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ASPECTS OF THE BIOLOGY OF SEA TURTLES IN THE MID-ATLANTIC BIGHT.

A Dissertation

Presented to

The Faculty of the School of Marine Science The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

by

William C. Coles

UMI Number: 9921076

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APPROVAL SHEET

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DEDICATION

To my family.

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ACKNOWLEDGEMENTS

I would like to acknowledge everyone who provided advise, encouragement and support. Many thanks to my major advisor Jack Musick and committee members, Herb Austin, David Evans, Mark Patterson, and Ken Lohmann for the time, patience and effort they gave to the completion of this manuscript.

I would also like to thank all the ITNS faculty who gave assistance and allowed me, sometimes without their knowledge, access to computer services, without none of this would have been possible. Gloria Rowe, Louise Lawson and Jim Duggan are among the numerous VIMS staff, faculty and students who offered their help and encouragement. Thanks to my office mates Heidi Banford, Geoff White and Anne Sipe had to put up with all sorts of quirks. Thanks to my brother, Roy (Riox) Pemberton, in mind and spirit, if not genetics.

I owe a lot of thanks to Scott Kennedy for his enormous help in designing data acquisition routines for the Magnetic Resonance Imaging, which frequently pushed the instruments to their mechanical limit. Katya Prince for assistance in developing programs for numerically solving several problems. The Abingdon Volunteer Fire Company members, who had to learn to deal with a Californian, helped keep me sane.

I owe my success to my parents William A. and Alma Coles for the many days and nights they spent helping me begin my career in science. My spouse MaryJane Coles who put up with some of the darkest moments, and rejoiced in the lightest. Lastly I need to acknowledge my sons Duncan and Morgan who make this all worth while.

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ABSTRACT

I present here an investigation of several aspects of the biology of sea turtles in the mid-Atlantic Bight. During 19 years of data collection, included in this study, strandings have increased for all species of sea turtles in Virginia. Most sea turtle strandings occurred during the spring when juvenile turtles migrate into the Bay (Kemp's ridleys had a second significant stranding peak, during fall migration) along the Southern Bay and Virginia Beach Oceanfront. Sea turtles utilize the Chesapeake Bay as a feeding area when the water temperature approaches 20°C, and they leave after the water temperature drops below 20°C. Although some turtles have stranded at much lower temperatures.

The number of possible anthropomorphic interactions with turtles has increased as recreational boating & fishing has increased in popularity. The cause of death attributed to the largest number of strandings is boat and propeller damage. Commercial fishery interactions (entanglement) were second in importance, but such interactions, while usually resulting in turtles drowning, were less easily detected. The vast number of the strandings having an unknown cause of death maybe attributed to carcass decomposition and lack of observer training.

The VIMS data set provided the basis for morphometric analysis. Regressions calculated from the data often explain more than 90% of the variation in the measurements. These regressions may be used to estimate missing values required by State and Federal management agencies. The carapace morphology of loggerheads and Kemp's ridleys changes as they grow. The carapace flattens out in larger individuals, presumably to maintain a relatively constant amount of lift while swimming at higher cruising velocities. The extra lift may be needed by hatchlings because of their low swimming speed.

Using satellite imaging technology and sea turtle abundance and distribution data from coastal aerial surveys, off North Carolina, I confirmed a behavioral temperature range of 13°C to 29°C, which is well within previously established physiological limits and also encompass values recorded in the Chesapeake Bay.

Magnetic resonance imaging techniques, were used to image juvenile Kemp's ridley and loggerhead sea turtle heads. The location of magnetic particles in the sea turtle heads appears to be in the ethmoid, in the same region as in birds and fishes. The anomalies were bilaterally paired suggesting a possible use as a sensory system.

Results from an oxytetracycline injected adult loggerhead sea turtle show that bone rings are laid down on an annual basis. Examination of whole cross sections of the humerus suggests that the dorsal and ventral regions used for taking bone cores used in previous studies is inappropriate. The failure in other studies to detect growth rings may have been due to samples being taken from the dorsal surface of the bone. The lateral edges of the humerus should be used for future oxytetracycline studies. Growth rates and ring deposition support previous data, supporting the notion that sexual maturity may occur over a very large size range.

Aspects of the Biology of Sea Turtles in the

Mid-Atlantic Bight.

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INTRODUCTION

Sea turtles spend nearly all of their lives in the ocean making their study logistically difficult. After leaving the beach as hatchlings, turtles' return only for nesting, and on occasion for basking (Musick & Limpus 1997, Spotila et al. 1997). This life history makes basic life science studies difficult. Much of the turtles' biology is inferred from these brief terrestrial periods. I present here an investigation of several aspects of sea turtle biology from the Mid-Atlantic Bight.

Juveniles of <u>Eretmochelys</u> <u>imbricata</u> (hawksbill), <u>Chelonia mydas</u> (green), <u>Dermochelys coriacea</u> (leatherback), <u>Lepidochelys kempii</u> (Kemp's ridley), and <u>Caretta caretta</u> (loggerhead) sea turtles have been recorded in the Chesapeake Bay and coastal waters of Virginia (Musick 1988).

Hawksbill, green, and leatherback turtles are uncommon in the Bay. Neither hawksbill or green sea turtles have a significant impact on the ecology of the Chesapeake Bay nor does the Bay influence their ecology, due to their rarity. The leatherback may have a larger influence on food chain dynamics than is currently understood (Hood, R. 1997. Personal Communication. Biomass of primary consumers in the Chesapeake Bay. Horn Point, MD) because of its dietary

preference for primary consumers (jellyfish, salps, and other gelatinous organisms (Bjorndal 1997), which seasonally can make up a significant proportion of the biomass in the Bay (Personal Observation, VIMS: Trawl Survey).

Kemp's ridleys are the second most abundant sea turtle in the Bay and are generally recorded as stranded during migration (Lutcavage & Musick 1985). Ridleys utilize shallow habitats around the margins of the Bay, foraging almost exclusively on blue crabs (<u>Callinectes sapidus</u>) (Musick 1988) adjacent to the deeper loggerhead habitat.

The most common turtle in the Chesapeake Bay is the loggerhead sea turtle. It is estimated, from aerial surveys that between 3,000 and 10,000 loggerheads inhabit the Bay during the summer, feeding on benthic invertebrates, primarily horseshoe crabs (<u>Limulus</u> <u>polyphemus</u>) (Byles, 1988; Musick, 1988; Lutcavage & Musick, 1985). These turtles come from two populations, as determined by mitochondrial DNA analysis, 58% from the Georgia/South Carolina populations and 42% from the Florida population (Norrgard, 1995).

The Virginia Institute of Marine Science (VIMS) started collecting data on sea turtles in Virginian waters in 1979. VIMS collects and maintains several different

types of sea turtle data: stranding records, aerial surveys, satellite telemetry, nesting, and diving behavior. Using these data, long term (>5 years, the life of a Ph.D. Student) trends (size classes of juveniles, numbers of dead (spatial and temporal distributions), growth rates of recaptured turtles, correlations with other data sets (e.g. water temperature, satellite derived sea surface temperatures) can be identified, and generalizations of the biology of the juveniles can be inferred.

Adult and juvenile loggerhead turtles migrate along the east coast of North America from summer feeding grounds in and around the Chesapeake and Delaware Bays to wintering areas off the Florida coast (Keinath & Musick 1991 a,b; Byles 1988; Keinath et al. 1987). Loggerheads caught in pound nets at the mouth of the Potomac River, on the Chesapeake Bay, and transported to Back Bay National Wildlife Refuge (BBNWR) (on the VA-NC border) have been caught in the same pound net, just weeks later (Jett, F. 1995. Personal Communication. Recapture of flipper tagged sea turtles. Ophelia, VA). The turtles' mechanism of navigation required for migration and homing is unknown. Loggerhead eye morphology indicates that turtles are myopic when not in direct contact with water (Ehrenfeld 1966), suggesting that stellar, or visual cues are not important

for navigation. Hatchling loggerheads appear to orient with respect to magnetic fields (Lohmann & Lohmann 1996a,b, 1994a,b, 1993, 1992; Light et al. 1993; Lohmann 1991; Lohmann et al. 1990). Magnetite, a naturally occurring biomineral may be used as part of a neural transducer and has been identified in the green sea turtle dura (Perry et al. 1981). New non-invasive magnetic resonance imaging techniques that have identified magnetic particles in tissue (Coles 1994) can be used to localize particles in sea turtles.

The analyses presented in this study will feature: 1) Distributions of stranded sea turtles, and correlations with water temperature, using the VIMS pier data as surrogate data for Chesapeake Bay water temperature, 2) Descriptive morphology of sea turtles' including estimates for missing measurements, weight estimates from carapace curvature, and Reynolds numbers of sea turtles, 3) Analysis of satellite sea surface temperatures and sea turtle location (from aerial surveys), 4) Magnetic resonance imaging to locate magnetite particles in sea turtle heads, 5) Further validation of skeletochronology as an important tool in studies of sea turtle age and growth.

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Trends of Sea Turtle Strandings in the Chesapeake Bay and Surrounding Waters.

Introduction

Loggerhead, Kemp's ridley and leatherback sea turtles are frequently seen in the Chesapeake Bay during the summer (Byles 1988, Musick 1988, Lutcavage & Musick 1985). The Virginia Institute of Marine Science (VIMS) started collecting data on sea turtles in Virginian waters in 1979. There is now a continuous eighteen year sea turtle stranding data set of Virginian marine turtles, from which long term trends may be determined. A network of trained volunteers from state, federal, local and private organizations collect data from stranded and incidentally captured sea turtles. VIMS is the central repository for all Virginian sea turtle data, which is distributed to the National Marine Fisheries Service (NMFS). These data are archived and maintained at VIMS, currently in a Microsoft Access format; the raw data are also archived. A host of water parameters are monitored at the VIMS, Gloucester Point campus. A sampling station was first established in the 1940s, on the VIMS Ferry Pier, when daily water temperature maxima and minima were recorded. In early 1985 six minute recordings of temperature and salinity were begun. Average values were computed from 240 daily points. The six minute data monitoring has been continuous, except for sensor failure, from 1985 to present

(Anderson, G. 1998. Personal Communication, History of the VIMS pier sampling station. Gloucester Point, VA). VIMS pier water temperatures will be used as a surrogate for Bay temperatures.

The purpose of this study is to investigate patterns relating sea turtle stranding records to water temperature, spatial and temporal trends and population structure of marine turtles in the Chesapeake Bay and nearby coastal waters.

Materials & Methods

All sea turtles, either live (sick, injured, or incidentally captured by fishermen) or stranded dead documented by the VIMS program, were assigned an identification number based on the date the turtle was discovered or captured (a turtle receives a new identification number each time it is captured, or recaptured). The identification number assigned to the turtle is recorded as "MT-YYMMDD##" where "MT-" signifies that it is from the VIMS marine turtle data collection, (YY) the year of capture, (MM) the month, (DD) the day of discovery and (##) the number of the turtle caught that day. For example, identification number MT-98061203, indicates the turtle was the third turtle recovered on the 12th of June, 1998. Data in the Microsoft Access[™] database was checked for errors against the original data sheets. Weekly, monthly and yearly average numbers of sea turtle strandings were calculated by species.

Water temperature data recorded from the VIMS pier was used as surrogate data for Bay temperature (VIMS: Temperature Data, 1997). The VIMS data are the only continuous water temperature data set available for the 1979 to 1997 period. Temperatures were compiled and checked to remove sensor and recording errors. Daily,

weekly, monthly and yearly averages were computed from recorded temperature values.

Temperature interactions were determined for loggerhead, Kemp's ridley, and leatherback sea turtles. Weekly temperature means and variance, for weeks with and without sea turtles, and weekly mean temperatures of first and last stranding were compared by Students t- and Ftest's (Zar 1984).

Locations of recorded strandings were grouped into 7 regions: Maryland/Delaware Ocean, Maryland Bay, Eastern Shore Bay, Western Bay, Southern Bay, Eastern Shore Ocean and Virginia Beach Ocean (Figure 1). Stranding frequencies were plotted and analyzed by month and year.

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Figure 1. Map (Mercator projection) of the lower Chesapeake Bay and Mid-Atlantic bight, broken into 7 stranding regions: Western Bay, Southern Bay, Virginia Beach Ocean, Eastern Shore Ocean, Eastern Shore Bay, Maryland/Delaware Ocean and Maryland Bay.



Results

The yearly number of loggerhead (Figure 2), Kemp's ridley (Figure 3) and leatherback (Figure 4) strandings were plotted by year from 1979 to 1997. A simple linear regression was computed for each species and the slope of the regression is presented on the graphs. The slope indicates the general state of turtle strandings (increasing, decreasing, or constant) on a yearly basis. The graphs show that leatherback strandings occur at a low relatively constant rate (Figure 4) and numbers of Kemp's ridley strandings have been slowly increasing at a rate of about 1 turtle a year (Figure 3) since 1979. In contrast the number of stranded loggerheads has been increasing at a rate of 3 turtles/year (Figure 2).

