Mercury in loggerhead sea turtles, *Caretta caretta*: Developing monitoring strategies, investigating factors affecting contamination, and assessing health impacts

by

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ABSTRACT

This study investigates various aspects of mercury contamination in loggerhead sea turtles along the coast of South Carolina, Georgia, and north Florida. The validity of using blood and scute scrapings for non-lethal routine monitoring of mercury in loggerhead sea turtles is addressed. These tissues are also used to determine if this population is affected by potentially harmful levels of mercury or exhibits life history and geographic variations in mercury contamination. Isotope dilution cold vapor inductively coupled plasma mass spectrometry was used for total mercury determination in a suite of tissues collected from 6 dead stranded loggerheads and in blood and keratin from 34 wild captures. Dead turtles exhibited high variability among individuals (mean of CVs for each tissue = 86.9%) and among tissues (0.037 µg/g in spinal cord to 2.326 µg/g in keratin). The mercury content in each monitoring tissue (blood, keratin and skin) was regressed against mercury content in each internal tissue (liver, kidney, muscle and spinal cord). Blood proved to be the most accurate overall predictor of internal mercury burden (mean $r^2 = 0.943$). Keratin was a better predictor of liver but slightly less effective for other tissues (mean $r^2 = 0.873$). Mercury content in blood and keratin from live captures ranged from 0.005-0.188 µg/g and 0.061-2.837 µg/g respectively. Mercury levels in these tissues were highly correlated (linear regression $r^2 = 0.926$, P = 0.0001), and increased significantly with body weight ($r^2 = 0.173$, P = 0.016, $r^2 = 0.187$, P = 0.012).

The residuals from this regression provide an index of comparison between recent

mercury intake (as measured in the blood) relative to average past intake (as measured in

keratin). This index of recent exposure (IRE) was modeled in a stepwise multiple

regression with 12 biological and environmental parameters. Proximity of capture site to

major river mouth was the only significant effect (P = 0.0102). This suggests there is an

elevation of bioavailable mercury in nearshore habitats where terrestrial and

anthropogenic impacts are high relative to sites distant from major river outflow. In light

of the recent evidence for foraging site fidelity, this environmental variability may be

amplified in loggerhead tissues over time and explain the high variability among

individuals and occasional highly contaminated turtle seen in this and previous studies.

Hematology and immune function assay of selected individuals reveals a negative

relationship between blood mercury concentration and total white blood cell count

(Spearman Rho, -0.8827, P = 0.0198) and b-cell proliferation (Spearman Rho, -0.7273, P

= 0.0112) suggesting these concentrations of mercury may be capable of

immunosuppression in loggerheads. Blood mercury concentration was also negatively

correlated to plasma sodium concentration, indicating that the intracellular/extracellular

homeostasis of this ion may be disrupted by mercury at these concentrations.

INTRODUCTION

Sea turtle and human populations have been interacting with one another for millennia in tropical, subtropical, and temperate regions around the globe. The nature of this interaction historically has been subsistence and commercial harvesting of sea turtles for meat, eggs and other useful or desirable turtle products, such as turtle shell. In modern times subsistence harvest and local markets still have a significant impact in less developed nations. While these activities are prohibited in many nations, other anthropogenic factors such as fisheries bycatch and degradation of nesting beaches have become the largest contributors to the endangered status of sea turtles (Lutcavage *et al.* 1997).

Global populations of sea turtles have been drastically reduced from their historical numbers. The most abundant sea turtle species along the coastline of the southeast United States is the loggerhead sea turtles, *Caretta caretta*. The subtropical and temperate distribution of this species creates more contact with highly developed and industrialized nations where beachfront development and marine pollution are most prominent. This distribution also exposes loggerheads to incidental bycatch of subadults and adults in commercial shrimp trawls in the Atlantic, where populations north of Cape Canaveral, Florida appear to be continuing to decline (National Research Council 1990). This species is on the Council on International Trade in Endangered Species (CITES)

Appendix I list of prohibited items of international trade and is currently classified as a threatened species by the U.S. Department of Interior.

Like many long lived, late maturing species, loggerheads provide a challenge to population biologists and resource managers. After a pelagic phase that last around 10 years (Bjorndal *et al.* 1994, Zug *et al.* 1995, Chaloupka 1996), they return to the neritic zone and adopt a demersal lifestyle. In the southeastern United States, these subadult and adult loggerheads utilize estuarine and nearshore habitats, foraging primarily on crabs, molluscs, and horseshoe crabs*,* and occasionally fish, vegetation, sea anemones, sea whips, sea pens, jellyfish and barnacles (Lutcavage and Musick 1985, Dodd 1988, Burke *et al.* 1993, Plotkin *et al.* 1993). Loggerheads may not reach sexual maturity until 25-30 years of age (Chaloupka and Musik 1997). As a result assessing the population size using conventional nest counting techniques creates a considerable time lag during which the subadult portion of the population is not accounted for. This makes it difficult for resource managers to gauge the success of conservation efforts such as turtle excluder devices that were mandatorily required in the U.S. beginning in 1988. In order to address this problem, the National Marine Fisheries Service funded a multiyear project to establish an index of relative abundance for sea turtles in the waters from South Carolina to northern Florida (NMFS Grant # NA07FL0499). This in-water trawl survey was designed and implemented by the South Carolina Department of Natural Resources (SCDNR). Standardized sampling methodology was used to allow comparison of the abundance of subadult and adult sea turtles utilizing these coastal waters from year to year.

Many anthropogenic factors have been implicated in the decline in sea turtle populations. Some forms of pollution, such as plastics and other nonbiodegradable debris, have been shown to directly contribute to sea turtle mortality through ingestion and entanglement (Balazs 1985). However, the role of chemical pollutants in sea turtle health is largely unknown. There have been numerous examples of the detrimental effects of toxicants on wildlife. More recent investigations have shown that exposure to environmental stressors at low concentrations can impact health in more subtle ways, such as endocrine disruption, immune function impairment, and genotoxicity. Environmental contaminants have been listed as one of the possible factors contributing to the development of the viral infection fibropapillomatosis in sea turtles through reduced immune function (Balazs and Pooley 1991).

Due to their threatened status and elusive lifestyle, researchers seldom have access to large numbers of free-ranging sea turtles. This has resulted in a scarcity of baseline information on blood parameters routinely used in animal health assessment. This lack of data on what a healthy sea turtle blood profile should look like makes assessing the condition of wild, captive, or rehabilitated turtles difficult. The SCDNR abundance survey provides an opportunity to establish baseline data on blood analytes, hematocrit, total protein, glucose and white blood cell types and counts. In addition to these basic health parameters the genetic stock structure, sex ratios, contaminant loads and immune function are being investigated. Measuring all contaminant concentrations and the numerous biological endpoints they may affect is an overwhelming task. This study begins this process by focusing on one toxin of particular interest, mercury, and its relationship to some of the aforementioned health endpoints.

There is increasing awareness of potentially harmful levels of mercury in aquatic food chains. Mercury has been identified as one of the most serious environmental threats to the well being of fish and wildlife in the southeastern United States (Facemire *et al.* 1995). The prevalence of mercury in food such as fish and marine mammals has prompted concerns for the health of subsistence fishermen and the general population who regularly consume fish. This is especially true for young children and pregnant women where low exposure to methylmercury can impair mental development. The use of mercury in some occupations has also focused attention on the risk to humans. Among the more well-known examples of human methylmercury poisoning is the Minamata Bay, Japan incident from the 1950's. Many people died, and many more suffered neurological and developmental impairment after consuming fish contaminated with mercury from local industrial sources (Hamada and Osame 1996). Mercury often drives the issuance of state and local fish consumption advisories warning the public of potentially harmful mercury levels in fish from local bodies of water. In Canada over 97% (~2570) of all fish consumption advisories in 1997 were from mercury contamination (EPA 2001). In the United States, methylmercury contamination accounted for 79% (~ 2200) of all fish and wildlife consumption advisories in 2000 (EPA 2001). The overt toxic effects of this metal have been demonstrated on a wide variety of taxa, including impaired growth and development, reduced reproductive success, liver and kidney damage, and neurological damage. Death can occur at doses from 0.1-0.5 μg/g body weight/day (EPA 1997).

Mercury is a metal that occurs naturally in the environment at low concentrations and can be liberated from rock by geothermal activity or erosion. In some cases local geological phenomena can be responsible for elevated mercury concentrations

in biota. Shilts and Coker (1994) documented a case in which mercury contamination in trout was attributed to volcanic bedrock which lay under a lake. However, sources of mercury can often be traced to either point source pollution or to the vast amounts of atmospheric mercury released into the environment by industrial activity. Some well-known anthropogenic sources of mercury pollution include fossil fuel combustion, refuse incineration, nonferrous metal production, iron and steel production, and the chlor-alkali industry (Hutton and Symon 1986). The result has been an increase in the global atmospheric concentration of mercury by a factor of 2-5 since the beginning of the industrial era. The subsequent transport and wet and dry deposition of this ubiquitous atmospheric mercury has served as a major source of contamination in a variety of ecosystems on a very broad spatial scale (Engstrom and Swain 1997).

Mercury undergoes a number of biochemical and geochemical transformations in the environment. From a toxicological standpoint, the most important of these is the conversion of inorganic Hg^{2+} into the most toxic form, methylmercury (MeHg). The most important pathway for methylation in the aquatic environment is sulfate-reducing bacteria at oxic-anoxic interfaces in sediments and in wetlands (Gilmour *et al.* 1998). The majority of the methylation is thought to occur within these bacteria, by either enzymatic or nonemzymatic pathways, with methylcobalamine serving the active methyl donor to a Hg^{2+} ion (Ridley *et al.* 1977). These Hg^{2+} ions presumably enter the bacteria as neutral dissolved species such as $HgCl_2$ or HgS (Benoit *et al.* 1999). In a nationwide survey of both coastal and lacustrine habitats, South Carolina wetlands were recently identified as having one of the highest methylation efficiencies in the United States (Krabbenhoft *et al.* 2001). Methylmercury comprises the vast majority of the mercury

acquired by organisms (Boudou and Rebeyre 1985), since unlike Hg^0, methylmercury is lipid soluble. This allows efficient retention in tissues and causes difficulty in excreting it from the body.

The aforementioned characteristics allow mercury to become concentrated in some organisms. Concentrations of methylmercury in fish commonly exceed those in ambient surface water by a factor of 10^6 to 10^7 (Boudou and Ribeyre 1997). Bioaccumulation occurs when the mercury intake of an organism exceeds its ability to excrete it. This results in the older, larger individuals in a population, and long-lived species to be most likely to accumulate potentially harmful levels of mercury. Mercury has also conclusively been shown to biomagnify through the food chain with great efficiency (Bryan 1984). Biomagnification is the transfer of a substance from one trophic level to the next, resulting in the highest concentrations occurring in species that feed high in the food web. The combination of bioaccumulation and biomagnification accounts for the elevated mercury concentrations often seen in consumers in aquatic systems. Evidence of harmful mercury burdens has been documented for fish (Wiener and Spry 1996), piscivorous birds (Barr 1986, Fimreite 1974), mink (Wobeser and Swift 1976), and otter (Wren 1985). Other species such as cetaceans and pinnipeds also accumulate high concentrations of mercury but the effects of these concentrations are not well understood.

The threatened status and long life span of the loggerhead makes it an ideal candidate for further mercury studies. The existing literature on mercury in loggerheads is limited. There is one study from the southeast U.S. on eggs (Stoneburner *et al.* 1980), one study from the Gulf of Mexico on eggs (Alam and Brim 2000), and three loggerheads

from the Gulf of Mexico using blood and keratin (Presti 1999). Five other studies from the Mediterranean, Japan and Australia used liver, kidney and muscle tissue obtained from a total of 36 stranded individuals (Sakai *et al.* 1995, Storelli *et al.* 1998a, Storelli *et al.* 1998b, Godley *et al.* 1999, Gordon *et al.* 1998). In these studies, mercury concentrations were lower in loggerheads than in many other top predators (Figure 1). However the physiological importance of these concentrations in loggerheads is unknown.

Data from the previously mentioned studies were generated using a variety of analytical techniques, featured small sample sizes, were highly variable, and were collected in regions quite distinct from the present study area. Therefore, the existing data do not adequately present a clear understanding of mercury contamination in loggerheads in the southeast U.S. The first step in addressing this issue is to determine the degree of contamination that loggerheads in this area experience. The typical approach to determining contaminant burdens in sea turtles has been to obtain tissues opportunistically from fresh-dead sea turtles found stranded on the beach. However this approach usually yields small sample sizes due to limited access to suitable specimens. Another problem this strategy creates is a bias toward sampling individuals that were physiologically compromised by illness or traumatic injury, or that may have undergone post-mortem changes that could effect the toxin concentrations in some tissues.

When dealing with endangered or threatened species, these tissues cannot be collected by sacrificing live animals or subjecting them to invasive sampling techniques. The alternative is to develop non-destructive monitoring techniques that allow the collection of informative toxicological data while not compromising the animal. This

strategy has been utilized with mixed success on feathers from seabirds (Furness and Hutton 1979, Thompson et al 1990, Bearhop et al 2000), dermal scutes and blood from alligators (Yanochko *et al.* 1997), skin from snakes (Burger, 1992), blood and carapace from snapping turtles (Golet and Haines 2001) and more recently with sea turtles (Keller in press, Orvik 1997, Presti 1999, Sakai 2000). Both Orvik (1997) and Presti (1999) analyzed heavy metals in the blood of Kemp's ridleys, and Presti (1999) also analyzed keratinized scutes from the carapace. Keller (in press) used blood for analysis of organochlorines and pesticides in sea turtles. Target tissues for contaminant analysis should be easily collected, sufficiently accumulate the toxin in question, and provide reliable and repeatable information about the toxin in the individual or ecosystem from which it came. Mercury is distributed among body tissues and organs based on its affinity for these tissues and the metabolic function of the organs. Knowing the relative proportions of this toxin in various tissues is crucial in interpreting monitoring efforts and understanding the potential for health impacts.

This study analyzed tissues from stranded individuals (blood, keratin, liver, kidney, muscle, spinal cord, and skin) and blood and keratinized scutes from live turtles captured in the field. Tissues obtained by non-destructive methods provide a rare look at the levels of mercury found in healthy individuals in the wild instead of relying solely on stranded turtles that may not be representative of the population. This combined approach allows comparison of blood and keratin from live and dead turtles to assess if these two groups do have different levels of contamination. Analyzing potentially useful monitoring tissues and internal tissues from the same individual was used to determine if these monitoring tissues are indeed effective predictors of the mercury concentrations in

physiologically important organ systems. The larger sample size from live captures can then be interpreted in a meaningful way. If blood and keratin are to be used in this capacity, these tissues must also be evaluated for sampling reproducibility on a temporal and spatial basis using a well-described methodology that allows repeatable monitoring.

Another facet of this study was made possible by the abundance of live captures available and the wealth of data from collaborative efforts that were generated for each individual. Relationships between mercury contamination and life history characteristics, blood parameters, and health endpoints were investigated to assess how mercury affects loggerhead health. The capture sites for live turtles varied in their geographic distribution and represent a variety of physical and biological conditions. A geographic information system (GIS) was used to supplement standard statistical techniques in analyzing these spatial trends and capture site characteristics relative to mercury contamination.

OBJECTIVES

- Establish baseline values for mercury contamination in loggerheads of the western north Atlantic

- Determine the validity of using blood and scute scrapings as a non-lethal means for monitoring mercury in loggerheads

- Investigate how life history characteristics and habitat utilization affect the accumulation of mercury

- Evaluate the impact of Mercury in wild loggerheads on selected health parameters

METHODS

Field Methods

Strandings

Internal tissues were collected from six stranded loggerheads in cooperation with the South Carolina Department of Natural Resources and the North Carolina Wildlife Resources Commission. Only tissues collected within a suitable time after death were considered for sampling. This included individuals that were euthanized or classified as "Code 1", indicating a fresh-dead stranding. A stranding report (Sea Turtle Stranding and Salvage Network) accompanied each animal documenting the GPS coordinates, date of the stranding event, and any notable abnormalities present. A thorough post-mortem exam was performed after transporting each carcass to the appropriate facility. South Carolina turtles were necropsied at the SCDNR Donnely Site or the Grice Marine Laboratory in Charleston, South Carolina. North Carolina turtles were necropsied at the NMFS Laboratory in Beaufort, North Carolina. Body weight, head width, body depth, and curved and straight carapace length and width were recorded to the nearest centimeter. An external exam detailed all wounds (new and old), epizootics, plastron, carapace, skin, mouth, eyes, cloaca and general body condition. All major organs, and the respiratory and muscular systems were examined, and the sex determined. The

gastrointestinal system was opened from the trachea to the cloaca to examine gut

contents, anthropogenic interaction (e.g. fishing hooks or plastic), and any abnormalities.

Each turtle was classified as an acute mortality, chronic mortality, or unknown, based on

the overall findings of the necropsy.

All instruments used for tissue collection were cleaned using high purity water

and isopropanol prior to use, and clean non-talc latex gloves were worn by personnel at

all times. All tissues were stored at -80°C except blood, which was stored at -20°C to

prevent cracking of the vacutainer tubes. Blood, keratin and skin were collected first,

followed by muscle, liver, kidney, nerve, and brain. The keratin collection was identical

in location and procedure to that described later for live turtles. Blood collection

followed the protocol established for live turtles when possible. If blood could not be

obtained from the cervical sinus, it was extracted directly from the heart with a vacutainer

and needle or syringe. Skin (0.1-0.2 g) was collected from the right front flipper. A

small area on the posterior margin of the right front flipper, and a marginal scute near the

selected area, were cleaned using distilled water, ethanol and wipers. The flipper was

positioned so that the cleaned surface of the flipper was pressed against the cleaned

marginal scute. A 4 mm or 6 mm biopsy tool was then used to remove 4-8 plugs of skin

tissue by pressing perpendicular to the flipper, using the marginal scute as a back-stop.

Skin plugs from each turtle were stored in a labeled polypropylene sample bottle.

Muscle, liver and kidney were removed using a clean stainless steel surgical

scapel and stainless steel forceps, and stored in labeled polyethylene sample bags.

Approximately 200 g of muscle was taken from the pectoralis of the left forelimb, a 200

g section of liver was removed from the ventral surface of the organ, and the entire left kidney was removed.

A stainless steel knife with plastic handle was used to remove the muscle surrounding the backbone and expose the vertebral column in the neck. Cleaned garden clippers were used to cut out a section of the vertebral column (4-7 vertebrae). The nerve cord was then removed using clean stainless steel forceps by pulling from one end and then the other until it came free. The entire brain was removed from 4 individuals by opening the scull with a hacksaw and separating the brain from the attached nerves using a scapel. Nerve and brain were stored in labeled polypropylene sample bottles at.

<u>Live Captures</u>

Blood and keratin samples were obtained from 34 live loggerheads captured in the SCDNR sea turtle index of abundance study (NMFS Grant # NA07FL0499) in July and August, 2001. Individuals selected for mercury analysis were captured in 5-16 m of water from Winyah Bay, South Carolina, to Fernandina Beach, Florida. Research vessels approximately 70 feet in length pulled double-rigged turtle trawls. These are flat nets with an 18 meter head rope and a 20 meter foot rope equipped with mud rollers and a tickler chain. These types of nets are typically used by the Army Corps of Engineers for turtle surveys during channel dredging projects, and have a stretched-mesh size of 20 cm to reduce bycatch. Tow times were limited to 30 minutes, measured from the time the rigs are set to the beginning of retrieval. Sampling commenced shortly after sunrise and ended just before sunset. Duration of cruises ranged from 1 to 5 days, and upon return all

samples were transferred to the South Carolina Marine Resources Research Center in Charleston.

Water temperature, GPS position, and depth were recorded for all tows. A small mesh try-net (5 cm) was also pulled at each station to determine the potential prey present at the capture site. The try-net was deployed immediately after the large rigs were set and towed for 15 minutes. This catch, as well as the bycatch from the large trawls, were processed in an exhaustive work-up. An aggregate weight and count were determined for all species. Commercially and recreationally important species and elasmobranchs were also individually measured.

Turtles captured in tows were processed immediately, followed by a work-up of the bycatch. Personnel involved with the handling of turtles and collection of samples wore clean, non-talc latex gloves at all times. Once removed from the tail-bag of the trawl net turtles were transferred to the "turtle chair" if deemed in healthy condition. The turtle chair places the turtle in a head-down position at approximately 110° from the deck, encouraging quiescence and speeding the blood drawing process. Five mL of blood for mercury analysis were drawn from the dorsal cervical sinus (Owens and Ruiz 1980) using a 22 gauge syringe and vacutainers containing sodium or lithium heparin. Blood samples were immediately placed in a -10°C freezer onboard until transfer to -20°C storage. Additional blood was drawn and processed in the appropriate manner for a variety of analyses performed by collaborating scientists. These include DNA analysis to identify each turtle's haplotype (J. Quattro, University of South Carolina), testosterone assay to determine gender (D. Owens, College of Charleston), organochlorine and pesticide analysis (J. Keller, Duke University) and lymphocyte proliferation assays (M.

Peden-Adams, Medical University of South Carolina). As part of a general health assessment, basic blood parameters were determined for each individual. Hematocrit counts were determined using standard hematocrit tubes and centrifuge, total protein content was measured by refractometer, and blood glucose determined using a handheld glucose meter. Selected samples were also subjected to a CBC comprehensive reptilian profile by Antech Inc. (Atlanta, Georgia, U.S.A.). Blood chemistry values from Antech were measured using a Hitachi 747-200 and total and differential white blood cell counts were counted by hand from blood smears.

