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Monitoring Mercury in the Loggerhead Sea Turtle, *Caretta caretta*

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The validity of using blood samples and keratinized scutes for nonlethal routine monitoring of mercury (Hg) in loggerhead sea turtles, *Caretta caretta*, is evaluated in the context of how effectively these matrixes predict internal tissue Hg burdens and the different temporal scales of exposure they represent. Total Hg (THg) was measured in blood and scutes collected from live captures ($n = 34$) and liver, kidney, muscle, spinal cord, blood, and scutes collected from freshly stranded loggerhead turtles ($n = 6$) along the coast of the southeastern United States. Linear regressions between monitoring compartments and internal tissues from stranded animals were all statistically significant ($r^2 > 0.805$, $p < 0.015$) but varied in their utility as a predictive tool depending on which tissues were paired. Blood was an effective predictor of THg in muscle ($r^2 = 0.988$, $p < 0.0001$) and spinal cord ($r^2 = 0.988$, $p < 0.0001$), while scute was the most accurate predictor of THg in liver ($r^2 = 0.948$, $p = 0.0010$). The strength of the relationship between tissues types is believed to reflect the similarity in the temporal scales they represent and the variability in the fraction of methylmercury present. The stability of Hg in the scute matrix makes this tissue preferable for approximating long-term exposure, while blood Hg levels can be affected by recent changes in Hg intake. THg levels in blood and scutes from live captures were highly correlated (linear regression $r^2 = 0.926$, $p < 0.0001$) and increased significantly with body mass ($r^2 = 0.173$, $p = 0.016$ and $r^2 = 0.187$, $p = 0.012$ respectively), further supporting that there is a component reflecting long-term accumulation of Hg in these matrixes. We also present a novel technique using the residuals from the blood-scute regression as an index of recent exposure (IRE). The interpretation of this value is derived from the comparison between the most recent Hg intake (which contributes to the Hg measured in the blood) relative to the average past intake (which is recorded in the scute). A stepwise multiple regression revealed a significant positive relationship between the IRE and the proximity of the capture site to the nearest major industrial river mouth ($p = 0.0102$). This suggests that there is an elevation of bioavailable Hg in nearshore habitats where terrestrial influences and anthropogenic impacts are high. Seasonal foraging site fidelity and the variability in environmental Hg may explain the high intraspecific variability and occasional highly contaminated turtle seen in this and previous studies.

Introduction

The cycling of mercury (Hg) in the environment and the impacts of this highly toxic and prevalent contaminant on humans and wildlife has been studied extensively since the 1950s. The continuing concern about the harmful effects of Hg is reflected in recent statistics citing Hg as responsible for over 97% of all fish consumption advisories in Canada in 1997 and 79% of all fish and wildlife consumption advisories in the United States in 2000 (*1*). Research to better understand this issue has focused primarily on aquatic systems where inorganic Hg (IoHg) is most easily transformed into the more toxic and bioavailable methyl Hg (MeHg). While studies of lacustrine ecosystems have provided much of the available literature, there is an increasing emphasis on Hg contamination in coastal and estuarine environments. The unique conditions that exist in estuaries make these water bodies major repositories for riverborne and watershed derived Hg (*2*). On the basis of recent findings, South Carolina wetlands rank among the highest in the nation for methlylation efficiencies (*3*) and have the most miles of Hg-impaired waterways in the country (*2*).

The loggerhead sea turtle, *Caretta caretta*, is the most abundant sea turtle along the coast of the southeast United States. Subadult and adult loggerhead turtles utilize estuarine and nearshore habitats and forage on crustaceans, mollusks, fish, and a variety of other benthic invertebrate prey (*4*). The body of literature on Hg in other aquatic consumers is extensive, including studies on cetaceans, mink, otter, birds, and fish (*5*). Despite the threatened and endangered status of sea turtles globally, the existing literature on Hg in loggerhead turtles is sparse (*6*−*13*) and there is currently no information on post-hatchling sea turtles in the western Atlantic.

Some forms of pollution, such as plastics and other nonbiodegradable debris, contribute directly to sea turtle mortality through ingestion and entanglement (*14*), but the role of chemical pollutants in sea turtle health is largely unknown. Environmental contaminants have been identified as one of the possible factors contributing to the development of the viral infection fibropapilloma (FP) in sea turtles by reducing immune function (*15*). This debilitating and often fatal disease occurs most commonly in near-shore waters, areas adjacent to large human populations, and areas with low water turnover (*15*, *16*). Environmental conditions in such areas are favorable for methylation, and in the areas of highest disease prevalence (Florida Bay and the Indian River Lagoon, FL and Oahu, HI) elevated levels of Hg have been documented (*17*−*19*). The increasing prevalence of FP and other clinical diseases in sea turtles merits further investigation of the potential role of contaminants in their etiology. Thus far, the ability to investigate the role of contaminants in sea turtle health has been impeded by a lack of clinical data, by limited access to appropriate samples, and by the absence of a proven method for assessing the contaminant

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status of live animals that may or may not exhibit disease symptoms. Wildlife toxicologists commonly promote the use of nonlethal sampling techniques for assessing the contaminant status of an individual, population, or ecosystem. From a conservation standpoint, this is a favorable approach for any species and is often obligatory when dealing with specially protected species, such as sea turtles. In the present study, we discuss the validity of using two tissues for monitoring Hg that can be collected noninvasively from sea turtles: blood and keratinized scutes.

Blood Hg kinetics is a complex process that has been the topic of numerous pharmacokinetic and toxicological studies. Pharmacokinetic models developed in mammals and birds indicate that ingested MeHg is absorbed into the blood on the order of hours (*20*−*22*). After the concentration peaks, there is an initial phase of rapid decline (several days) where the majority of the dose is distributed to other organs and tissues. This is followed by a terminal phase of much slower elimination that may last several months (*23*, *24*). Despite the temporal variability that could be introduced by these transient pulses, correlations between Hg concentrations in blood and less dynamic tissues (*21*, *25*−*29*) suggest there is a more stable component to the Hg signature in the blood that reflects long-term exposure. The mechanism behind these trends may be an equilibrium between the MeHg, and to a lesser extent IoHg, in blood and other tissue compartments such as muscle, liver, kidney, and nervous tissue (*30*, *31*). Therefore it appears that the Hg signature in blood is comprised of a combination of (i) a transient component reflecting the dietary Hg intake over the previous days to weeks and (ii) a more stable component related to the Hg concentration in other tissues and reflecting the total body burden from more long-term accumulation. Intuitively, the relative importance of these two components would depend on the magnitude of the dietary pulses relative to the body burden of Hg at the time of sampling.