The bulk of the yearly loggerhead, Kemp's ridley and leatherback sea turtle deaths occurs in the spring of the year (Figures 5, 6, 7) (Coles & Musick 1998). Graphs of Kemp's ridley (<u>Lk</u>) (Figure 6), loggerhead (<u>Cc</u>) (Figure 5) strandings and mean water temperatures distinctly show a primary stranding period occurring in the spring when the water temperature approaches 21°C (<u>Lk</u>: $s^2 = 1.6$) and 19°C (<u>Cc</u>: $s^2 = 1.9$) which usually occurs sometime in May. Kemp's ridley deaths drop to a near zero value during the middle

Figure 2. The number of loggerhead (<u>Caretta caretta</u>) strandings in the Mid-Atlantic Bight and Chesapeake Bay by year, from 1979 to 1997 and a simple linear regression are plotted. The slope of the regression line is provided, and represents a change in strandings per year. The slope identifies an increasing trend in the state of loggerhead deaths.



Figure 3. The number of Kemp's ridley (Lepidochelys kempii) strandings in the Mid-Atlantic Bight and Chesapeake Bay by year, from 1979 to 1997 and a simple linear regression are plotted. The slope of the regression line is provided, and represents a change in turtle strandings per year. The slope identifies a general increasing trend in the state of Kemp's ridley deaths.



Figure 4. The number of leatherback (<u>Dermochelys coriacea</u>) strandings in the Mid-Atlantic Bight and Chesapeake Bay by year, from 1979 to 1997 and a simple linear regression are plotted. The slope of the regression line is provided, and represents a change in turtle strandings per year. The slope identifies a steady but slightly increasing trend in the state of leatherback deaths.

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Figure 5. Mean number of loggerhead (<u>Caretta caretta</u>) strandings (bars) and mean water temperature (°C) (line) by week from 1979 to 1997.



Figure 6. Mean number of Kemp's ridley (<u>Lepidochelys</u> <u>kempii</u>) strandings (bars) and mean water temperature (°C) (line) by week from 1979 to 1997.





Figure 7. Number of leatherback (<u>Dermochelys coriacea</u>) strandings (bars) and mean water temperature (°C) (line) by week from 1979 to 1997.



of the summer (Figure 6), while loggerheads maintain a low level of strandings throughout the summer (Figure 5).

The mean water temperature for the last recorded stranding of the year for a Kemp's ridley is 19° C (s² = 5.7; Table 1, Figure 8) and for a loggerhead is 16° C (s² = 4.4; Table 2, Figure 9). The lag between the time turtles die and the time they strand on the beach increases variance seen in the estimated autumn exiting (fall, southerly migration) water temperature. These stranding temperatures agree with satellite sea surface temperature preferences (Coles 1998).

Leatherbacks (Figure 7) and green turtles do not occur in sufficient numbers to determine if there are multiple stranding peaks throughout a single season. Mean water temperatures of first, last stranding and mean water temperature for weeks with presence, and absence of both leatherback (Figure 10, Table 3) and green (Table 4) turtles were calculated. The small numbers of strandings precluded additional analysis, because there are many years with no strandings of either species.

VIMS sea turtle stranding data records information from both live (usually detailed) and dead turtles. Dead turtles were necropsied whenever possible to determine the state of health (parasites, fat content, etc.) and the

Figure 8. Plot of water temperature (°C) of first (blue line) and last (red line) stranding of Kemp's ridley (Lepidochelys kempii) from 1979 to 1997.



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Figure 9. Plot of water temperature (°C) of first (blue line) and last (red line) stranding of loggerhead (<u>Caretta</u>) <u>caretta</u>) from 1979 to 1997.



Figure 10. Plot of water temperature (°C) of first (blue line) and last (red line) stranding of leatherback (Dermochelys coriacea) from 1979 to 1997.



cause of death. Despite the attempts to determine the cause of death, the cause could not be determined in a majority of stranded turtles because of the advanced state of decomposition (Table 5). A significant number of the total records came from live turtles that were recovered from pound nets. There are no identifiable trends in the cause of death data for loggerheads, Kemp's ridleys or leatherbacks, in part due to the lack of detailed cause of death data.

Almost all the turtles recovered (live or dead) were juveniles, determined by carapace length and/or internal exam. There was no size frequency shift in loggerhead strandings between months (Figure 11) or years (Figure 12). Each month and year had a similar distribution of stranding lengths. Kemp's ridleys on the other hand showed a length frequency shift from small to large turtles as the season progressed (Figure 13), although there was no pattern of length frequency changes between years (Figure 14). There was insufficient data to draw any conclusions for green or leatherback turtles.

All the verified strandings in the VIMS data base were grouped into 7 regions: Maryland & Delaware Ocean (MDO), Maryland Bay (MB), Eastern Shore Bay (ESB), Western Bay (WB), Southern Bay (SB), Eastern Shore Ocean (ESO) and

Figure 11. Tip to Tip (T-T) length frequency graph of loggerhead sea turtles (<u>Caretta caretta</u>) by month for the years 1979, 1980, 1987-1997.



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission. Figure 12. Tip to Tip (T-T) length frequency graph of loggerhead sea turtles (<u>Caretta</u> <u>caretta</u>) by year.



Figure 13. Tip to Tip (T-T) length frequency graph of Kemp's ridley sea turtles (<u>Lepidochelys kempii</u>) by month for the years 1979, 1980, 1987-1997.



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission. Figure 14. Tip to Tip (T-T) length frequency graph of Kemp's ridley sea turtles (<u>Lepidochelys kempii</u>) by year.



Virginia Beach Ocean (VBO) (Figure 1). The trends in loggerhead stranding location viewed by year (Figure 15) show a steadily increasing number of deaths in all areas in recent years. Similar trends are seen for Kemp's ridleys (Figure 16). Loggerhead and Kemp's ridley data show particularly high yearly stranding numbers in the SB and VBO regions. Stranding frequencies by month clearly show that strandings peak in all regions in June, and that VBO also has a fall (October) peak (Figures 17, 18) (large for Kemp's ridleys, and small for loggerheads). The large June peak corresponds to the water temperature increase that occurs as the turtles migrate into the Bay. The fall VBO peaks correspond to times the turtles are migrating out of the Bay. Leatherback and green turtle deaths do not occur in sufficient numbers to determine yearly or monthly trends. They tend to strand in the same areas, SB, WB and VBO, as the loggerheads and Kemp's ridleys (Figure 19). The lack of Kemp's ridley turtles in the MDB and MD/DE regions is due to the lack of awareness and any semblance of a sea turtle stranding program until the mid 90's.

Figure 15. Frequency of loggerhead sea turtle (<u>Caretta</u>) <u>caretta</u>) strandings by location and year.



Figure 16. Frequency of Kemp's ridley sea turtle (Lepidochelys kempii) strandings by location and year.



Figure 17. Frequency of loggerhead sea turtle (<u>Caretta</u>) <u>caretta</u>) strandings by location and month for the years 1979, 1980, 1987-1997.



Figure 18. Frequency of Kemp's ridley sea turtle (<u>Lepidochelys kempii</u>) strandings by location and month for the years 1979, 1980, 1987-1997.



Figure 19. Frequency of leatherback sea turtle (<u>Dermochelys coriacea</u>) strandings by location for the years 1979, 1980, 1987-1997.



Discussion

Due to the nature of the data collected, we can only make broad generalizations about many of the trends identified. One problem with the data set is spatial discrepancies of effort in reporting turtle strandings because beaches are not equally patrolled (marshy areas receive less coverage than broad sandy beaches). Additionally stranding coverage was more complete in some years than others because of fluctuations in the availability of resources and funds (low funds or resources makes for low numbers of records). In many cases the only information recorded was the date that a dead turtle was reported, unless there was a good chance the turtle was a Kemp's ridley or leatherback, species were not determined. As a result some general trends may be identified, but specific nuances may remain hidden.

The identification number assigned to stranded turtles represents the discovery date, not the date of death. Death may have occurred days or even weeks prior to discovery (a newly dead turtle will tend to sink; as it decomposes the gas produced will cause the turtle to float; floating turtles are then blown ashore). This accounts for the spurious sightings of turtles during the fall and

winter, when water temperatures are well below that of the lethal minimum temperature (decomposition takes longer).

The driving force behind the large yearly fluctuations in stranding numbers (Figures 2, 3, 4) make the trend line at best a simple first order approximation. The fluctuations may be driven by multiple factors including fishing mortality, recreational boat interactions, water temperature, or other unidentified environmental factors and stranding coverage.

One of the major factors determining the presence of sea turtles in the Bay is water temperature. As the water temperature approaches 20°C turtles start to enter the Bay. The mean weekly stranding numbers of loggerhead (Figure 5), Kemp's ridley (Figure 6) and leatherback (Figure 7) sea turtles show that most of the years' strandings come in the spring, when the turtles first enter the Bay. It is not surprising that the weekly water temperature means with turtles (loggerhead, $t_{0.05(1)941} = 12.14$; Kemp's ridley, $t_{0.05(1)941} = 2.33$; where nomenclature for t is probability of a Type 1 error, 1 or 2 tailed test, degrees of freedom n-2) are significantly higher than those weekly means without turtles (Tables 1, 2, 3). Although the magnitude of the water temperature doesn't correlate with the total numbers of turtles in the Bay there is a threshold temperature,
20°C, which is a signal that can be easily monitored. The plots of water temperature for first and last strandings of the year (Figures 10, 11, 12) clearly supports our temperature findings.

Many sea turtles entering the Bay early in the spring are in poor health, emaciated and heavily encrusted with *Chelonibia* barnacles (Belmund 1988, Bellmund et al. 1987, Lutcavage & Musick 1985). These compromised turtles enter the Chesapeake Bay early in the season and in a physiologically weakened state. A sharp thermal lens exists in the Bay until late spring, which keep the turtles in the upper water column, away from benthic food sources. The delay in feeding further depletes the turtle's energy reserves. In this weakened state, turtles may confine their activities to the warm surface water until the thermal lens has broken down, making benthic food sources available.

In the 1870's, fishing techniques significantly changed as pound nets were introduced to the Chesapeake Bay (Reid 1955). The pound and other nets can entangle and drown physiologically weakened turtles (later in the year when turtles have replenished their energy reserves, they are better able to avoid pound nets) (Bellmund et al. 1987, Byles 1988, Musick 1995, Musick et al. 1985, Lutcavage & Musick 1985). More recently, recreational fishing and boating has increased. The increase of boat traffic and recreational fishing in the spring (fish, as well as turtles, migrate into the bay) increases the number of interactions with turtles. The turtles' natural avoidance response to aerial or surface stimulus is to dive (Wyneken et al. 1994). If a thermal lens is present, turtles maybe forced through it, cooling the body. Reducing the body core temperature further physiologically compromises the turtle. These cold turtles which are less active may drift into nets, become entangled and drown. Turtles that arrive later in the spring enter an environment where food is immediately available (thermal lens has dissipated). They can quickly replenish their energy reserves and are able to avoid nets (Bellmund et al. 1987).

Loggerheads and Kemp's ridleys have different stranding patterns (Figures 5, 6). This difference is reflected in the different habitat types the turtles use during the summer. Kemp's ridleys are generally found in shallow, less than 5 meters depth, protected grass beds, and are removed from most commercial fishing activities. Loggerheads utilize the edges of channels in water depths of 5 to 13 meters (Byles 1988, Musick 1988, Musick & Limpus 1997). The loggerheads are exposed to more anthropogenic

interactions than Kemp's ridleys, which account for the larger number of loggerhead strandings during the midsummer.

In the fall as the Kemp's ridleys start to migrate out of the Bay there is a second, smaller stranding peak (Figure 6) presumably due to the turtles reentering areas where the number of human interactions increases. It is clear that to reduce Kemp's ridley mortality in Virginia, efforts should focus on the spring and fall migration periods. The Kemp's ridleys do not seem to be particularly susceptible to fishing or other stresses while feeding in the Bay. Loggerhead strandings do not exhibit this secondary peak; stranding numbers just dwindle to zero as the water temperature drops. Leatherback stranding numbers exhibit a large spring peak, with a fall peak as well, although the numbers are too small to draw definitive conclusions (Figure 7).