Keratin was collected from loggerheads for mercury analysis. The keratin was scraped from the outermost edge of scutes within a standardized area comprised of the eight most posterior marginal scutes of the carapace. Scutes sampled were those most free of fouling organisms, and those that appeared to have keratin of sufficient thickness and texture to provide a sufficient sample mass while minimizing the risk of penetrating through the keratin layer. This most often occurred where the keratin from the dorsal and ventral surfaces of a scute meet. This area can form a relatively thin edge, especially on the posterior corner, where the keratin and underlying bone can be discriminated. This avoided scraping too deeply and causing injury to the turtle and it also prevented contaminating the sample with untargeted tissues. The 2 cm of carapace dorsal and ventral to the edge of these scutes were cleaned of sloughing keratin and epiphytic/epibiotic organisms using a plastic scrubbing pad, rinsing liberally with high purity distilled water and then isopropanol. Cellulose based cleanroom wipers, distilled water and isopropanol were then used to remove any remaining foreign matter and debris. A disposable stainless steel biopsy tool was used to obtain 0.2-0.5 g of superficial keratin

from the prepared areas by moving the tool parallel to the edge being sampled. This yielded small shavings or splinters of keratin < 1 mm in thickness that were dropped directly into a polyethylene sample bag which was stored at -10˚C until it was transferred to -20°C at the end of the cruise. All non-disposable tools were rinsed with high purity water and isopropanol prior to use.

Extensive morphometrics followed the collection of blood, including straight and curved carapace length and width, head width, body depth, tail length, and weight. A physical examination was also performed describing old and new injuries, barnacle and other parasite load, behavior, condition of nares, eyes, mouth, cloaca, and other noteworthy characteristics. Hand-written diagrams and photographs were used to document any abnormal features. Two Inconel flipper tags and one subcutaneous magnetic PIT tag were attached to each individual before its release, and all captures were scanned for previous tags.

Laboratory Methods

Sample Processing

Liver, kidney and muscle were processed in the laboratory under Class 100 clean room conditions. The exterior of each piece of tissue was trimmed away on a Teflon cutting board using a titanium knife to eliminate surface contaminants and non-target tissues. The remaining sample was rinsed using high purity deionized water, transferred to Teflon bags and placed in liquid nitrogen until frozen solid. They were then

pulverized using a sledge hammer (also wrapped in Teflon) and the homogenized sample transferred to a polyethylene sample bottle for storage at -80°C.

Nerve and brain tissues were processed under a HEPA filter vertical laminar flow hood. Tissues were rinsed with high purity deionized water and trimmed on a Teflon cutting board using a titanium knife. The ends of the nerve cord where contamination was most likely were discarded, along with the posterior portion of the brain and the base of the optic nerves. The samples were then sectioned into pieces weighing approximately 0.07 g. The protocols described here and for the other internal tissues were designed to provide a homogenous sample for mercury analysis. Obtaining the sample for analysis from pulverized or sectioned tissue samples minimizes the opportunity for random sampling error due to heterogeneity of the tissues and non-uniform distribution of mercury through these tissues.

Analytical Technique

Total mercury concentration in tissues was determined using isotope dilution cold vapor inductively coupled plasma mass spectrometry (ID-CV-ICPMS) at the National Institute of Standards and Technology (NIST), Charleston, South Carolina. ID-CV-ICPMS has several advantages over other analytical techniques used for mercury. Unlike atomic absorption and atomic fluorescence spectrometry, ICPMS can utilize the high accuracy technique of isotope dilution (Montaser 1998). This method is often used in the certification of standard reference materials because of the sources of error are well understood. Injecting the mercury as a cold vapor instead of a nebulized solution further

serves to increase accuracy, sensitivity, and element selectivity, while reducing the memory effects and wash-out times (Christopher 2001).

The stable isotopes for many elements occur in nature at known ratios. The natural abundances of the four most abundant isotopes of mercury are, 16.9% for ^{199}Hg, 23.1% for ^{200}Hg, 13.2% for ^{201}Hg, and 30.0% for ^{202}Hg. ^{201}Mercury was selected for enrichment, and a known quantity of this isotope was added to a tissue sample. This "spiked" sample was then completely dissolved, and the spike and natural isotopes allowed to completely equilibrate during sample digestion process. The ratio of ^{201}Hg and the reference isotope, ^{202}Hg, in solution was then measured by ICPMS. The deviation of this ratio from that found naturally, and the known mass and concentration of spike added, allow calculation of the total µg of mercury in the unknown sample. The isotope dilution equation is as follows:

$$(\mu g\ Hg\ in\ sample) = (\mu g\ Spike)\ \frac{(Natural\ Atomic\ weight)}{(Spike\ Atomic\ weight)}\ \frac{(201_S - 202_S\ R_M)}{(202_X\ R_M - 201_X)}$$

where 201_S and 202_S refer to the atom fractions of ^{201}Hg and ^{202}Hg in the spike solution, and 201_X and 202_X refer to the atom fractions in the sample that occur naturally. R_M refers to the measured ratio of ^{201}Hg/^{202}Hg. The mercury concentration in the sample is then calculated from the µg of mercury in the sample and the mass of the sample digested.

Reagents

High-purity nitric acid was purchased from Fisher Scientific (Suwanee, GA). Tin chloride, hydrochloric acid and potassium dichromate were purchased from JT Baker

(Phillipsburg, NJ). For the isotope dilution experiments, 100 ng/g and 1 µg/g mercury

spike solutions in 10 % (mass fraction) HNO_3 were created from 98.11% ^{201}Hg enriched

solid mercury (II) oxide obtained from Oak Ridge National Laboratory (Oak Ridge, TN).

NIST Standard Reference Material SRM 3133 Mercury Spectrometric Solution obtained

from NIST (Gaithersburg, MD) was used as the calibrant. All sample and standard

solutions were diluted with high quality water obtained from a Millipore (Bedford, MA)

deionization station capable of producing 18 M.-cm resistivity water.

Spike Calibration

An approximately 100 ng/g ^{201}Hg spike solution was prepared from solid mercury

(II) oxide using 3% HNO_3 and 0.5% $K_2Cr_2O_7$ as the diluent. This solution stabilizes the

mercury in solution, minimizing the loss of elemental mercury and subsequent change in

concentration. The ^{201}Hg enriched spike solution was periodically calibrated using SRM

3133 Mercury Spectrometric Solution, which contains natural isotopic ratios. Two

quantitative dilutions of approximately 100 ng/g mercury were made from this calibrant

and preserved in 5% HNO_3. Each of these natural solutions of known concentration were

then quantitatively mixed with the enriched spike solution to yield a $^{201}Hg/^{202}Hg$ ratio of

approximately 2:1. Four of these spike calibration solutions were prepared (two from

each natural solution), and the isotopic ratios measured by ICPMS. This reverse isotope

dilution procedure yields four values for the spike concentration, with the mean serving

as the working spike concentration. The spike solution was periodically recalibrated

using freshly prepared natural solutions and replaced when exhausted. Since the mercury

concentration in the liver was expected to be considerably higher, a spike solution of

approximately 1 µg/g was used for this tissue. This spike solution was quantitatively

diluted to 100 ng/g, and the calibration routine performed on this solution. The dilution

factor was then used to determine the exact concentration of the spike added to the

sample. All quantitative dilution and mixes were done by mass difference on a Sartorius

MC 210 S five-place balance.

Sample Preparation

Tissue samples were analyzed in 32 analytical batches from January 24 to May 9,

2002. Each batch consisted of four loggerhead tissues, one control, and one procedural

blank. This experimental design allows the analyst to detect error due to incorrect spike

calibration, contamination, mass discrimination, and systematic error associated with

laboratory conditions and reagents. Running a control and blank with every batch

prevents ambiguity on when error was introduced, allowing appropriate measures to be

taken to correct the suspected problem before confounding future data. The µg of

mercury in the blank was subtracted from both tissue samples and controls to correct for

the small contamination contributed by reagents and atmospheric deposition in the lab.

The mass of sample analyzed ranged from 0.08-0.8 g, depending on the mercury

concentration anticipated for each type of tissue and the mass of tissue available. Target

masses of the various tissues are as follows: keratin ~ 0.08 g, skin ~ 0.1 g, blood ~ 0.8 g,

liver ~ 0.2 g, muscle ~ 0.3 g, kidney ~ 0.3 g, nerve ~ 0.2 g, brain ~ 0.3 g. The mass of

spike delivered ranged from 0.5-1.0 g, and was designed to obtain an isotopic ratio that

would minimize the propagation of random error associated with over or under spiking

the sample. This will be discussed in more detail later. Approximately 0.4 g of SRM

2976 Trace Elements and Methylmercury in Mussel Tissue (total mercury = 0.0610 ± 0.0036 µg/g) was used as a control for all tissues. This control tissue provided a reasonable matrix match for all loggerhead tissues analyzed except for blood. Using control samples close in matrix to the unknowns maximizes the similarities in the isobaric interferences that can effect isotopic ratio measurement (Fassett and Paulsen 1989). If these molecular ion interferences exist then inaccurate measurement of the control would result, indicating a similar error is occurring in the unknowns. Matrix matching the control to unknowns also helps determine if sample digestion and measurement methodology is sound. The control for 7 loggerhead blood batches was approximately 0.8 g of SRM 966 Trace Metals in Bovine Blood. The mass of spike used for the procedural blank was reduced to 0.068 g to prevent over-spiking and the subsequent increase error propagation.

Samples were digested and equilibrated in a Perkin-Elmer (Shelton, CT) Multiwave microwave oven at the highest possible temperatures (up to 300°C) and pressures (up to 8 MPa). Tissues were delivered to quartz microwave vessels using Teflon-coated spatulas (SRM 2976, liver, kidney and muscle), polyethylene weighing boats (keratin, skin, nerve and brain), or poured directly from the tube (SRM 966 and blood). SRM 2976, liver, kidney, and muscle were weighed on a Mettler Toledo PG503-S three-place balance, and all others weighed on a Sartorius MC 210 S five-place balance. A known mass of enriched ^{201}Hg spike and high purity nitric acid (5 mL) was added to each microwave vessel. Following digestion, the vessels were vented and diluted to 40 mL (blank diluted to approximately 25 mL) with high purity water and transferred to polyethylene bottles. All digestions were successful in completely

dissolving tissue samples to ensure equilibration of natural and spike isotopes. Subsequent non-quantitative dilutions were performed to obtain a ^{201}Hg concentration of approximately 0.3 ng/g for injection into the ICPMS. Each vessel was rinsed with 400 mL of high purity water after each batch and subjected to a microwave cleaning digestion with 5 mL of HNO$_3$. This was followed by another high purity water rinse of 600 mL and drying at a HEPAfiltered work station hood to prepare for the next batch.

ID-CV-ICPMS Measurements

After proper dilution, the digested samples were ready for analysis. Separate peristaltic pump tubes powered by a four-channel peristaltic pump delivered the sample and reductant solution to a gas-liquid separator. The reductant solution, which was 10% SnCl$_2$ in 7% HCl in water, served to reduce all mercury in solution to elemental Hg^0, allowing liberation to the gaseous phase. The gas-liquid separator consisted of a glass chamber with a high surface area post to receive the mixed solution. A stream of argon gas (250 mL/min) directed into the chamber stripped the Hg^0 out of solution, allowing cold vapor transfer of the sample via Viton tubing directly to the ICPMS injector line. A mass flow controller (AALBORG Model GFC 171, Greenwich, CT) controlled with Labview software and National Instruments (Austin, TX) data acquisition hardware regulated gas flow through the gas-liquid separator (Christopher 2001).

A Thermo Elemental PQ3 ICPMS using typical ICP power and gas flows was used for measurement of isotope abundances. Time resolved analysis (TRA) mode was used for data acquisition. This yielded a profile of the counts per second measured for each mercury isotope over a designated data collection window of 240 seconds. Injection

of each sample was preceded by 30 seconds of baseline isotopic measurements from a 5% HNO_3 wash solution with $SnCl_2$ reductant. These baseline values represent the background noise present in the gas-liquid separator, and were used to correct the signal for each sample prior to calculating the $^{201}Hg/^{202}Hg$ ratio. The $^{201}Hg/^{202}Hg$ ratio for samples, controls, and blanks were calculated from the mean of eight integration windows of 10 second durations. If necessary, the placement of windows was manually changed to avoid occasional anomalies in the sample delivery (e.g. air bubbles). These events were not common, but could be observed in the form of large positive or negative spikes in the signal. All data were corrected for detector dead time and mass discrimination bias.

Field Blank

The previously stated experimental design details the use of the optimal analytical techniques and equipment, validation of each batch of analytical samples with certified standard reference material, and the use of high purity reagents. Further measures were also taken to evaluate the quality of laboratory and field methods. An experiment was designed to evaluate the blood collection protocol used in the field. Since the vacutainer blood tubes being used were not trace metal certified, the potential for mercury contamination from the lithium heparin (LiHep) or sodium heparin (NaHep) anticoagulant, rubber stopper, the inside of the tube, or the needle was unknown. If this mercury contribution was significantly higher than the standard procedural blank, then a field blank correction value would be required. LiHep vacutainer tubes and double-ended needles (n = 5) were selected from the same lots used in field sampling. High

purity water (5 mL) was pulled into each tube and inverted 10 times to dissolve the anticoagulant. Approximately 1 g from each tube was used to mimic blood sample aliquots, and the five field blanks run in one analytical batch to determine the total mercury concentration. The same procedure was then performed using NaHep vacutainer tubes (n = 5) and these mean values compared to the mean of procedural blanks from the next five batches prepared in the same laboratory room.

To provide a worst-case scenario, this experiment was modified to determine the total mercury available in the tubes using a HNO_3 solvent instead of water. HNO_3 (5 mL) was pulled into LiHep and NaHep vacutainers (n = 2 for each). The tubes were vigorously shaken for 15 seconds and the entire contents digested and analyzed for mercury. A standard procedural blank and control were run in the same batch.

Reproducibility

Various reproducibility exercises were performed to evaluate the consistency of the analytical technique and homogeneity of samples. Aliquots of candidate SRM 1947 Lake Michigan Fish Tissue (n = 4) were analyzed using SRM 1946 Lake Superior Fish Tissue (certified at 0.433 ± 0.009 µg/g) as a control. Aliquots of SRM 2976 (n = 4) were analyzed with no control (two blanks were included) since this is a fully certified reference material. These SRM and candidate SRM materials were fully homogenized using standard NIST procedures. Batches were also run with loggerhead blood (using SRM 966 as a control) and keratin (using SRM 2976 as a control). Aliquots of keratin were run from loggerheads CC2146 and CC4067 (n = 4 for each), and aliquots of blood from CC2146 (n = 4).

Lymphocyte Proliferation

This analysis was performed by the lab of Dr. Margie Peden-Adams at the Medical University of South Carolina. A brief description is included in this manuscript for reference. Peripheral blood was collected in 5 mL lithium heparin vacutainer tubes from the dorsal cervical sinus. Blood was kept cool in the refrigerator or on ice until processed in the lab. All blood samples were processed within 32 hr of collection. The blood was centrifuged for 25 minutes at 500 rpm (42 g) to separate the plasma and the lymphocytes in the buffy coat from the red blood cells. The buffy coat and plasma were removed and placed in a sterile 15 mL centrifuge tube and centrifuged again for 5 minutes at 1500 rpm (377 g). The plasma was then pipetted off of the white blood cell pellet. The white blood cell pellet was resuspended in 1 mL RPMI-1640 (RPMI, 5%FBS, 10 mM NEAA, 100 nM sodium pyruvate, 10 mM HEPES, and 50 μg/mL Penicillin/Streptomycin). This was recentrifuged at 1500 rpm (377 g) for five minutes to wash off any remaining plasma. The supernatant was poured off and the cells were resuspended in 1 mL of RPMI. The number of viable white blood cells was determined via trypan blue exclusion.

Samples were diluted to 1.8×10^6 cells/mL and plated in a 96-well plate (1.8×10^5 cell/well). To determine T-cell blastogenesis, 2.5 μg Concanavalin A/mL of culture was added to three wells from each sample. To determine B-cell blastogenesis 10 μg of Lipopolysaccharide/mL of culture was added to three wells from each sample and 200 ng of PDB (12,13-phordbol-dibutyrate)/mL of culture was added to another three wells from

each sample. The other three wells of each sample received 100 μL of RPMI as a control.

Plates were incubated in a 30°C incubator with 5% carbon dioxide for four days. Then, 100 μL of 0.05 μCi tritiated thymidine/mL RPMI was added to each well. The plates were then incubated under the same conditions for another 16 hours. After incubation the cells were harvested using a Packard 96-well plate harvester, and the samples were counted on a Packard TopCount scintillation counter. The results are reported as a ratio of the control, the Stimulation Index (SI = cpm stimulated/cpm unstimulated).

Statistics

All statistics were performed using SAS Institute's JMP 3.26. Arcview 3.2 was used to generate spatial parameters, maps, and inverse distance weighted surface interpolation.

RESULTS

Method Validation

The isotope dilution technique for mass spectrometry is often chosen for certifying standard reference materials because of its high accuracy and precision. Utilizing this analytical technique minimizes the introduction of uncertainty from the analytical method itself, allowing natural trends in the contaminant data to be more easily detected, even at low concentrations. Combining this technique with a rigorous experimental design using a method blank and standard reference material for every batch of four unknown samples ensures the highest degree of confidence in these measurements. Workers often run a reference material to validate the accuracy of their analytical method only once or twice during the course of a study that may last months. This could allow random or systematic error to affect a large portion of the data undetected. The NIST certified SRM2976 and SRM966 have 95% confidence intervals of ± 3.6 ppb and ± 1.6 ppb respectively. Out of the 32 analytical batches in which these samples were run, departure from these uncertainty values occurred in only four batches for a mean value of only 1.2 ppb (1.8%) (Figures 2-3).

Replicate analysis of NIST standard reference materials revealed excellent reproducibility. SRM 1947 and SRM 2976 are both fully homogenized, so the variability

in the measurements of multiple aliquots is an approximation of the precision of the

analytical method for this type of tissue matrix. The coefficient of variation (CV) for

these materials was 1.07% for SRM 1947 and 1.36% for SRM 2976 (Figure 4). Blood

and keratin samples from loggerheads were not homogenized and subject to variability

due to sampling and processing techniques. Since these tissues could potentially be used

for monitoring, reproducibility exercises were also performed on selected samples.

Despite freezing for storage and thawing for analysis, whole blood mixed with

anticoagulant yielded a CV equal to that of the homogenized SRM tissues (1.17%)

(Figure 4). This suggests that the sampling, storage, and processing of these blood

samples did not introduce any additional variability beyond that inherent to the analytical

method. The variability in keratin samples obtained from the carapace scutes of two

individuals was 6.23% and 8.1% (Figure 4). Subtracting the mean CV for the SRMs

from the mean CV for the keratin samples leaves 5.95% of additional variability

introduced by the heterogeneity of mercury in the keratin and the collection protocol.

Field sampling equipment and protocols were designed to prevent contamination

of samples. However, the vacutainer tubes selected for the collection of blood were not

trace metal certified due to prohibitive cost and collaboration with a larger project. The

potential for mercury contamination from this source was determined using field blanks

for LiHep and NaHep vacutainers. The mean μg mercury in each field blank (n = 5) was

below the mean of the normal procedural blanks (n = 26) that were prepared in the same

lab room (Figure 5), indicating that contamination from the vacutainers was not an issue.

The lower mercury concentration in field blanks is probably an artifact of the procedural

blanks having a larger sample size that spans many different batches and spike

calibrations. Analysis of the total mercury available in the vacutainers tubes using HNO_3 and vigorous shaking also detected no substantial mercury content (Figure 5).

Stranded loggerheads

The compartmentalization of mercury among tissues and organs from stranded loggerheads revealed patterns similar to that reported in the literature. The mean mercury concentrations among tissues varied over an order of magnitude with keratin>liver> kidney>skin>muscle>blood>spinal cord (Table 1). Variability among individuals was also high, as seen in the standard errors of the mean tissue concentrations (Table 1). The mercury concentrations were log-transformed to meet assumptions of homoscedasticity and normality and a two-way ANOVA was performed using log-transformed mercury concentrations, tissue type, and individual. The model was highly significant ($r^2 = 0.932$, $P < 0.0001$) and found significant differences among tissues ($P < 0.0001$) (Figure 6) and individuals ($P = 0.0001$) (Figure 7).

The tissue differences reflect the variable affinity of mercury for proteins in different tissues and the metabolic functions of these organs. One interesting note is that the mercury concentrations in blood, spinal cord, and brain were not significantly different (Figure 6). Overall mercury burdens among individuals were not significantly different except for one turtle (CCS4), which had consistently higher mercury concentrations in all tissues (Figure 7).

Linear regressions were performed between mercury in each monitoring tissue and each internal tissue from stranded loggerheads to determine the relationship between

these tissue compartments. Residuals for all regressions met assumptions of normality

based on the Shapiro-Wilks test except for the keratin-muscle regression (Shapiro-Wilks

test for normality, P = 0.022). Given the robustness of linear regression analysis, and for

consistency, the excepted data were not log-transformed. All regressions were significant

(P < 0.043) for each monitoring tissue. The r^2 values indicate that blood was the most

effective predictor of the mercury burden in internal tissues, followed by keratin and then

skin (Table 2). Due to the higher variability and more invasive collection protocol

necessary for skin, it appears to be a less than ideal monitoring tissue. Future discussion

of monitoring tissues will be limited to blood and keratin. Liver and kidney mercury

concentrations (Figure 8) were more difficult to predict than spinal cord and muscle

mercury concentrations (Figure 9).