Scutes are the hard keratinized plates that armor the carapace of sea turtles in the family Chelonidae and other aquatic and terrestrial turtles. This proteinaceous keratin originates from growing regions at the basal infoldings of the epidermis and forms a series of nonliving keratin layers deposited one on top of the other, as shown in Figure 1 (*32*). Previous work on seabird feathers has shown that Hg avidly binds keratin proteins (*33*) and that UV radiation, heating (37.8 °C), freezing (−6.7 °C), and weathering for eight months has less than a 10% effect on the Hg concentrations (*34*). So scutes can be noninvasively collected and should be stable enough to be used for live or dead animals that have been exposed to diverse conditions with a minimal risk of sample degradation. Loggerhead turtles periodically shed a whole scute or superficial layers of keratin (personal observation). The exact rate at which this keratin is grown and sloughed is unknown, but the persistence of commensal barnacles (*Chelonibia testudinaria*) on the carapace suggests these scutes may represent growth over several years. The Hg bound to a growing scute should be determined by the blood/keratin binding coefficient and the Hg concentration in the blood at that time, providing a historical record of the blood Hg concentration. This same reasoning has been applied to the use of human hair to reconstruct Hg exposure over the time of hair growth (*35*) and to monitor Hg exposure in human populations (*36*, *37*).

Several workers have proposed using turtle scutes to measure Hg in sea turtles (*38*, Kemp's ridley; *7*, loggerhead turtles), but none have addressed the potential heterogeneity of Hg in scutes and the impact that the sampling methodology may have on the results. Workers have analyzed samples of superficial keratin mixed from various locations on the carapace but no comparisons to physiologically important tissues were reported (*38*). Others have established a correlation between Hg in the carapace to the total body burden, but the overall mass of this tissue compartments suggests that more than just keratinized scutes were used in the analysis (*7*). The present study describes a defined and reproducible sampling methodology for using scutes, assesses the potential sampling error inherent to blood and scutes, and discusses how effectively these matrixes predict Hg levels in specific internal tissues. We also introduce a novel concept that uses the relative Hg concentrations in blood and scute compartments to gain insight into the exposure history for an individual.

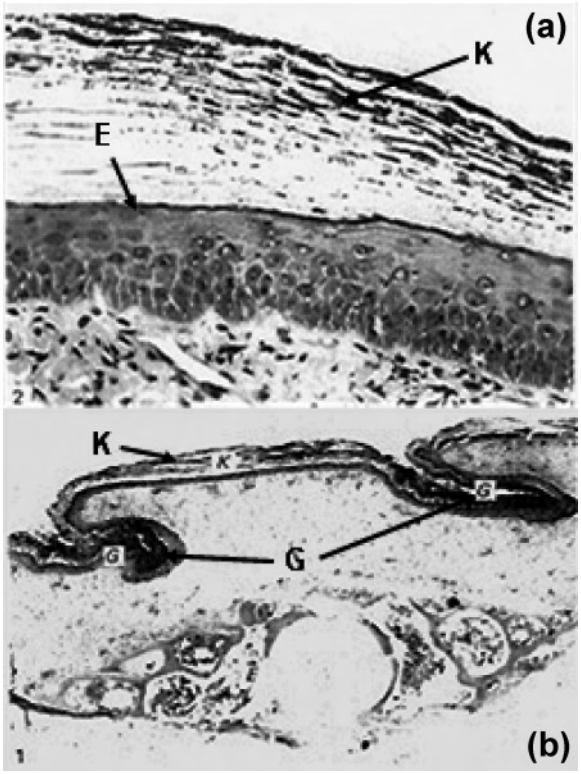

FIGURE 1. Transmission electron micrograph of a sea turtle scute cross-sections showing (a) the epidermis (E) and the layers of keratin deposition (K) and (b) a lower magnification image showing the growing regions (G) where keratinocytes become pyknotic and migrate into the scute. Reprinted from ref *32* with permission from Blackwell Publishing Ltd.

Methods

Field Methods. Blood and scute samples were obtained from 34 live loggerhead turtles captured in the South Carolina Department of Natural Resources (SCDNR) sea turtle index of abundance study (National Marine Fisheries Service Grant NA07FL0499) in July and August 2001 under permit from the National Marine Fisheries Services Endangered Species Division. Large-mesh trawl nets were towed in coastal waters 5−16 m in depth from Winyah Bay, South Carolina, to Fernandina Beach, Florida according to a stratified random sampling design. A subsample of captured loggerheads were selected for Hg analysis to provide a representative distribution of size classes and geographic location. Blood samples were collected from the cervical sinus (*39*) using evacuated test tubes containing sodium or lithium heparin. Scute samples were collected from the eight posterior marginal scutes of the carapace after thoroughly cleaning the area with a plastic scrubbing pad, clean-room wipes, high-purity water and 2-propanol. Keratin was scraped from the radial edge, where the dorsal and ventral surfaces form a thin edge and the keratin and underlying tissue can be discriminated. A disposable stainless steel biopsy tool was used to obtain

0.2−0.5 g of the scute by moving the tool parallel to the edge. This yields splinters of keratin ≈1 mm in thickness representing the entire depth of scute deposition. A suite of biological and environmental data was also recorded for each capture.

Samples of liver, kidney, muscle, spinal cord, scute, and blood were collected from 6 stranded loggerhead turtles from May 2001 to February 2002 in conjunction with the SCDNR and the North Carolina Wildlife Resources Commission (NCWRC). Samples were only collected from animals that were discovered freshly dead or required euthanization, and post-mortem and morphometric exams were performed. Blood and scute samples were collected using the same protocols used in live-captures, except a single blood sample that was collected directly from the heart. All samples were stored at −20 °C until processed and then transferred to −80 °C, except blood, which remained at −20 °C.

Laboratory Methods. Liver, kidney, and muscle samples were processed in a Class 100 clean room. The exterior surfaces of each piece of tissue were trimmed away and the remaining sample (≈100 g) was frozen in liquid nitrogen in a Teflon bag and then pulverized. Spinal cord was processed under a HEPA filter vertical laminar flow hood. The ends of the spinal cord were trimmed away and the remaining sample sectioned into pieces weighing approximately 0.07 g. All tissues were trimmed using a titanium knife and Teflon cutting board and were rinsed in high-purity deionized water.