The causes of sea turtle mortality previously identified by Lutcavage (1981) are: Pound net entanglement, boat or propeller damage, haul seine, long line, rod and reel, mutilation, crab pot entanglement and natural predation. The present data set lumped these into commercial fishing (Net/crab line entanglement), boat/propeller, hook & line fishing, malicious mutilation,

and natural causes (predation & illness) (Table 5). In 1980 at least 30% of the stranded turtles died due to pound net hedging entanglement, and it is likely that more turtles were tangled and drowned than were reported (Lutcavage 1981). However the percentage of net related deaths for the period 1979-1983 was only 18.6 (Bellmund et al. 1987). In contrast there are only 2.4% confirmed entanglements of loggerhead turtles throughout the whole period (Table 5). This suggests that there was a change in techniques used to identify entangled turtles. It is likely that entanglement deaths are and have been vastly underreported, in part because the turtles have decomposed beyond the point that a cause of death can be determined at the time of discovery and regular surveys of sea turtles entangled in nets have been discontinued. The general consensus remains that commercial fishing has a great influence on the numbers of sea turtle deaths (Coles & Musick 1998, Terwilliger & Musick 1995), although the cause of death for the vast majority of strandings is unknown.

Previous studies have shown that sub adult turtles are the preponderant size class in the Bay (Lutcavage 1981, Byles 1988). If we make the assumption that turtle strandings represent a random sample of the population, then these stranding results support those conclusions,

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(Figures 14, 16). The loggerhead population structure of the is uniform both annually and inter-annually (Figure 13, 14) with a majority of the loggerheads being in the 50-80 cm range. The Kemp's ridley population does not show the same pattern. The monthly standing data show unexplained shifts in the turtle's population structure as the year progresses (June-Sept.) (Figure 15). It is possible that this pattern is due to random perturbations in the total number because of the small number of Kemp's ridleys recorded on a yearly basis (Figure 16). Therefore the strandings in an individual year can have a large influence on monthly trends. Chesapeake Bay loggerheads come from multiple nesting populations (Norrgard 1995). These nesting areas cover a vast geographic region along the Atlantic coast, from Virginia to Florida. The large numbers and distribution of loggerheads decreases the effects of random perturbations, or the lack of hatchling success from a single local event on an individual beach.

All the verified strandings in the VIMS data base were grouped into 7 regions: Maryland & Delaware Ocean (MDO), Maryland Bay (MB), Eastern Shore Bay (ESB), Western Bay (WB), Southern Bay (SB), Eastern Shore Ocean (ESO) and Virginia Beach Ocean (VBO) (Figure 1). Loggerhead and Kemp's ridley data show high yearly stranding numbers in

the SB and VBO regions. The higher stranding numbers from SB and VBO possibly represent spatial discrepancies in reporting effort, because all beaches are not patrolled equally. The SB and VBO are primarily areas with wide sandy beaches, heavily used by both local residence and tourists (especially when turtles are present). Other areas of the coast are not uniformly covered. Large areas are not easily accessible, even by boat (mud flat and marsh), and so are not regularly patrolled, if at all. The Eastern Shore has the least consistent coverage of any region, which is reflected in the low stranding numbers. Maryland numbers are low because they do not have the same number of turtles in their region as is seen in the lower Bay, and their coverage is sporadic like most of the marshy areas in the Bay.

Recently there has been a large increase in the number of strandings in the SB, the beaches of Fisherman's Island, Kiptopeke State Park and Sunset Beach areas of Northampton County. It is likely that this increase is due to an increase in commercial fishing, particularly the spring gill net fisheries, where large meshed gill nets are used (Terwilliger and Musick 1995). Although since 1995 there has been a dramatic increase in boat traffic due to the construction of the new Chesapeake Bay Bridge Tunnel (CBBT)

span from Fisherman's Island to the north (CBBT) tunnel island.

This study of sea turtle stranding data demonstrates several major points. 1) Juvenile sea turtles enter and utilize the Chesapeake Bay as a feeding area when the water temperature approaches 20°C, and they leave after the water temperature drops below 20°C. 2) The cause of death attributed to the largest number of strandings is boat and propeller damage because such damage is easy to recognize. Interactions (entanglement) with commercial fishing gear were second in importance, but such interactions, while usually resulting in turtles drowning, may be less apparent. The vast bulk of the strandings are of unknown cause of death due to decomposition and lack of observer training. 3) The size composition of loggerheads in the Bay is uniform both between and within years. Kemp's ridleys show a lot more variation in their size composition within and between years. Analysis of this data has reenforced the importance of uninterrupted support for longterm monitoring projects. In addition, detailed data on the location of fishing effort and seasonality is needed to test for correlations between fishing activities and sea turtle strandings.

Table 1. Temperature statistics, calculated from weekly temperature averages, for Kemp's ridley (Lepidochelys kempii) (Lk) sea turtles. Calculations encompass the nineteen season period (1979 to 1997). For each year, the mean temperature of weeks with the first and last stranding record and all weeks with and without stranding records were used for calculations. Mean water temperatures with Kemp's ridley strandings is greater than the mean temperature without Kemp's ridley strandings ($t_{(0.05,2,941)} = 2.33$).

	First	Last	With Lk	w/o Lk
Mean Water Temp.	20.9	19.1	22.1	15
Standard Deviation	1.6	5.7	5	8.3
Min. Water Temp.	17.9	7.2	4.2	-0.6
Max. Water Temp.	23.6	28.1	29	29.5
Number of data points	19	19	153	790

Table 2. Temperature statistics, calculated from weekly temperature averages, for loggerhead (<u>Caretta caretta</u>) sea turtles (<u>Cc</u>). Calculations encompass the nineteen season period (1979 to 1997). For each year, the mean temperature of weeks of the first and last stranding record and all weeks with and without stranding records were used for calculations. The mean water temperature with Loggerheads is significantly higher than the water temperature without loggerheads ($t_{0.05,(1),941} = 12.14$).

	First	Last	With Cc	w/o Cc
Mean Water Temp.	18.7	15.7	21.5	10.5
Standard Deviation	1.9	4.4	6.2	6.2
Min. Water Temp.	15.3	10.8	-0.6	0.5
Max. Water Temp.	22.5	26.9	29.5	28.5
Number of data points	19	19	483	460

Table 3. Temperature statistics, calculated from weekly temperature averages, for leatherback (<u>Dermochelys</u> <u>coriacea</u>) sea turtles (<u>Dc</u>). Calculations encompass the nineteen season period (1979 to 1997). For each year, the mean temperature of weeks of the first and last stranding record and all weeks with and without stranding records were used for calculations. There was insufficient data to meaningfully compare temperatures.

	First	Last	With Dc	w/o Dc
Mean Water Temp.	22.9	21.6	23.6	15.6
Standard Deviation	2.6	6.1	3.99	8.3
Min. Water Temp.	18.4	10.7	10.7	-0.6
Max. Water Temp.	27.4	27.6	28.6	29.5
Number of data	17	16	66	877
points				

Table 4. Temperature statistics, calculated from weekly temperature averages, for green (<u>Chelonia mydas</u>) sea turtles (<u>Cm</u>). Calculations encompass the nineteen season period (1979 to 1997). For each year, the mean temperature of weeks of the first and last stranding record and all weeks with green turtle and without green turtle stranding records were used for calculations. There was insufficient data to meaningfully compare temperatures.

	First	Last	With Cm	w/o Cm
Mean Water Temp.	23.5	13.3	19.5	15.3
Standard Deviation	3.4	5.2	5.5	8.8
Min. Water Temp.	18.1	6.4	6.4	-0.6
Max. Water Temp.	27.3	20.9	27.3	29.5
Number of data	9	6	22	966
points				

Table 5. Frequency of sea turtle strandings lumped by cause of mortality. The data represents a total for years 1979, 1980, 1987, 1988, and 1990-1997. Loggerhead (<u>Caretta</u> <u>caretta</u>) (<u>Cc</u>), Kemp's ridley (<u>Lepidochelys kempii</u>) (<u>Lk</u>), and leatherback (<u>Dermochelys coriacea</u>) (<u>Dc</u>) data are presented. With the exception of live and sick turtles recovered (primarily from pound nets) the data represents turtle mortality. The causes of sea turtle mortality identified by Lutcavage (1981) were lumped into commercial fishing (net/crab line entanglement), boat/ propeller, hook & line fishing, malicious mutilation (hammer, knife, gunshot) and illness or natural causes.

	Cc	Lk	Dc
Missing/Unknown	1443	111	34
Boat/Propeller	157	5	13
Entanglement	49	6	5
Hook & Line	3	0	0
Malicious	27	0	0
Illness/Natural	41	3	0
Live, Incidental	358	57	2
Capture			

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Morphometrics of Sea Turtles in Virginia.

Introduction

Anatomical measurements have been made on stranded and live Kemp's ridley (Lepidochelys kempii) and loggerhead (Caretta caretta) sea turtles in the Commonwealth of Virginia since 1979. Often stranded turtles are disarticulated, pieces are missing, or the turtle's position, condition, location make measurements unreliable or impossible. Occasionally the only piece of the turtle that can be reliably measured is the head. Often there is a need to accurately convert between one or more measurements for various biological and physical analyses.

The objective of this study is to provide a morphometric analysis of loggerhead and Kemp's ridley sea turtles from Virginia and provide regression equations to allow for conversion from one kind of measurement to another.

Materials and Methods

Since 1979, measurements of stranded sea turtles found in Virginian waters, have been made by members of the Virginian sea turtle stranding network. Straight measurements (S) were made with either one or two meter calipers, curved measurements (C) were made with fibrous measuring tapes. All measurements were made by trained volunteers and recorded on the Virginia Institute of Marine Science (VIMS) sea turtle stranding forms.

Carapace measurements taken are: Notch to Notch (NN), Tip to Tip (TT), Width (CW) and Notch to Tip (NT). The NT measurement was only recently added to fulfill a National Marine Fisheries Service (NMFS) sea turtle stranding network requirement. Head length (HL) and head width (HW) measurements are also made (Figure 1). Plastron measurements are taken when available (width without bridge (PW), width with bridge (PWB), and length (PL)) (Figure 2). Carapace lengths and widths are made to the marginal edge of the carapace and recorded as both curved (C) and straight (S) measurements. Straight measurements require that observers have calipers, which are not available to all volunteer observers, and are frequently not recorded. All plastron and head measurements are made with calipers making them the least

Figure 1. Line drawing of the carapace of a marine turtle, showing the location of carapace and head measurements made. The measurements are: (TT) - Tip to Tip, (NT) -Notch to Tip, (NN) - Notch to Notch, (HL) - Head Length, (HW) - Head Width and W - Carapace Width.



Figure 2. Line drawing of the plastron of a marine turtle, showing the location of plastron measurements made. The measurements are: (PW) - Plastron width, without Bridge, (PWB) - Plastron Width with Bridge and (PL) - Plastron Length.



frequent measurements recorded. Curved measurements are simply made with a tape measure and are the most frequent measurements recorded. These data were entered into a Microsoft Access™ database and all data used for analysis were checked with original data sheets. Forty-eight loggerhead hatchling measurements were provided from a study of loggerhead nesting parameters (Jones, W. 1997. Personal Communication. Hatchling sea turtle data. Virginia Institute of Marine Science, Gloucester Point, VA).

Weight estimates are generally derived from a corresponding volume measure, generalized as length cubed, (1³), of the organism (Schmidt-Nielsen 1985, Calder 1984). Turtle lengths and widths are reliable measurements. Turtle depth (dorso-ventral length) is not recorded. Rapid decomposition after death and bloating also make turtle height an unreliable measurement. This makes the 1³ estimate for weight inappropriate.

The shape, curvature, of the carapace (how domed it is) should be a good indicator of the weight of the turtle. Curvature (k) is generally defined as $\partial \theta / \partial s$ (change of angle (θ) divided by the change of arc length (s)). By observation of the cross section shape of the carapace we assume that the first order curvature of the sea turtles carapace can be approximated as a circle. That circle can

be defined by the arc (curved carapace width) and chord (straight carapace width). The radius (r) of the circle and angle (described by the arc and chord) can be numerically solved from the relationship arc/chord = $(r*\theta)/(r*sin(\theta))$. Conveniently, circles have constant curvature, which simplifies the equation for curvature to k = 1/r (Shenk, 1984). The curvature was regressed against weight and curved notch to notch carapace length for loggerheads and Kemp's ridleys.

For each regression, the original data set was sorted and filtered to eliminate unknown, unreliable species identification and unpaired data. Simple linear regressions for lengths and widths for loggerheads and Kemp's ridley sea turtles were calculated for all measurement combinations. The significance of regression coefficients were tested with F-tests ($\alpha = 0.05$) (Zar 1984).

Results and Discussion

Morphological measurements made on vertebrates generally involve measurements along the long and occasionally the short axis of the structure (Hall 1962). By consistently taking these measurements we are able to provide estimates and corrections for missing and suspicious data.