Live captured loggerheads

The blood and keratin samples collected from 34 live captures provides a much

larger sample size than is usually possible when relying solely on stranded animals.

Mean mercury concentration in the keratin (0.461 ± 0.080 µg/g) was 16 times higher than

in the blood (0.029 ± 0.005 µg/g). Like the stranding data, the ranges are very large due

to the presence of one individual (CC2151) with considerably higher mercury

concentrations in both blood and keratin (Figure 10).

Linear regression analysis between mercury concentrations in blood and keratin

(Figure 10) reveals a strong correlation for these tissues each individual (r^2 = 0.926, P =

0.001). The Shapiro-Wilks test confirms the residuals from this regression meet

assumptions of normality (P = 0.3960). Omitting the highly contaminated individual

from the analysis still results in a highly significant regression (r^2 = 0.604, P = 0.0001).

Keratin and blood both show a significant increase (P = 0.0119 and P = 0.0162

respectively) in mercury concentration with body weight (Figure 11). Two-way ANOVA

on mercury concentration in keratin with gender (Figure 12), and haplotype showed no

significant relationships.

Interpreting tissue concentrations

The results reported above suggest that the mercury concentration in the blood of

loggerheads is related to keratin as well as other internal tissues. Similar results in blood

from other species were reported by Henny *et al.* (2002) and Golet and aines (2001).

Since the mercury content of these latter tissues is not likely to change significantly over

the short term (weeks to months) it stands to reason that at least a portion of the total

mercury present in the blood is relatively persistent. It is likely that this component of

the total blood mercury is in equilibrium with other tissue storage locations.

In contrast, pharmacokinetic studies on mercury in the blood of humans show that

after a brief exposure to mercury vapor the blood mercury concentration increases

dramatically within 5 hours and then decreases rapidly toward the baseline values from

before the exposure. The half time of this decline was calculated at approximately 5

days, and nearly 70% of the mercury had left the blood compartment after 14 days

(Barregard and Sallesten 1992). The mercury intake is via the diet for predators such as

loggerheads, and would appear in blood as doses associated with the mass and mercury

concentration of each prey item consumed. The absorption of mercury into the blood stream through the digestive tract would be slower than inhalation, and would depend on the digestive rate of the species. A freshwater turtle, the red-eared slider (*Trachemys scripta elegans*), shows a peak in plasma amino acid concentration 24 hours after being fed fish (Herbert and Coulson 1975). Since the proteins in the prey are where the large majority of the mercury is bound, this gives a rough estimate of the time required for mercury to be absorbed into the blood for turtles. Given the difference in metabolism, the rate at which this mercury is eliminated from the blood in turtles is likely to be slower than for humans, but probably still on the order of weeks.

Therefore it appears that the mercury signature in blood is comprised of (1) a baseline value related to other tissues and reflecting the total body burden from long-term accumulation and (2) a transient component reflecting the mercury intake over the previous several weeks. When sampling blood for contaminants the relative contributions of these two sources of mercury must be considered when interpreting results.

The carapace of sea turtles in the family Chelonidae is covered in a layer of hard keratinized plates known as scutes. This layer of proteineceous keratin originates from growing regions at the basal infoldings of the epidermis. Mature cells containing keratin fibrils migrate, develop desmosomal attachments to neighboring cells, and eventually become pyknotic (Solomon *et al.* 1986). The result is a series of non-living keratin layers deposited one on top of the other, shown in Figure 13 (Elkan and Cooper 1980). This protective layer is impervious to water and salts even at pressures as high as 300 lb/in^2 (Stokes and Dunson 1982). Appelquist *et al.* (1984) showed that UV radiation, heating

(100°F), freezing (-20°F), and weathering for eight months had only a 10% effect on the mercury concentration in the keratin of bird feathers. Therefore, this non-living body part provides a potential means to non-invasively collect contaminant data and is stable enough to be collected from live or dead animals that have been exposed to diverse conditions.

Loggerheads periodically shed a whole scute down to the epidermis, or sometimes slough only superficial layers of keratin (personal observation). The rate at which this keratin is grown and sloughed is unknown. However the occurrence of large commensal barnacles (*Chelonibia testudinaria*) on the carapace of loggerheads suggests the time scale is on the order of a year or more. The sample of keratin scraped from the marginal scutes of the carapace in this study is a cross-section that includes the oldest layers of keratin (most superficial) and the more recent layers (deeper). These splinters are digested completely for analysis, providing an average mercury concentration for the period required for the growth of these layers. Since blood is the medium for transport of mercury through the body, one would expect that the amount of mercury bound to growing keratin would be related to the concentration available in the blood. This assumption is supported by the strong relationship between mercury concentrations in the blood and keratin for each individual. Therefore, the mercury concentration in keratin samples obtained from these loggerheads reflects the average blood mercury concentration during the previous year or more. This same reasoning has been applied using human hair to reconstruct mercury exposure over the time of hair growth (Wheatley et al 1979).

The strong regression between the mercury concentrations in blood and keratin (Figure 10) confirms the relationship that would be expected from a physiological standpoint. The residuals from this regression indicate whether the blood mercury concentration is high or low relative to what is predicted based on the keratin mercury concentration (Figure 14). Given the temporal scales which these two tissues represent, the residual value for each individual provides a comparison of the recent mercury intake relative to the average mercury intake over the previous year or more (Index of Recent Exposure - IRE). A positive IRE indicates high recent intake relative to the average previous exposure, and a negative IRE indicates lower recent intake. The IRE can then be used to determine the impact of biological and environmental variables that relate to short-term mercury exposure.

Geographic variation

A backward stepwise multiple regression was performed using the IRE and 5 biological (body weight, packed cell volume, total blood protein, blood glucose, and gender) and 6 environmental (water depth, water temperature, distance to land, distance to nearest river or inlet, distance to nearest major river, and major river of association) parameters. The model was set to initially reject all parameters from the model with $P > 0.1$. This resulted in the rejection of all parameters except distance to nearest major river. The IRE for turtles caught closer to these river mouths was significantly higher than the IRE of individuals caught further away ($P = 0.012$) (Figure 15). This trend is displayed graphically by using the IRE values and an inverse distance-weighted surface interpolator

in Arcview (Figure 16). Turtles associated with all major river systems except the Waccamaw exhibit this trend.

Correlation analysis between the IRE and bycatch data revealed no significant relationships between recent mercury exposure and abundance of type of prey present at the capture sites.

Health Impacts

The clinical determination of the relationship between a given toxin and some aspect of immune response is a widely used technique to determine the dose response and type of impact. A correlative relationship between the immune response and the mercury concentration present in the sample was used to establish possible effects of this toxin on the immune system. The assays reported here investigated the proliferation of T-cells and b-cells in response to mitogens known to illicit a positive response in other species. Figure 17 shows that two non-parametric measures of association detected a significant negative correlation between blood mercury concentration and b-cell proliferation (Spearman Rho, -0.727, $P = 0.011$, Kendall Tau b, -0.600, $P = 0.010$).

Non-parametric analysis of blood mercury concentration and 20 parameters from the Antech comprehensive blood chemistry analysis also yielded two significant correlations. Figure 18 shows the significant negative correlation between blood mercury concentration and total white blood cell count (Spearman Rho, -0.883, $P = 0.020$, Kendall Tau b, -0.788, $P = 0.032$). There was also a highly significant negative correlation

between blood mercury and sodium concentrations (Spearman Rho, -0.986, P = 0.0003, Kendall Tau b, -0.966, P = 0.007), seen in Figure 19.

Considering the sample size and method of capture it is fair to assume the tissues collected from wild loggerheads are a representative subsample of the population in this region. Due to difficulty in determining the cause of death, samples from stranded turtles are somewhat more difficult to interpret in terms of the physiological condition of the animal and how that may relate to contaminant burden. Mercury contamination in like tissues from live captures and from strandings were compared using analysis of covariance for mercury concentrations, straight carapace length, and blood and keratin mercury concentrations. Straight carapace length was included to account for variation due to the difference in the size structure of these two groups (Figure 20). The ANCOVA showed significantly higher mercury concentrations in blood (P = 0.0018) and keratin (P = 0.036) from strandings than from live captures (Figure 21).

DISCUSSION

Monitoring strategies

The issue of mercury pollution in the environment is an area of research that continues to grow on many fronts. Our understanding of the transport and biogeochemical cycling of mercury has improved greatly in recent years. However the extent and impact of mercury contamination in wildlife is more poorly understood. The potential of a given species or population to be at risk for accumulating high concentrations of mercury can be generally predicted. These predictions are based on our knowledge of the cycling of mercury in the environment and its transfer through the food web.

The mercury concentrations reported for stranded loggerheads in this study are most similar to loggerheads from the Mediterranean Sea (Figure 22). The geometric mean of liver mercury concentration from this study was 0.523 µg/g. The liver mercury concentrations in two studies in the south Adriatic Sea (Storelli *et al.* 1998a, Storelli *et al.* 1998b) were 0.42 µg/g and 0.70 µg/g (geometric means). Another study from the north coast of Cyprus (Godley *et al.* 1999) found liver mercury concentrations of 0.602 µg/g (median). Liver mercury concentrations from Sakai (1995) in Japan (1.51 µg/g, geometric mean) and Gordon *et al.* (1998) in Australia (0.015 µg/g, arithmetic mean)

were considerably higher and lower (respectively) than the present study (Figure 22). These differences are probably a result of many biotic and abiotic characteristics specific to each region, including geology, habitat type, water chemistry, prey, and anthropogenic impacts. Comparison of mercury concentrations in these loggerheads to other aquatic consumers shows they rank low relative to cetaceans, pinnepeds, and seabirds (Figure 1). This is due largely to the higher trophic level at which these species feed, and their more piscivorous diet. The physiological significance of these concentrations for any given taxa is difficult to determine, and will be discussed in more detail later.

One factor rarely mentioned that must impact the bioaccumulation of toxins acquired through diet is the rate of metabolism. More active, faster growing, homeothermic species require a higher caloric intake, and concomitant mercury intake, than a slow growing, ectothermic species like the loggerhead that becomes less active on a seasonal basis. The rate of elimination of mercury from the body is also a significant factor in the accumulation of this toxicant. The major routes of elimination in birds and mammals are the binding of mercury into growing feathers and hair and excretion via the feces (Wiener *et al.* 2003). The plumage of seabirds can contain over 70% of the total body burden of mercury (Braune and Gaskin 1987) which can subsequently be removed via molting. The hard keratinized dermal layer that armors the body in loggerheads is homologous to feathers in birds and hair in mammals. The disulphide bonds in these keratin proteins avidly bind mercury (Crewther *et al.* 1965), and accumulate higher concentrations than any other tissue that was analyzed (Table 1). This non-living tissue serves as a sink for methylmercury, thus reducing the body pool available to the nervous, hepatic, and renal systems where damage can occur.

While the molting cycles in birds is well understood, little is known about the rates of growth and shedding of scutes in loggerheads. Amphibians and reptiles both shed their skins, but unlike snakes, chelonians may only lose individual shields or pieces of skin (Elkan and Cooper 1980). Of the 199 loggerheads captured in the 2001 SCDNR trawl survey, 58% had some degree of shedding recorded in the physical examination. The majority of this was minor "scaling" where small, thin layers of keratin were peeling away. This occurred in isolated patches or covered large portions of the carapace, plastron, or head. Other common observations included large flakes of keratin or whole scutes that were loosely attached or recently shed from the carapace.

This shedding serves to rid loggerheads of their often dense epibiotic communities as well as removing the highly concentrated mercury deposited there. Determining the importance of this route of mercury elimination is difficult without a more quantitative understanding of the rate of shedding. In the absence of this information, the relative importance of the major tissue and organ compartments to the overall body burden of mercury is informative. Biometric data from Sakai *et al.* (2000) was used to estimate the weight of liver, kidney, and muscle relative to total body weight. Out of 26 different organs and tissues, 72-82% of the body burden of mercury was found in these three compartments (Sakai 2000). The estimated tissue/organ weights and the mercury concentrations from stranded loggerheads in this study were then used to calculate the total mercury contained in each of these compartments. The contribution of the keratin compartment was determined by removing all scutes from the carapace of one stranded individual. These scutes comprised 1% of the total body weight, and an additional 0.3% was added to account for the scutes of the plastron, head, and limbs that

could not practically be removed. Based on these weights and the mercury

concentrations from the four major compartments for mercury, the scutes contained

approximately 12% of the body burden of mercury (Table 3). This is equal to half of the

mercury stored in the liver where accumulation is usually the highest. The scute

compartment contains less mercury than the 70% reported in the plumage of some

seabirds, but even conservative estimates of shedding rates would suggest this is a

significant route of elimination for this species.

The distribution of mercury in various tissue compartments is useful information

in the context of the body's mercury budget. This knowledge is also necessary in the

design and interpretation of monitoring protocols using non-lethal sampling techniques.

The results from this study show there is surprisingly good correlation among different

tissues and organs from the same individual. The strong regression between blood and

keratin from live captures suggests that these tissues may be reliable indicators of the

overall mercury contamination (Figure 8.1). The trend of bioaccumulation in blood and

keratin is further evidence that these tissues reflect the overall body burden. Finally,

significant regressions between blood and keratin and internal tissues from dead

loggerheads show these tissues are indeed good predictors of contaminant loads in

important organ systems (Figure 7). The equations from these regressions provide a way

to estimate the mercury concentrations in the liver, kidney, muscle, and spinal cord of

live captures using the mercury concentrations from the blood and keratin collected from

these individuals. The relatively small sample size and strong leverage exerted by the

one highly contaminated stranded turtle urge caution in using these regression equations

to predict exact mercury concentrations in internal tissues. The relationship between

mercury concentrations in the blood and keratin and in the internal tissues appears to weaken when predicting differences of the scale seen in the five individuals at the lower end of the curve (Figure 7). However, the consistently high mercury levels found in all tissues from the highly contaminated individual demonstrates that blood and keratin are effective predictors of physiologically relevant differences in mercury contamination.

The similarity of the mercury concentrations in the blood and brain supports previous evidence that mercury is able to pass freely across the blood-brain barrier that is responsible for regulating the exposure of sensitive brain tissues to unwanted analytes (Chang and Hartman 1972, Steinwall and Olsson 1969).   From a management perspective, this means a blood sample is sufficient to determine the approximate mercury concentration in the brain. These data also suggest that spinal cord mercury concentrations can be used to approximate brain mercury concentrations in necropsy cases where brain samples are not available due to head trauma or other sampling complications.

To assess the consistency of these predictive tools, regression equations from the stranding data were used to predict the mercury concentrations in internal tissues of the larger live capture data set. A prediction of the mercury concentration in the liver, kidney, muscle, and spinal cord was generated for each individual using both the blood mercury concentration and the keratin mercury concentration. These values were labeled by tissue type (liver, kidney, muscle or spinal cord) and the source of the prediction (blood or keratin) and a factorial two-way ANOVA was performed. The model was significant ($r^2 = 0.612$, $P < 0.0001$) due to expected differences among the tissue types ($P <0.0001$). However the prediction source effect ($P = 0.558$) and the interaction term ($P =$

0.986) were not significant. This indicates that the predictions using blood and keratin for the mean body burden and mean tissue concentration of these loggerheads were not statistically different (Figure 23). Furthermore, statistical analysis using a paired T-test revealed no significant difference between the values predicted by the blood mercury concentration and keratin mercury concentration on an individual basis (liver P = 0.886, kidney P = 0.233, muscle P = 0.092, nerve P = 0.373).

The agreement between predictions from blood and keratin is encouraging. There are several consideration concerning which tissue is the better monitoring tool. Strictly based on the r^2 values, the blood mercury concentrations are slightly more indicative of the internal mercury burdens than keratin (Table 2). However this could be an artifact of the lower magnitude of the blood concentration and resulting lower error sums of squares when these points deviate from the expected value. The lower blood concentrations are also responsible for lower standard errors in the predicted internal tissue concentrations (Figure 23). Predictions of internal mercury burdens were generally lower when using the blood mercury concentration than when using the keratin mercury concentrations. This is probably due to the difference in the ratios of mercury concentrations in the keratin and blood in dead turtles versus live turtles. In live turtles the mercury concentration in keratin is on average 15.9 times higher than the blood, whereas in strandings the mean ratio is only 10.8. This higher blood concentration in the strandings results in lower conversion factors for internal tissues, and subsequently a lower predicted value when this conversion is applied to live captures.

It is unclear whether this difference can be attributed to an increase in the blood mercury due to physiological processes occurring prior to death or post-mortem changes.

One simple explanation for the higher blood concentration in strandings could be post-mortem dehydration. Freeze drying to determine dry weight conversions for blood from strandings and live captures could be performed to evaluate this possibility. Alternatively, the mobilization of mercury stored in muscle and fat could contribute to blood mercury concentrations. This would be most probable in the case of a chronically ill animal that is not feeding and is metabolizing these tissues for energy. The mean mercury concentration in the fat of loggerheads is approximately 16 ppb (Sakai *et al.* 2000) and the mean mercury concentration in muscle is 155 ppb (present study). Reported mercury concentrations in potential loggerhead prey species include 170 ppb for blue crab and 50 ppb for scallop (U.S. Food and Drug Administration website). This suggests that metabolizing loggerhead body tissues probably would not enrich the blood with mercury any more than their typical food sources. Furthermore, the only severely emaciated turtle sampled had a keratin/blood mercury ratio of 19.8 compared to a mean of 9.3 for turtles with a normal body condition. This suggests that the blood mercury concentration may actually be decreased when turtles are emaciated. The possible relationship between higher mercury concentration in blood from strandings and the health of these individuals will be discussed later.

The sampling reproducibility for blood (1%) was better than it was for keratin (6%) due to the more homogenous distribution of mercury in blood samples. Several workers have proposed using turtle scutes to monitor mercury (Presti 1999; Kemp's ridley, Sakai *et al.* 2000; loggerheads, Golet and Haines 2001; snapping turtles) but none have addressed the potential heterogeneity of mercury in scutes and the impact that the sampling methodology may have on the results. Golet and Haines (2001) removed one

posterior marginal scute for analysis, Presti (1999) mixed very superficial keratin scraped haphazardly from various locations on the carapace, and it is not clear what portion of the carapace Sakai *et al.* (2000) used for analysis. Sakai *et al.* (2000) reported that 15% of the body weight was comprised of the carapace, implying that more than scutes were included in this compartment. Despite efforts to standardize collection techniques in this study, there is still some variability among aliquots from the same individual. The layered growth pattern and multiple growing regions in the scutes (Figure 13) make this variability difficult to control. The thickness of the scutes varies depending on their location on the carapace and which part of the scute is measured (medial or lateral, anterior or posterior) (personal observation). Therefore, keratin layers collected at different depths and location on the carapace may reflect different periods of deposition in the turtle's history. The strong correlation between mercury concentration in blood and keratin suggest that the slow accumulation of mercury in this species may render these differences negligible. Future efforts to assess the potential variability from this source will require the use of a laser ablation ICPMS or radio frequency glow discharge MS system. This will allow a profile to be generated of the relative mercury concentrations at different depth layers in the scute, and at different scute locations on the carapace.

While spatial heterogeneity of mercury is not an issue with blood, the dynamic nature of mercury in blood provides challenges. Studies using blood for contaminant analysis have had mixed results. Studies on alligators (Yanochko *et al.* 1997) and Kemp's ridley sea turtles (Presti 2000) found no correlations between mercury concentrations in the blood and other tissues or with size. However, Golet and Haines

(2001) reports a significant regression between mercury concentrations in blood and muscle in snapping turtles (P = 0.0004, r^2 = 0.32). In the present study blood mercury concentrations varied significantly with liver, kidney, muscle, and spinal cord. However, the regressions with liver and kidney were more variable than with muscle and spinal cord (Table 2). Blood and feathers (and presumably scutes) contain nearly 100% methylmercury (Thompson and Furness 1989), and muscle and nerve tissue are also predominantly methylmercury. However the liver and kidney contain larger fractions of inorganic mercury due to the demethylation of mercury that occurs in these organs. This inorganic mercury is accumulated and stored as Hg-Se-protein complexes and as insoluble HgSe (tiemannite) granules (Martoja and Berry 1980, Nigro 1994, Cavalli and Cardellicchio 1995, Palmisano *et al.* 1995). This difference in the form of mercury may explain the stronger relationship between mercury concentrations in blood, muscle, and spinal cord compared to blood, liver, and kidney. This is supported in a study by Henny *et al.* (2002) using tissues from cormorants. They found a highly significant regression between mercury in the blood and methylmercury in the liver of cormorants (P < 0.0001, r^2 = 0.83). In the same samples the regression between blood mercury and liver inorganic mercury was also statistically significant but much more variable (r^2 = 0.32).

