

INORGANIC ELEMENTS IN GREEN SEA TURTLES (*CHELONIA MYDAS*):
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Abstract: Inorganic elements from anthropogenic sources have entered marine environments worldwide and are detectable in marine organisms, including sea turtles. Threatened and endangered classifications of sea turtles have heretofore made assessments of contaminant concentrations difficult because of regulatory restrictions on obtaining samples using nonlethal techniques. In the present study, claw and skin biopsy samples were examined as potential indicators of internal tissue burdens in green sea turtles (*Chelonia mydas*). Significant relationships were observed between claw and liver, and claw and muscle concentrations of mercury, nickel, arsenic, and selenium ($p < 0.05$). Similarly, significant relationships were observed between skin biopsy concentrations and those in liver, kidney, and muscle tissues for mercury, arsenic, selenium, and vanadium ($p < 0.05$). Concentrations of arsenic, barium, chromium, nickel, strontium, vanadium, and zinc in claws and skin biopsies were substantially elevated when compared with all other tissues, indicating that these highly keratinized tissues may represent sequestration or excretion pathways. Correlations between standard carapace length and cobalt, lead, and manganese concentrations were observed ($p < 0.05$), indicating that tissue concentrations of these elements may be related to age and size. Results suggest that claws may indeed be useful indicators of mercury and nickel concentrations in liver and muscle tissues, whereas skin biopsy inorganic element concentrations may be better suited as indicators of mercury, selenium, and vanadium concentrations in liver, kidney, and muscle tissues of green sea turtles. *Environ Toxicol Chem* 2014;33:2020–2027. © 2014 SETAC

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INTRODUCTION

Metals and other trace elements (hereafter, “inorganic elements,” inclusive of transition metal cations, metalloids, and inorganic anion species) from anthropogenic sources have entered marine environments, including those contaminants that drain from waterways of the continental United States into the Gulf of Mexico [1,2]. Inorganic element concentrations have been reported for sediments [3,4], sea grass (*Thalassia testudinum*) [5], juvenile red snapper (*Lutjanus campechanus*) [6], oysters (*Crassostrea virginica*) [7], and bottlenose dolphins (*Tursiops truncatus*) [8], among other organisms and environmental samples collected from the Gulf of Mexico. However, concentrations of inorganic elements in sea turtles from the Gulf of Mexico have not yet been well characterized [9]; L.M. Orvik, 1997, Master’s thesis, Texas A&M University, College Station, TX, USA). Because all sea turtle species found in the Gulf of Mexico are listed as threatened or endangered under the Endangered Species Act [10], more comprehensive assessments of sea turtle exposure to inorganic elements and other contaminants are warranted.

Green sea turtles (*Chelonia mydas*) are classified as threatened in the Gulf of Mexico; but in other areas of North America where breeding populations exist (e.g., Florida’s Atlantic coast, Mexico’s Pacific coast), they are classified as

endangered [10]. Internationally, green sea turtles are classified as endangered by the International Union for Conservation of Nature and listed in Appendix I of the Convention on International Trade in Endangered Species of Flora and Fauna due to extensive subpopulation declines worldwide [11,12]. Population declines have been attributed to intentional harvesting of eggs and turtles, fishing by-catch, habitat degradation, disease, and environmental contaminants [11,13].

Pesticides, inorganic elements, and polychlorinated biphenyls have been detected in tissues and eggs of green sea turtles [14–16]. Contaminant concentrations at which adverse effects occur have not been established [14,17]. Several studies have determined inorganic element concentrations in tissues of opportunistically collected sea turtles (e.g., those that have become stranded, were cold-stunned, or were obtained from fishing by-catch) [17–22]. These studies reported concentrations of mercury, cadmium, chromium, lead, selenium, cobalt, manganese, strontium, molybdenum, antimony, barium, thallium, vanadium, zinc, and copper in liver, kidney, muscle, fat, blood, and gastrointestinal (GI) tract contents [19,23–31]. In light of the protected status afforded to sea turtles by the Endangered Species Act and the International Union for Conservation of Nature, development and validation of minimally invasive tissue sampling techniques would facilitate research on exposure to contaminants among sea turtles without adversely impacting these charismatic marine organisms.