Linear regression equations, coefficient of determination (r^2) sample sizes, and F-test are shown for Kemp's ridleys (Table 1) and loggerhead (Table 2). The high coefficient of determination $(r^2$, an indication of the accuracy of the predictions) shows that most of the variation in turtle measurements is dependent on the size of the turtle. This makes these regression equations excellent estimators for erroneous and missing measurements. The high coefficient of determination of these regressions is similar to those presented for green turtles (Chelonia mydas) (Bjorndal and Bolten 1989).

All linear regressions were found to be significant ($\alpha = 0.05$). From scatter plots and regression equations of Kemp's ridley or loggerhead sea turtles (Table 1, 2), there is only one morphological population, of each species, present in Virginian waters. This is expected for Kemp's ridleys because they all come from the same breeding

population (The entire population of Kemp's ridleys nest in one restricted area (Rancho Nuevo) in Tamaulipas, Mexico), making them a truly panmictic species.

Virginian loggerhead sea turtles come from two populations, as determined by mitochondrial DNA analysis (Norrgard 1995), 58% from the Georgia/South Carolina nesting populations and 42% from the Florida nesting population. There are no distinguishing morphological features seen in our data set.

The greatest variability (lowest r^2) occurred for the linear regressions involving head measurements. Allometric equations ($y = a * x^b$, or log(y) = log(a) + b*log(x)) have two important terms, a- the intercept at unity, and bslope of the regression line. Customarily the original data is log transformed to handle biological scaling problems (Schmidt-Nielsen 1985, Calder 1984, Vogel 1988). In most cases the r^2 accounted for over 90% of the variability. The cases of lower r^2 the power transformation did not appreciably improve the values. Although this wasn't unexpected for the carapace and plastron comparisons, the carapace head measurements were also linear. Most of the variation may be explained by differences in individual measurer's techniques. Carapace measurements are explicit, because measurements are made to

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the marginal edge of the shell. Head measurements are more subjective, requiring a familiarity of cranial anatomy, and experience, making cranial measurements more subject to observer bias.

Curvature of the carapace was calculated for loggerheads and Kemp's ridleys. The curvature was plotted against weight (Figure 3), and the curved carapace length (notch to notch) was plotted against curvature (Figure 4). These plots all exhibited significant power relationships for the loggerheads, and highly significant for the Kemp's ridleys. The high r² for the curvature-weight estimates show that using curvature to estimate weight (Figure 3) is as good as carapace length (Figure 5).

A turtle's carapace changes shape as it grows; which is seen as a decrease in the curvature of the carapace as the length increases (Figure 4). The decrease in curvature may be correlated to the increase in swimming speed as the turtle grows (Wyneken 1997). The faster the turtle swims, the greater the lift created by a highly domed carapace. Therefore the turtles carapace likely changes shape in order to optimize lift and drag for the turtle's "cruising" velocity. Wind tunnel/flow tank studies of the lift and drag of different carapace shapes will validate these preliminary numbers.

Figure 3. Scatter plot, trendline and allometric scaling equations of curvature and weight for loggerhead (<u>Caretta</u> <u>caretta</u>) (a) and Kemp's ridley (<u>Lepidochelys kempii</u>) (b) sea turtles. The coefficient of determination (r^2) for the equations and number of data points are also presented.



Figure 4. Scatter plot, trendline and allometric scaling equations of carapace length (notch to notch) and curvature for loggerhead (<u>Caretta caretta</u>) (a) and Kemp's ridley (<u>Lepidochelys kempii</u>) (b) sea turtles. The coefficient of determination (r^2) for the equations and number of data points are also presented.



Figure 5. Scatter plot, trendline and allometric scaling equations of carapace length (notch to notch) and weight for loggerhead (<u>Caretta caretta</u>) (a) and Kemp's ridley (<u>Lepidochelys kempii</u>) (b) sea turtles. The coefficient of determination (r^2) for the equations and number of data points are also presented.



Without data from lift and drag experiments we can identify certain traits of the turtle as it moves through the water. These can be determined by looking at the Reynolds number (Re), which is "the nearest thing to a completely general guide to what is likely to happen when a solid and a fluid encounter each other" (Vogel 1989). The Reynolds number is a non-dimensional number representing the ratio of inertial and viscous forces and is the index from which different flows can be compared.

Hatchling and juvenile sea turtles live in a world of moderate Re, ranging from 1.1×10^4 to 4.6×10^4 (Wyneken 1988, 1997). Reynolds numbers were calculated from mean sea water density and viscosity values, Virginian loggerhead turtle lengths and swimming speeds from Wyneken (1997). These values ranged from $(1.17 \times 10^4$ to 1.57×10^4) for hatchlings to $(1.9 \times 10^4$ to 3×10^5) for migrating adults. The turtle's operate at a lower Re than calculated for many other migratory species (Wyneken 1997, Vogel 1981). This low number may be a result of the turtle's ectothermic physiology, not being able to maintain elevated metabolic rates. The turtles may compromise for the low efficiency by increasing their apparent lift by the curvature of the carapace.

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It is apparent that the analyses provided herein may provide a useful tool for the study of physical and mechanical aspects of sea turtle biology. The regression equations may be used to accurately estimate measurements that are missing or to check for errors in data sets.

Acknowledgments

This project was funded, in part, by the Virginia Coastal Resources Management Program of the Department of Environmental Quality through Grant # NA37000287-01 of the National Oceanic and Atmospheric Administration, Office of Ocean and Coastal Resource Management, under the Coastal Zone Management Act of 1972, as amended. Parts of this project were funded by Virginia Game and Inland Fish-Non Game Program. Thanks are due to all the past and present volunteers who assisted in collecting and maintaining the Virginia sea turtle stranding data.
Table 1. Linear regressions of Kemp's ridley (Lepidochelys kempi) sea turtle measurements, from VIMS stranding data. Comparisons of; straight (S) and curved (C) carapace [notch to notch (NN), tip to tip (TT), notch to tip (NT) and width (W)] plastron [width (PW), width with bridge (PWB) and length (PL)] and head [length (HL) and Width (HW)], measurements are shown with the respective r^2 , N and F values.

Equation	r^2	N	F
S:NT = (1.0114 * S:NN) + 0.1091	0.9995	33	64901.9
S:NT = (0.9894 * S:TT) - 0.0853	0.9994	33	50483.5
S:NT = (1.0548 * S:W) + 0.885	0.9794	33	1475.8
S:NT = (1.7944 * PW) - 0.7996	0.9560	30	608.3
S:NT = (1.2971 * PWB) 1.8584	0.9603	31	701.9
S:NT = (1.3485 * PL) - 1.4587	0.9929	31	4078.4
S:NT = (0.9645 * C:NT) - 0.6732	0.9964	29	7577.4
S:NT = (0.9723 * C:NN) - 0.6629	0.9965	31	8160.2
S:NT = (0.9333 * C:TT) + 0.1921	0.9983	32	18003.9
S:NT = (0.9757 * C:W) - 0.9297	0.9868	32	2244.8
S:NT = (3.7484 * HL) + 2.6424	0.8626	31	182.1
S:NT = (5.2462 * HW) - 5.5652	0.9748	30	1082.0
S:NN = (0.9744 * S:TT) + 0.0182	0.9985	107	69971.5
S:NN = (1.0095 * S:W) + 2.0497	0.9740	107	3931.8
S:NN = (1.5999 * PW) + 2.8336	0.9755	91	3550.9
S:NN = (1.2404 * PWB) + 2.3647	0.9589	98	2241.2
S:NN = (1.3084 * PL) - 1.1344	0.9843	98	6018.7
S:NN = (0.9539 * C:NT) - 0.7591	0.9963	30	7638.9
S:NN = (0.9395 * C:NN) + 0.1749	0.9884	102	8520.8
S:NN = (0.9203 * C:TT) + 0.2497	0.9971	102	34785.5
S:NN = (0.9456 * C:W) - 0.4784	0.9852	101	6594.7
S:NN = (4.04057 * HL) - 1.8933	0.8961	93	785.2
S:NN = (5.1518 * HW) - 6.2388	0.9367	95	1377.2
S:TT = (1.0473 * S:W) + 1.6005	0.9841	115	7011.5
S:TT = (1.6434 * PW) + 2.8298	0.9756	90	3519.3
S:TT = (1.2627 * PWB) + 2.6965	0.9558	98	2077.4
S:TT = (1.3351 * PL) - 0.9583	0.9844	98	6038.7
S:TT = (0.9763 * C:NT) - 0.6351	0.9951	30	5685.7
S:TT = (0.9646 * C:NN) + 0.1189	0.9882	103	8477.2
S:TT = (0.9467 * C:TT) + 0.1710	0.9946	110	19831.6
S:TT = (0.9732 * C:W) - 0.6606	0.9830	109	6171.6
S:TT = (4.1740 * HL) - 2.2045	0.9010	94	836.8
S:TT = (5.2428 * HW) - 6.0491	0.9102	101	1004.0

Table 1. Cont.	1		
Equation	r^2	N	F
S:W = (1.6114 * PW) + 0.4235	0.9854	92	6056.9
S:W = (1.2197 * PWB) + 0.7102	0.9654	99	2707.7
S:W = (1.2791 * PL) - 2.4885	0.9765	99	4022.8
S:W = (0.8881 * C:NT) - 0.2669	0.9629	30	727.1
S:W = (0.9148 * C:NN) - 1.1002	0.9781	103	4501.2
S:W = (0.8929 * C:TT) - 0.8779	0.9860	110	7631.6
S:W = (0.9261 * C:W) - 1.9901	0.9902	110	10886.0
S:W = (3.9855 * HL) - 3.5264	0.8992	94	820.6
S:W = (5.2043 * HW) - 8.6776	0.9461	98	1684.7
PW = (0.7339 * PWB) + 0.7122	0.9705	96	3090.4
PW = (0.8008 * PL) - 1.9357	0.9627	95	2400.4
PW = (0.5456 * C:NT) - 0.1231	0.9717	29	925.8
PW = (0.539 * C:NN) + 0.1981	0.9531	94	1868.6
PW = (0.5261 * C:TT) + 0.3249	0.9587	94	2134.9
PW = (0.5455 * C:W) - 0.3402	0.9622	93	2319.1
PW = (2.5456 * HL) - 3.1271	0.8894	89	699.9
PW = (3.0601 * HW) - 4.1417	0.9162	92	983.7
PWB = (1.037 * PL) - 2.1966	0.9726	100	3476.0
PWB = (0.7489 * C:NT) - 1.9566	0.9561	30	609.5
PWB = (0.7317 * C:NN) - 0.6591	0.9490	98	1788.0
PWB = (0.7215 * C:TT) - 0.7232	0.9813	96	4938.0
PWB = (0.7498 * C:W) - 1.7232	0.9818	96	5072.7
PWB = (3.1181 * HL) - 1.9205	0.8644	94	586.3
PWB = (4.1600 * HW) - 6.5932	0.9371	93	1356.7
PL = (0.7178 * C:NT) + 0.5441	0.9898	30	2717.9
PL = (0.7248 * C:NN) + 0.7531	0.9820	97	5174.3
PL = (0.6968 * C:TT) + 1.2659	0.9879	99	7907.2
PL = (0.7148 * C:W) + 0.8015	0.9754	99	3839.7
PL = (3.0103 * HL) + 0.1450	0.8825	94	690.9
PL = (3.9067 * HW) - 3.4840	0.9293	96	1235.9
C:NT = (1.0197 * C:NN) - 0.4582	0.9990	38	36300.3
C:NT = (0.9683 * C:TT) + 0.7832	0.9973	39	13821.7
C:NT = (1.0347 * C:W) - 1.4689	0.9799	39	1807.4
C:NT = (4.0122* HL) + 0.8863	0.9195	27	285.4
C:NT = (5.3311 * HW) - 4.2515	0.9544	29	565.0
C:NN = (0.9541 * C:TT) + 0.9754	0.9968	144	44761.6
C:NN = (0.9978 * C:W) - 0.3205	0.9820	149	8000.8
C:NN = (4.2114 * HL) - 1.3080	0.8979	93	800.5
C:NN = (5.4299 * HW) - 6.2595	0.9184	96	1058.0

Table 1. Cont.			
Equation	r^2	N	F
C:TT = (1.0357 * C:W) - 1.0623	0.9813	155	8012.4
C:TT = (4.4261 * HL) - 2.6348	0.9115	95	957.9
C:TT = (5.0631 * HW) - 2.1138	0.9190	104	1157.4
C:W = (4.2013 * HL) - 0.4133	0.8969	95	808.7
C:W = (5.2919 * HW) - 4.4371	0.9412	103	1617.7
HW = (0.7512 * HL) + 1.1749	0.8883	88	683.6

Table 2. Linear regressions of loggerhead (<u>Caretta</u> <u>caretta</u>) sea turtle measurements, from VIMS stranding data. Comparisons of; straight (S) and curved (C) carapace [notch to notch (NN), tip to tip (TT), notch to tip (NT) and width (W)] plastron [width (PW), width with bridge (PWB) and length (PL)] and head [length (HL) and Width (HW)], measurements are shown with the respective r², N and F values.