If the less toxic inorganic mercury reserves are immobile, then mercury concentrations in the blood and scutes would not reflect this portion of the historical exposure and the correlations between these compartments should be weaker. So generally speaking, blood and keratin will be better predictors of the total mercury in the liver and kidney at low concentrations when the % methylmercury is high. The limited data for methylmercury in loggerheads shows that liver total mercury concentrations <

0.6 µg/g have ~60% methylmercury, and > 0.6 µg/g have ~28% methylmercury (Storelli *et al.* 1998). This trend should be realized when interpreting mercury concentrations, but does not diminish the utility of using blood and scutes for monitoring purposes. Since methylmercury is the form that is responsible for toxicity in wildlife, the stronger relationship between mercury concentrations in blood or scutes and methylmercury in other tissues is actually beneficial in approximating the physiologically important portion of the mercury burden.

Another consideration when using blood for contaminant analysis is what portion of the total blood mercury signature is derived from recent dietary intake versus a relatively stable baseline reflecting long-term exposure. This duality of the blood mercury concentration is evident in the data from the present study. A significant relationship between blood mercury concentration and body weight in live captures (Figure 11) indicates the bioaccumulatory effects of long term exposure are reflected in the blood. The correlation between blood and other tissues also supports the assertion that the blood mercury concentration reflects more than just recent exposure (Figures 8-10). However there is much variation in the blood mercury concentration not explained by either age/size or by the mercury concentration in other tissues. A significant portion of this variation is explained by the proximity of the foraging grounds where the turtles were caught to major river outflow (Figure 15-16). This effect indicates that recent exposure (presumably through diet) also contributes to the total blood mercury signature. This geographic variation in mercury exposure will be discussed in more detail in a subsequent section.

This interpretation agrees with published work on the dynamics of mercury in blood. Based on pharmacokinetic studies in humans, it is clear that individual doses of mercury are absorbed and redistributed by the blood rather quickly. The mean half-times are around 5 days, and after 30 days, the blood mercury concentrations are back to baseline (Barregard *et al.* 1992, Sandborgh-Englund *et al.* 1998). Dosing studies on seabirds have estimated the mercury half-time in the blood to be between 40-65 days (Monteiro 1996). Positive relationships between stable isotope concentrations (δ^{15}N, which reflects trophic position) and total mercury in the blood of seabirds indicates that diet can explain variation in blood mercury concentrations. However it is interesting to note that this relationship was present in chicks but not adults (Bearhop *et al.* 2000). Adult blood mercury was several times higher than in chicks and there was no correlation between mercury concentrations and δ^{15}N. This is probably due to long-term exposure in adults contributing to the total blood mercury signature and confounding the relationship between the transient dietary mercury component and by δ^{15}N.

The relative magnitude of these two temporal components must be considered when interpreting blood contaminant data. In the case of loggerheads, several pieces of evidence indicate that long-term exposure is more important in determining the overall blood mercury concentration than recent exposure. (1) The significant relationship between the total blood mercury concentration and body weight indicates that blood reflects long-term bioaccumulation (Figure 11). One alternative explanation for this relationship is that larger turtles feed on more highly contaminated prey or consume more prey per unit of body weight. However, there is no evidence of this based on trophic studies. Furthermore, larger mature loggerheads typically forage farther offshore away

from terrestrial sources of mercury and significantly reduce foraging during the mating season when these samples were collected. (2) The relationship between mercury concentrations in blood and keratin from live captures shows there is little deviation of the instantaneous blood mercury concentration from the long-term average blood mercury reflected by the keratin (Figure 10). If transient pulses of mercury from dietary sources comprised the majority of the total blood mercury signature this relationship would be weaker. (3) Elevated mercury concentrations in internal tissues and organs from strandings were accompanied by an elevated mercury concentration in the blood. Since mercury concentrations in the tissues and organs could not fluctuate rapidly, the agreements with the blood mercury concentration suggests little fluctuation in this compartment as well.

The conclusions from the present study may not be applicable to other species. For example, blood mercury concentrations in marine mammals may reflect more recent intake. Marine mammals prey mostly on fish, therefore dietary doses of mercury would be much higher than for loggerheads. As shown by Henny *et al.* (2002), blood mercury concentrations correlate more strongly to liver methylmercury than liver total mercury. Since the higher total mercury burdens in the liver and kidney of marine mammals means lower % methylmercury, blood mercury concentrations would not reflect the inorganic mercury loads in these long-term storage sites. Further work is needed to better understand the relative importance of dietary versus body burden sources of mercury in blood. Stable isotope analysis of blood samples would provide valuable information toward answering this question. Pharmacokinetic studies would also be very useful. This would involve serial blood draws from a captive animal placed on a mercury-free

diet followed by known doses of mercury. This would allow determination of the time required for mercury redistribution and the relative magnitude of the two components of the blood mercury signature.

Life history and geographic effects

The only previous data available on mercury in sea turtle blood were from two studies in the Gulf of Mexico. The mean blood mercury concentration from three loggerheads was 0.015 µg/g, and mean keratin concentration was 0.236 µg/g (Presti 1999), which are nearly half the concentrations found in the present study. This difference may be due to the young age of the individuals (29.3-57.0 cm straight carapace length) sampled in the Gulf of Mexico compared to those in this study (50.2-94.9 cm straight carapace length). Mercury concentrations in blood from Kemp's ridley sea turtles, *Lepidochelys kempi*, from the Gulf of Mexico were comparable, but slightly lower, than in the present study (0.018 µg/g, n = 106, Orvik 1997, 0.027 µg/g, n = 100, Presti and 2000).

The present study provides the first evidence of bioaccumulation of mercury in loggerheads (Figure 11). The abundance survey which provided blood and keratin from live captures yielded a large sample size with excellent representation from all subadult and adult size classes. All tissues collected from strandings showed a positive relationship with body size, but were not statistically significance. Further discussion in the life history and geographic effects section will be limited to the live capture data set. Bioaccumulation of mercury has also been shown in other sea turtles species. Orvik

(unpublished thesis, 1999) showed a significant ($P < 0.0001$) regression between blood

mercury concentration and straight carapace length in 106 Kemp's ridleys from the Gulf

of Mexico. Sakai (2001) reported significant correlations between mercury

concentrations in liver and kidney and straight carapace length in hawksbill sea turtles,

Eretmochelys imbricata.

The trends stated above indicate that loggerheads take in mercury faster than they

are able to eliminate it. In addition to normal depuration, and special routes of excretion

such as shedding scutes, another potential route of elimination is reproduction. Female

cetaceans have been shown to dump significant amounts of toxins into calves through

mobilization of lipid stores and lactation (Aguilar *et al.* 1999). This often results in

females having contaminant loads much lower than males of the same age. Female

loggerheads mate once every two or three years and lay three or four clutches per mating

season. Each clutch usually has over 100 eggs which weigh over 30 g each (Hirth 1980,

Van Buskirk and Crowder 1994). Despite depositing approximately 10 kg of biomass

each mating season, sexually mature sizes of male and female loggerheads from the

present study have almost identical mercury concentrations in both blood and keratin

(Figure 12).

There are several papers investigating contaminant levels in eggs, mostly from the

perspective of using them as a proxy for contamination in the adults (Stoneburner *et al.*

1980, Gordon *et al.* 1988, Sakai 1995, Godley *et al.* 1999). These studies suggest either a

very large range in egg mercury concentrations depending on the population, or

discrepancies in the analytical methods. Among the more recent studies, Sakai (1995)

found the mean egg mercury concentrations in Japan from 5 loggerheads to be 5.5 ng/g.

The total excretion of mercury via eggs from one mating season was estimated at < 5% of the total body burden, and Sakai (1995) suggested this was not a major route of mercury elimination. Godley *et al.* (1999) disagrees, stating that eggs may be a potentially important long term route for eliminating metals such as mercury. Data from the present study comparing mature males and females suggests the latter view may be flawed.

Gender does not appear to have an impact on long-term mercury accumulation. However reproductive behavior in mature adults could potentially affect mercury accumulation during the mating and nesting season. Both males and females decrease or stop feeding during reproductively active periods (cite). Loggerhead males in North America peak in activity for the mating season in March and April. Females have a more prolonged period of reproductive activity lasting until their last clutch is laid, typically some time in July. Since the adults in this data set were captured in July and August, one would expect the adult females to have a lower recent intake of mercury than males. Though not statistically significant, the mean IRE in females was over three times lower than the IRE in males (-0.0032 and -0.001) caught at comparable distances from terrestrial sources of mercury (27 and 26 miles from major industrial rivers). Four out of the five females had negative IRE's, suggesting low recent intake of mercury, and one had a highly positive IRE. The latter female with relatively high blood mercury could be explained by her being in a reproductively inactive year, and therefore foraging normally. It is impossible to tell from testosterone levels whether these females (or males for that matter) are post-reproductive for the year or taking a year off. Their reproductive status could only be conclusively determined by ultrasound or laporoscopy, but these trends in the IRE agree with what would be expected based on our knowledge of sea turtle biology.

The trend of large inter-individual variation in mercury load is common among the existing studies on mercury in sea turtles. The range in blood mercury concentrations in the present study is 5 to 188 ppb. Neither gender nor haplotype seem to be related to mercury loads. Genetic differences in the ability to metabolize and eliminate mercury probably exist on some scale, but they were not evident using these techniques. The size of the individual accounts for some of the variability, but there is considerable deviation from this trend and neither of the highly contaminated individuals were particularly large.

Based on the current knowledge of loggerhead life history, habitat utilization is a likely source of considerable variation in mercury exposure. Loggerheads utilize a wide variety of habitats after settling into their benthic lifestyle. The subadult and adult size classes sampled in this study typically utilize habitats from brackish rivers, bays, and sounds out to the edge of the continental shelf break. Within these habitats they are known to consume a wide variety of prey.

Loggerheads in the study area are known to migrate seasonally in search of warmer waters, either south or further offshore. This mobility makes establishing relationships between mercury contamination and environmental parameters more complicated than sampling shellfish or residents of isolated lacustrine habitats. Their foraging patterns are not very well understood, but recent data from North Carolina and South Carolina (SCDNR unpublished data, Avens in press) provide strong evidence of foraging site fidelity. These data (Avens) show subadults displaced from foraging grounds return to the same sites from which they were capture that same season. Tag-recapture data (SCDNR) also suggest subadults return to the same regions in subsequent seasons.

The contribution of mercury from winter foraging grounds in south Florida, the

Bahamas, or the Caribbean to long-term accumulation is unknown. Considering this

migratory behavior and the apparent relationship of blood and keratin mercury

concentrations to long-term accumulation, it is not surprising that there was no

relationship between the capture location and mercury concentrations in these tissues.

However, isolating the recent intake of mercury in the blood by normalizing by the

keratin mercury concentration makes comparing the recent mercury intake and

environmental parameters possible. This approach would require an individual to forage

in the same area for only a couple of weeks for the blood to reflect the available mercury

in prey from a given area.

The significant relationship between the IRE and the proximity of the capture site

to the nearest major river provides compelling evidence there is an environmental

gradient of mercury in the neritic zone in the study area (Figures 15-16). The gradient is

assumed to be a result of higher mercury loads in loggerhead prey near terrestrial sources

of mercury. Collecting and analyzing selected prey species caught as bycatch from the

same tows would be labor intensive, but would be a very interesting follow-up to these

results. However, the reduction in the biomagnification of mercury at these lower trophic

levels may make detecting differences in contamination difficult. Other large predators

that inhabit the coastal environment (sharks, king mackerel, grouper/seabass) may reveal

similar patterns and could be targeted for future investigation.

There are several processes that could explain this geographic trend, both natural

and anthropogenic. These samples were collected from over 640 kilometers of the

southeast U.S. coastline. These river systems were chosen as reference points due to the

presence of major point-sources of mercury pollution (coal burning power plants, steel mills, chlor-alkali plants). While mercury is generally considered to be a ubiquitously distributed pollutant, the form of mercury being released determines the range of atmospheric transport. Gaseous elemental mercury (Hg^0) makes up the majority of what is emitted into the atmosphere and has global-scale transport and atmospheric residence times of about 1 year. However particulate and reactive gaseous mercury (ionic Hg^{2+}, $HgCl_2$, $Hg(NO_3)_2H_2O$) comprise most of the remainder and have maximum travel distances of only tens of kilometers and short atmospheric residence times (Mason *et al.* 1994). Therefore the particulate and reactive gaseous mercury released from these point sources would be expected to cause some local elevation of mercury in water and soil through wet and dry deposition, and a subsequent increase in biota after methylation.

The industrial rivers used in this analysis also drain some of the largest watersheds in the study area. With the exception of the Altamaha and St. Mary's Rivers, the rivers used in this analysis represent all major river discharges from Georgetown, South Carolina to Jacksonville, Florida. Broad-scale atmospheric deposition and natural geological sources of mercury all contribute to the total contaminant load drained from these watersheds. Surface waters and sediments of wetlands draining these watersheds receive the runoff from a sizable area, and may be prone to concentrating mercury. Since the conditions in wetlands are among the most favorable for mercury methylation (Krabbenhoft *et al.* 2001), these waters would be expected to have far more bioavailable mercury than oceanic water. The higher IRE in loggerheads utilizing habitats near these river mouths is in agreement with what would be expected based on the current understanding of the biogeochemical cycling of mercury. Furthermore, a recent study

(Hyland *et al.* 2003) investigating heavy metal contamination in sediments off the coast of Georgia also found higher levels of mercury, arsenic, and lead in nearshore waters (Figure 24). This provides evidence for the environmental variation in contaminants that allows the biomagnification of mercury to occur in these areas.

The live loggerheads sampled in this project came from a large latitudinal range, but were all captured from 1.4-9.4 miles from land. The stretches of coastline with relatively little river outflow were where IRE values were the lowest. However, sampling individuals utilizing habitats further offshore was impossible due to restraints in the methodology of the sea turtle abundance survey. Future comparison of turtles utilizing the deep water habitat of the shelf break to turtles foraging in estuaries would provide an interesting look at the differences in contaminant loads in these two distinct ecosystems. Comparison of pelagic juveniles to recent recruits into benthic habitats would also contribute to the understanding of the accumulation of mercury in this species through its very distinct life stages.

While the abundance and bioavailability of mercury in the environment determines the degree of contamination at lower trophic levels, prey selection is often more important in determining contamination for higher level consumers. For example, toothed whales that feed on fish and other marine mammals accumulate orders of magnitude more mercury than planktivorous species (Woshner *et al.* 2001). Stomach contents of loggerheads range from jellyfish and soft corals, which probably have very low levels of mercury, to potentially more highly contaminated species such as fish, crab, and whelks. However, there were no correlations between the IRE and the species composition and abundance of the bycatch at the capture sites. No patterns were

discernible regardless of the degree of taxonomic grouping or which species were included in the analysis as potential prey. This could be partially due to the fact that the presence and abundance of a given prey species does not necessarily imply it is being ingested. Gastric lavage would be an effective way to remedy this problem. Using stable isotope analysis of ^{15}N would also allow the discrimination between differences in the trophic level at which the turtles have been feeding.

Health impacts

Immunotoxicity

The immune system can serve as a very sensitive indicator of toxic insults (Luster and Rosenthal 1993). Immunotoxicity can occur at concentrations well below concentrations associated with overt toxicity, but it is more difficult to identify in the etiology of health problems because it serves only a facilitative role in the onset of the apparent medical condition. The prevalence of fibropapillomatosis in near-shore waters, areas adjacent to large human population, and areas with low water turnover (Limpus and Miller1990, Balazs and Pooley 1991) suggests that environmental stressors may play a role in the pathogenesis of this disease. The present study shows a significant negative correlation between blood mercury concentration and lymphocyte proliferation (Figure 17). This finding is consistent with published immunotoxic effects of mercury, including mercury-induced decreases in lymphocyte proliferation in both humans (Zelikoff *et al.* 1994) and whales (DeGuise *et al.* 1996). Total white blood cell counts are also significantly lower in individuals with higher blood mercury concentration (Figure 18).

Although causal relationships cannot be established with this type of field study, the negative relationships between blood mercury concentration and both b-cell proliferation and total white blood cell count suggest that immunosuppression is occurring in this wild population of loggerheads. Alternatively, the effects observed here could be from other contaminants or environmental stressors whose concentrations correlate with mercury. PCB's are also known immunosuppressors, and may have a geographic gradient similar to mercury. The actual health impact of this degree of suppression is not yet understood, but this decrease in fitness may increase the susceptibility of loggerheads to viruses such as fibropapillomatosis or opportunistic infection resulting from normally non-fatal injuries.

<u>Overt toxicity</u>

These data agree well with previous studies suggesting that despite their long life-span, sea turtles generally do not accumulate mercury as prolifically as more piscivorous species like marine mammals and seabirds (Figure 1). However the concentrations at which overt toxicity and immunotoxicity occur may vary depending upon the species and tissue in question. There have been no clinical studies on the effects of any contaminants on sea turtles using blood or other tissues. Other than one recent study pertaining to organochlorines and pesticides (Keller, in press), there are also no existing correlative field studies comparing contaminants to any sort of biomarkers for health indices in live sea turtles. Due to the current lack of work in this taxon, these results must be placed in the context of the body of literature available for other taxa.

The majority of loggerheads in this study had blood mercury concentrations below levels considered toxic in other species. However, blood mercury concentrations for one stranded turtle (0.306 µg/g) and one live capture (0.188 µg/g) were within or near the 0.2-0.5 µg/g range that may cause smptoms of methylmercury poisoning in humans (Wheatley *et al.* 1979). The corresponding high mercury concentration in other tissues from these two highly contaminated turtles suggests these high mercury levels in the blood are persistent, and represent chronically high concentrations. Many of the symptoms of methylmercury poisoning are neurological and behavioral and would be difficult to quantify even for a captive sea turtle. The highly contaminated live turtle appeared to be healthy (normal hematocrit, total protein and glucose) and of normal body weight, suggesting that any possible neurological effects were not affecting its foraging behavior. The post-mortem exam of the stranded turtle with high mercury levels revealed hemorrhaging around the cerebellum, but the implications of this are unclear.

In vertebrates the liver is typically the organ with the highest concentration of mercury. It is thought that the liver serves as a storage and detoxification site for mercury, converting the highly toxic methylmercury into less harmful inorganic mercury (Henny *et al.* 2002, Storelli *et al.* 1998a). The highest concentration found in liver tissue in this study was 1.34 µg/g (also from the aforementioned stranded turtle). The liver of this individual was described as congested with spider-liker blood vessels during the post-mortem exam. This liver total mercury concentration is considerably lower than values often reported for sea birds and marine mammals (Figure 1). Previous studies have shown a decrease in % methylmercury in the liver occurs at total mercury concentrations above 110 µg/g in striped dolphin (Storelli *et al.* 1998), 10 µg/g in Beluga

whales (Becker *et al.* 2000), around 7.5 μg/g in various seabird species (Thompson *et al.* 1990), and less than 1 μg/g in loggerheads (Storelli *et al.* 1998). These points approximate the threshold concentrations at which a dose dependent demethylation of mercury occurs to protect against mercury toxicity. This suggests that either loggerheads are much more efficient at demethylating mercury and consequently do so at even low concentrations, or they have a higher sensitivity to methylmercury than their homeothermic counterparts and must begin demethylation at a much lower concentration. Whether either of these explanations is accurate requires further investigation.

Like all marine osmoregulators, sea turtles have evolved mechanisms to cope with their hypertonic environment. In this case an organ known as the salt gland located behind the eyes plays a major role in the concentration and excretion of major ions such as sodium, chloride, and magnesium (Nicholson and Lutz 1989, Hudson and Lutz 1985). The strong negative correlation between blood mercury concentration and sodium concentration in the plasma may indicate disruption of ionoregulation at the cellular level. It has been demonstrated that mercury interacts with phospholipids monolayers and model membranes of human erythocytes (Rabenstein and Isab 1982). Mercury in red blood cells can inhibit Na^+/K^+-ATPase via membrane sulfhydryls (Massaro 1997) and decrease transmembrane sodium gradients in muscle cells (Massaro 1997). Intracellular concentrations of sodium are generally very low relative to extracelluar concentrations in vertebrates. The cellular damage described above may inhibit the cell's ability to maintain the large electrochemical gradient for sodium by decreasing the selective permeability of the cell membrane and disrupting active ion pumping. This would cause

an increase in the sodium in red blood cells and a corresponding decrease of sodium in the plasma similar to that observed here.

The results reported here show significant negative relationships between mercury and several indicators of immune function and plasma sodium concentration. This suggests that mercury does have physiological effects even at the relatively low concentrations found here. Assessing the overall health impacts of these physiological trends is beyond the scope of this project. Assessing growth rates, fecundity, or other measures of fitness would require controlled experiments. Based on the physical condition of the live captures, it does not appear that the mercury burden experienced by these individuals has a profound impact on their health. However liver damage and neurological impairment would not be evident from the blood chemistry and physical exams performed. The decrease in immune response is another effect that may not express itself until challenged by a pathogen.

It is interesting that mercury concentrations in blood and keratin from dead turtles was significantly higher than in live turtles (Figure 16). This trend is opposite of what would be expected from the size distribution of these two groups (live SCL = 72.1 cm, dead SCL = 61.9 cm) and the bioaccumulatory effects previously presented. When considering the dynamic nature of blood, the trend in this tissue could be an artifact of post-mortem changes such as dehydration. However the mercury bound to keratin has been shown to be very resistant to a variety of treatments considerably more rigorous than that experienced by the loggerhead carcasses (Appelquist *et al.* 1984). This included extreme ranges in heat, UV radiation, and weathering maintained over extended periods.

Therefore the mercury concentrations in scutes from strandings can legitimately be compared to scutes from live captures.