Total Hg (THg) concentration (based on wet mass) in tissues was determined using isotope dilution cold vapor inductively coupled plasma mass spectrometry (ID-CV-ICPMS). This analytical method has been previously described in detail (*40*), and is briefly summarized here. Isotopically enriched ^{201}Hg spike solution was prepared and calibrated using NIST SRM 3133 Hg Spectrometric Solution. The spike was then added quantitatively to a mass of sample (0.1−0.8 g, depending on tissue type and anticipated Hg concentration) to yield an isotopic ratio (^{201}Hg/ ^{202}Hg) that minimizes random error propagation. Samples were then 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) using quartz microwave decomposition vessels and high-purity nitric acid (Fisher Scientific, Suwanee, GA). The digestant was mixed with a $SnCl_2$ and HCl reductant solution in a gas−liquid separator, allowing cold vapor transfer of the resulting Hg^0 in a stream of argon to the ICPMS injector. A VG Elemental Plasma Quad 3 ICPMS (United Kingdom) using typical ICP power and gas flows was used in time-resolved analysis mode for measurement of isotope ratios.

Tissue samples were analyzed in 32 analytical batches from January 24 to May 9, 2002. Each batch consisted of four loggerhead tissues, one NIST Standard Reference Material (SRM) used as an external control, and one procedural blank. NIST SRM 2976 Mussel Tissue (Trace Elements & Methylmercury) Freeze-dried (THg certified value = 0.0610 ± 0.0036 μg/g) was used as the control with all tissues except blood. NIST SRM 966 Toxic Metals in Bovine Blood (THg certified value = 0.0294 ± 0.00161 μg/g) was used as the control with blood batches to provide a close matrix match. The 32 measurements of these SRMs departed from the certified values by an average of 1.56 ng/g over all analytical batches, and the mean procedural blank was 0.13 ± 0.029 ng/g. Reproducibility exercises were performed using aliquots of homogenized material (SRM 2976 and candidate NIST SRM 1947 Trace Metals in Lake Michigan Fish Tissue) analyzed within one analytical batch. There was excellent reproducibility within each batch (n = 4), with RSD values of 1.36% for SRM 2976 and 1.07% for candidate SRM 1947.

Field blanks were used to evaluate the blood collection protocol used in the field. The mean Hg concentration in field blanks (n = 5) was below the mean of the normal procedural blanks (n = 26), indicating field contamination was not an issue. Reproducibility experiments were also conducted on potential monitoring tissues (blood and scutes) to assess the variability introduced by heterogeneity of Hg in these tissues. This consisted of analysis of four replicate samples of unprocessed blood (1 individual) and scutes (2 individuals) within the same analytical batch.

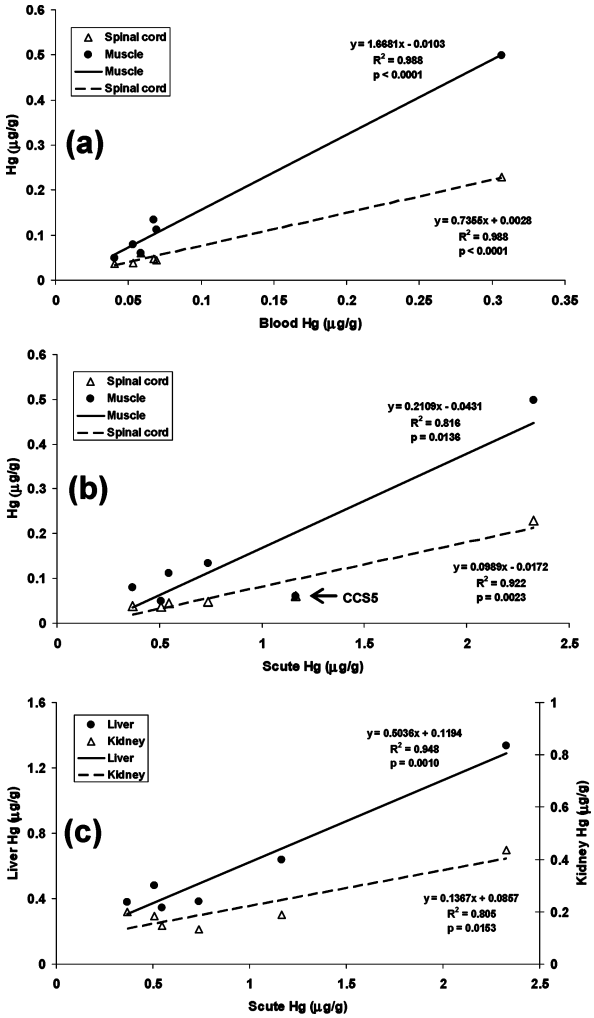

FIGURE 2. Linear regressions between THg in potential monitoring matrixes (blood and scutes) and internal tissues collected from stranded loggerhead sea turtles (n = 6).

Index of Recent Exposure. A value that will be referred to as the Index of Recent Exposure (IRE) was used as the response variable in several statistical analyses. The IRE for each individual is equal to its residual value from the linear regression between blood and scute Hg concentrations (Figure 3b). The IRE for each turtle represents the recent Hg intake relative to the historical exposure for that individual. A positive IRE indicates high recent intake (represented by blood) relative to the average previous exposure (represented by scutes), and a negative IRE indicates lower recent intake. This interpretation arises from the different temporal scales which these two matrixes reflect. The IRE can then be used to assess the impact of biological and environmental variables that may relate to short-term Hg exposure.