	·····	·	
Equation	<u>r</u> ²	N	F
S:NT = (1.0015 * S:NN) + 1.0292	0.9904	137	13859.9
S:NT = (0.9724 * S:TT) + 0.8243	0.9964	135	36513.2
S:NT = (1.3028 * S:W) - 4.9568	0.9474	142	2522.6
S:NT = (1.3028 * S:W) - 4.9568	0.9474	142	2522.6
S:NT = (2.0222 * PW) - 2.8684	0.9091	107	1049.6
S:NT = (1.3674 * PWB) + 0.8718	0.9330	108	1475.4
S:NT = (1.3078 * PL) - 0.6725	0.9718	110	3719.6
S:NT = (0.9427 * C:NT) - 1.4867	0.9815	125	6509.7
S:NT = (0.9583 * C:NN) - 1.6041	0.9749	125	4778.9
S:NT = (0.9106 * C:TT) + 0.0072	0.9818	124	6597.7
S:NT = (1.0123 * C:W) - 2.0314	0.9611	127	3089.8
S:NT = (3.9176 * HL) + 4.7321	0.7801	123	429.3
S:NT = (4.5714 * HW) + 4.5690	0.8333	125	614.8
S:NN = (0.9537 * S:TT) + 0.8376	0.9944	729	128427.7
S:NN = (1.282 * S:W) - 5.1640	0.9291	746	9747.1
S:NN = (2.0411 * PW) - 5.0732	0.9264	561	7039.6
S:NN = (1.4031 * PWB) - 2.3775	0.9447	569	9693.4
S:NN = (1.2884 * PL) - 0.7228	0.9576	607	13648.1
S:NN = (0.9447 * C:NT) - 2.7372	0.9808	120	6014.2
S:NN = (0.9440 * C:NN) - 1.7551	0.9844	623	39187.0
S:NN = (0.8964 * C:TT) - 0.2891	0.9867	615	45373.0
S:NN = (0.9810 * C:W) - 1.4943	0.9422	621	10081.9
S:NN = (4.0851 * HL) + 1.4958	0.8090	644	2718.7
S:NN = (4.515 * HW) + 2.7783	0.8818	653	4855.6
S:TT = (1.2579 * S:W) - 1.6522	0.9523	861	17160.3
S:TT = (2.0553 * PW) - 3.1383	0.9723	604	21107.5
S:TT = (1.5059 * PWB) - 4.7664	0.9558	565	12171.1
S:TT = (1.3391 * PL) - 0.9875	0.9890	658	58862.4
S:TT = (0.9812 * C:NT) - 3.1977	0.9785	120	5359.9
S:TT = (0.9834 * C:NN) - 2.3128	0.9810	617	31817.8
S:TT = (0.9385 * C:TT) - 1.0703	0.9882	674	56261.2
S:TT = (1.0491 * C:W) - 3.6637	0.9414	676	10828.8
S:TT = (4.257 * HL) + 1.1280	0.8151	658	2892.1
S:TT = (4.6421 * HW) + 3.3347	0.8624	696	4350.9

Table 2. Cont.			
Equation	r^2	N	F
S:W = (1.6003 * PW) - 0.2057	0.9758	616	24793.1
S:W = (1.052 * PWB) + 4.1898	0.9645	571	15460.8
S:W = (1.0329 * PL) + 2.0646	0.9627	672	17298.5
S:W = (0.6888 * C:NT) + 5.1722	0.9345	126	1769.9
S:W = (0.6766 * C:NN) + 6.7057	0.9348	625	8926.2
S:W = (0.6409 * C:TT) + 8.0865	0.9221	673	7945.0
S:W = (0.7429 * C:W) + 4.5269	0.9425	694	11352.1
S:W = (2.9975 * HL) + 8.1742	0.7798	664	2344.3
S:W = (3.3832 * HW) + 8.4473	0.8247	701	3288.5
PW = (0.6749 * PWB) + 1.8802	0.9370	613	9092.5
PW = (0.6368 * PL) + 1.7793	0.9693	662	20867.0
PW = (0.4186 * C:NT) + 4.1176	0.8593	126	757.1
PW = (0.4336 * C:NN) + 3.5152	0.9227	551	6553.2
PW = (0.4144 * C:TT) + 4.0314	0.9290	553	7204.8
PW = (0.467 * C:W) + 2.7114	0.9158	564	6110.5
PW = (1.8635 * HL) + 5.0699	0.7622	591	1887.9
PW = (2.0827 * HW) + 5.4327	0.8081	597	2505.9
PWB = (0.8757 * PL) + 3.3611	0.9310	640	8613.2
PWB = (0.592 * C:NT) + 5.3357	0.8744	124	849.7
PWB = (0.6181 * C:NN) + 4.2277	0.9285	562	7269.9
PWB = (0.5941 * C:TT) + 4.9201	0.9167	583	6393.6
PWB = (0.6734 * C:W) + 2.4488	0.9451	574	9842.9
PWB = (2.6779 * HL) + 5.5512	0.7009	626	1462.4
PWB = (2.9209 * HW) + 6.6250	0.7224	649	1684.0
PL = (0.7066 * C:NT) + 0.6502	0.9574	127	2810.4
PL = (0.7010 * C:NN) + 1.3794	0.9637	598	15803.2
PL = (0.6687 * C:TT) + 2.3884	0.9458	640	11142.3
PL = (0.7487 * C:W) + 0.42	0.9317	644	8764.5
PL = (3.0214 * HL) + 3.7566	0.7710	643	2157.9
PL = (3.3763 * HW) + 4.0988	0.8282	668	3209.5
C:NT = (1.0163 * C:NN) - 0.0476	0.9927	273	36627.4
C:NT = (0.9832 * C:TT) + 0.3518	0.9937	273	42540.9
C:NT = (1.1012 * C:W) - 2.0372	0.9673	284	8350.3
C:NT = (4.0252 * HL) + 7.6576	0.7203	140	355.4
C:NT = (4.4788 * HW) + 9.7739	0.6925	142	315.3
C:NN = (0.9537 * C:TT) + 1.0795	0.9922	1347	171339.8
C:NN = (1.0475 * C:W) + 0.1857	0.9570	1400	31122.1
C:NN = (4.1048 * HL) + 6.2790	0.7606	639	2024.3
C:NN = (4.5783 * HW) + 7.0042	0.8369	655	3350.0

Table 2. Cont.			
Equation	r^2	N	F
C:TT = (1.0712 * C:W) + 0.6252	0.9660	1590	45057.4
C:TT = (4.3443 * HL) + 4.6388	0.7570	671	2083.9
C:TT = (4.9638 * HW) + 3.7465	0.8837	657	12315.5
C:W = (3.8533 * HL) + 7.2637	0.7782	671	2347.6
C:W = (4.6592 * HW) + 2.9498	0.8926	828	6868.3
HW = (0.7166 * HL) + 2.394	0.7260	760	2008.7

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Sea Surface Temperature Preferences of Sea Turtles

off North Carolina

Introduction

Juvenile Loggerhead (<u>Caretta caretta</u>) and Kemp's ridley (<u>Lepidochelys kempii</u>) sea turtles use the Middle Atlantic Bight as an important foraging area (Byles 1988, Keinath et al. 1987, Lutcavage & Musick 1985). Sea turtles enter the Middle Atlantic Bight during the spring and migrate out of the Bight in the fall after the first winter storms, and move to the south of Cape Hatteras along the North Carolina coast (Byles 1988, Keinath 1993, Musick & Limpus 1997). The zeitgebers (cue for beginning migration) for sea turtle migration are poorly understood, although temperature may have the greatest influence.

Aerial surveys of turtle distribution in the southern the Middle Atlantic Bight over North Carolina waters indicate that sea turtles may not be randomly distributed. Their positions may be restricted by water temperature (Epperly et al. 1995, Lutcavage & Musick 1985). Lutcavage & Musick (1985) noted that most loggerhead sea turtles entered the Chesapeake Bay in the spring when temperatures rose to 18°C and departed in the fall when temperatures fell below 18°C. Some cold stunned turtles were found in the Bay at temperatures as low as 8°C (Musick unpublished data). Epperly et al. (1995) noted that sea turtles in the ocean off North Carolina occurred mostly at temperatures

above 11°C. But, the correlation between the location of sea turtles and sea surface temperatures or fronts is poorly understood.

The objective of the present paper was to determine whether a correlation existed between temperatures and turtle locations, utilizing archived satellite derived images of sea surface temperatures (SST) and historical VIMS aerial survey data, which determined positions of sea turtles.

Materials & Methods

Sea turtle abundance and distribution data from 10 aerial surveys, performed for the U.S. Department of the Interior, Minerals Management Service (OCS Study MMS 95-0024) was collected between 8 May 1991 and 17 Sept. 1992. The surveys were flown in a DeHavilland U-6A Beaver at an altitude of 152 m at a velocity of 128 km/hr (Byles 1988, Keinath 1993). Surveys were flown in a saw-toothed pattern along the Outer Banks of North Carolina with individual flight lines being approximately 28 km long, extending at least to the thermal edge of the Gulf Stream, south of Cape Hatteras (Figure 1). All transects in a survey were flown the same day.

Survey data included: the flight transect location (North, Middle, South), time of turtle sighting, beginning and ending transect positions (determined by LORAN) and times of each flight line (Keinath et al. 1987). These data were initially used to determine population densities and provide a quantitative assessment of the standing stock of sea turtles along the North Carolina Coastline.

Each sea turtle position along the transect was calculated from the airplane's mean velocity along the flight line and the difference between the sighting time and transect start time. The turtles positions and

transect lines were overlaid on the satellite images for temperature analysis (Figure 1).

The sea surface temperatures (SST) were measured by the Advanced Very High Resolution Radiometer (AVHRR), mounted on the NOAA-11 polar orbiting weather satellite. Only images acquired within 12 hours of the respective aerial survey were used for analysis. Multichannel atmospheric correction algorithms, capable of producing SST estimates accurate to 1°C in cloud free images, are used to process the raw satellite data (Epperly et al. 1995, Cornillon et al. 1987, Maul 1985, Robinson 1985). The digital images were mapped on Mercator projections with 1.47 km/pixel resolution at 35°N latitude. Processed images were obtained through the National Oceanic and Atmospheric Administration (NOAA) Computer-based Coastal Observation and Analysis of Sea Temperatures (CCOAST) project.

Flight lines were digitally reconstructed using latitudes and longitudes of each transect as endpoints, with NOAA's Interactive Digital Image Display Analysis System (IDIDAS) imaging software (NOAA 1991). The turtle positions along the flight lines were calculated and also plotted (Figure 1).

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Figure 1. Mercator projection of a satellite image of Cape Hatteras North Carolina for 7 April 1992. An overlay of the eighteen flight transects (white lines) are grouped (six lines per group) into north, middle and south groups. Each group was continuously flown, with observing breaks between groups. The turtle locations are shown as black points along the flight lines. The large white and black regions, temperature discontinuities, are caused by cloud cover and depend on the thickness of cloud. Turtles along the northern transects (influenced by cloud cover) were not included in the calculations. The color bar at the top shows temperatures in degrees Celsius.



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The SST for each pixel along the flight lines was recorded from the image. A 3x3 pixel average of SST's was also made for each pixel on the flight line. Comparisons between the flight line SST and the averaged SST were used to filter SST affected by the edges of clouds and clouds with an area smaller than 1.5 km². If by, paired t-test, the flight line temperature was significantly different from the 3x3 average the flight line data was discarded. Satellite images that did not cover the entire sampling area were also discarded. The means and variances of flight line and sea turtle SST's were graphed and analyzed.

Results and Discussion

Initial image analysis showed that cloud bands in the images had large temperature changes across their boundaries. Image pixels that lay on the boundary of or inside a cloud did not accurately portray the real SST. The effect of cloud cover was to artificially lower the temperature in the image, often to sub-zero temperatures. Flight data sets were eliminated if any transect in that set was obscured by cloud cover. Therefore, for the data used in our analysis, there was no difference between the averaged and non-averaged flight temperature means (Paired t-test: t = 1, DF = 1,000, a = 0.05). The similarity between the two temperature groups indicate that there was very little, if any influence of cloud cover in the images analyzed.

Data were analyzed in two groups, cold (winter; 15 Nov, 7 Apr.92 and 21 Jan.92) (Figure 2) and warm (summer; 6 Aug.91, and 13 Sep.91 and 17 Sep.92) (Figure 3). The limited data is an artifact of the limited number of aerial surveys and availability of adequate (cloud free and complete) satellite images.