There are several possible explanations for individuals with higher mercury burdens having higher mortality rates. (1) Mercury could be a contributing factor to their mortality. In light of the immunotoxicity data presented, the most plausible mechanism for this explanation is a decrease in the ability to cope with attacks from pathogens. It is also possible that neurological impairment changes behavior in a way that increases mortality (e.g. decrease in visual acuity or response time for preventing boat strikes, entanglement, or drowning in trawls) (2) Individuals with a preexisting physical ailment may be less efficient in eliminating mercury from their bodies. This would result in more rapid accumulation of mercury in these turtles that eventually strand for other reasons. (3) Factors positively correlated with mercury accumulation could contribute to mortality. The geographic trends reported in the present study show higher mercury bioavailability near major rivers. It is very likely that other anthropogenic pollutants such as pesticides, PCBs, and other heavy metals share a similar distribution and may act cumulatively (and possibly synergistically). Concentrations of natural stressors such as parasites, mycotic, microbial, and viral pathogens may also be concentrated in these areas. The incidence of boat strikes, entanglement, and drowning by trawl would also increase in inshore or nearshore waters. These factors could create a bias in the strandings toward turtles that utilize these more highly contaminated areas.

There is probably some truth in each of the explanations offered above. It is unlikely that the mercury burdens in these dead turtles were high enough to cause organ failure or fatal methylmercury poisoning. In light of the immunotoxicity data, it is quite

possible that individuals with higher mercury burdens were more susceptible to infection from normally non-fatal injuries. The three stranded turtles with the highest mercury concentrations in the keratin and spinal cord (CCS4, CCS5, and CCS8) did have either fresh or old wounds from boat strikes. These three keratin mercury concentrations rank 1, 2, and 5 among live and dead turtles. CCS4 also had brain hemorrhaging that is consistent with bacterial encephalitis (George *et al.* 1995). This animal was found floating alive, but moribund, and died shortly after being taken in for treatment. Whether the high mercury levels played a role in the etiology of these symptoms is unknown.

Future efforts to investigate the role of contaminants in sea turtle health would benefit from more thorough necropsies to determine the cause of death. Increasing the sample size of strandings (and possibly expanding sampling efforts to less fresh strandings by collecting only keratin samples) would also be prudent to minimize the effects of sampling error. Coordination with sea turtle rehabilitation facilities would also prove useful. Blood samples obtained upon entry and release would provide an unbiased comparison to healthy live captures and possibly elucidate the relationship of contaminant levels to overall health

CONCLUSIONS

Loggerhead sea turtles are a species at risk from a number of anthropogenic

effects, with chemical pollutants playing an uncertain role. This study provides the first

data for mercury contamination in loggerheads in the western north Atlantic. This

population is similar in contaminant load to loggerheads in the Mediterranean despite the

potential for higher contamination in that region from the high population density, semi-

enclosed basin, and geothermal activity. It is likely that the aquatic habitats of South

Carolina, Georgia, and northern Florida have less total mercury input than the

Mediterranean, but higher methylation efficiencies, making the accumulation of mercury

in these populations comparable.

Monitoring programs in wildlife toxicology are usually concerned with the health

of the selected species, its use as a food source, or employing the target as a sentinel

species to monitor the health of the ecosystem from which it was collected. Regardless

of the motivation, measuring contaminant concentrations in easily collected tissues must

be accompanied by additional data that allow for the meaningful interpretation of these

values. Informative monitoring of contaminant risks to wildlife requires the tissues

analyzed to be of physiological importance, or to adequately predict contaminant

concentrations in organs that are physiologically important. The results presented here

suggest that either blood or scutes can be used to approximate the mercury concentrations

in muscle and spinal cord in loggerheads. While mercury concentrations in blood and

scutes do reflect large differences in the liver and kidney total mercury concentrations,

these tissues are more difficult to predict. This is because the insoluble inorganic

mercury stored in the liver and kidney is not readily remobilized and therefore is not in

equilibrium with the blood. Measurement of methylmercury in liver and kidney would

probably reveal that blood and scutes more closely approximate these concentrations.

Generally speaking, the use of blood for contaminant analysis may be appropriate for

some species and not for others. Workers must consider the relative contributions of

recent dietary intake and long-term exposure, the dynamics of the contaminant in the

blood over time, the relationship of the contaminant concentration in blood to other

tissues of interest, and the form of the contaminant in the blood and other tissues of

interest. These factors will determine if the blood contaminant concentration reflects the

chronic exposure of the animals sampled or the short-term intake in a given region.

Loggerheads captured in the wild show a significant increase in mercury

contamination with size. This shows that the accumulation of mercury exceeds the rate

of elimination in this region. The shedding of the mercury-rich scutes that armor the

body is a significant route of elimination for this metal, but off-loading into eggs does not

appear to reduce the mercury loads in mature females. The habitat utilization of the

demersal life-stage of the loggerhead has a significant impact on the short-term mercury

intake. Higher recent exposure was detected in individuals captured closer to the mouths

of major industrial river systems. This meso-scale geographic gradient in mercury

contamination suggests that local terrestrial sources of mercury are contributing to the

accumulation of mercury in predators in the near coastal zone. This spatial variation in

environmental mercury combined with the inter-annual foraging site fidelity of loggerheads may explain the high variability in mercury burdens among individuals. The two highly contaminated loggerheads could be returning to heavily polluted sites to forage seasonally. Determining if similar patterns exist for commercially or recreationally harvested species that accumulate mercury more prolifically (king mackerel, sea bass) could have implications for fish consumption advisories. The sea turtle abundance survey is likely to be repeated in decades to come to assess changes in the loggerhead population status. Repeating the sampling protocol developed for this study will provide an effective and reproducible way to monitor long-term trends in mercury contamination in loggerheads and in our near-coastal ecosystem as a whole.

The tissue concentrations found in the present study were generally below the mercury toxicity thresholds reported for these tissues in other species. The exceptions are the highly contaminated stranded turtle (CCS4) and highly contaminated live capture (CC2151). These individuals had blood mercury concentrations that are in or near the range that causes symptoms of methylmercury poisoning in humans. Approximate brain and liver concentrations for these individuals are below the concentrations shown to have detrimental effects on other species. The available data on these types of toxicity thresholds show a tremendous amount of variability between species and individuals, and are largely limited to mammals, fish, and birds. They also do not adequately address the effects of chronic low-level exposure, reproductive success, and behavioral effects such as predator avoidance and prey capture. These behavioral effects have been documented for fish at concentrations well below levels that would cause normal toxicity (Wiener and Spry 1996).

Some of the more subtle effects of mercury exposure measured in this study reveal that even low concentrations of this highly toxic metal can impact physiological processes. There is a decrease in sodium concentration in the plasma portion of the blood with increasing mercury concentration that may suggest a disruption in the homeostasis of intracellular and extracellular sodium concentrations. There are also two independent measures of immune health that are negatively correlated with blood mercury concentrations. The total white blood cell count and lymphocyte proliferation both decrease with increasing mercury concentration. A compromised immune system would render these animals more susceptible to infections such as fibropapillomatosis, whose occurrence shares the inshore distribution shown for mercury (and probably other contaminants) in this study. This would also increase the severity of opportunistic infections in individuals that have suffered from injuries or ingested water. Scute mercury concentrations were significantly higher in strandings than in live captures, and the three strandings with the highest scute mercury concentrations did suffer from boat strike injuries varying in severity. However confounding factors, a lack of pathological data, and a small sample size make drawing conclusions about the role of mercury here hypothetical.

Clinical studies on sea turtles are unlikely due to their threatened status, but toxicological work on surrogate reptilian species would improve our understanding of their contaminant resistance. This study provides the first large survey of mercury contamination in free-ranging loggerheads and valuable information for approximating concentrations in important organ systems. Using these data as a reference will allow laboratory toxicity tests to be placed in a realistic context for wild populations.

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TABLES

Table 1. Total mercury concentrations in *Caretta caretta* tissues from this study and
previous studies. Reported values are arithmetic means in µg/g (wet mass) unless
otherwise stated.

	Present study, South Carolina, Georgia, Florida, U.S.					Presti (1999), Texas, U.S.		
	Strandings (n = 6)			Live captures (n =34)		Loggerheads (n = 3)	Kemps Ridley (n = 100)	
	Mean	*SE*	*Geo. Mean ± 1 SD*	*Mean*	*SE*	*Mean*	*Mean*	
Keratin	0.941	0.299		0.461	0.087	0.236	0.920	
Blood	0.099	0.042		0.029	0.008	0.015	0.027	
Skin	0.255	0.106						
Spinal cord	0.076	0.031		*Geo. Mean ± 1 SD*			*Median*	*Mean ± SE*
Muscle	0.155	0.070		**Sakai et al. (1995), Japan**	**Storelli et al. (1998a), Mediterranean**	**Storelli et al. (1998b), Mediterranean**	**Godley et al. (1999), Mediterranean**	**Gordon et al. (1998), Australia**
Kidney	0.214	0.046		n = 7	n = 12	n = 4	n = 5	n = 6
Liver	0.594	0.155	0.523 ± 0.379	1.51 ± 2.93	0.42 ± 0.26	0.7 ± 0.32	0.602	0.015 ± 0.006

Table 2. r^2 values for regressions between mercury concentrations in tissues from stranded loggerheads. Liver mercury concentration was best explained by keratin mercury concentration, and blood mercury concentration is the best predictor of the overall body burden.

	Liver	Kidney	Muscle	Spinal cord	Mean
Blood	0.892	0.904	0.988	0.988	0.943
Keratin	0.948	0.805	0.816	0.922	0.873
Skin	0.695	0.759	0.959	0.873	0.821
Mean	0.845	0.822	0.921	0.928	0.879

Table 3. The relative importance of the four major tissue compartments for the deposition and storage of mercury was determined. The % body weight for liver, kidney and muscle were from biometric data from Sakai (2000) and % body weight for scutes was determined in the present study. Body burden values were calculated for four stranded loggerheads using the mercury concentrations in the tissues and the mass of the tissues determined by the animals body weight and the % of the body weight these tissues represent.

	Liver	Kidney	Muscle	Scutes
% body wt	2.7%	0.8%	27.2%	1.3%
Mean Hg conc. (μg/g)	0.708	0.252	0.172	1.091
% of Hg body burden	23.2%	2.4%	62.1%	12.2%

FIGURES

Figure 1. Comparison of total mercury concentrations (wet mass) in livers from aquatic consumers. Bars represent upper and lower ranges and numbers in parentheses are sample size. Loggerhead sea turtles generally have lower liver mercury burdens than more piscivorous taxa such as marine mammals and seabirds.
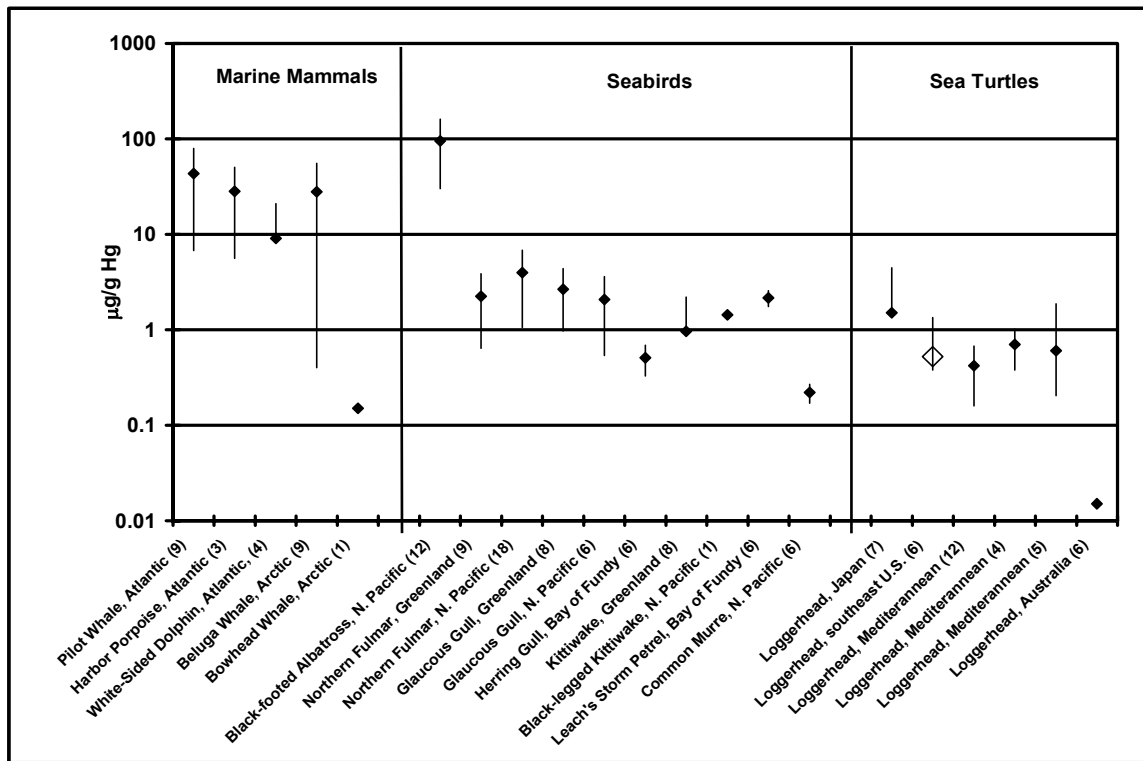
Figure 2. Loggerhead tissues were analyzed in 32 analytical batches from Jan. 24-May 9, 2002 at the NIST laboratory in Charleston, SC. Analytical batches consisted of four turtle tissues samples, a NIST certified standard reference material, and a method blank. SRM2976 (Trace Metals in Mussel Tissue, 0.061 ± 0.0036 µg/g Hg) was used for was used for method validation with all tissues except blood. The analytical method shows a high degree of accuracy, with departure from the 95% confidence intervals occurring in only 3 batches for a mean value of only 1.5 ng/g.
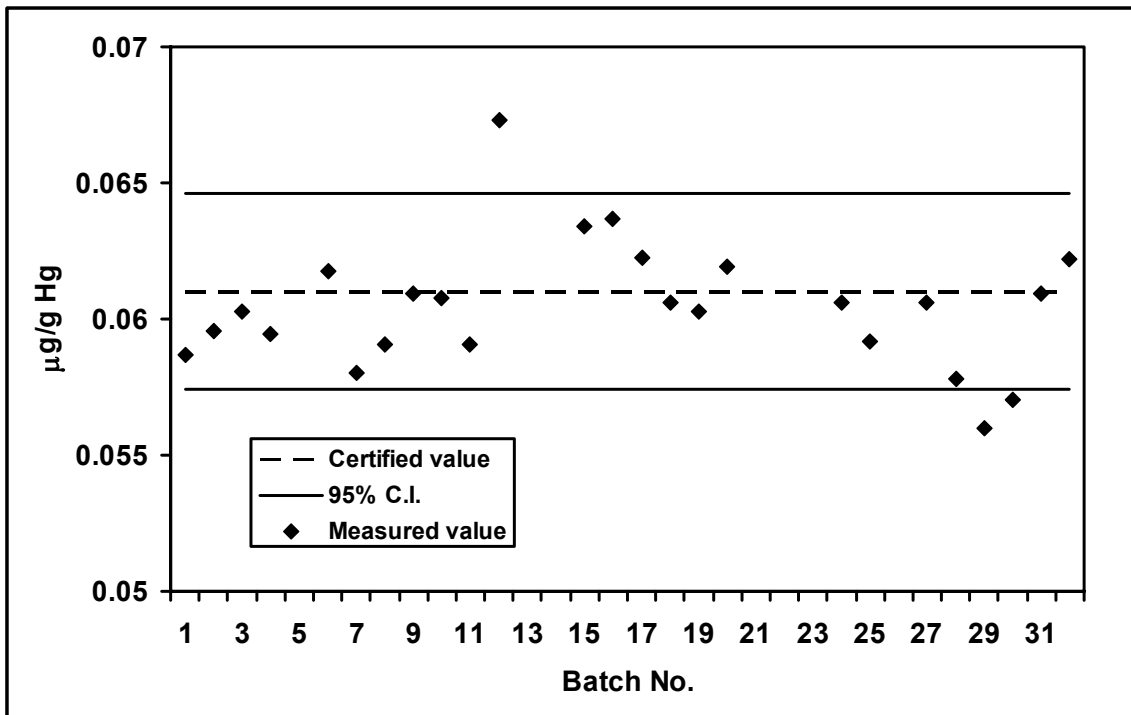
Figure 3. Loggerhead tissues were analyzed in 32 analytical batches from Jan. 24-May 9, 2002 at the NIST laboratory in Charleston, SC. Analytical batches consisted of four turtle tissues samples, a NIST certified standard reference material, and a method blank. NIST SRM966 (Toxic Metals in Bovine Blood-Level 2, 0.0294 ± 0.00161 µg/g Hg) was used for method validation with blood. The analytical method shows a high degree of accuracy, with departure from the 95% confidence intervals occurring once by only 0.4 ng/g.
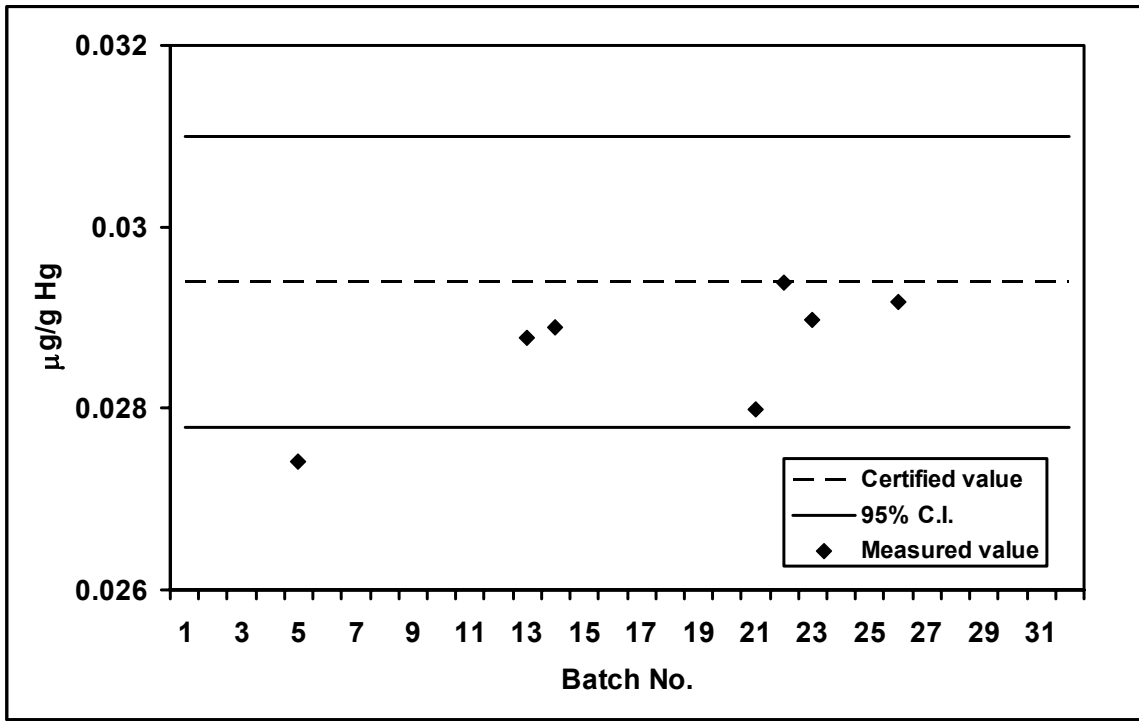
Figure 4. Four aliquots of homogenized NIST SRM1947 Lake Superior Fish Tissue and SRM2976 Trace Metals in Mussel Tissue were analyzed within one batch to determine the reproducibility for the analytical method. Two keratin samples and one blood sample from loggerheads were analyzed to determine additional variability introduced by heterogeneity of mercury in the sample and the collection protocol. Reported values are the coefficient of variation for each sample.
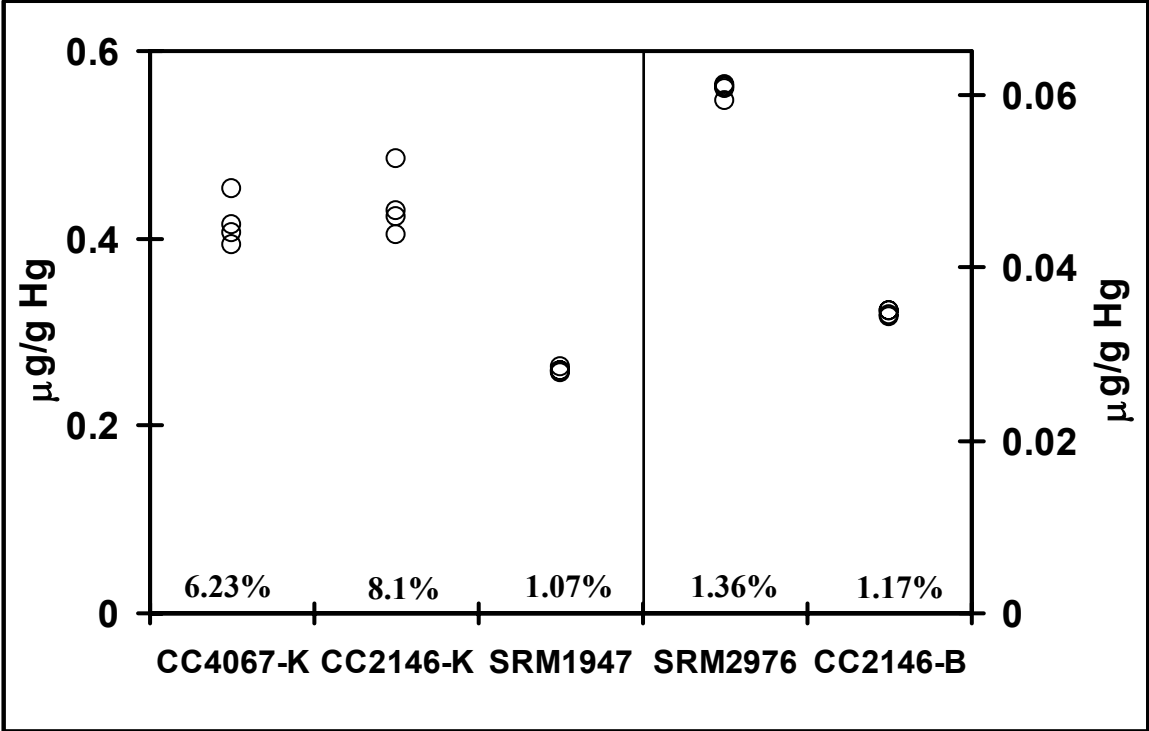
Figure 5. Mean ng mercury in field blanks was below mean ng mercury for procedural

blanks prepared under the same conditions. NaHep and LiHep vacutainers washed with