Statistics. All statistics were performed using commercially available software (SAS Institute's JMP 3.26). Linear regression analysis was used to compare different tissues from the same individual and assess any trends of bioaccumulation. A Shapiro-Wilk test for normality was used to confirm that the residuals from all parametric tests conformed

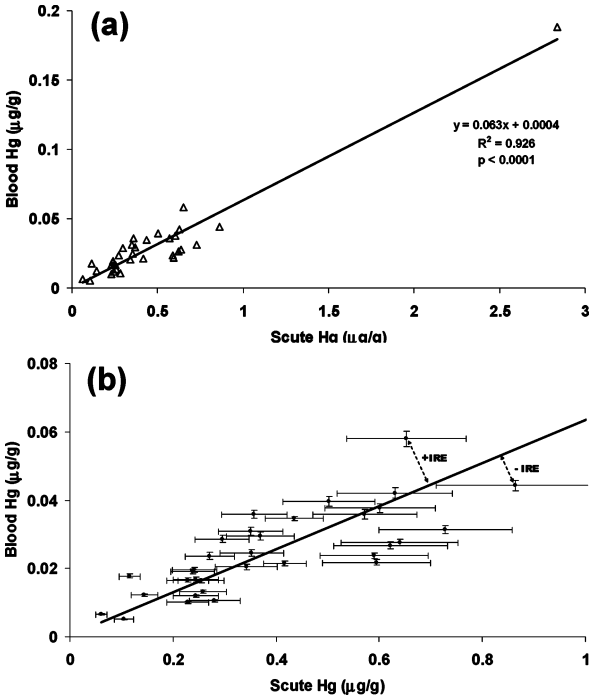

FIGURE 3. (a) Linear regression of THg in blood and scutes collected from live captures ($n = 34$). (b) Magnification of the lower end of the regression showing graphically the Index of Recent Exposure (IRE) and the expanded uncertainty bars.

to assumptions of normality. Assumptions of homoscedasticity for ANOVAs were tested using several standard methods, i.e., the O'Brien, Brown-Forsythe, Levene, and Bartlett tests for homoscedasticity. The IRE was used as the response variable in a backward-stepwise multiple regression. The model analyzed the relationship of the IRE with five biological parameters (body weight, hematocrit, total blood protein, blood glucose, and gender) and six environmental parameters (water depth, water temperature, distance offshore, distance to nearest river mouth or inlet, distance to nearest major industrial river mouth, and the identity of the most closely associated major watershed) that could relate to Hg intake. The model was set to reject all parameters with $p > 0.1$. Expanded uncertainty values were calculated for blood and scutes from live captures according to the Guide to the Expression of Uncertainty in Measurement (*41*, *42*). These uncertainty values combine the sampling RSD, analytical RSD, and additional sources of uncertainty originating from calibration standards and isotopic spike solutions, and were used to bracket the measured concentrations in the regression between blood and scutes to inspect the impact of this uncertainty on the calculation of the IRE. Commercially available geographic information software (ESRI's Arcview 3.2 with Spatial Analyst Extension) was used to generate spatial parameters, maps, and the inverse distance weighted surface interpolation.

Disclaimer. Certain commercial products are identified in this paper to adequately specify the procedures outlined. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology.

Results

Loggerhead blood exhibited reproducibility (one individual, $n = 4$, RSD = 1.17%) comparable to the analytical reproducibility determined for NIST SRMs used as controls in the study (RSD = 1.2%). This confirms that Hg is homogeneously distributed in the blood, minimizing the risk of spatially driven sampling error when using this compartment for monitoring.

TABLE 1. Total Hg (μg/g wet mass) in Loggerhead Turtle Tissues[a]

tissue type	strandings ($n = 6$)	live captures ($n = 34$)
blood	0.099 ± 0.042 (0.040-0.306)	0.029 ± 0.008 (0.005-0.188)
scutes	0.941 ± 0.299 (0.368-2.326)	0.461 ± 0.087 (0.062−2.837)
spinal cord	0.076 ± 0.031 (0.037-0.229)	
muscle	0.155 ± 0.070 (0.049-0.499)	
kidney	0.214 ± 0.046 (0.132-0.436)	
liver	0.594 ± 0.155 (0.346-1.336)	

[a] Mean ± 1 standard error, range.

The mean RSD for scute samples was 7.17%, reflecting the higher heterogeneity of Hg in this matrix. Adjusting the total RSD for the scute samples by the analytical reproducibility leaves approximately 6% of variability that is accounted for by the nature of the tissue and the sampling methodology. This variability most likely results from the layered growth patterns that the keratinized scutes exhibit (Figure 1) and slight inconsistency in both the Hg concentration at various depositional layers and the scute depth to which the collection penetrated.

The compartmentalization of Hg among tissues and organs from stranded loggerhead turtles revealed patterns similar to those reported in the literature for sea turtles and other vertebrates. Concentrations varied over an order of magnitude, with the lowest concentrations found in the blood (0.099 ± 0.042 μg/g) and spinal cord (0.076 ± 0.031 μg/g) and the highest concentration in the scutes (0.941 ± 0.299 μg/g) (Table 1). There was also high variability among individuals, with one highly contaminated individual (CCS4) having significantly higher mean tissue burdens than all other animals (two-way ANOVA with tissue type and individual, $p < 0.0001$).

Linear regressions of Hg concentrations in monitoring compartments (i.e., blood and scutes) against internal tissue concentrations revealed statistically significant relationships for all tissue pairs ($r^2 > 0.805$, $p < 0.015$) (Figure 2). Due to the strong influence exerted by the highly contaminated individual in determining the significance of some of these tissue pairs, the practical utility of using these monitoring compartments as a predictive tool depends on which tissues were paired. Blood Hg concentration proved to be an excellent predictor of muscle and spinal cord Hg ($r^2 = 0.988$, $p < 0.0001$, $r^2 = 0.988$, $p < 0.0001$). The relationship between Hg in scutes and muscle and spinal cord was more variable ($r^2 = 0.816$, $p = 0.0136$, $r^2 = 0.922$, $p = 0.0023$), primarily due to one individual (CCS5) with high scute Hg concentrations relative to that in muscle and spinal cord. The liver Hg burden was most effectively predicted by scutes ($r^2 = 0.948$, $p = 0.0010$) and the kidney Hg concentration was the most variable internal tissue relative to monitoring matrixes, with positive correlations being driven entirely by the highly contaminated individual. A paired t test between Hg concentrations in spinal cord and brain ($n = 4$) indicated these tissue compartments were not significantly different (mean difference 0.0047 μg/g, t-ratio 0.721, $p = 0.523$), suggesting spinal cord is representative of the central nervous system as a whole.