Nonlethal, minimally invasive tissue sample collection techniques are needed so that internal tissue residue concentrations can be assessed in living sea turtles. Such techniques have been established for many other vertebrate species; notable

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examples are assessments of a wide variety of chemicals in fingernails and hair from humans [32,33] and eggs and feathers from birds [34,35]. Among reptilian species, chorioallantoic membranes have been used to provide estimates of polychlorinated biphenyl exposure in alligators [36,37] and loggerhead sea turtles (*Caretta caretta*) [38], caudal scutes and eggs were indicators of mercury exposure among crocodiles [39], and eggs have been used to assess exposure to organochlorine pesticides among Morelet's crocodiles [40,41]. Tail clippings have also been reported to be useful indicators of contaminant exposure among snakes [42,43]. Several different tissues from terrestrial, freshwater, and marine turtle species have been examined as indicators of inorganic element exposure, including eggs of green sea turtles [22,44]; scutes of loggerhead, black (*Chelonia mydas agassizi*), and Kemp's ridley sea turtles [11,17,21,45,46]; and blood, claws, and scutes of several terrestrial and freshwater turtle species [20,47,48].

For nonlethal, minimally invasive techniques to provide meaningful and useful information, establishment of strong relationships between potential indicator tissue concentrations and internal target tissue concentrations is critical [49,50]. In the present study, inorganic element concentrations were determined for external and internal tissues of green sea turtles collected opportunistically from Laguna Madre at Padre Island National Seashore. The primary objective was to examine the utility of external tissues (front flipper claw clippings and skin biopsies) as indicators of internal tissue (liver, kidney, and muscle) inorganic element concentrations.

MATERIALS AND METHODS

Tissue collection

Tissues (liver, kidney, cardiac blood, GI tract contents, muscle, flipper containing claw and skin) were obtained from 12 cold-stunned juvenile green sea turtles collected from the Laguna Madre at Padre Island National Seashore from 2 February to 12 February 2011. Samples were shipped frozen to Texas Tech University (Lubbock, TX, USA) on 29 March 2011 and stored at -80°C until sample digestion. All tissues were collected under US Fish and Wildlife Service permit TE840727-3 and Texas Parks and Wildlife Department permit SPR-0190-122. Sex and standard carapace length (SCL) were determined at the time of necropsy. Tissues were obtained from 5 male and 7 female sea turtles with mean ($\pm$ standard error [SE]) SCL of 49.9 ± 2.0 cm (range, 46.2–56.4 cm) and 52.6 ± 3.9 cm (range, 42.7–74 cm), respectively.

Skin biopsies were obtained from the dorsal surface of the shoulder region using an 8-mm diameter biopsy punch after the skin surface was rinsed with 18 MOhm water to remove external surface contamination. The biopsy punch was detergent-washed and rinsed sequentially with tap water, 10% nitric acid, and deionized water, between samples. As in other studies [51,52], 2 skin layers, dermis and epidermis, were identified using a Leica MZ9.5 stereomicroscope (Leica Microsystems). Connective tissue was removed with a scalpel so that only dermis and epidermis skin layers remained. Claws were removed from the flippers using a detergent-washed, hardened steel animal claw clipper. Claws were subsequently rinsed in 18 MOhm water to remove external surface contamination.

Tissue digestion

Tissues (0.5 ± 0.01 g wet wt for all but claw and skin biopsies, which were digested in their entirety) were weighed in a 50-mL polypropylene centrifuge tube. Skin biopsies were air

dried for 24 h inside a fume hood in the centrifuge tubes in which they were digested. Methods for tissue digestion were modifications of those performed by Rainwater et al. [39]. Briefly, 3 mL of 1:1 v/v nitric acid:hydrochloric acid (Fisher Scientific) was added to each tube. Tubes were placed in a $90 \pm 5^{\circ}\text{C}$ water bath (Isotemp 210; Fisher Scientific) and digested for approximately 1 h until the solution was clear. Digested samples were removed from the water bath and cooled for 15 min. Following addition of 1.5 mL of 30% hydrogen peroxide (Fisher Scientific) to each tube, all digests were returned to the water bath for approximately 45 min. Digests were then removed from the water bath and centrifuged at 3000 g for 30 min on a Beckman Allegra 6R centrifuge to remove undigested fats. Supernatants were brought to 50-mL volume with 18 MOhm water and placed in new 50-mL polypropylene centrifuge tubes for analysis.

The following quality assurance and control samples were digested and analyzed in the same fashion with each set of sample tissues: 1 duplicate of a randomly selected sample for each tissue, 0.25 ± 0.01 g of National Research Council Canada DORM-2 dogfish muscle certified reference material for trace metals, 0.25 ± 0.01 g National Research Council Canada DOLT-3 dogfish liver certified reference material for trace metals, and reagent blanks.