We identified an upper thermal limit as well as a lower limit to preferred turtle temperatures. The minimum SST where sea turtles were found was 13.3°C (also the mean

Figure 2. Temperature vs. frequency of temperature "pixels" plot of satellite image pixels along flight line transects, for 15 Nov. 1991, 7 Apr. 1992 and 21 Jan. 1992. The number above each bar represents the number of turtles seen at that temperature. The asterisk indicates the mean flight temperature. Percentages indicate the percent of the temperatures above or below that point. It is clear that the turtles prefer water temperatures above 13°C.





Figure 3. Temperature vs. frequency of temperature "pixels" plot of satellite image pixels along flight line transects, for 6 Aug. 1991, 13 Sep. 1991 and 17 Sep. 1992. The number above each bar represents the number of turtles seen at that temperature. The asterisk indicates the mean flight temperature. Percentages indicate the percent of the temperatures above or below that point. It is clear that the turtles prefer water temperatures below 29°C.



Sea Surface Temperature Frequencies and Sea Turtle Counts for Warm Months

of all observations), where the lowest observed SST was 4.9°C (Figure 2, Table 1). The warm temperature data suggest that turtles preferred temperatures below 29°C (Figure 3, Table 2), This difference suggests that sea turtles were not geographically randomly distributed, but stayed within a preferred temperature range.

The ability to sample all water temperatures available to turtles along transects allows for a rigorous determination of a preferred temperature range. The available temperature range for the turtles to occupy, during this study (May 1991 to September 1992), was 4.9°C to 32.2°C, but turtles were only observed in water from 13.3°C to 28°C. The lower limit we observed is higher than the low temperature (11°C) determined by Epperly et al.(1995) and is higher than the lower exposure limit (10°C, when cold stunning occurs) (Schwartz, 1978). Our observed upper limit is well below the lethal limit for hatchlings (33°C) and below the upper lethal limit for turtles' (37.5°C) (Faulkner and Binger 1927).

This study also suggests that the turtles' preferred temperature range is seasonally variable. During the summer turtles were all found in water warmer than the maximum winter turtle temperature, even though cooler waters were available. There was, during each sampling

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day, a wide range of water temperatures available but the turtles were only found in small portions of the range. The turtles undoubtedly have the ability to move into regions of preferred temperature. It is not unreasonable to assume that they would move to cooler or warmer water if the temperature approaches their thermal limits. This shift of preferred temperature ranges could be better understood with more intensive aerial surveys during the turtles' migration periods. Table 1. Summary of Sea Surface Temperatures (SST) observed by satellite image overlay of aerial transects off Cape Hatteras, North Carolina. SST along the flight lines were recorded. The percentage of SST above, below and within the range of SST that sea turtles were observed are also summarized. These flight dates (15 Nov.91, 7 Apr.92 and 21 Jan.92) were considered "winter" flights.

	Observed SST (°C)	SST with Turtles
Mean SST	13.3	15.9
Standard Deviation	3.3	1.4
Minimum SST	4.9	13.3
Maximum SST	21.4	21.1
Number of Observations	1121	25

	SST Range (°C)	<pre>% of Observed SST in range</pre>
Below Turtle Presence	4.5-13.2	39.6
Turtles Present	13.3-21.1	60
Above Turtle Presence	21.2-21.4	0.4

Table 2. Summary of Sea Surface Temperatures (SST) observed by satellite image overlay of aerial transects off Cape Hatteras, North Carolina. SST along the flight lines were recorded. The percentage of SST above, below and within the range of SST that sea turtles were observed are also summarized. These flight dates (6 Aug.91, 13 Sep.92 and 17 Sep.92) were considered "summer" flights.

	Observed SST (°C)	SST with Turtles
Mean SST	26.5	26.7
Standard Deviation	2.5	1.4
Minimum SST	9	21.9
Maximum SST	32.2	28
Number of Observations	1120	17

	SST Range (°C)	<pre>% of Observed SST in range</pre>
Below Turtle Presence	9-21.8	3.1
Turtles Present	21.9-28	84.7
Above Turtle Presence	28.1-32.2	12.2

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Using Magnetic Resonance Imaging to Locate Magnetic Particles in Sea Turtle Heads.

Introduction

Many organisms have the ability to detect and use the earth's ambient magnetic field (Blakemore 1982, Light et al. 1993, Lohmann 1991, Wiltschko & Wiltschko 1988, Beason & Semm 1991), although the mechanism is understood only for bacteria, which possess a form of passive orientation called magnetotaxis. The chains of magnetite in bacteria orient parallel to the magnetic lines of flux, passively orienting the cells in fields as small as 50 micro Tesla (20% of the earth's field) (Frankel & Blakemore 1981). When disturbed the bacteria orient along the magnetic isocontours and swim to the bottom of ponds and bays where optimal living conditions exist. Because bacteria lack a nervous system, magnetotaxis contributes little to our understanding of the perception and utilization of magnetic information by multicellular organisms.

Magnetite is an inorganic iron-oxide that has the strongest magnetic field of any naturally occurring material (Kirschvink 1983) and may be involved in magnetic field transduction to the nervous system (Beason et al. 1990, Perry et al. 1985, Kirschvink 1983). Magnetite has been localized to the ethmoid region in bobolinks (Beason 1989b, Beason & Brennan 1985) and salmoniformes (Walker et al. 1988) and to the anterior of the dura mater in green

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sea turtles (Perry et al. 1981, 1985). The magnetite concentrations are large enough to theoretically detect changes of less than 200 nanno Tesla (0.5% the magnitude of the earth's field) (Beason & Semm 1987, Yorke 1981).

Behavioral experiments have shown that salmon (<u>Oncorhynchus</u> spp.) (Chew & Brown 1989, Quinn et al. 1981), alligators (<u>Alligator mississipiensis</u>) (Rodda 1984), hatchling loggerhead sea turtles (<u>Caretta caretta</u>) (Light et al. 1993, Lohmann & Lohmann 1994, Lohmann 1991), homing pigeons (<u>Columba livia</u>) (Keeton 1974, Walcott & Green 1974), and bobolinks (<u>Dolichonyx oryzivorus</u>) (Beason 1989a, Beason & Nichols 1984) all respond predictably to changes in the magnetic field, indicating that they are using the magnetic field for navigation. The mechanism responsible for this behavior is unknown.

Sea turtles on land appear to use visual cues to find the sea (Ehrenfeld, 1968, 1966; Mrosovsky & Kingsmill 1985; Mrosovsky 1970; Witherington 1991). In contrast hatchlings in shallow water near shore orient seaward by swimming into oncoming waves (Lohmann & Lohmann 1992, Lohmann et al. 1990, Wyneken et al. 1990). Hatchling sea turtles also appear to orient themselves with respect to the earth's magnetic field. Hatchling turtles react to both the inclination angle and magnitude of the earth's magnetic

field (Light et al. 1993, Lohmann 1991, Lohmann & Lohmann 1993, 1994a,b, 1996a,b,c). An inclination angle of 60° caused the hatchlings to swim in a southerly direction and an angle of 30° caused the hatchlings to swim northeasterly (Lohmann & Lohmann 1996 a,b). These inclination angles correspond to the northern and southern edges of the North Atlantic gyre. It is unknown if post-neonate loggerhead sea turtles retain the ability to utilize magnetic fields.

Adult and juvenile loggerhead turtles migrate along the east coast of North America from summer feeding grounds in the Chesapeake and Delaware Bays to wintering areas off the Florida coast (Byles 1988, Keinath et al. 1987, Keinath & Musick 1991 a,b, Musick & Limpus 1997). The turtles' eye morphology suggests they are myopic when not in direct contact with water (Ehrenfeld 1966), which indicates that stellar, or visual cues are not important for navigation.

Loggerheads caught in pound nets in the Potomac River, on the Chesapeake Bay, and transported to Back Bay National Wildlife Refuge (BBNWR) (on the VA-NC border) have been recaptured in the same pound nets weeks later (Jett, F., 1995, Personal Communication, Recapture of flipper tagged sea turtles. Ophelia, VA). The magnetic navigating ability of the hatchling sea turtles may be responsible for the homing ability of the juveniles and adults. Oceanic

behavior has been studied in large juvenile and adult sea turtles using doppler shift based satellite telemetry (ARGOS, 1996). Doppler shift tracking technology is limited in its use for tracking marine animals, due to the narrow time windows that data can be recovered by the satellite, and the general lack of precision in determining position (Personal Observation), which creates a lot of variability.

Whether juvenile and adult turtles can use the earth's magnetic field for orientation and navigation is unknown. The location of the mechanism allowing sea turtle hatchlings to navigate using the earth's magnetic field is also unknown, although the ethmoidal region is thought to contain a transducer (Beason 1989b, Beason & Semm 1987, Walker et al. 1988, Yorke 1981).

Historically methods for determining the presence of magnetite in tissue required digestion of tissue, concentration of remaining particulate by centrifugation, and use of electron or X-ray diffraction analysis for identification. Location of iron particles can be established with a series of histological procedures. These procedures have major drawbacks: 1) diffraction analysis results in the destruction of surrounding tissue so locations cannot be determined, and 2) histological

techniques are not specific to magnetite. These problems can be solved with Magnetic Resonance Imaging (MRI).

Internal imaging with MRI started in 1973; since then, medical imaging applications have blossomed. Soft tissue anatomy has been studied with MRI, because it is a noninvasive and nonionizing modality (Bradley and Tosteson 1981). Current MRI techniques can determine the presence and location of magnetic anomalies without tissue damage (Coles 1994, 1990).

Magnetic Resonance Imaging (MRI) can locate magnetic particles within tissue, non-intrusively (Coles 1994). Although sea turtle adults are too large to image even in whole body MRI machines, preserved heads from collections can be used to determine magnetite locations, without using the invasive techniques used for identifying magnetite in green turtle heads (Perry et al. 1981). MRI information can be used in conjunction with more traditional techniques to determine the location of a mechanism for magnetic transduction in sea turtles.

Materials & Methods

Kemp's ridley, <u>Lepidochelys kempii</u>, (<u>Lk</u>) and loggerhead, <u>Caretta caretta</u>, (<u>Cc</u>) sea turtle heads were obtained from euthanised animals. The heads were preserved in 10% formalin (made with glass distilled deionized water). Prior to both fixation and imaging the heads were rinsed with deionized water and 10M HCL in an attempt to remove magnetic and other contaminants.

The size of the head and ethmoid volumes precluded the use of the 9.4 T General Electric (GE) Omega 400WB NMR Imaging Spectrometer (the maximum sample size for the magnet is 4 cm diameter), used for previous bird head experiments (Coles 1994). The turtle heads were sealed in plastic bags filled with enough formalin, to fill voids, and retain moisture. Each head was arranged within an 8 cm diameter "bird cage" coil and placed in the bore of a GE CSI-II NMR imaging spectrometer, operating at 2 T. This spectrometer had been used to identify biogenic magnetite in magnetotactic bacteria (<u>Aquaspirilum magnetotactum</u>, MS-1), suspended in gelatin samples (Coles 1994).

For each echo time (TE) (the time between the initial rf excitation of the sample and the recording of the signal "echo" from the sample) used (15, 17 and 25 ms), two gradient recalled image sequences (a sequence is composed

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of 2 interleaved 8-image series) were made to locate anomalies (Table 1). Each image slice in a series was either 1 mm (<u>Lk</u>) or 1.5 mm (<u>Cc</u>) thick with a field of view of either 70 mm (<u>Lk</u>) or 100 mm (<u>Cc</u>) and was separated from adjacent slices by 1 mm (<u>Lk</u>) or 1.5 mm (<u>Cc</u>), allowing for the interleaving. The variation in slice thickness and separation was due to the difference in the size of the turtle heads used; the smaller head allowed us to cover the same regions as the large head with thinner slices. The heads were imaged, covering the ethmoidal region of the head from the nasal openings to posterior of the orbits. A spin echo image sequence with TE = 25 ms was created to help identify internal structures of the heads.

The spin echo sequence has a refocusing pulse, at a pulse time (Θ) , which is dependant on the resonance frequency (ω) , where $\Theta = \omega(TE/2)$, giving the image better detail, but less susceptibility to magnetic gradients (Coles 1994). The gradient recalled sequence does not have a refocusing pulse, which makes it susceptible to magnetic inhomogeneity within the sample.

GE Omega software was used to acquire, sum acquisitions and save the images to disk. Separate routines were used to convert the image data from the proprietary GE format to a raw data format on a Sun Sparc 2

workstation. The raw 256 X 256 data was downloaded via the Internet to a Apple Power Macintosh® 6100/60 where the images were converted to Tag Image File Format (TIFF) and analyzed using the public domain NIH Image program (Version 1.61) (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Only anomalies that met the criteria established by Coles (1994) were recorded and measured: (1) the anomaly size increased between gradient recalled TE values; (2) the boundaries of the anomaly were distinct and well defined; (3) the anomaly was either not present in the spin echo images or was smaller in the spin echo than in the short echo time gradient recalled images (Coles 1994).