The strong regression between the Hg concentrations in blood and scutes from live captures ($r^2 = 0.926$, $p < 0.0001$) suggests that the temporal fluctuation in the blood Hg concentration is small relative to the average historical blood Hg concentration reflected by the scutes (Figure 3a). The

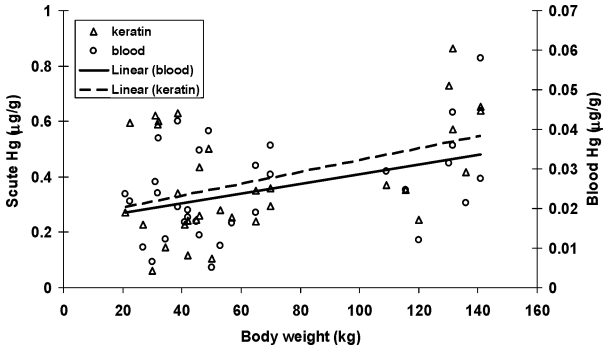

FIGURE 4. Relationship of Hg concentration in blood and scutes to body mass ($r^2 = 0.173$, $p = 0.016$ and $r^2 = 0.188$, $p = 0.012$, respectively) for live captures ($n = 33$). The anomalously high individual was omitted from this analysis.

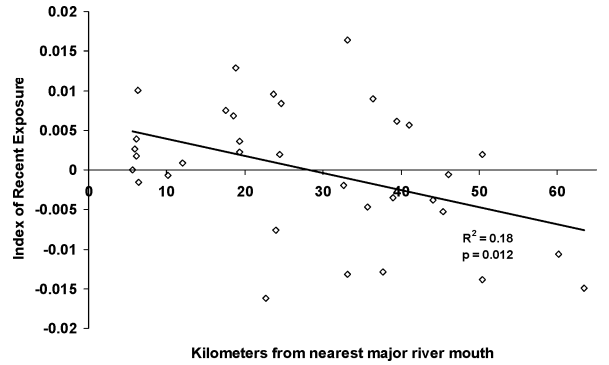

FIGURE 5. Relationship of the Index of Recent Exposure to the proximity of the capture site to the nearest major river mouth.

live capture data set also contained one individual (CC2151) with considerably higher (≈16x) Hg levels in both tissues. Omitting CC2151 from the analysis still results in a highly significant regression ($r^2 = 0.604$, $p < 0.0001$) with nearly identical slope and intercept parameters. Both blood and scutes also reflect a significant increase in Hg concentration with body mass (considered here as a proxy for age) ($p = 0.0119$ and 0.0162 respectively). This trend may indicate that these matrixes effectively detect long-term trends in bioaccumulation present in this population of loggerhead turtles (Figure 4). The unique circumstances that resulted in the high Hg accumulation in CC2151 (see Discussion section) prompted the omission of this individual from the bioaccumulation analysis. Loggerheads ranged from 21 to 141 kg (50.2−94.9 cm straight carapace length), with 16 females, 17 males, and 1 undetermined.

All of the variables included in the stepwise multiple regression (body weight, hematocrit, total blood protein, blood glucose, gender, water depth, water temperature, distance offshore, distance to nearest river mouth or inlet, distance to nearest major industrial river mouth, and the identity of the most closely associated major watershed) with the IRE were rejected from the model as insignificant, except for the distance between the capture site and the nearest major industrial river mouth. These river systems were chosen based on the presence of major point sources of Hg (coal-fired power plants, steel mills, or former chlor-alkali plant location). Individuals captured closer to these river mouths had significantly higher IRE values ($r^2 = 0.18$, $p = 0.0120$), suggesting a higher relative dietary intake of Hg (Figures 5 and 6). This effect is presumed to result from higher levels of bioavailable Hg in these areas, and the subsequently higher Hg concentrations in prey consumed by loggerhead turtles foraging in these areas. Applying the expanded uncertainty to these measurements did not have a substantial effect on the direction and magnitude of the IRE that was determined for each individual (Figure 3b).

Discussion

Nonlethal Estimation of Hg Tissue Burdens. The results from this study show that the potential monitoring matrixes (blood and scutes) provide a reasonable approximation of Hg burdens in important loggerhead tissues using the nonlethal collection techniques presented here. THg in muscle and spinal cord were reliably predicted by either blood or scute THg, while scute THg proved to be most effective predictor of liver THg. For several of the tissue pairs (blood-kidney, scute-kidney, blood-liver) there is poor linearity at the lower end of the concentration range. Reanalysis of these particular data without the highly contaminated individual shows that the significant r^2 and p values for these regressions are driven by the consistently high Hg concentrations in all tissues of this individual. This suggests that for these tissue pairs the monitoring matrixes may provide a gross approximation of internal Hg burdens but have otherwise poor predictive abilities. It must also be noted that blood collected post-mortem is susceptible to dehydration which can artificially inflate the concentrations of analytes. Higher blood Hg/scute Hg ratios in strandings compared to live captures (0.11 vs 0.06 respectively) suggests some relative elevation in blood collected from strandings that could be due to post-mortem dehydration, remobilization of Hg from other tissues due to trauma or illness, or higher recent dietary intake. Therefore the pairwise regressions using blood from strandings may underestimate the Hg in internal tissues, while scutes will generally provide a less biased estimate. These limitations and the relatively small sample size for strandings are created by the difficulty in obtaining suitable samples from a federally protected species such as sea turtles. While these caveats preclude using these inter-tissue relationships to make precise predictions of Hg concentrations, these results suggest that blood and scute samples can be used to approximate toxicologically important differences in Hg exposure in future health assessment and toxicological studies on this difficult to study and protected species.

Mercury species were not measured in the current study, but the proportion of IoHg and MeHg that typically prevails in each tissue type is one factor that could affect the strength of the correlations between various tissue pairs (Figure 2) (*28*, *29*, *43*). The mercury in blood and muscle in seabirds is nearly 100% MeHg (*43*, *44*), and nerve tissue is also predominantly MeHg (*45*; 80−90%). The % MeHg in scutes is probably comparable to that reported for bird feathers (nearly 100%), or slightly lower, as has been reported for mammalian hair (65−99%). In contrast, the liver and kidney typically contain larger fractions of IoHg due to the demethylation that is believed to occur in these organs (*46*). Evidence suggests that IoHg can be stored in the liver as Hg−Se−protein complexes and as insoluble HgSe (tiemannite) granules (*47*−*50*). The regressions between monitoring tissues and either liver or kidney had higher variability and more positive y-intercepts compared to regressions with muscle or spinal cord (Figure 2). Similar results were found with snapping turtles, showing significant correlations between blood and scute Hg concentrations with muscle and no correlation between liver and any other tissue (*28*). This may reflect the stronger equilibrium of MeHg among tissues, while the large fractions of IoHg in the liver and kidney are less available for equilibrium with the blood, and persist even when blood THg values approach zero. This interpretation is also supported in a study using tissues from cormorants (*29*). They found a highly significant regression between THg in the blood and MeHg in the liver of cormorants ($p < 0.0001$, $r^2 = 0.83$). In the same samples, the regression between blood THg and liver IoHg was still