Mercury analysis

Mercury (Hg) analyses were performed using a CETAC M-8000 quick trace cold vapor atomic absorption mercury analyzer equipped with a CETAC ASX-520 autosampler and quick trace software. A single amalgamation method, which uses a gold trap, was used to address matrix effects and allow for lower detection limits [48]. The method detection limit and limit of quantification for mercury can be found in Supplemental Data, Table S1. As previously noted, standards were also digested in the same manner as the samples along with instrument duplicates, while $1.0 \mu\text{g/L}$ Hg check standards were performed every 10 samples for quality control and assurance. Concentrations were calculated as mg/kg of tissue and corrected based on recovery percentages of DOLT-3 within each set of analyses due to lower Hg recovery percentage of ($\text{mean} \pm \text{SE}$) $83.8 \pm 3.9\%$ for DOLT-3. Concentrations were calculated as mg/kg of wet tissue for GI tract contents, liver, kidney, muscle, cardiac blood, and flipper claw, and they were calculated as mg/kg of dry tissue for skin.

Inorganic element analysis

Analysis of tissue digests for 16 inorganic elements (vanadium, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, strontium, molybdenum, cadmium, antimony, barium, thallium, and lead) were performed using an Agilent 7500cs series inductively coupled plasma-mass spectrometer (ICP-MS) with an octopole reaction system equipped with an Agilent ASX-50 Series ICP-MS Autosampler and Agilent Chemstation software. The following elements (atomic mass) were used as internal standards: scandium (45) for vanadium, chromium, manganese, cobalt, nickel, copper, zinc, and arsenic; indium (115) for selenium, strontium, molybdenum, cadmium, antimony, and barium; and terbium (159) for thallium and lead. Method detection limits and limits of quantification for these inorganic elements can be found in Supplemental Data, Table S1. For instrument quality control purposes, instrument duplicates and check standards were performed every 10 samples. Coefficients of determinations for calibrations were deemed acceptable when 0.99 or greater. Concentrations were

calculated as mg/kg of wet tissue for GI tract contents, liver, kidney, muscle, cardiac blood, and flipper claw, and they were calculated as mg/kg of dry tissue for skin. No concentration corrections were made for these 16 inorganic elements because their recoveries were close to the certified concentrations with a recovery percentage of (mean $\pm$ SE) $92.5 \pm 8.0\%$ for DOLT-3. Quality assurance and quality control results are presented in the Results section. In circumstances in which there were fewer than 3 detections, standard error of the mean could not be calculated, and only the means of detectable concentrations are presented.

Statistical analyses

Model II major axis regression [53] was used to determine relationships between internal tissue (liver, kidney, and muscle) and claw or skin concentrations for each inorganic element irrespective of sex. Model II major axis regression takes into consideration deviations from both independent and dependent variables, whereas a model I ordinary least squares regression takes into consideration deviations from only the dependent variable [54]. Model II major axis regression was used because approximately equal random variation (i.e., not controlled by the researcher) was assumed in both independent and dependent variables [55]. Tissue concentrations in green sea turtles were a result of environmental exposure, so random variation in concentrations was expected. However, variation was assumed to be equal for independent and dependent variables, so major axis regression was the most appropriate model II regression method [54]. Analysis of covariance (ANCOVA) was used to determine if internal tissue (liver, kidney, and muscle) inorganic element concentrations were different between male and female turtles using claw or skin inorganic element concentrations as covariates. Concentrations were log base 10 transformed [$\log_{10}(\text{concentration} + 1)$] to meet assumptions of normality associated with ANCOVA and model II major axis regression analyses. Regressions and ANCOVA analyses were performed only when at least 6 data points were available. Pearson product-moment correlations were performed between all possible combinations of concentrations in tissues and SCL to examine influence of age on inorganic element internal tissue burdens. An alpha level of 0.05 was set for all statistical analyses. All

statistical analyses were performed in R version 2.13.1 statistical software [56].

RESULTS

Tissue concentrations

Concentrations of arsenic, chromium, copper, strontium, vanadium, and zinc in most tissues were elevated (i.e., > 1 mg/kg) relative to other inorganic element concentrations. Thallium was below the method detection limit in all samples (Table 1). Mean concentrations of arsenic, barium, chromium, manganese, selenium, strontium, vanadium, and zinc were higher in claws than all other tissues. Mean concentrations of antimony, copper, and mercury were highest in liver tissues. Mean copper concentration in liver tissue was more than 8 times greater than that of any other tissue. Mean concentrations of cadmium and cobalt were highest in kidney tissues (Table 1).