HNO_3 yielded slightly higher, but still negligible, amounts of mercury.
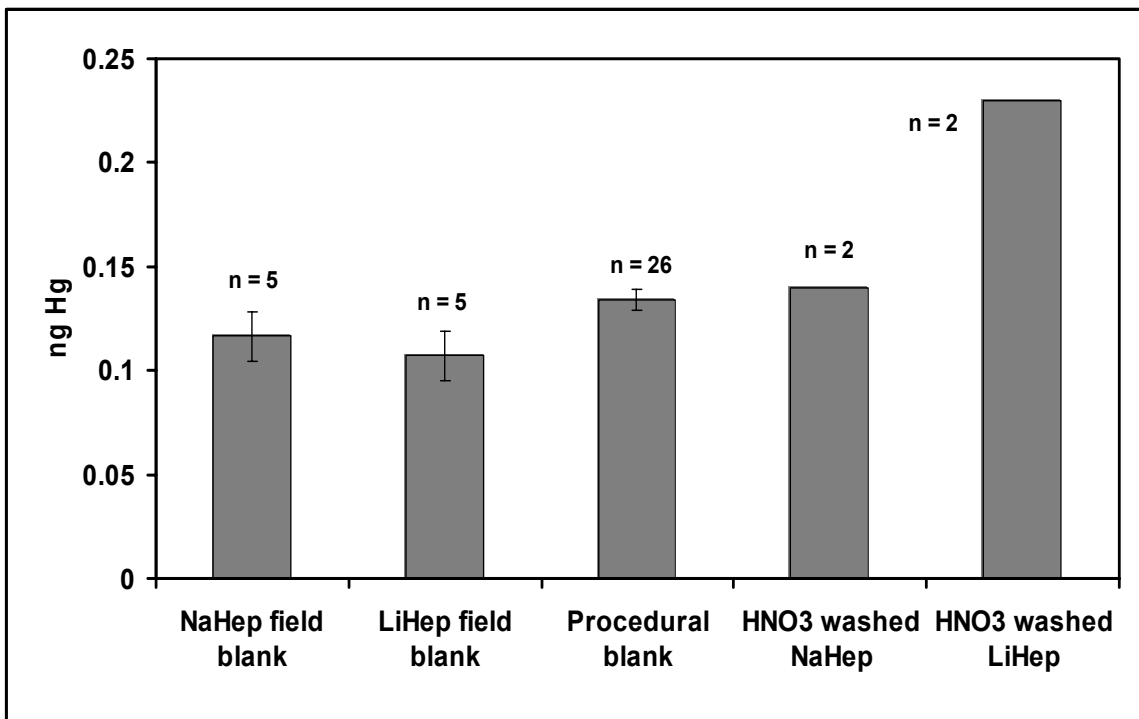
Figure 6. Total mercury concentrations for tissues collected from stranded loggerheads (mean ± SE). Data were log transformed and two-way ANOVA with tissue type and individual was performed ($r^2 = 0.932$, $P < 0.0001$). Significant differences ($P < 0.05$) according to pairwise contrasts are indicated by tissues with different letters.
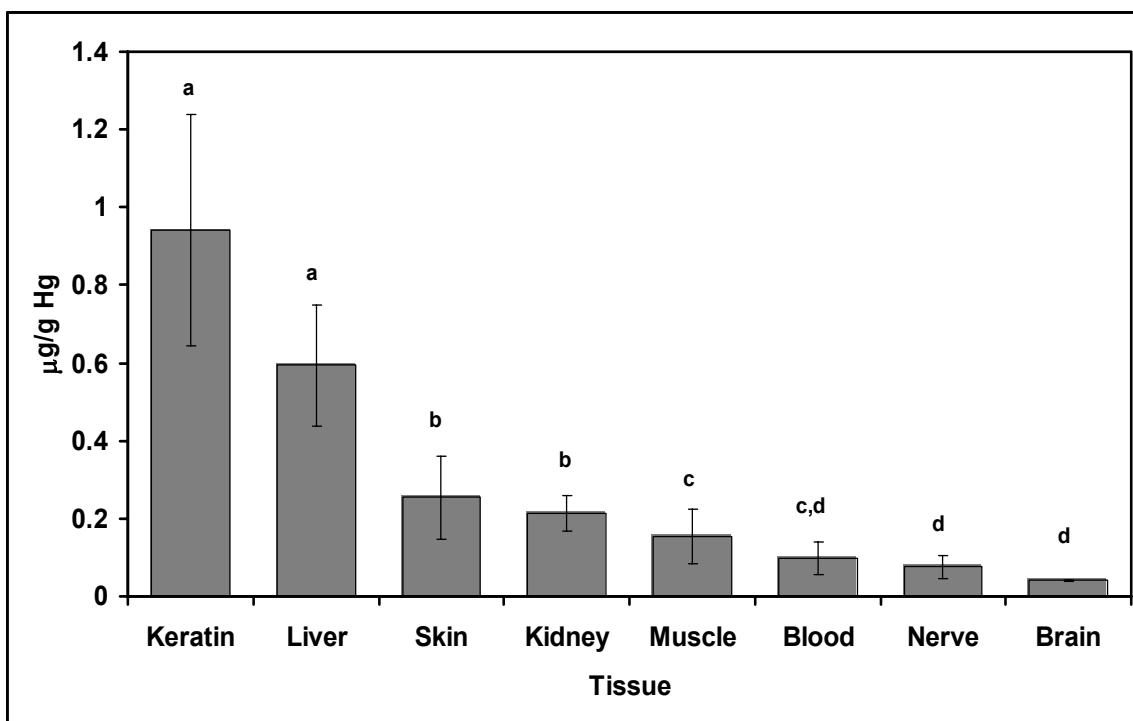
Figure 7. Mean mercury concentrations (± SE) for all tissues from each stranded individual. CCS4 had a significantly higher (P < 0.05) mercury body burden than all other turtles.
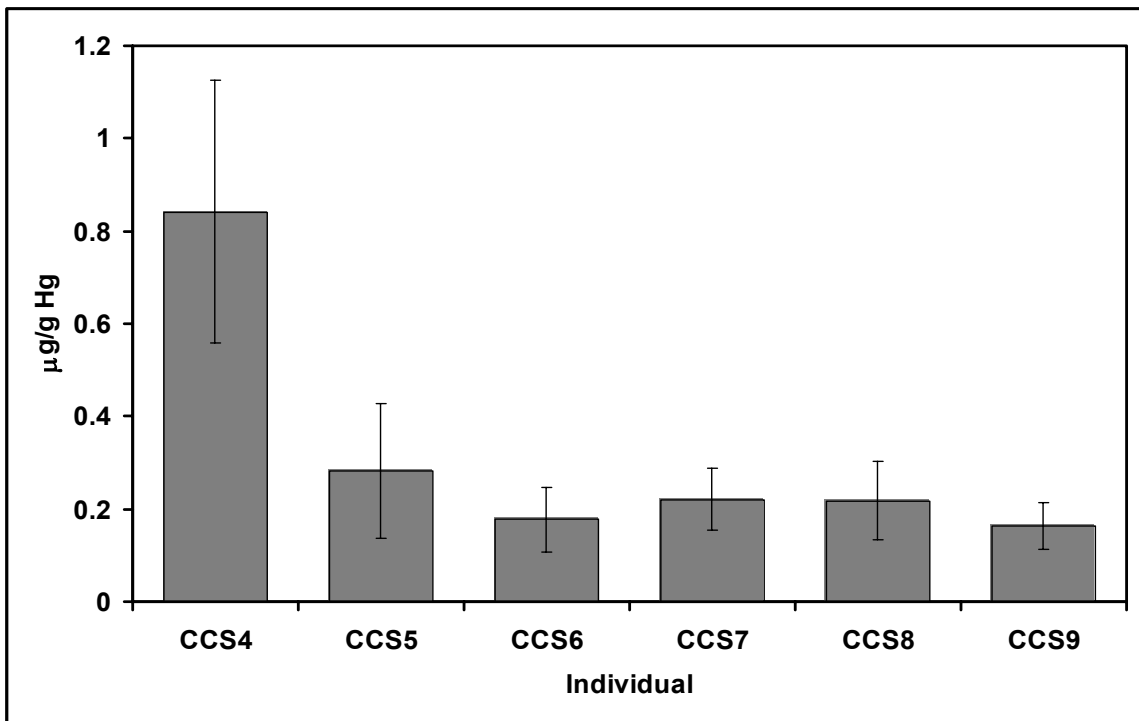
Figure 8. Blood and keratin reflect large scale differences in the mercury loads in liver

and kidney, but are less effective at predicting concentrations in these tissues than in

spinal cord and muscle. This is most likely due to the larger fraction of inorganic

mercury in the liver and kidney for long term storage after demethylation.
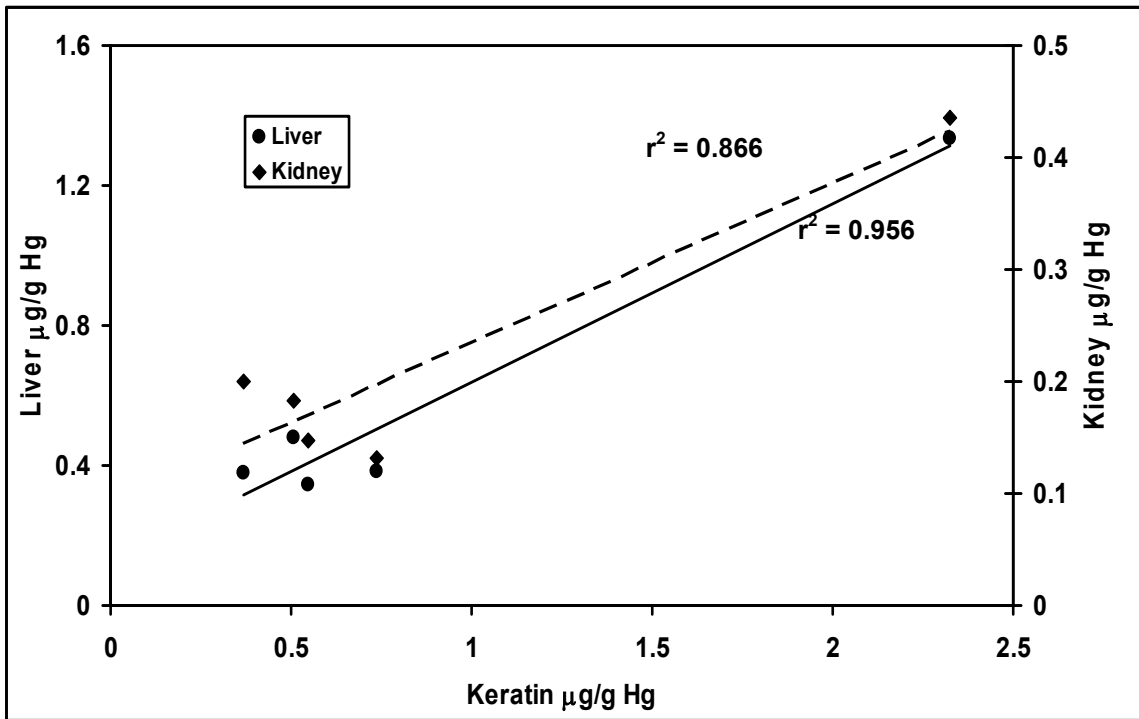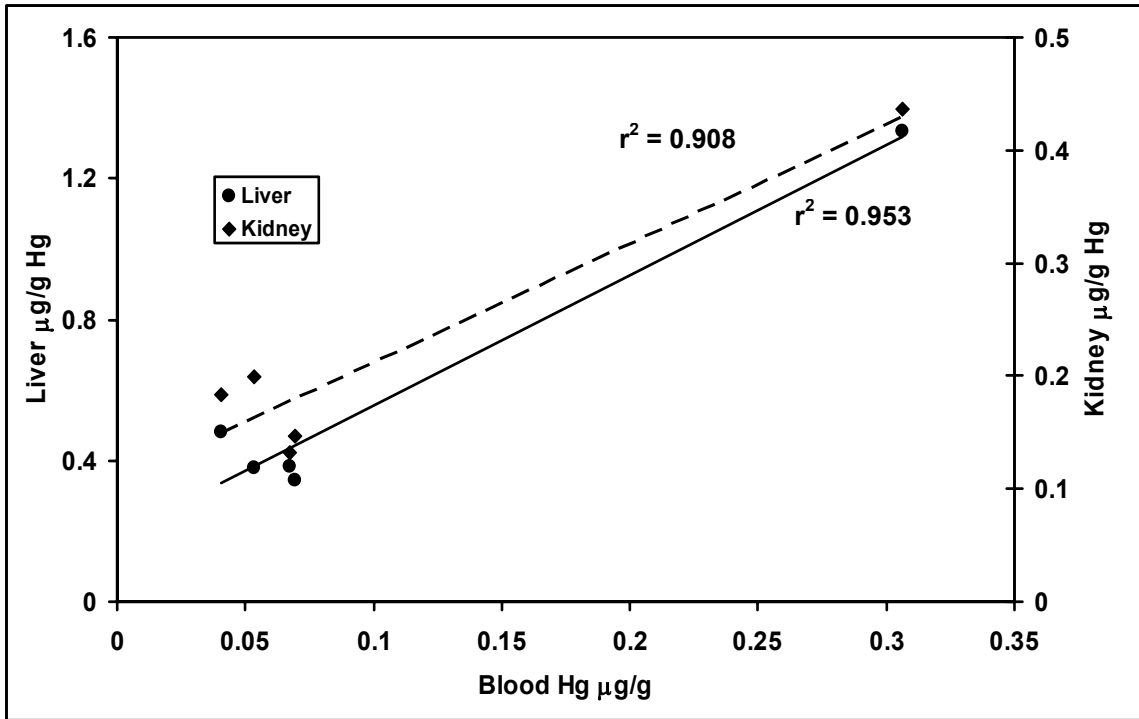
Figure 9. Mercury concentrations in blood and keratin are strongly correlated to mercury in spinal cord and muscle tissues. This suggests that blood and scute scraping are reliable tools for monitoring mercury contamination in loggerheads.
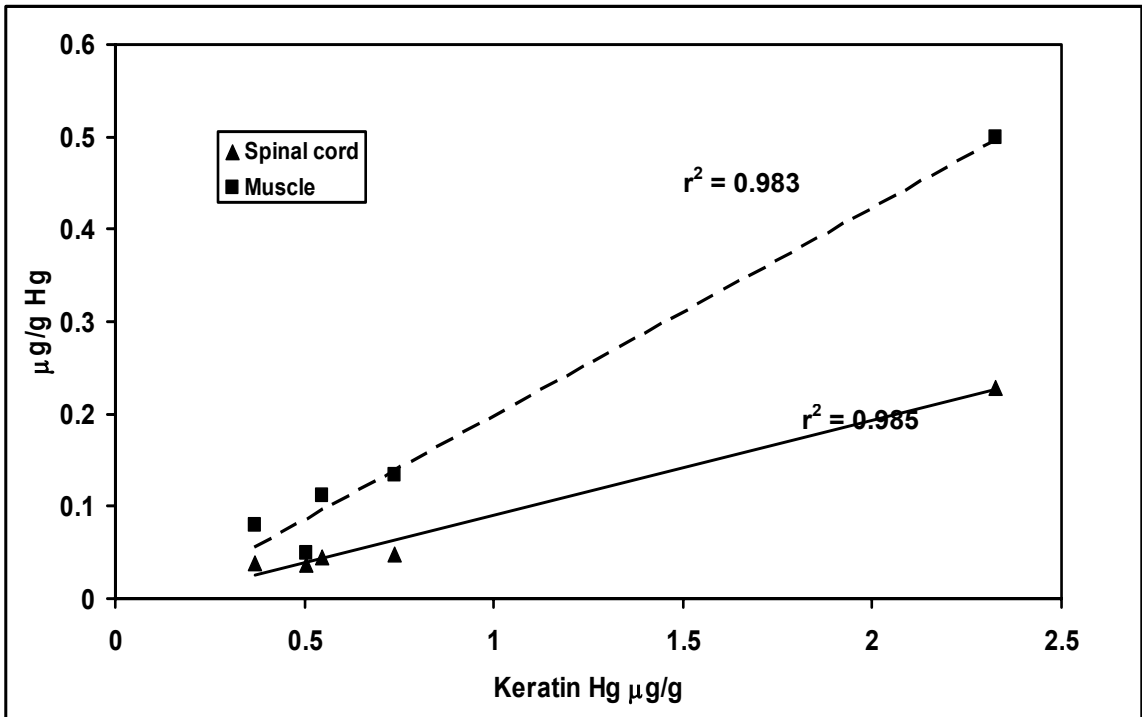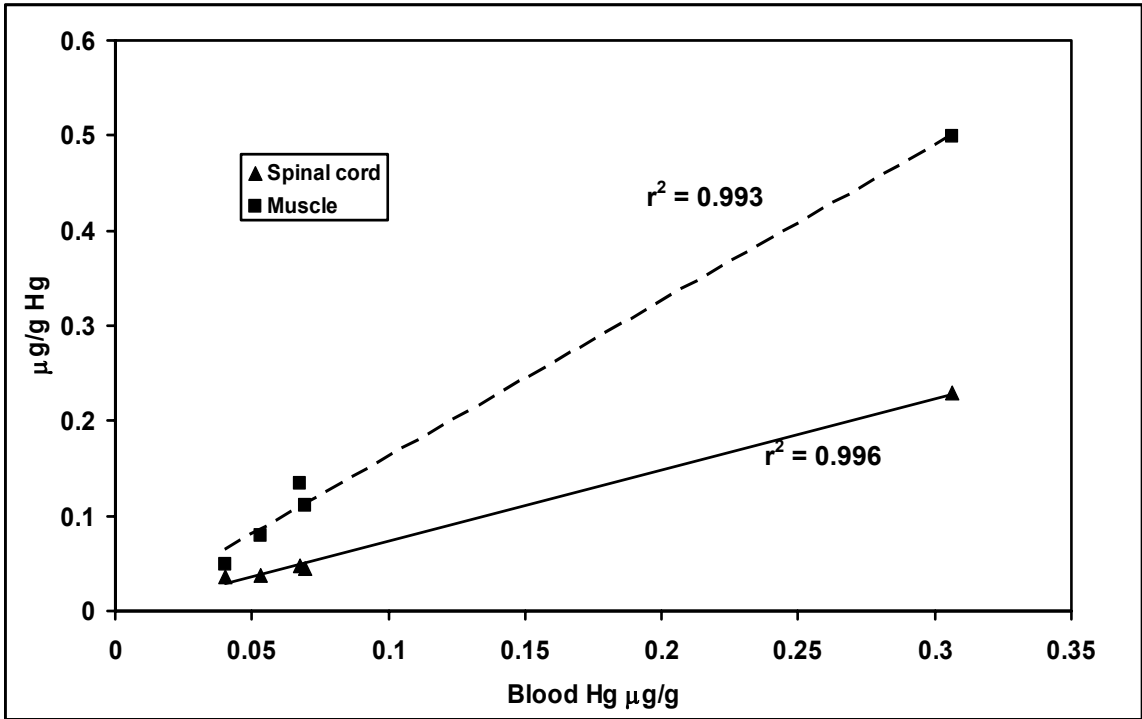
Figure 10. Linear regression of mercury in blood and keratin from each live capture is highly significant (r^2=0.926, P=0.0001).
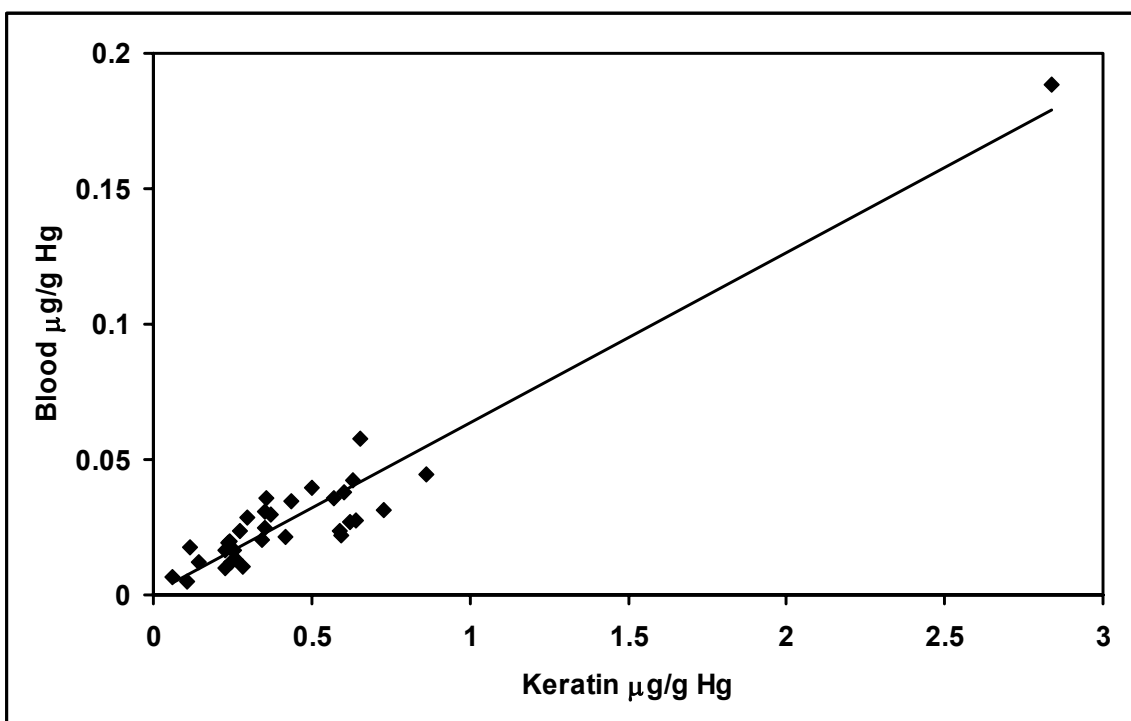
Figure 11. Bioaccumulation of mercury as a function of body weight is significant for both blood and keratin ($r^2 = 0.173$, P = 0.016 and $r^2 = 0.188$, P = 0.012 respectively) for live turtles.