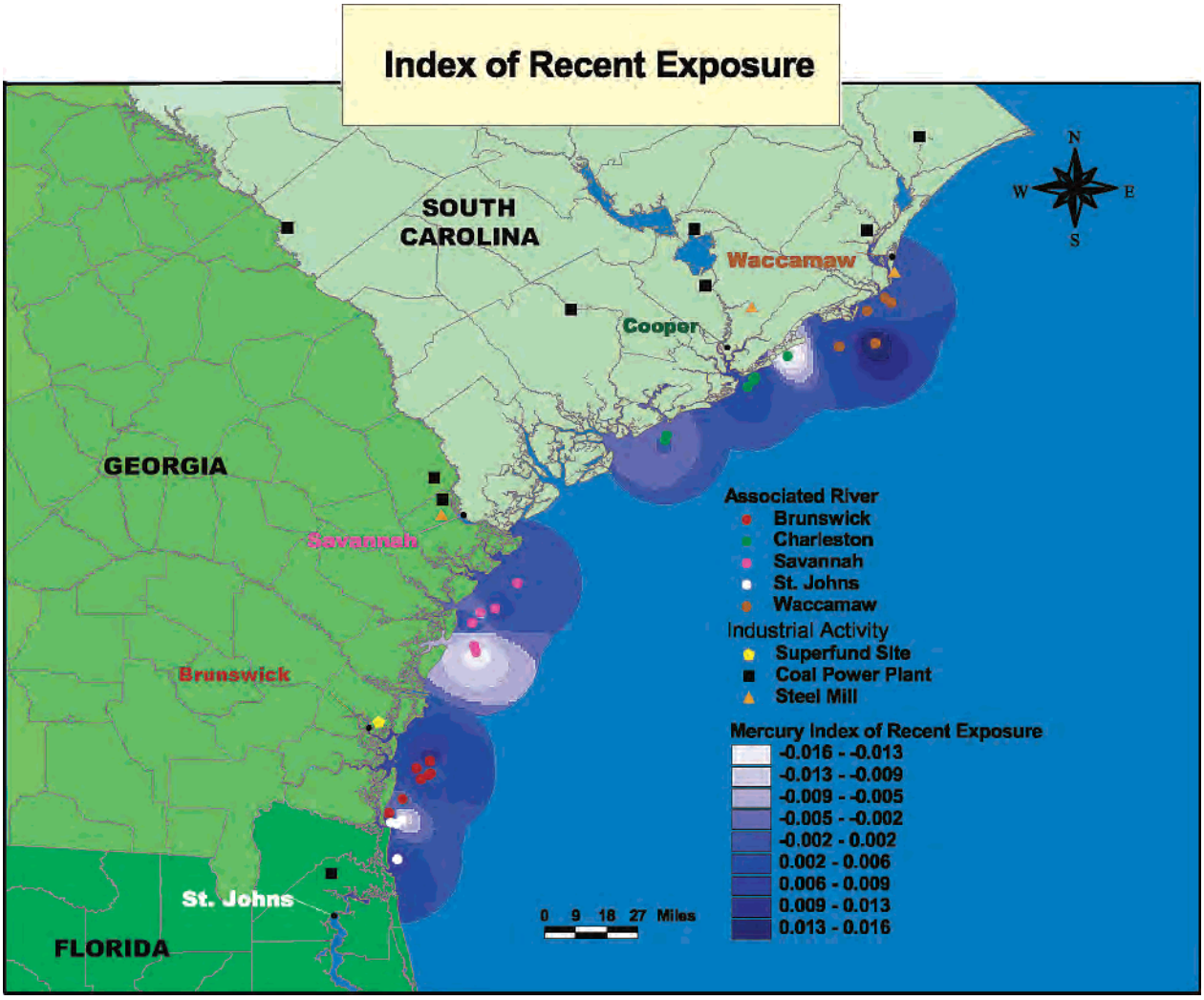

FIGURE 6. Points represent capture of loggerhead sea turtles by trawl, color-coded by the river system with which they were most closely associated. The geographic trend in the index of recent exposure (IRE) is displayed using an inverse distance weighted surface interpolation map. Low IRE values, indicated by light colors, are most prominent intermediate to major river outflow, with higher IRE values generally occurring closer to these watersheds. Industrial point sources of mercury in the study area are also shown.

statistically significant but much more variable ($r^2 = 0.32$). So generally speaking, blood and scutes may provide an approximation of MeHg tissue concentrations, which is the most toxic and physiologically important portion of the Hg burden. Considering the dose-dependent demethylation of Hg in the liver reported by several authors (*9*, *29*), these monitoring matrixes will better predict the THg in the liver at low concentrations, and may underestimate THg in this organ (and to a lesser extent in the kidney and brain) when higher concentrations are present. Quantification of both THg and MeHg in organs where demethylation occurs will improve our understanding of the distribution and metabolism of Hg in chelonians. Further work is also needed to determine if the small fractions of IoHg in the blood or scutes is correlated with the IoHg in the liver or kidney.