Arsenic, barium, chromium, nickel, strontium, vanadium, and zinc concentrations were highest in claw and skin biopsies, whereas antimony, cadmium, cobalt, and copper concentrations were generally highest among liver, kidney, or muscle relative to other tissues. With the exceptions of antimony, lead, manganese, molybdenum, and strontium, inorganic element concentrations of GI tract contents were among the lowest concentrations found in all tissues. Cardiac blood concentrations of all inorganic elements had similarly low relative tissue concentrations (Figure 1).

Recovery percentages for mercury analyses (mean $\pm$ standard error) were $75.8 \pm 7.1\%$ for DORM-2, $83.8 \pm 3.9\%$ for DOLT-3, and $105.8 \pm 1.1\%$ for the $1.0\text{-}\mu\text{g/L}$ check standard, and $102.8 \pm 2.3\%$ for the $1.0\text{-}\mu\text{g/L}$ blank spike. Relative difference percentages for instrument and sample duplicates for mercury analyses (mean $\pm$ SE) were $1.5 \pm 1.6\%$ and $15.8 \pm 12.3\%$, respectively. Recovery percentages for other inorganic element analyses were (mean $\pm$ SE) $92.5 \pm 8.0\%$ for DOLT-3 and $107.5 \pm 2.7\%$ for check standards. Relative difference percentages for instrument and sample duplicates (mean $\pm$ SE) were $7.9 \pm 10.5\%$ and $0.6 \pm 29.1\%$, respectively. Higher relative difference percentages for sample duplicates often were associated with higher values when GI tract contents were used as sample duplicates.

Table 1. Mean ($\pm$ standard error) inorganic element concentrations in gastrointestinal (GI) tract contents, liver, kidney, muscle, cardiac blood, and claw in mg/kg of wet tissue, and skin tissues in mg/kg of dry tissue of green sea turtles (*Chelonia mydas*) collected in the Laguna Madre near Padre Island National Seashore, Texas, USA, from 2 February to 12 February 2011^a

Inorganic analyte	GI tract contents	Liver	Kidney	Muscle	Cardiac blood	Flipper claw	Skin
Antimony	0.046 (2) ^b	0.093 $\pm$ 0.019 (9)	LOQ	0.031 (1) ^b	LOQ	LOQ	LOQ
Arsenic	3.02 $\pm$ 0.35	2.71 $\pm$ 0.25	3.37 $\pm$ 0.61	6.86 $\pm$ 1.07	2.57 $\pm$ 0.26	22.7 $\pm$ 7.6	17.6 $\pm$ 1.5
Barium	0.953 $\pm$ 0.176	0.056 $\pm$ 0.003 (5)	0.201 $\pm$ 0.084 (6)	MDL	0.060 $\pm$ 0.003 (3)	7.12 $\pm$ 1.90	3.80 $\pm$ 0.42
Cadmium	0.063 $\pm$ 0.006	0.904 $\pm$ 0.127	3.07 $\pm$ 0.61	0.043 (1) ^b	0.102 $\pm$ 0.013	MDL	MDL
Cobalt	0.065 $\pm$ 0.005 (7)	0.273 $\pm$ 0.065	0.595 $\pm$ 0.057	MDL	0.065 $\pm$ 0.006 (8)	MDL	LOQ
Copper	0.576 $\pm$ 0.097	37.1 $\pm$ 7.3	3.24 $\pm$ 1.11	0.651 $\pm$ 0.091	4.48 $\pm$ 1.13	0.941 $\pm$ 0.118 (4)	3.88 $\pm$ 0.37
Chromium	6.48 $\pm$ 0.68	4.35 $\pm$ 0.11	4.28 $\pm$ 0.18	4.80 $\pm$ 0.12	3.79 $\pm$ 0.07	84.3 $\pm$ 29.4	46.9 $\pm$ 4.7
Lead	0.236 $\pm$ 0.047 (10)	0.107 $\pm$ 0.029 (11)	0.215 $\pm$ 0.111 (9)	MDL	0.063 $\pm$ 0.008 (10)	1.54 (1) ^b	LOQ
Manganese	2.65 $\pm$ 0.43	2.31 $\pm$ 0.18	1.27 $\pm$ 0.17	0.239 $\pm$ 0.047	0.283 $\pm$ 0.042	4.22 $\pm$ 1.76	1.15 $\pm$ 0.23 (6)
Mercury	0.0023 $\pm$ 0.0002	0.081 $\pm$ 0.015	0.023 $\pm$ 0.003	0.012 $\pm$ 0.003	0.008 $\pm$ 0.001	0.070 $\pm$ 0.017	0.027 $\pm$ 0.004
Molybdenum	0.305 $\pm$ 0.028 (11)	0.189 $\pm$ 0.013	0.076 $\pm$ 0.007 (9)	0.015 (1) ^b	LOQ	1.12 (1) ^b	LOQ
Nickel	0.083 $\pm$ 0.011	0.151 $\pm$ 0.032	0.066 $\pm$ 0.007	0.616 (1) ^b	0.211 $\pm$ 0.080	0.003 $\pm$ 0.001	MDL
Selenium	0.358 $\pm$ 0.026	1.65 $\pm$ 0.15	0.881 $\pm$ 0.089	0.865 $\pm$ 0.085	0.728 $\pm$ 0.065	2.96 $\pm$ 0.82	2.01 $\pm$ 0.18 (11)
Strontium	10.6 $\pm$ 1.7	0.972 $\pm$ 0.111	1.31 $\pm$ 0.12	0.336 $\pm$ 0.039	0.468 $\pm$ 0.022	58.2 $\pm$ 21.5	3.00 $\pm$ 0.21
Thallium	MDL	MDL	MDL	MDL	MDL	MDL	MDL
Vanadium	2.11 $\pm$ 0.21	1.24 $\pm$ 0.02	1.17 $\pm$ 0.02	1.35 $\pm$ 0.04	1.09 $\pm$ 0.02	23.6 $\pm$ 8.8	13.9 $\pm$ 1.4
Zinc	3.49 $\pm$ 0.40	35.0 $\pm$ 3.3	27.9 $\pm$ 5.7	16.0 $\pm$ 2.2	11.4 $\pm$ 0.6	146 $\pm$ 15	43.8 $\pm$ 4.6

