fixed for flood fluke examination in the laboratory. The intestine is then tied at its entrance to the pelvic inlet, removed from the cavity and set to one side for further examination. The esophagus, duodenum, jejunum and large intestine are then opened along the entire length of the gastrointestinal tract. Lesions are noted and parasites carefully collected with their numbers and locations recorded. The mesentery suspending the intestine is examined. The pancreas and the spleen are removed for sectioning. The spleen is antedorsal to the caecum within the mesocolon. It is slate to blue-grey, egg-shaped and smooth.

Lungs and Heart

The trachea is a white tube lying ventral to the esophagus and held open by a series of incomplete cartilaginous rings. It is normally unobstructed. Just below the heart, it bifurcates to form paired bronchi with enter the lungs. The paired lungs should be dark pink, partially air-filled and of a soft, expansible consistency. On cut section they are dry. Both lungs are covered by a rather thick grayish-blue sac (pleura). Their pink color is only apparent after removal from the pleura.

The trachea can be grasped and freed from underlying tissue. While holding the trachea, the lungs, which lie dorsally against the carapace, can be dissected free with the heart and great vessels. They should be removed <u>in toto</u> to simplify identification of the great vessels. The heart can then be removed from the lungs and examined. Alternatively, however, if it is easier for the prosector, the heart may be removed from the lungs while still within the animal. On the anterior pericardial sac, often obscured by yellow fat, are the thyroid and parathyroid glands. They are translucent and red but are difficult to see so a wide excision of the tissue is needed to assure their removal. Just lateral to these organs and the sac is the paired pinkish-grey lobular thymus. When the pericardial sac is cut, be careful to note the amount, consistency and color of the pericardial fluid. A mid-dorsal opening of the aorta will expose the aortic valves. The ventricle may be opened by an incision from its attachment to the right atrium thorough the apex, to the left atrium. The arterio-ventricular valves can then be examined and the incision continued into each atria.

The trachea should be opened and followed to the bronchial bifurcation. The lung parenchyma can be examined by

following the respiratory tracts and by transverse incisions through the tissue. Changes in color, texture and the presence of parasites are noted and sections collected.

A possible, but not certain sign of drowning is a thick, tenacious, persistent white (or slight pink) foam which can be expressed from the nostrils by pressure on the throat or is present within the trachea, bronchi and on cut section of the lungs. In addition, the lungs are greatly expanded and contain a watery fluid. When removed and placed on the autopsy table, they will collapse and fluid will run from them freely. Vomitus may be found in the trachea and bronchi. It should be noted that these signs have not been demonstrated to be caused by drowning in sea turtles.

Urogenital System and Adrenal

The ovaries lie on each side of the posterior pleuroperitoneal cavity and can be removed with their unattached oviducts by incising the mesovarium. Note should be taken of the presence and exact numbers of eggs and degree of calcification. As the oviducts are followed posteriorly, the urinary bladder is found just anterior to the cloaca. There are no accessory cloacal bursae in sea turtles. The bladder, its cloacal orifice and the oviductal openings into the cloaca are examined. At this point, the ureters are observed and followed forward to the paired dorsal kidneys.

By careful dissection, the cloacal opening and rectum can be freed, pulled upward and forward. Then the attachments of the urogenital system and the kidney can be severed and the complete system removed from the abdominal cavity. The kidneys and bladder should be removed <u>in toto</u> with the ovary and oviducts, incising just posterior to their cloacal attachment. The kidneys are dark red to black, covered by a grey-blue peritoneum and multi-lobed. All organs should be examined for masses, changes in color or consistency, exudates and parasites.

In the male, the cloaca can be opened laterally to expose the penis (this organ may be difficult to find in juvenile turtles; sex determination in younger animals usually depends on histological examination of gonadal tissue). In either sex, such an incision will expose the orifices of the bladder and cloaca anteriorly. The testes and epididymides occupy the same area as the ovaries and their ducts.

The internal and chromaffin tissues (adrenal cortex and medulla) are not discrete and are on the ventral surface of the kidney. It is wise therefore, to use wide excision when removing the kidneys and to save renal peritoneum for location of these structures.

Connective Tissue, Skeletal Muscle and Osseous System

The major pectoral and pelvic muscle masses should be carefully palpated for evidence of mass lesions, and if necessary, incised and examined.

The shoulder and hip joint should be opened and articular cartilaginous surfaces and synovial fluid examined, noting the color and consistency of both cartilage and fluid. Both front flippers should be disarticulated at the shoulder joint and removed in toto for determination of presence or absence of internal head start tags. Whenever possible, roentgenographies of the extremities should be obtained for delineation of skeletal and joint trauma or the presence of lesions. Disarticulated limbs should either be frozen or preserved in 10% buffered formalin, and then submitted for age determination studies. When possible, the cervical spine, consisting of eight mobile vertebrae should be disarticulated from the head and from the first thoracic vertebrae, which is firmly fixed to the anterior portion of the carapace.

Central Nervous System and Organs of Special Sense

The head is then disarticulated. Using a Stryker or hack say, the dorsal calvarium of the head can be removed. This requires a transverse cut through the bone just posterior to the orbits followed by two cuts at right angles to the first cut through the dorso-lateral calvarium extending posteriorly to its ending. The only structure then holding the roof of the skull will be the underlying supraoccipital process. This can be reached with a pair of shears just above the attachment of the vertebral column to the posterior skull.

When all cuts have been made, the calvarium may be lifted away revealing three structures: two large lobulated salt glands postorbitally, to each side, and the cut dorsal surface of the supraoccipital process. If two incisions are made through the lateral portions of this process, it too may be lifted away revealing the small, elongate brain. The pineal gland is just under the roof of the skull dorsal to the

pituitary and located in the middle of a line drawn behind the eyes.

Turning the head upside down, the brain will be held only by cranial nerves, olfactory tracts and optic nerved which may be carefully cut with scissors. Examine the brain and fix it in 25% formalin. The pituitary, lying below the brain in a bony depression, is removed with the brain.

The eyes should be removed with curved scissors, cutting within the orbits and severing the optic nerves, then fixed in 10% formalin. A transverse cut made just posterior to the nostrils and joined by a frontal cut below the nostrils allows removal of the nares and examination of the nasal canals. Incise the tympanic membrane over the ear cavity and remove the slender columella bone which is attached to the skin and extends medially to the inner ear. It may not be possible to remove in bone in toto, but even a partial specimen can be valuable for age determination.

A portion of the cervical spinal cord should be removed and preserved. It is indeed difficult to obtain the whole cord and/or thoracic lumbar sections which require a ventral approach, hence this tissue is not obtained routinely.

Determination of Cause of Death

In general, cause of death of a sea turtle carcass will not be discernible from gross necropsy observations. Apparent exceptions to this are obvious signs of trauma, such as bullet wounds, severe lacerations, massive hemorrhage and decapitation; but evidence such as this might have been produced postmortem. The determination of cause of death will require histopathological examination and analysis in some cases. In other cases, the determination may be impossible because causes of death have not been adequately researched.

The determination of cause of death is dependent, along with other factors, on the knowledge of diseases and pathological conditions occurring in that species. Information regarding diseases, their prevalence, and the clinical manifestations of disease and pathological conditions in sea turtles is minimal at present. Data are being collected rapidly, but interpretation often relies on comparisons with similar observations in higher vertebrates, which may or may not be valid."

Regardless, whenever possible, a qualified veterinarian or a qualified veterinary medical diagnostic clinic or laboratory should be employed to perform sea turtle necropsies, and analyses and interpretation of pathological, histological toxicological, and microbiological observations and data should be done by qualified clinical veterinarians.