Impacts of Temporal Variability on Monitoring. The temporal scales that these tissues reflect raises the separate, yet related, issue of how best to implement monitoring strategies. If Hg intake is constant then models predict blood MeHg and IoHg should eventually reach a steady-state equilibrium (*30*). However, as with many species, the migratory and foraging behaviors of sea turtles would be expected to contribute to deviations from steady state equilibrium. When using blood for contaminant analysis, one needs to consider the relative contributions of transient dietary pulses of the analyte versus the more stable component reflecting equilibrium with other tissue storage compartments. Blood Hg levels are generally considered to be an indicator of dietary Hg intake, but the correlation between Hg concentrations in blood and other tissues supports the assertion that the blood Hg concentration reflects more than just the immediate exposure (Figures 2 and 3). The significant relationship between blood Hg concentration and body mass in live captures (Figure 4) also suggests that blood reflects bioaccumulation from long-term exposure. Alternatively, this trend in the blood Hg levels could be due older loggerheads recently consuming prey that are higher in mercury. The loggerheads sampled in the present study (50−95 cm) are in their benthic life stage and forage on a wide range of benthic invertebrates that changes on the basis of prey availability seasonally and geographically. While there is a significant diet shift when loggerheads recruit to benthic habitats from their juvenile pelagic stage (thought to occur around 40−50 cm), there is no evidence in the literature to suggest there is a size-related shift in diet during the benthic life stage (*4*). An increase in Hg concentration with size is also seen in the scutes, indicating that the elevation in blood Hg levels in older loggerheads is a persistent condition. Examples of higher blood Hg levels as a function of age are rare in the wildlife literature, despite overwhelming

evidence of Hg bioaccumulation in other tissues (*5*), and a general agreement in the pharmacokinetic literature that MeHg is transferred bi-directionally between blood and other tissue compartments (*23*, *24*). However, examples of blood bioaccumulation have been documented in public health studies (*51*, *52*), which benefit from large sample sizes and use multivariate models that can account for strong confounding factors such as recent fish consumption. Bioaccumulation of Hg in sea turtles has also been reported in blood from Kemp's ridley sea turtles (*Lepidochelys kempi*) in the Gulf of Mexico (*53*) and in liver and kidney from hawksbill sea turtles (*Eretmochelys imbricata*) in Japan (*7*). The intermediate trophic position of sea turtles would make dietary pulses of mercury in the blood small relative to more piscivorous species, decreasing sampling error related to temporal variability. The relatively small temporal fluctuations in blood Hg levels, slow growth, and long-life span may contribute to the detection of Hg bioaccumulation in blood from these species.

Despite the relationships described above, there is considerable variability in blood Hg levels that is unrelated to size or other tissue storage compartments. Variation in the blood Hg as a function of foraging location was demonstrated using the IRE, probably reflecting changes on a scale of weeks. A more extreme, and prolonged example of the potential for changes in blood Hg relative to other tissues is seen in data from one of the stranded turtles (CCS5). CCS5 was a severely and chronically emaciated animal with virtually no fat stores, extreme muscle atrophy, and an empty gastrointestinal tract. Metabolizing fat and muscle would be expected to mobilize Hg stored in these tissues, possibly elevating the Hg level in the blood. However the blood Hg/scute Hg ratio for CCS5 is 0.05 compared to a mean of 0.11 for data from the other strandings, suggesting there was a decrease in this individual's blood Hg levels. This decrease is likely a result of prolonged aphagia that results in a cessation of dietary Hg input from prey. In the short-term, the quantity of Hg remobilized from fat (16 ng/g, *7*) and muscle (155 ng/g, present study) may not be significantly higher than that acquired from prey such as blue crabs (150 ng/g, *54*) during normal foraging. In the long-term it appears that the result is an increase in depuration or a redistribution of mercury away from the blood compartment. Blood Hg concentrations relative to muscle and spinal cord were consistent among all stranded individuals, suggesting the decrease in blood Hg for CCS5 was accompanied by a corresponding decrease in muscle and spinal cord (Figure 2a). This relative decrease is not observed in either scutes or liver (Figure 2b), where Hg is deposited in a metabolically inactive keratin matrix or is bound in relatively immobile IoHg complexes. This case exemplifies why scutes may be more effective than blood at approximating long-term Hg exposure. This is particularly important in cases where trauma or illness have significantly altered the metabolic condition or migratory or reproductive behavior result in short-term changes in foraging behavior.

Despite efforts to standardize collection techniques in this study, there is still a 6% RSD among aliquots of scute from the same individual. The layered growth pattern and multiple growing regions in the scutes (Figure 1) may be responsible for this variability. Scute thickness varies depending on carapace location and which part of the scute is measured (medial or lateral, anterior or posterior). Therefore, keratin layers collected at different depths and location on the carapace may reflect different periods of deposition in the turtle's history. Although the slow bioaccumulation of Hg in this species may render these differences negligible, we still recommend that sampling methods for turtle scutes be standardized. Techniques such as radio frequency glow discharge MS, laser ablation ICPMS, or secondary ion MS will be used in the future to investigate trace metal variability laterally across the carapace and as a function of depth within a scute, allowing further refinement of the sampling methodology.

Scutes may inherently have a slightly higher sampling error than blood, but the stability and depositional characteristics of this matrix make it a more reliable indicator of the long-term Hg exposure of an individual. Scutes are particularly attractive for sampling strandings where changes in the blood before and after death could alter results. Conversely, scute samples alone could tend to underestimate the recent intake of an individual that could relate to acute MeHg toxicity or short-term environmental exposure. The ease of collection, negligible impact to the animal, and complementary nature of blood and scutes merits the collection of both monitoring compartments when possible.

Index of Recent Exposure. Loggerhead turtles make extensive seasonal migrations in search of warmer waters, either south or further offshore. Intuitively, this mobility results in exposure to different contaminant regimes, making relationships between Hg contamination and environmental parameters difficult to establish. Since blood is more indicative of short-term exposure than other matrixes, it would seem to be the most likely candidate for investigating factors operating on a relatively short time scale. However, there are very few examples of this type of relationship in the literature. Positive correlations have been reported between stable isotope concentrations (δ^{15}N, which relates to trophic position of prey, and δ^{13}C, which relates to nearshore versus pelagic habitats) and THg in the blood of seabirds, suggesting that diet and habitat utilization can explain variation in blood Hg concentrations (*22*). It is interesting to note that in a related study a correlation between δ^{15}N and Hg was present in chicks but not adults (*55*). Blood THg concentrations in adults were several times higher than in chicks and had no correlation to δ^{15}N. This is probably due to long-term exposure in adults contributing to the overall blood Hg signature and confounding the relationship between the dietary Hg component and the δ^{15}N that share a similarly short time scale. The IRE is presented here as a method for isolating the recent Hg intake by normalizing the instantaneous blood Hg concentration by the historical average blood Hg represented by the scutes. Using the scute Hg concentration as a benchmark serves to separate recent exposure from other factors such as long-term bioaccumulation and differences in physiology, which would apply to both compartments. Since previous short-term fluctuations in blood Hg are also reflected in scute deposition, the IRE simply compares the relative magnitude of the current exposure to the average exposure during the period of scute deposition.