^aValues in parentheses represent number of turtles in which inorganic analyte was detected.

^bStandard errors not calculated because inorganic analyte was detected in fewer than 3 turtles.

LOQ = below limit of quantification; MDL = below method detection limit (calculated as $2 \times$ blank response).

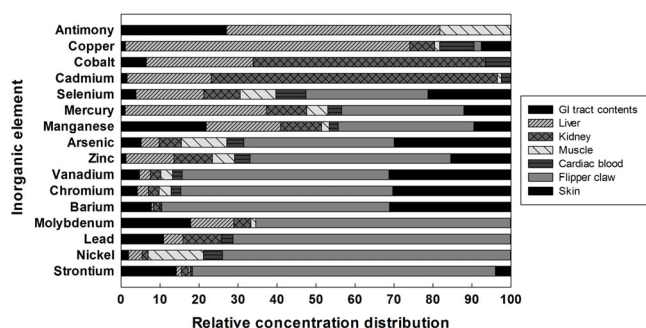


Figure 1. Relative distribution of inorganic element concentrations in gastrointestinal (GI) tract contents, liver, kidney, muscle, cardiac blood, claw, and skin tissues of green sea turtles (*Chelonia mydas*) collected from Laguna Madre at Padre Island National Seashore, Texas, USA, from 2 February to 12 February 2011.

Internal tissue relationships with claw and skin

Significant positive relationships between claw and internal tissues and skin and internal tissues were observed between log-transformed concentrations of specific inorganic elements (Table 2; Figures 2 and 3). Nickel and mercury concentrations were each significantly related between liver and claw tissues. Mercury, arsenic, and selenium were all related between muscle and claw tissues (Table 2, Figure 2). Significant positive relationships were observed with vanadium, selenium, arsenic, and mercury in liver and skin tissue comparisons; vanadium and selenium in kidney and skin tissue comparisons; and mercury, selenium, arsenic, and vanadium in muscle and skin tissue comparisons (Table 2, Figure 3). No other relationships were observed between internal tissue (liver, kidney, and muscle) and claw or skin concentrations (all $p \geq 0.06$). Additionally, there was no effect of sex on relationships between claws (all $p \geq 0.106$) or skin (all $p \geq 0.155$) and liver, kidney, or muscle

Table 2. Regression relationships of inorganic element concentrations among tissues of 12 opportunistically collected green sea turtles (*Chelonia mydas*) from the Laguna Madre at Padre Island National Seashore, Texas, USA, from 2 February to 12 February 2011