Anomaly sizes were measured by counting the pixels along both x- and y-axes of each anomaly (each pixel represents a 312.5 µm x 312.5 µm x 1 (or 1.5) mm volume of the sample). The mean of the x- and y-values was used for analyses. The sizes of individual anomalies were compared between echo times by Analysis of Variance (ANOVA) (Zar 1984).
Results

The Kemp's ridley and loggerhead samples contained anomalies in the ethmoid region. The ethmoidal anomalies were found in the same region (posterior half of the turbinate) in both Kemp's ridley and loggerhead heads. In many cases pairing (left and right) of anomalies was apparent (Figure 1). Many anomalies were not analyzed because they did not meet the criteria. Most frequently the boundary was indistinct or occurred outside the sample, making it an indeterminate size.

Anomalies were also observed in other regions of the head (eye socket, oral cavity and externally). These were caused by external contamination prior to fixation. Additional magnetic artifacts were apparent in the gradient recalled images. The anomalous effect was minimal in the spin echo images. This indicates that the anomalies were not caused by biological or physical structures (Figure 2, 3).

Statistics of the mean anomaly diameter measurements in pixels were calculated as a function of acquisition sequence (spin echo (SE) and gradient recalled echo time's (TE) 15 ms or 17 ms and 25 ms). The mean anomaly sizes in the samples increased significantly between spin echo and

Figure 1. Biomagnetic anomalies identified in Magnetic Resonance Images occurred in the ethmoid region of sea turtle head. Gradient recalled 15 ms (A), 25 ms (B) and Spin echo (C) sections from the head of a Kemp's ridley are shown.



A.





C.



Figure 2. Magnetic artifacts were apparent in several images. These anomalies were not recorded. This clearly shows the importance of magnetic cleanliness for magnetic resonance imaging. Gradient recalled 15 ms (A), 25 ms (B) and Spin echo (C) sections are shown.







Upper Jar

Lower Jan

Orbit

Orbit





gradient recalled echo (15/17 and 25 ms TE's) (single factor repeated measures ANOVA: F = 25.61, (k-1) = 2, (N-k) = 78, p < 0.05.

Discussion

These results show that loggerhead and Kemp's ridley heads contain magnetite. Many of the anomalies seen were probably due to biogenic magnetite and not contamination.

Changes in intensity, and therefore contrast, are normally caused by non-uniform densities of hydrogen protons in the sample. Contrast can also be caused by magnetic heterogeneity in the sample (Heiken et al. 1986, Saini & Ferrucci 1988, Stark et al. 1988), in this case the magnetic particles. The magnetic fields surrounding magnetite particles result in anomalies in the image that are greater than the size of the magnetite particles. Magnetite particles affect the phase relationship of neighboring protons. The protons near particles have different Larmor frequencies than protons farther away. During the 90° excitation pulse, hydrogen protons near the particle are not excited by the radio frequency pulse because they are at a different Larmor frequency than the protons farther away. The protons that are within the region of influence of the magnetite particle dephase at different rates depending on the distance from the particle. Those farther away would take longer to lose their phase relationship because the influence of the magnetite particles on the surrounding protons decreases as

 $1/r^3$, the decay rate of the magnetic field. The anomalies appear to grow in size as echo times increase because the precessions take longer to dephase farther from the particle (Coles 1994).

Black regions in the images are frequently correlated with structures such as bone or cartilage that contain little water and consequently produce little, or no, signal. These types of anomalies can be distinguished from anomalies caused by magnetite particles by several criteria. First, the location, size and shape of these structures are known and identifiable in the images. Secondly, their sizes do not significantly increase in size as echo times increase. Increase of anomaly sizes with increasing echo times is a characteristic of magnetic anomalies (Coles 1994). Air bubbles can also produce anomalies because they do not contain water and therefore produce no signal in NMR images. The bubble anomalies can be identified because they have distinct physical boundaries that are not affected by altering the echo time (Coles 1994).

Anomalies identified in the images are not correlated with known physical structures in the ethmoid. All anomalies were circular in shape and increased in size with longer echo times, an indication that they are caused by

magnetic particles. Many of the anomalies I measured are paired in the left and right turbinates, indicating that bilateral symmetry of the anomalies exists in loggerheads and Kemp's ridleys. It is unlikely that bubbles or other artifacts in a sample would be consistently paired. Bilateral symmetry of sensory organs is common in vertebrates, suggesting that a relationship exists between magnetite and the sensory system. Some anomalies were unpaired because one of the pair may have occurred in large regions of reduced, or no signal. This was especially noticeable in images with longer echo times (25 ms) where image heterogeneity was more pronounced.

Spin echo images (Coles 1994) were created to give better detail of the tissue structure surrounding the anomaly. Images produced with spin echo sequences either did not contain magnetic anomalies or they were much smaller than the same anomalies in gradient recalled (15 or 17 ms) images.

In conclusion, it is possible to visualize both geological (contaminants) and biogenic magnetite using MRI technology. The noninvasive nature of NMR imaging potentially can allow live animals to be used for both magnetite location (with NMR) and behavioral or physiological experiments. The short length of time it

takes to make a sequence of images (less than 20 min to acquire each sequence) makes this technique very attractive for future experiments. These results support the hypothesis that magnetite is present in the ethmoid regions in loggerhead and Kemp's ridley sea turtles, and that the location and arrangement (laterally paired) of magnetite suggest it could be involved with magnetic detection. It is also critical that samples be handled with care to prevent contamination.

Sequence	EZQ8V3	EZ8VC	MS16V1
Relaxation Time (ms)	600*	1800	1800-1400
Echo Time (ms)	15-25*	25	25
Field of View (mm)	70	70	100
Image Resolution	256	256	256
(pixels)			
Slice Thickness (mm)	1	1	1.5
NEX	128	64	32
Plane	1	1	1
Slice Offset (mm)	0	0	0
Slice Separation (mm)	2	2	3
Set Gain	0	0	0
Spec. Width	"12,000"	"12,000"	"12,000"
Trigger	0	0	0
Tip Angle (°)	70		
Pulse Duration (ms)	3.33		
Cycles	1		
Gphi	10		
# of Sinc		1	1
180 Thickness		2	2
Number of Slices	8	8	16

Table 1. Spin-echo (EZ8VC, MS16V1) and gradient recalled echo (EZQ8V3) image sequence parameters used to create sea turtle head images.

Table 2. Statistics of the mean anomaly diameter measurements in pixels as a function of spin echo (SE) and gradient recalled echo time's (TE) 15, 17 and 25 ms.. The mean anomaly sizes in the samples increased significantly between spin echo and gradient recalled echo (15/17 and 25 ms TE's) (single factor repeated measures ANOVA: F = 25.61, (k-1) = 2, (N-k) = 78, p < 0.05).

Loggerhead	SE	TE=15 ms	TE=25 ms
Mean (pixels)	2.8	5.7	6
Variance	1.08	3.07	8.04
Range	0-4	0-8	3-10.1
Number	3	6	7

Kemp's ridley	SE	TE=15 ms	TE=25 ms
Mean (pixels)	1.9	6.7	9.3
Variance	1.06	12.6	27.6
Range	0-3	2.5-13.5	4-19.5
Number	4	11	9

Kemp's ridley	SE	TE=17 ms	TE=25 ms
Mean (pixels)	4.5	9.5	10.4
Variance	6.5	8.45	14.7
Range	0-8	4.5-14	5-17
Number	10	11	11

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Introduction

Ectothermic vertebrates have cyclic growth rates with predominantly annual cycles (Chaloupka & Musick 1997). The cycle follows seasonal changes in temperature, reproduction and food availability, all of which affect growth rates. Skeletochronology has proven to be a reliable technique to determine age in a number of ectothermic vertebrates (fishes, amphibians and reptiles) (See; Zug et al. 1986). In sea turtles the long bones (humerus, radius, etc.) have growth patterns (rings) similar to what is seen in trees. These rings are compact (dark) during periods of slow growth (winter) and broader (lighter) during faster growing (summer) seasons. Ectotherms continue to grow throughout their lives, and the rate of growth is influenced by temperature, food availability, and age (Hainsworth 1981).

Oxytetracycline has been proven effective for time labeling bone in juvenile loggerheads (Klinger et al., 1997; Klinger & Musick, 1992; Klinger, 1988; Zug et al. 1984). The oxytetracycline is deposited in all calcifying structures at the time of injection into muscle tissue. This deposit, within the calcium matrix of the bone, fluoresces when exposed to ultraviolet light (Frost et al. 1961; Harris 1960; from Klinger et al. 1997).

Klinger & Musick (1992) found that juvenile loggerheads deposited annular growth rings. However, the longest recapture interval in the study was 2.94 years (a "tetracyclined" juvenile loggerhead from the Chesapeake Bay). We report here evidence from a sexually mature female loggerhead sea turtle, initially measured and injected with oxytetracycline in 1989 and recovered in 1997, that shows that loggerheads continue to lay down annual growth rings after maturity.

Methods and Materials

On June 20, 1989 a loggerhead sea turtle, incidentally captured on the Potomac River, Saint Mary's County, MD (Lat. 37° 58′, Long. 76° 20′), was measured, flipper-tagged and injected with 11 cc of oxytetracycline (LA-200 Liquamyacin®, Pfizer). The turtle was released the same day in the York River, Gloucester County, VA (Lat. 37° 14′ 47″, Long. 76° 30′ 23″) (VIMS: sea turtle database). The intramuscular oxytetracycline injection was administered on the ventral shoulder of a foreflipper (Klinger 1988).

On September 22, 1997 a stranded tagged turtle was recovered dead from Grandview Nature Preserve, Hampton, VA (Lat. 37° 06', Long. 76° 16' 30"). The turtle was measured, necropsied and buried on the beach. The fore flippers (and tags), head, stomach contents and ovaries were removed for laboratory analysis.

Humeri were dissected, fleshed and air dried before sectioning. The right humerus was sectioned (bone slices ranged from 0.2-0.5 mm thick) distal to the deltopectoral crest (Figure 1) with a diamond head saw. Lateral views of the bone slices were photographed under dissecting microscopes. Each view was photographed with both visible "white" (WL) and ultraviolet (UV) light.

Figure 1. Dorsal and ventral drawing of the right humerus of a loggerhead sea turtle (from Zug, et. al, 1984). The bone was sectioned distal to the deltopectoral crest.



Figure 2. Magnified white light (visible) image of a loggerhead sea turtle humerus. The tetracycline mark is not visible in this image. Growth rings are visible on the white light image, and can easily be counted.



Slides were digitized using a UMAX color scanner (Astra 1200s, H750) with Transparency Adapter (UTA-2A, H760) and analyzed with Adobe® PhotoDelux[™] (Version 1.1) on an Apple Power Macintosh® 6100/60. From the WL slides (Figure 2), growth rings were outlined as a separate "layer" (layer 2) (Figure 3). The UV slides (Figure 4) were then overlaid (layer 3), on the WL image and drawing and the edge of the bone and physical markers were aligned (Figure 5). The tetracycline mark was then traced as a 4th layer (Figure 6). The two image layers (layers 1,3) were then removed (opacity set to 0). The number of growth rings distal and proximal to the tetracycline mark were counted (Figure 7).

The Von Bertalanffy growth equation and curve, recreated from Klinger (1988), were compared to the age and measurements from this recapture. Figure 3. Magnified white light (visible) image of a loggerhead sea turtle humerus. Growth rings are visible and are traced as a separate overlaying layer.



Figure 4. Magnified ultraviolet light image of a loggerhead sea turtle humerus. The tetracycline mark is easily identifiable on the lateral edges of the bone. Dorsal and ventral surfaces of the humerus showed no evidence of a tetracycline mark.



Figure 5. Overlaid magnified white light (visible) (layer 1) and ultraviolet light image (layer 3) of a loggerhead sea turtle humerus. The opacity of both image layers was reduced to increase transparacy for aligning physical markers and the edge of the bone. The opacity of the growth ring traces (layer 2) was set to zero.



Figure 6. Magnified ultraviolet light image (layer 3) of a loggerhead sea turtle humerus. Opacity of the magnified white light (visible) and growth ring traces (layers 1 and 2) were set to zero. The tetracycline mark was traced as a separate overlaying layer (layer 4).


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Figure 7. Growth and Tetracycline (layers 2 and 4) traces of a magnified loggerhead sea turtle humerus. Opacity of the magnified white light (visible) and ultraviolet light image (layers 1 and 3) were set to zero. The number of growth marks both distal and proximal to the tetracycline mark can easily be counted.



Results

A tetracycline mark was easily identifiable on the lateral edges of the bone (Figure 8). Dorsal and ventral surfaces of the bone displayed highly compressed growth rings and no evidence of a tetracycline mark, even under magnification (Figure 2,4).