*The one highly contaminated turtle was omitted from this analysis
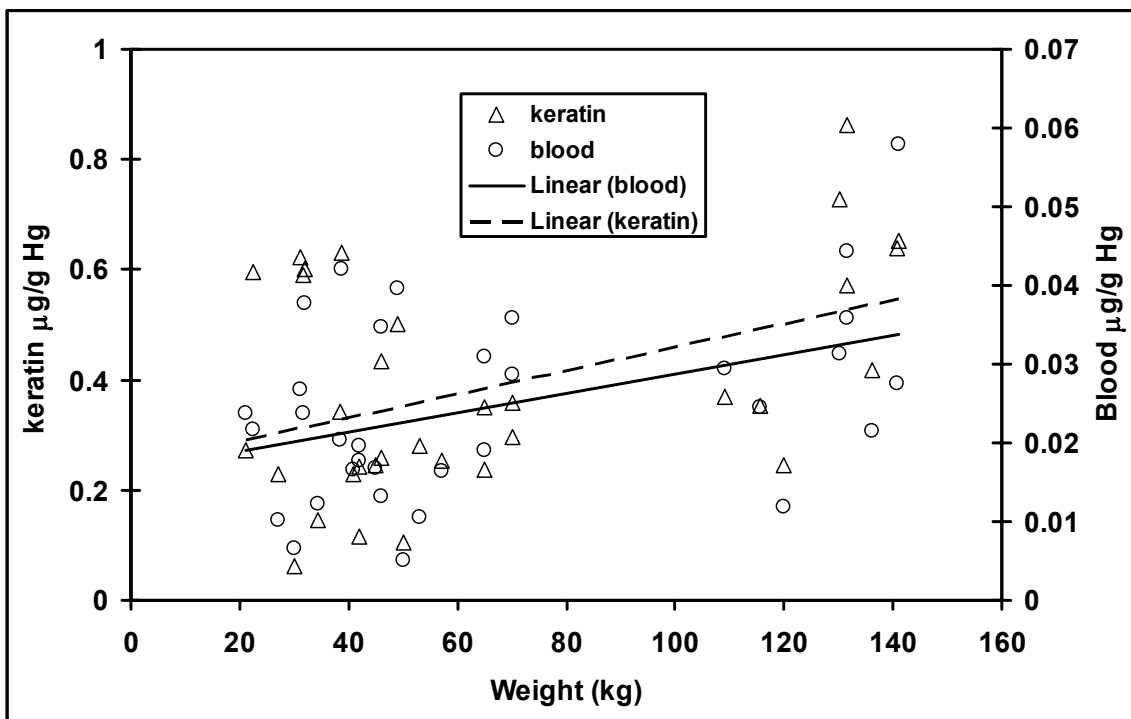
Figure 12. Multiple regression including gender found no significant difference in mercury burdens between males and females. Comparable sizes of each sex are presented here, showing similar mercury contamination for males and females at all size classes. For the sexually mature size class (100+ kg), this suggests that eggs are not a significant route of mercury elimination.
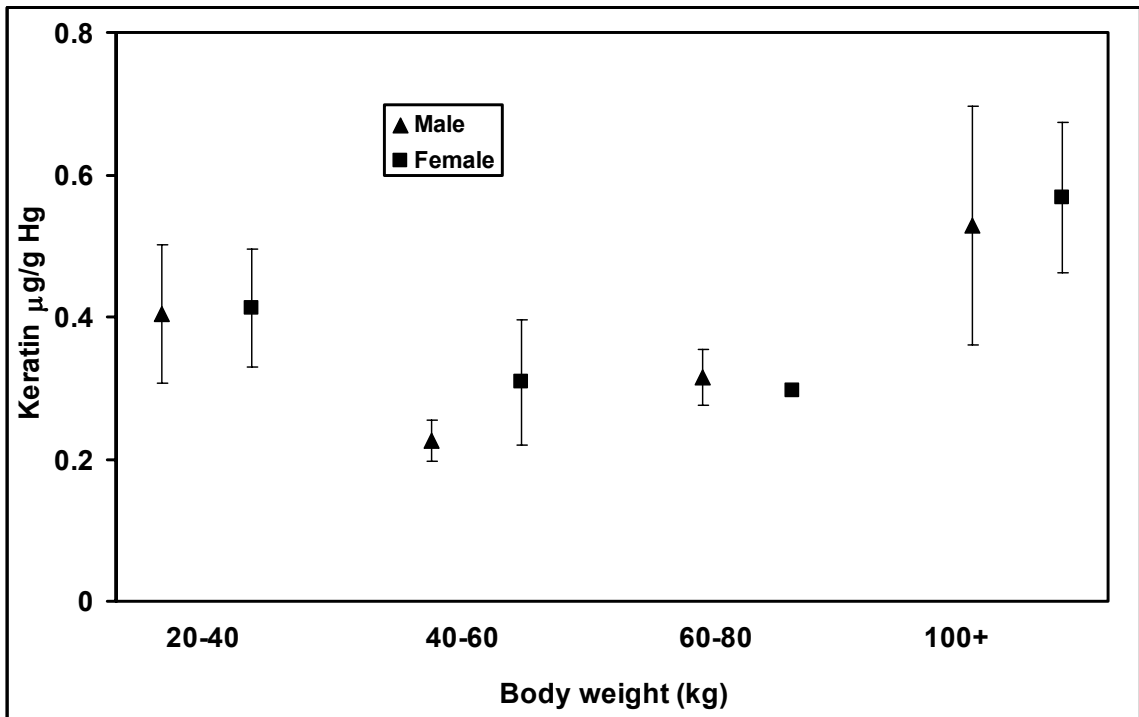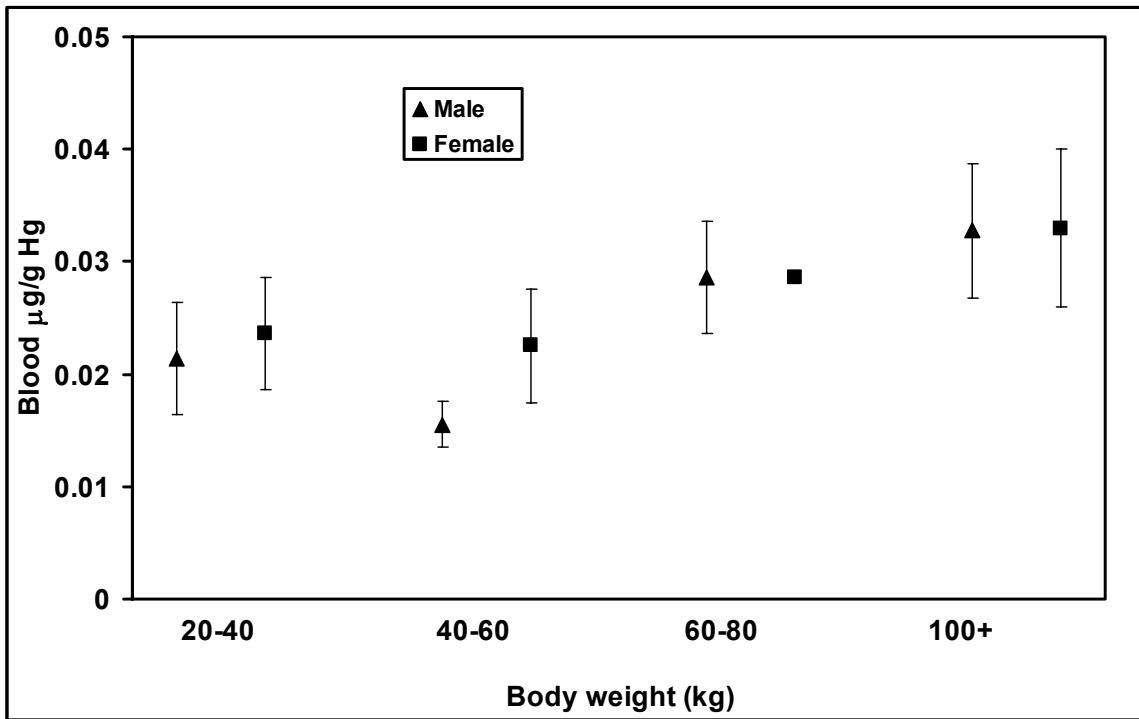
Figure 13. TEM image of the carapace of a loggerhead turtle. Image 1 at lower magnification shows the growing regions (G) where keratinocytes become pyknotic and are deposited in layers (K). Images 2 and 3 show higher magnification of the layers of deposited keratin superficial to the epidermal layer (E).
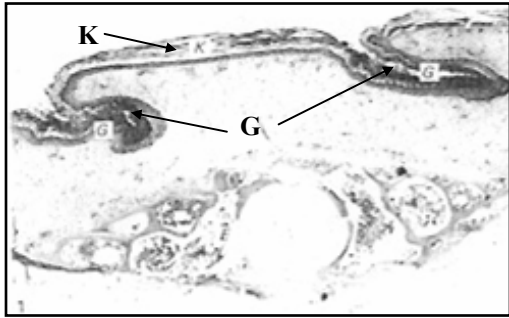
1
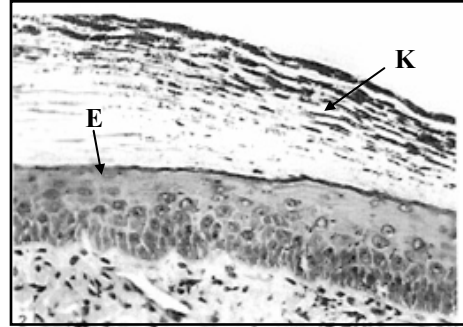
2
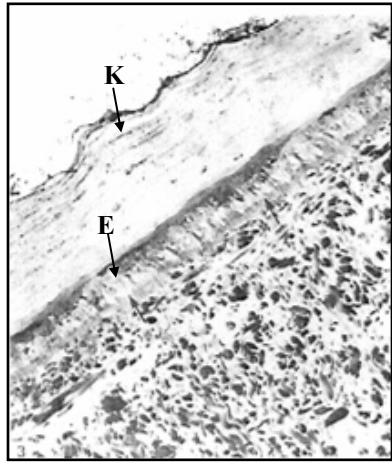
3

Figure 14. The residuals from the blood-keratin regression serve as an index of recent exposure (IRE) by comparing two tissues of differing temporal scales. Positive values represent higher recent mercury intake (as measured in the blood) relative to average past intake (as measured in keratin from carapace scutes).

Blood μg/g Hg

Keratin μg/g Hg

+ IRE

- IRE

+ IRE

- IRE
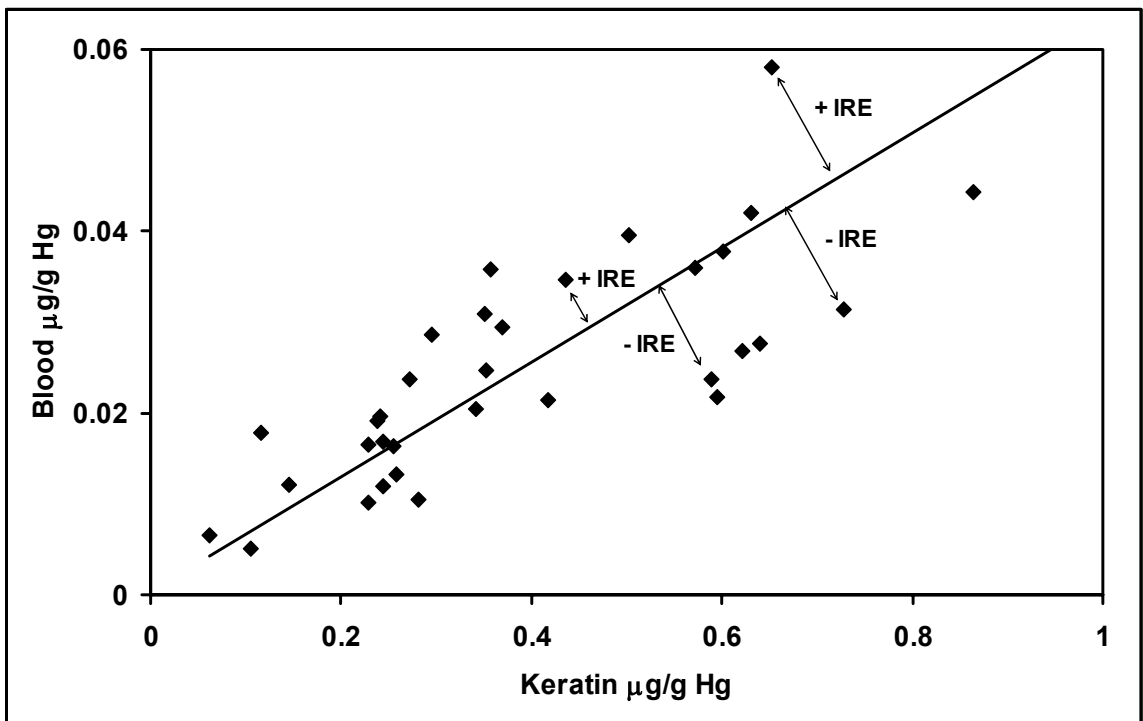
Figure 15. Mean IRE (± SE) for loggerheads captured < 20 miles (n = 18) and > 20 miles (n = 16) from major river mouths. Proximity to major river (P = 0.0120) was the only significant environmental or biological parameter from a stepwise multiple regression analysis of 12 parameters.
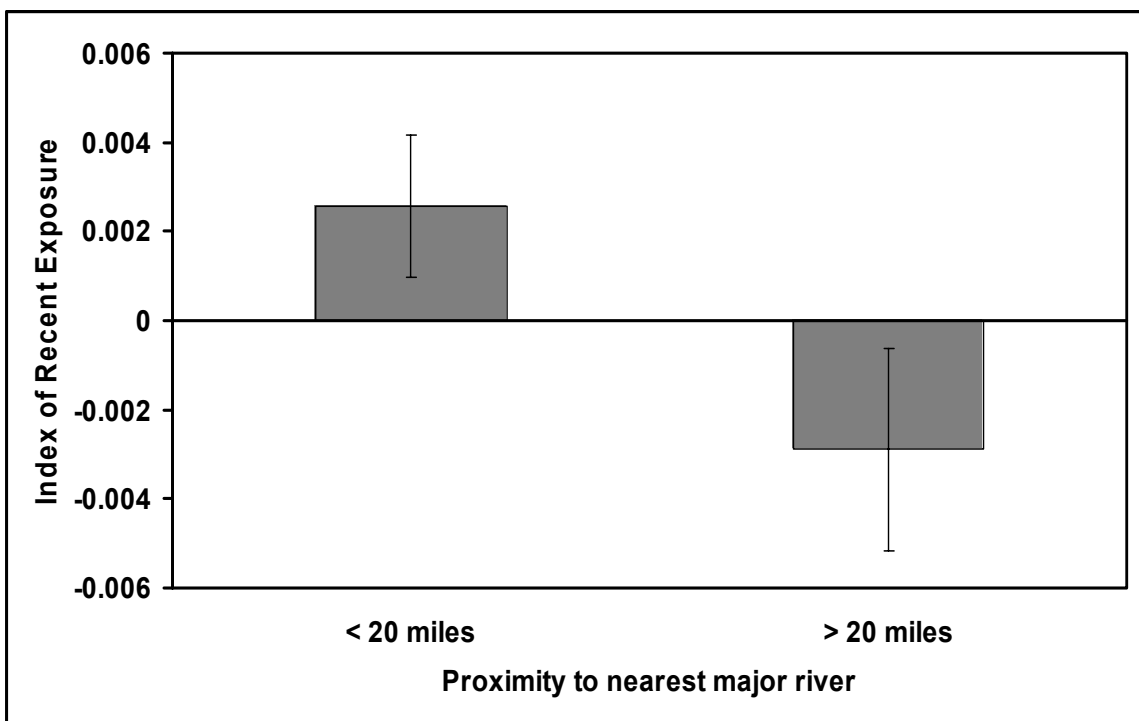
Figure 16. Points represent locations where *Caretta caretta* were captured by trawl during the SCDNR abundance survey in the summer of 2001. Turtles are grouped according to which major river they were associated. The trend in mercury exposure is displayed graphically by using the index of recent exposure values to create an inverse distance weighted surface interpolation map in Arcview. Low recent exposure (light colors) is most prominent away from major river outflow, with higher exposure (dark colors) generally occurring closer to major river outflow. These rivers are where mercury-polluting industrial activity is concentrated and they drain the greatest area of wetlands where methylation occurs most efficiently. The effect seen here is assumed to result from higher mercury concentrations in loggerhead prey in these areas.
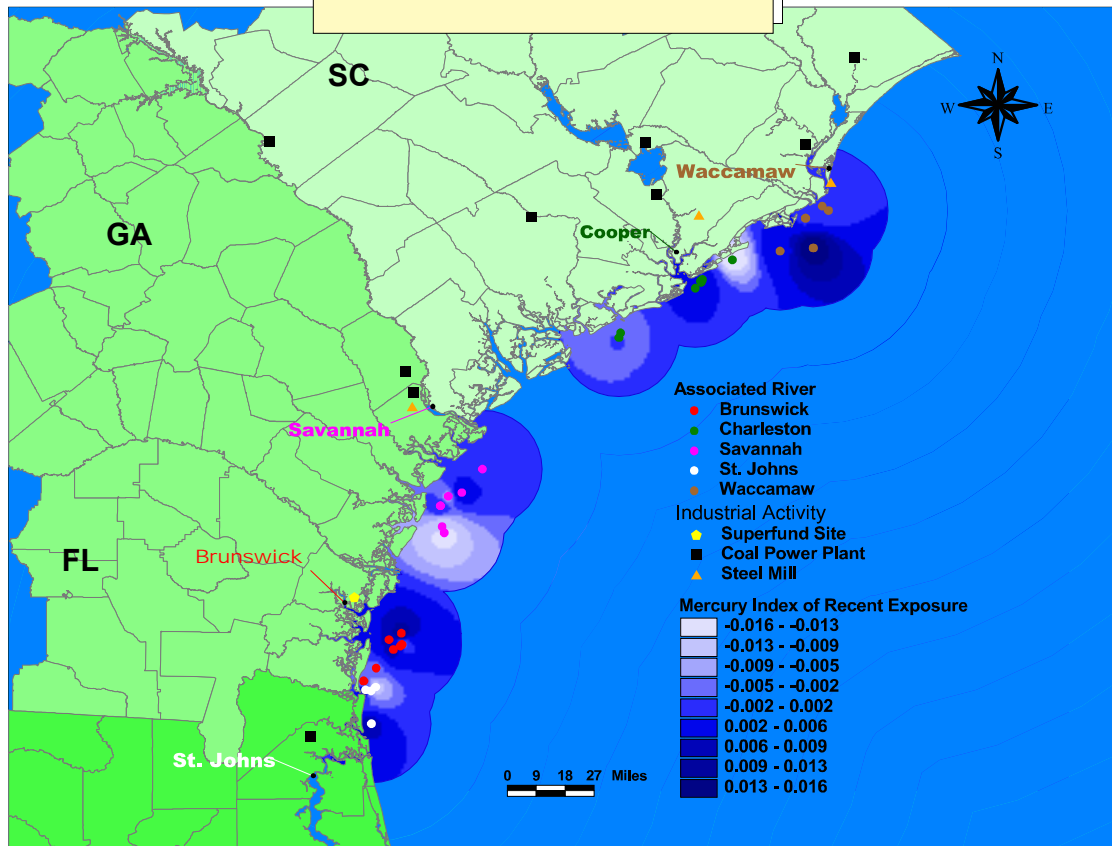
Index of Recent Exposure