The error bars in Figure 3b represent the expanded uncertainty for the blood and scute Hg concentrations and demonstrate the potential impact that measurement and sampling error could have on the calculation of the IRE. These 95% confidence intervals do not substantially affect whether an individual was determined to have a positive or negative IRE, but it must be noted that using a less precise analytical method may not have yielded the same results. The high precision achieved by isotope dilution CV-ICPMS maximizes the likelihood of detecting trace element trends in biological systems that are inherently complex and variable. The IRE was represented in the present study by the residuals from the linear regression between blood and scute Hg concentrations in the interest of statistical interpretation. However, using the ratio of the blood Hg/scute Hg concentrations as the response variable gives the same result and is conceptually similar.

Ratios of contaminant concentrations between tissues have been discussed in the literature in the context of converting from one tissue to another (*43*). Other workers have used the changing ratios of polycyclic aromatic com-

pound (PAC) concentrations in blood and internal tissues during different stages in exposure to demonstrate the dynamic and physiologically active role certain organs play in coping with PACs (*56*). However, to our knowledge a static nonliving keratin matrix has never been proposed as a reference concentration for blood to gain insight into the contaminant exposure history for an individual. The temporal scales represented by turtle scutes, mammalian hair, bird feathers, and reptilian skin vary tremendously. However, each of these matrixes could provide a similar, but unique, interpretation for Hg exposure based on its specific growth characteristics.

Similar blood and scute Hg concentrations between males and females suggests that gender may not be important in determining long-term Hg accumulation (*57*). However, differing reproductive behavior in mature adults during the mating and nesting season could potentially affect short-term Hg accumulation. Both males and females decrease or stop feeding during reproductively active periods (*58*). Loggerhead males in North America peak in activity for the mating season in March and April, then resume normal foraging. Females have a more prolonged period of reproductive activity lasting until their last clutch is laid, typically in mid or late 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 dietary intake of Hg 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). These adult males and females were caught at comparable distances from terrestrial sources of Hg (mean of 44 and 42 km from major industrial rivers), so the differences appear to be driven by the intake of Hg, not the availability. These trends in the IRE agree with what would be expected based on our knowledge of sea turtle biology.

The significant relationship between the IRE and the proximity of the capture sites to industrial rivers provides evidence of an environmental gradient of Hg in the neritic zone (Figures 5 and 6). Loggerhead turtles sampled in this project were all captured from 2.3 km to 15.1 km from land, but the stretches of coastline with relatively little river outflow are where IRE values were lowest (Figure 6). These results are assumed to be a result of higher Hg loads in loggerhead prey near terrestrial sources of Hg. There are several processes that could explain this geographic trend, both natural and anthropogenic. These samples were collected from over 640 km of the Southeast U.S. coastline. The river systems classified as industrial were chosen as reference points due to the presence of major point-sources of Hg pollution (coal-burning power plants, steel mills, chloroalkali plants). While Hg is generally considered to be a ubiquitously distributed pollutant, the form of Hg being released determines the range of atmospheric transport. Gaseous elemental Hg (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 Hg (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 (*59*). Therefore the particulate and reactive gaseous Hg released from these point sources would be expected to cause some local elevation of Hg in water and soil through wet and dry deposition, and a subsequent increase in biota after methylation.

The industrial rivers used in this analysis drain some of the largest watersheds in the stretch of coastline that makes up the study area. With the exception of the Altamaha and St. Mary's Rivers, the rivers used in this analysis represent all of the major river discharges from Georgetown, SC, to Jacksonville, FL. Broad-scale atmospheric deposition and natural geological sources of Hg all contribute to the 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 Hg. Since the conditions in wetlands are among the most favorable for Hg methylation, (*5*), this runoff would be expected to have far more bioavailable Hg than more oceanic influenced waters. The higher IRE in loggerhead turtles utilizing habitats near these river mouths is in agreement with what would be expected based on the current understanding of the biogeochemical cycling of Hg.

Contaminants and Sea Turtle Foraging Ecology. The foraging patterns of loggerhead turtles are not very well understood, but recent data from North Carolina and South Carolina (*60*, *61*) provide strong evidence of inter- and intra-seasonal foraging site fidelity. This behavior, coupled with environmental variation in Hg intake, may explain the high individual variability and occasionally highly contaminated turtle seen in this population and others (*6*, *10*, *11*). One individual (CC2151) in the live capture data set had blood and scute Hg concentrations 16 times higher than the rest, despite being well below the average size. While the source of these elevated Hg levels cannot be directly determined, CC2151 also had relatively high blood polychlorinated biphenyls (PCB) concentrations with a unique PCB profile (*62*). This PCB profile matched the congener profile of Aroclor 1268, which was produced exclusively at the Superfund site where a chlor-alkali plant was located in the Turtle/Brunswick River Estuary, GA (*63*). This estuary also suffers from severe Hg pollution and is located only 37 km from the capture location of the highly contaminated individual. The PCB data and consistently high Hg levels in both the blood and scutes provide strong circumstantial evidence that this estuary has served as a multi-season foraging site for this individual. While traceable compounds may not be available in most cases, the anomalously high Hg values reported in other sea turtle contaminant studies may also be related to point sources of Hg and foraging site fidelity.

These data suggest that blood and scutes provide a valid means for estimating Hg burdens in the loggerhead turtle, and perhaps other sea turtles. These nonlethal monitoring tools may also prove valuable for establishing estuarine and freshwater turtles as sentinel species for their respective environments. The technique of calculating an index value by normalizing blood Hg concentrations relative to scute Hg concentrations may be applicable to keratinized structures in mammals, birds, and other reptiles. This could provide additional data interpretation, particularly for assessing the recent environmental exposure of highly mobile or migratory species. Comparing the Hg concentrations in loggerhead turtles to other aquatic consumers shows they rank low relative to cetaceans, pinnepeds, and seabirds (*57*). Despite generally lower Hg concentrations, preliminary work has found negative correlations with several measures of immune health, suggesting these levels of Hg may be capable of inducing immunosuppression in free-ranging sea turtles (*57*). The emerging health concerns and endangered status of sea turtles make these monitoring techniques a useful means for further investigating trends in Hg contamination, and assessing the role of Hg in sea turtle health.

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Supporting Information Available

Six figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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