Identification of the growth rings surrounding the tetracycline mark determined that externally there were seven (dense) arrested growth rings; internally 2 rings were identified (Figure 7). This indicates that there were eight growing seasons, which correspond to the eight years between the time the animal was marked and released and the time it was recovered. The presence of a fluorescing growth mark, 8.3 years after the time of injection, and growth rings prior to the mark, shows that tetracycline can be used in long term growth studies in sea turtles and that growth rings continue to be deposited on an annual basis.

Figure 8. Ultraviolet light lateral view of a loggerhead sea turtle humerus. The tetracycline mark is easily identifiable on the lateral edges of the bone. Dorsal and ventral surfaces have compressed rings and are not visible.



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Discussion

Some studies indicated that tetracycline might not be appropriate for long term studies because some recaptured turtles showed no fluorescing ring (Klinger & Musick 1992), or growth marks (Bjorndal et al. 1998). Many studies have taken bone cores from the ventral surface of the humerus, through the same region as our cross sections. These short axis rings are compressed and often unreadable. The long axis rings are more reliable and should be used for analysis (Klinger 1988). Our study supports this opinion and suggests that a better location to take bone cores is along the sagittal axis of the bone.

One of the primary criteria for skeletochronology studies is that physiologically important environmental conditions (diet, food availability [feeding rates], temperture, etc.) are not constant (Bjorndal et al. 1998, Zug et al. 1986). It is the change in the conditions that cause a corresponding change in growth rate, and rate of bone deposition. It is important that validation studies not violate the criteria for its use.

The Von Bertalanffy growth curve (Klinger 1988) roughly fits the data recorded from this turtle (Figure 9, Table 1). The age at first capture, calculated from the Von Bertalanffy equation from a straight carapace length

Figure 9. A Von Bertalanffy growth curve (straight carapace length) for loggerhead sea turtles, calculated by Klinger (1988). Straight tip to tip measurements for the turtle are plotted along the curve. For these comparisons the measurements and corresponding age of first capture are presumed correct. It is clear that the curve roughly fits the data recorded from this turtle. The Von Bertalanffy equation $L_t = 111.9*(1-e^{(-0.076*(t+1.16))})$ calculates length from age in years.



(SCL) of 91.2 cm, was 21.08 years old. The SCL at recapture was 99.4 cm, for a growth rate of 0.99 cm/yr. This growth rate is significantly lower than the range of 1.86-4.02 cm/yr for turtles in the 90 cm class, and higher than the 1 meter size class (0.64-0.67 cm/yr), discussed by Klinger (1988, Klinger & Musick 1995) (Table 2). The turtles' intermediate growth rate suggests that it may have become sexually mature and started nesting earlier than predicted by the age growth curve. Miller (1997) cautions that size alone can not be used to determine the maturity of the turtle. The allocation of resources from growth to reproduction would account for the differences (Hainsworth 1981).

Some sea turtle measurements increase in size over 25 times from hatchling to adult; the humerus, for example, increases over tenfold in cross-section diameter (Zug et al. 1986). This means there is extensive resorption and deposition of the periosteal layers as the turtle grows (Klinger 1988, Zug et al. 1986).

In conclusion, long term tetracycline studies of sea turtles should not be dismissed. We have shown that clear, distinctive growth marks can be identified after 8 years in the wild, and growth marks continue to be deposited in

large mature sea turtles. Location of bone plug samples isa critical part of the success of using tetracycline as a skeletal marker. Bone plugs should be taken along the long axis of the humerus. Additional studies to determine optimal concentrations of oxytetracycline markers should continue. This study clearly shows the viability of oxytetracycline use as a skeletal marker for skeletochronology studies. Table 1. Statistics and calculated values (age, straight carapace length and growth rates) from the Von Bertalanffy growth curve for loggerhead sea turtles, calculated by Klinger (1988). For these calculations the measurements and corresponding age of first capture are presumed correct. (* indicates known values)

Date	20 Jun 1989	22 Sep 1997
Time (yr)		8.255*
Length (cm)	91.2*	99.4*
Growth (cm)		8.2*
Growth Rate (cm/yr)		0.99*
Age from Curve (yr)	21.08	27.67
based on Length		
Predicted Length (cm)		100.88
based on (Age1+Time)		
Time (yr) based on		6.59
Growth and Agel		

Table 2. Growth rate(s) from our tetracycline recapture and calculated mean growth rates, standard deviation and range for two size classes of loggerhead sea turtles, from Klinger (1988).

Size (class)	Sample Size	Mean Growth Rate(cm/yr)	Standard Deviation	Range
90.0-99.9	7	2.87	0.85	1.86-4.02
Recapture	1	0.99		0.99
100.0-110.0	2	0.66	0.02	0.64-0.67

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Aspects of the Biology of Sea Turtles in the

Mid-Atlantic Bight.

CONCLUSION

The objectives of this dissertation were to compile, review and analyze several different aspects of the biology of sea turtles that utilize the Chesapeake Bay as a seasonal foraging habitat. Many of these results substantiate, collaborate and refine previous research. I summarize the results from each study here:

Several trends in turtle stranding data were established. There were gross increases in numbers of stranded turtles for loggerheads (3 turtles/year), Kemp's ridleys (0.7 turtles/year) and leatherbacks (0.5 turtles/year). The cause of death for most (84%) of these turtles was either undetermined or unrecorded. Of the turtles with recorded causes of death the most significant interaction appeared to be with boats. Understandably this cause of turtle mortality is very hard to monitor and quantify. Over half of the turtle deaths each year occur in the spring when turtles migrate into the Bay (Kemp's ridleys have a second significant stranding peak during the fall migration). The number of possible anthropomorphic interactions with turtles has increased as recreational boating & fishing has increased in popularity. Future cause of mortality data may be enhanced by increasing volunteer training and network diligence.

The turtles that strand in the Bay are primarily juveniles of each species; reproductive adults rarely strand. Among loggerheads, stranded turtles' appear to be normally distributed, in terms of size, whereas the Kemp's ridleys have an erratic pattern. The difference may be explained by yearly hatchling success of the ridleys because of their panmictic evolution. Loggerheads are much less susceptible to localized stresses (beach erosion, storms, human and animal interactions) because they nest throughout the southern coastal United States.

During migration periods, sea turtle strandings occur most commonly on the Southern Bay and Virginia Beach, Ocean regions; at other times strandings are spread throughout the Bay. The spatial trends probably do not represent true stranding patterns for several reasons. First the beaches are not uniformly sampled. Many beaches are inaccessible, even by boat, because of marsh boundaries, bars and islands. Other beaches (Southern Bay and Virginia Beach areas) are wide sandy beaches that are used daily by thousands of people and so are extensively monitored.

Sea turtle morphology data from the VIMS data set provides a solid base for future studies. Regressions calculated from the morphology data often explain more than 90% of the variation in the measurements. The remaining

10% of variance can easily be explained by such factors as: (1) poor condition of the turtle when measured; (2) inexperience of measurers and procedural variations (hundreds of volunteers made measurements); (3) location and accessibility of animal when measured. There were no morphologic differences identified between the two genetic populations of juvenile loggerheads found in the Bay. The regression equations can serve as excellent estimators for missing and suspect data. Many missing values are required for State and Federal management agencies and future research.

The carapace morphology of loggerheads and Kemp's ridleys changes as the turtles grow. The carapace flattens out in larger individuals, presumably to maintain a relatively constant amount of lift while swimming. The velocity of hatchling turtles is much less than the adults (Wyneken 1997). To maintain the same lift the carapace would need to have a higher dome. The larger (faster) turtles are flatter, and so maintain a relative (constant) amount of lift. Extra lift may be needed in hatchlings because of their swimming inefficiency; their Reynolds numbers (1.2E4 to 3E5) are much lower than other aquatic migrants (Wyneken 1997, Vogel 1981). This might also explain the domed shape of the hatchling carapace.

The Magnetic Resonance Imaging (MRI) techniques developed by Coles (1994) were used to image juvenile Kemp's ridley and loggerhead sea turtle heads. The location of magnetic particles in the sea turtle heads appears in the same region as birds and fish. The anomalies appeared to be paired (left and right). This bilateral symmetry suggests a possible use as a sensory system. This suggests that the MRI can be used for determining positions of electromagnetic stimulus in neurological studies. Unfortunately we have also verified the sensitivity of the MRI to magnetic contamination. Future studies must take extra precautions against contamination.

There have been no studies on the natural preferred behavioral temperature preferences of marine turtles. Using satellite imaging technology we were able to confirm the temperature limits determined by others (Epperly et al. 1995, Schwartz 1978, Faulkner & Binger 1927). The turtles observed were within a range of 13°C to 29°C, well within the previously established physiological limits and the entrance and exit temperatures of turtles during migration into and out of the Bay.

There have been many studies of age and growth in ectothermic vertebrates using oxytetracycline as a

skeletochronological marker (Zug et al. 1986), but these studies have usually been of short (1-2 year) duration. As a result, questions have been raised as to whether oxytetracycline will stay in the bone matrix for longer periods and whether growth marks are deposited annually in older turtles (Bjorndal et al. 1998, Klinger & Musick 1992). The results from a turtle injected with oxytetracycline eight years before recapture suggest otherwise. Sampling whole cross sections of the humerous suggest that the area frequently used for taking bone cores (used in previous studies) is inappropriate, and that the lateral edges of the humerous, or perhaps other bones, should be used for long term studies. The growth rates and ring deposition support Klinger's (1988) data, although it suggests that sexual maturity may be occurring at an earlier age (smaller size) than previously believed.

There is a need for long term continuous monitoring of populations of threatened and endangered species, particularly in areas that concentrate animals such as; nesting beaches, juvenile nursery habitats. For example, for three weeks in the spring of 1998 a major stranding event, possibly precipitated by a spring fishery near the mouth of the Bay, went unnoticed (Coles & Musick 1998). By the time fishery managers (NMFS, VMRC) discovered what was

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happening the cause of the event had disappeared. During this period 100's of loggerheads and Kemp's ridleys had stranded. The lapse in monitoring is a conservation catastrophe and emphasizes the need for continuous data collection and monitoring. It is also important for these monitoring databases to have good and consistent data management. Fisheries and resource managers should recognize these issues and take steps to alleviate the problems, by providing adequate resources to manage and maintain these types of valuable monitoring data.

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Appendix 1. Recipes

Roix Sea Turtle Deluxe.

2 Onions	salt	brown flour
Parsley	pepper	butter
Thyme	cloves	Allspice
glass of Claret	or Madeira Wine	1 Sea Turtle

Kill the turtle at Daylight in the Summer (the night before in the Winter), and hang it up to bleed. After breakfast, scald it well and scrape the outer skin off the shell. Open it carefully, so as not to break the Gall. Break both shells to pieces and put them into the pot. Lay the fins, the eggs and some of the more delicate parts by. Put the rest into the pot with a quantity of water to suit the size of your family. Add 2 onions, parsley, thyme, salt, pepper, cloves, and allspice to suit your taste.

Simmer turtle fins in boiling water until tender and then skinned. Dip the fins in brown flour and fry them in butter. When they are nicely browned, add a little white wine. Simmer until tender.

About half an hour before dinner thicken the soup with brown flour and butter rubbed together. An hour before dinner, take the parts laid by, roll them in brown flour, fry them in butter, put them and the eggs in the soup; just before dinner add a glass of Claret or Madeira Wine.

Adapted from: A Herpetological Cookbook. Ed. Ernest A. Liner, 310 Malibou Blvd. Houma, Louisiana 70360, and The Williamsburg art of cookery, or Accomplished gentle-womans companion: Being a collection of upwards of 500 of the most ancient and approved recipes of Virginia cookery. Ed. Helen Bullock, Deets Press, Richmond, VA. St. cRoix Turtle Extravaganza.

Sea Turtle meat	Can Ro-Tel tomatoes
4 hardboiled eggs	2 minced bell peppers
1/2 cup flour	4 large minced onions
1/2 cup oil	Can tomatoes #2
6 cloves garlic	Thyme
4 stalks minced celery	Sweet basil
1/4 cup Parsley	water
Cayenne	3/4 cup scallion tops

Season turtle meat with salt and cayenne, brown thoroughly in hot oil. Remove meat and add the onions, stirring until deep brown and all pan drippings are absorbed. Add the tomatoes and cook down until browned, also. Add bell pepper, celery, garlic, thyme, sweet basil and water. Let simmer about 1 hour until vegetables are done. Add turtle meat and boiled eggs and cook slowly until turtle is done, about 2 hours. Add sherry, parsley and scallions last 5 minutes. Serve over rice.

Modified from: A Herpetological Cookbook. Ed. Ernest A. Liner, 310 Malibou Blvd. Houma, Louisiana 70360.







IMAGE EVALUATION TEST TARGET (QA-3)







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