Figure 17. Blood samples from 11 live loggerheads were treated with mitogens to assess immune function. Non-parametric correlations between blood mercury and b-cell proliferation were significant (Spearman Rho, -0.7273, P = 0.0112, Kendall Tau b, -0.6000, P = 0.0102).
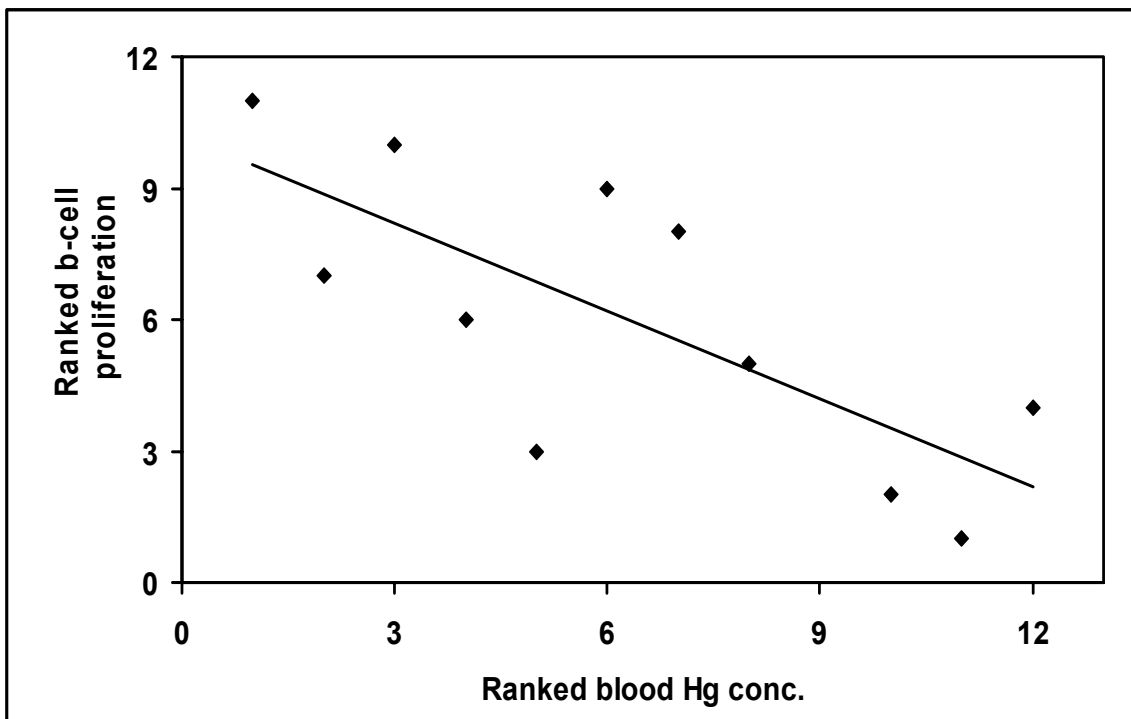
Figure 18. Blood samples from 6 live loggerheads were submitted for a comprehensive

blood chemistry profile and analyzed for total mercury content. Non-parametric ranked

correlations between blood mercury and 20 blood parameters revealed a significant

negative correlation with total white blood cell count (Spearman Rho, -0.8827, P =

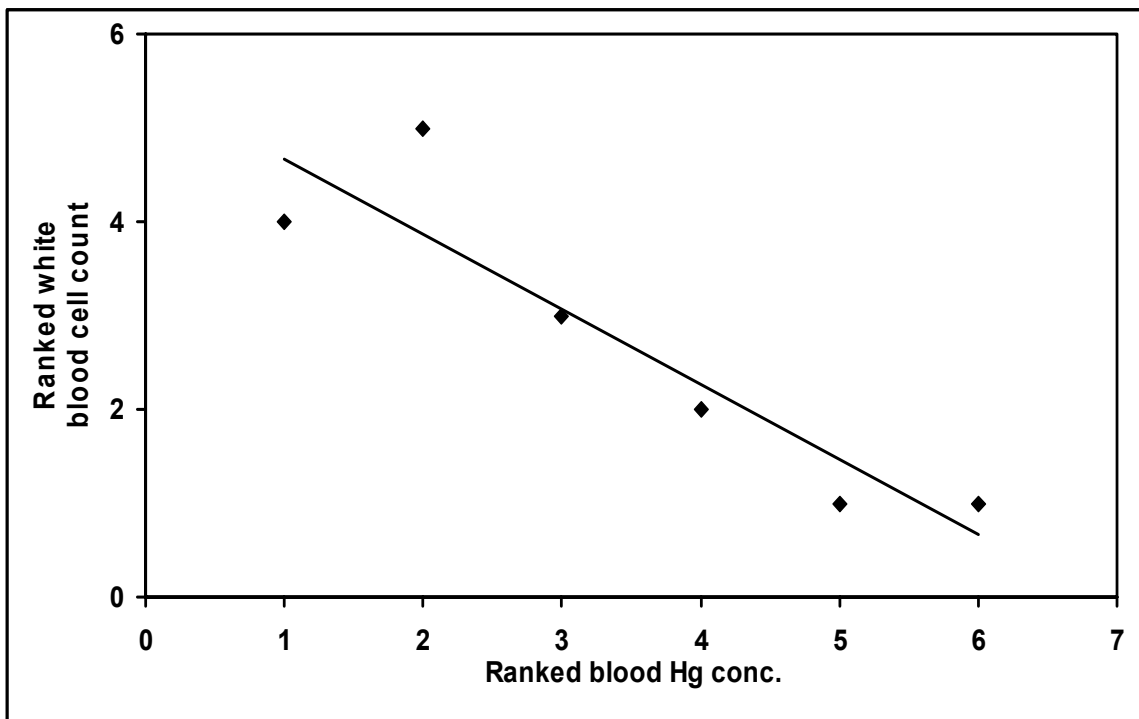0.0198, Kendall Tau b, -0.7877, P = 0.0321).

Figure 19. Blood samples from 6 live loggerheads were submitted for a comprehensive

blood chemistry profile and analyzed for total mercury content. Non-parametric ranked

correlations between blood mercury concentration and blood sodium concentration

revealed a significant negative correlation (Spearman Rho, -0.986, P = 0.0003, Kendall
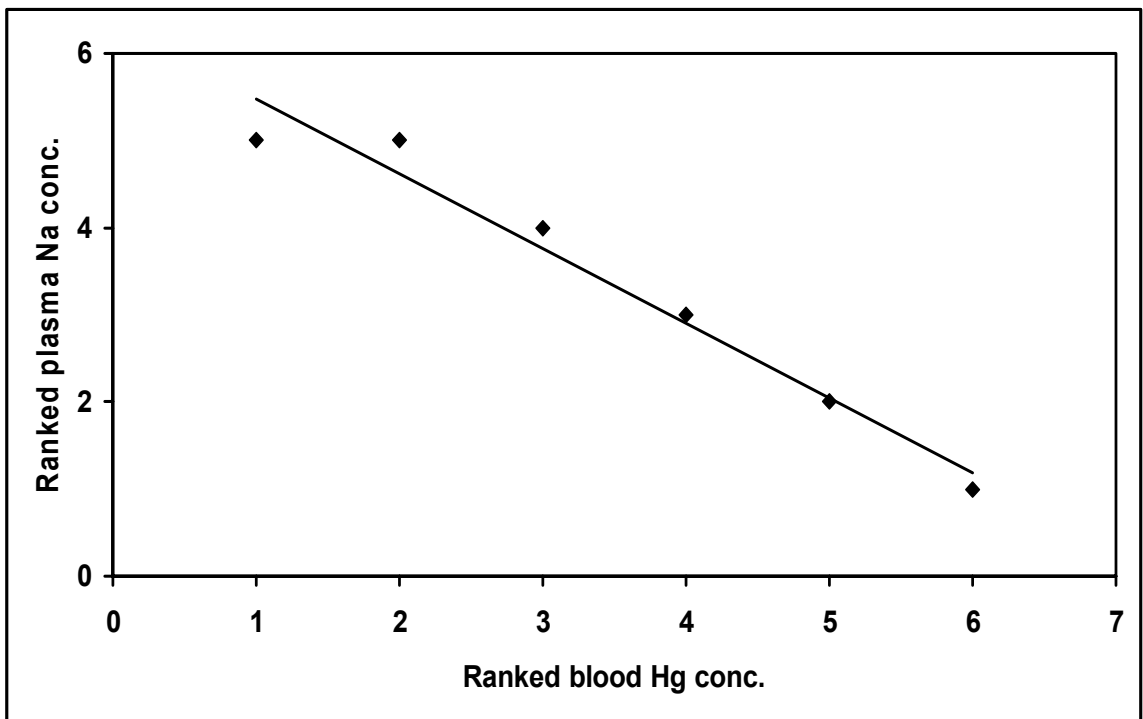
Tau b, -0.966, P = 0.007).

Figure 20. The size distribution of live turtles represents larger individuals than were sampled from strandings. Mean straight carapace length was 72.1 cm for live captures and 61.9 cm for stranding. Because of the disparity in size between these two groups, straight carapace length was included as a covariate in the statistical comparison between their contaminant levels.
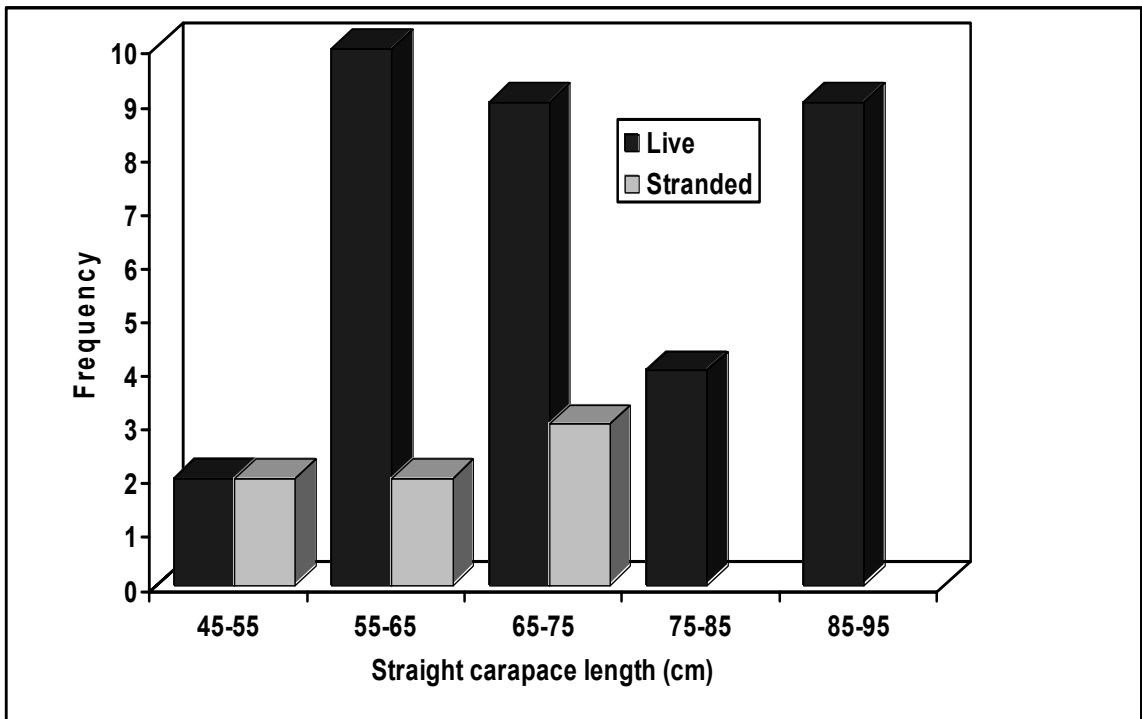
Figure 21. ANCOVA with condition (live or dead), straight carapace length and mercury in blood and keratin indicates stranded loggerheads have significantly higher contamination in blood (P=0.0018) and keratin (P=0.036). Error bars represent mean mercury concentration ± 1 SE.
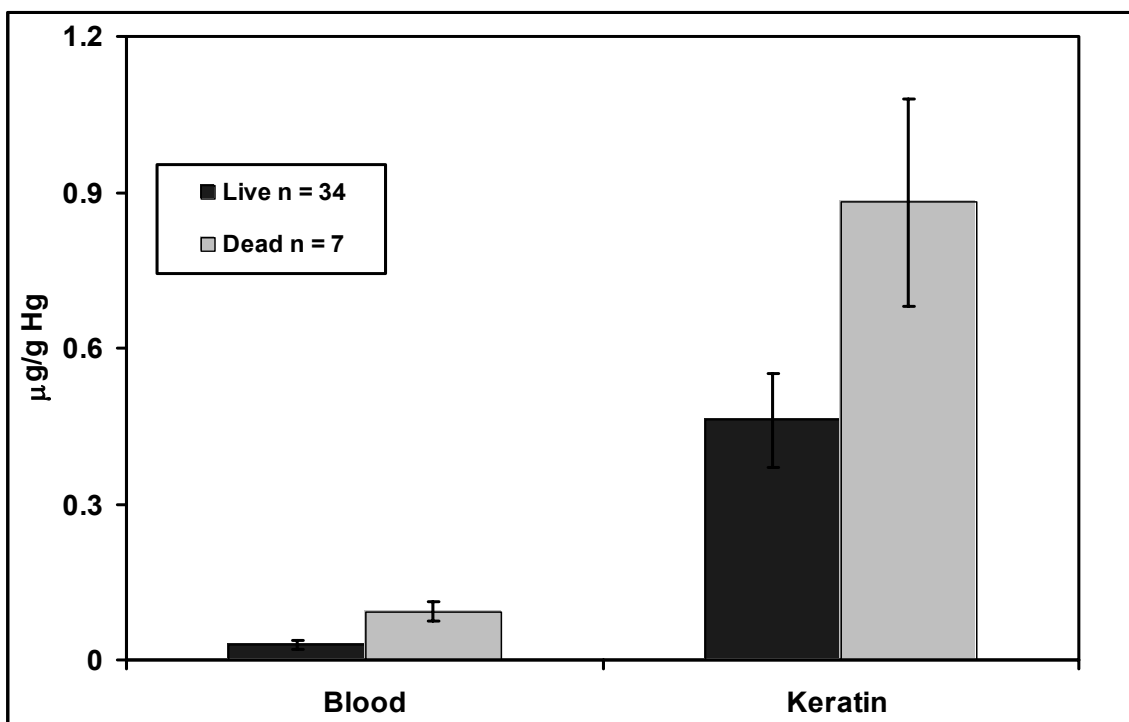
Figure 22. Summary of liver total mercury concentrations for loggerheads from different regions. Contamination in loggerheads from the southeast U.S. is most comparable to populations in the Mediterranean.

Southeast U.S.
Present study

Japan
Sakai et al. (1995)

Adriatic
Storelli et al (1998a,1998b)

Adriatic

North Cyprus
Godley et al. (1999)

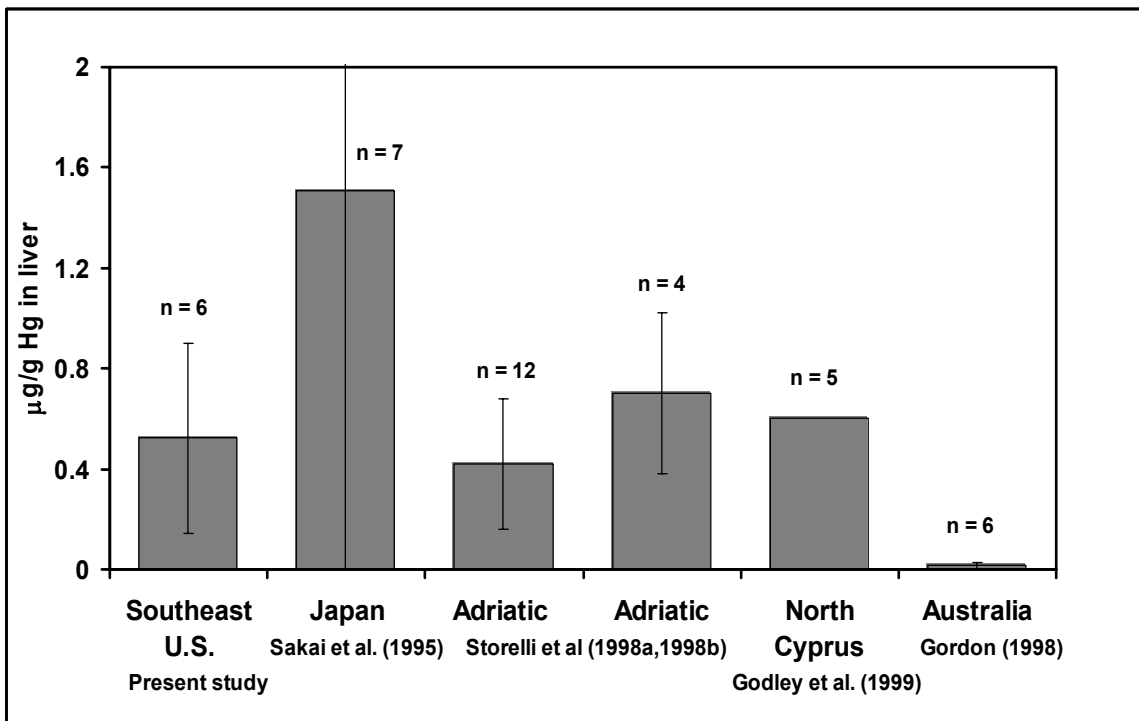Australia
Gordon (1998)

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Figure 23. Predictions of the mercury concentrations in internal tissues of 34 live

loggerheads were generated using their blood and keratin mercury concentrations and the

regression equations from the stranded turtles. Mean mercury concentrations from the

two prediction sources for each tissue (P = 0.986) and for the overall body burden (P =

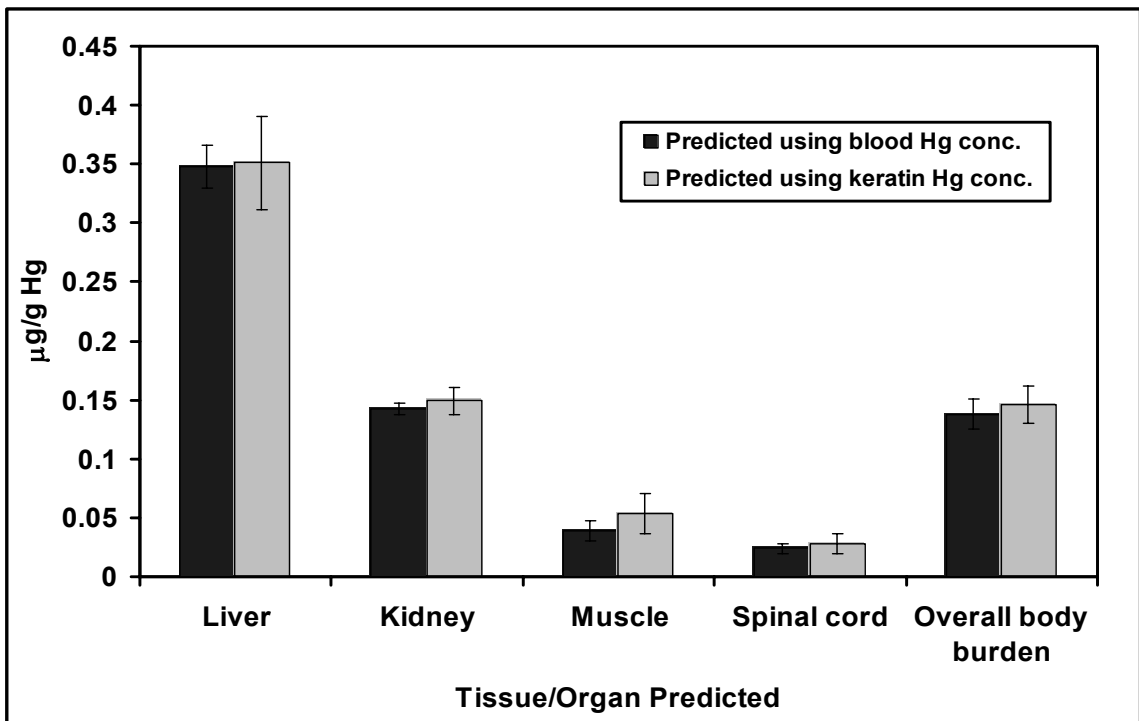0.558) were not significantly different.

Figure 24. Sediment cores from three transects off the coast of Georgia were analyzed for total mercury concentrations (Hyland 2002). Most stations had sediment mercury concentrations below the 2 ppb detection limit, but higher concentrations were found closer to shore.

Mercury in Coastal Sediments

Sapelo Sound

Doboy Sound

Altamaha River

Sediment stations

Mercury ppm
- 0 - 0.002
- 0.002 - 0.003
- 0.003 - 0.005
- 0.005 - 0.006
- 0.006 - 0.008
- 0.008 - 0.01
- 0.01 - 0.011
- 0.011 - 0.013
- 0.013 - 0.015

0   5   10   Miles