Regression ^a	Inorganic analyte	Slope ^b	Intercept ^c	r^2	p value	n
Claw vs						
Muscle	Hg	0.164	0.0005	0.730	< 0.001	12
Liver	Hg	0.822	0.010	0.453	0.017	12
Liver	Ni	28.5	0.028	0.844	0.003	7
Muscle	As	0.689	-0.020	0.311	0.030	11
Muscle	Se	0.173	0.173	0.255	0.047	12
Skin vs						
Muscle	Hg	0.778	-0.004	0.800	< 0.001	12
Liver	Hg	5.19	-0.026	0.462	0.015	12
Liver	As	0.707	-0.328	0.390	0.030	12
Muscle	As	2.36	-2.11	0.389	0.030	12
Muscle	Se	0.763	-0.087	0.698	0.001	11
Liver	Se	1.02	-0.060	0.445	0.025	11
Kidney	Se	0.703	-0.056	0.369	0.048	11
Liver	V	0.082	0.255	0.525	0.008	12
Kidney	V	0.060	0.266	0.361	0.039	12
Muscle	V	0.114	0.239	0.291	0.035	12

^aEach regression represents the relationship between the indicated tissue/inorganic analyte combination and claw or skin tissue concentrations of the same analyte.

^bSlopes have units of $\log_{10}[(\text{tissue analyte}) + 1] / \log_{10}[(\text{claw or skin analyte}) + 1]$.

^cIntercept values have units of $\log_{10}[(\text{tissue analyte}) + 1]$.

concentrations of any inorganic elements. Significant, positive correlations were observed between SCL and liver concentrations of cobalt ($r = 0.730$, $p = 0.007$) and lead ($r = 0.893$, $p < 0.001$). Manganese concentrations in cardiac blood were negatively correlated to SCL ($r = -0.594$, $p = 0.042$).

DISCUSSION

Liver, kidney, and muscle tissue concentrations of cadmium, chromium, copper, mercury, lead, and zinc in green sea turtles from the Gulf of Mexico were generally lower than concentrations observed in previous studies of green and Kemp's ridley sea turtles from the Gulf of Mexico [9]. However, concentrations of most elements were within the same order of magnitude [9]. Notable exceptions were chromium concentrations in kidney and muscle, which were 100 times and 10 times greater, respectively, compared with those reported by Wang [9]. Similarly, inorganic element concentrations in liver, kidney, and muscle tissues from the present study were generally lower, but within the same order of magnitude, than those of green sea turtle tissues from other regions of the world, including the Mediterranean Sea, East China Sea (Japan), South China Sea (China), and Hawaii (USA) [23,25,27,57]. Liver and kidney chromium concentrations observed in the present study were 4 times to 6 times greater than concentrations from green sea turtles in Hawaii and China [27,57].

Comparison of inorganic element tissue concentrations among sea turtles collected from different geographic regions is complicated by several factors. Green sea turtles occur worldwide in the Atlantic, Pacific, and Indian Oceans, as well as the Mediterranean and Black Seas [11,12]. They are highly mobile, often undertaking long-distance migrations between foraging grounds and nesting beaches separated by 1000 km or more [13] and spanning a wide range of habitats from sea grass beds to open ocean [10,11,13]. As green sea turtles mature, their diet shifts from an omnivory, consisting of juvenile fish, invertebrates, and crustaceans, to herbivory, comprised mainly of sea grass [13]. Therefore, green sea turtle exposure to inorganic elements and other contaminants may vary between feeding and nesting grounds, and age-related changes in diet may explain differences in concentrations observed across studies.

Other studies have evaluated correlations between sea turtle age and inorganic element concentrations in tissues and SCL, which varies with age and sexual maturity in green sea turtles [13]. Negative correlations between inorganic element concentrations in tissue and SCL have been reported for green and hawksbill turtles [23,26,29]. Kampalath et al. [26] postulated that such negative correlations may be attributed to changes in diet during maturation from juvenile to adulthood [26]. However, some inorganic element concentrations in blood and carapace tissues of Kemp's ridley sea turtles from the Gulf of Mexico are reported to be positively correlated with SCL [9]. In the present study, positive correlations were observed between SCL and cobalt and lead concentrations in liver, but negative between SCL and manganese concentrations in cardiac blood. Although only 3 inorganic elements were correlated with SCL, the number of green sea turtles (12) in the present study, their juvenile status, and their limited SCL range (42.7–74.0 cm) may not have been sufficient to effectively assess size-related correlations with inorganic element tissue concentrations in green sea turtles. Age- and size-related correlations with tissue element concentrations may also vary depending on factors such as turtle species, maturity, and geographic region.

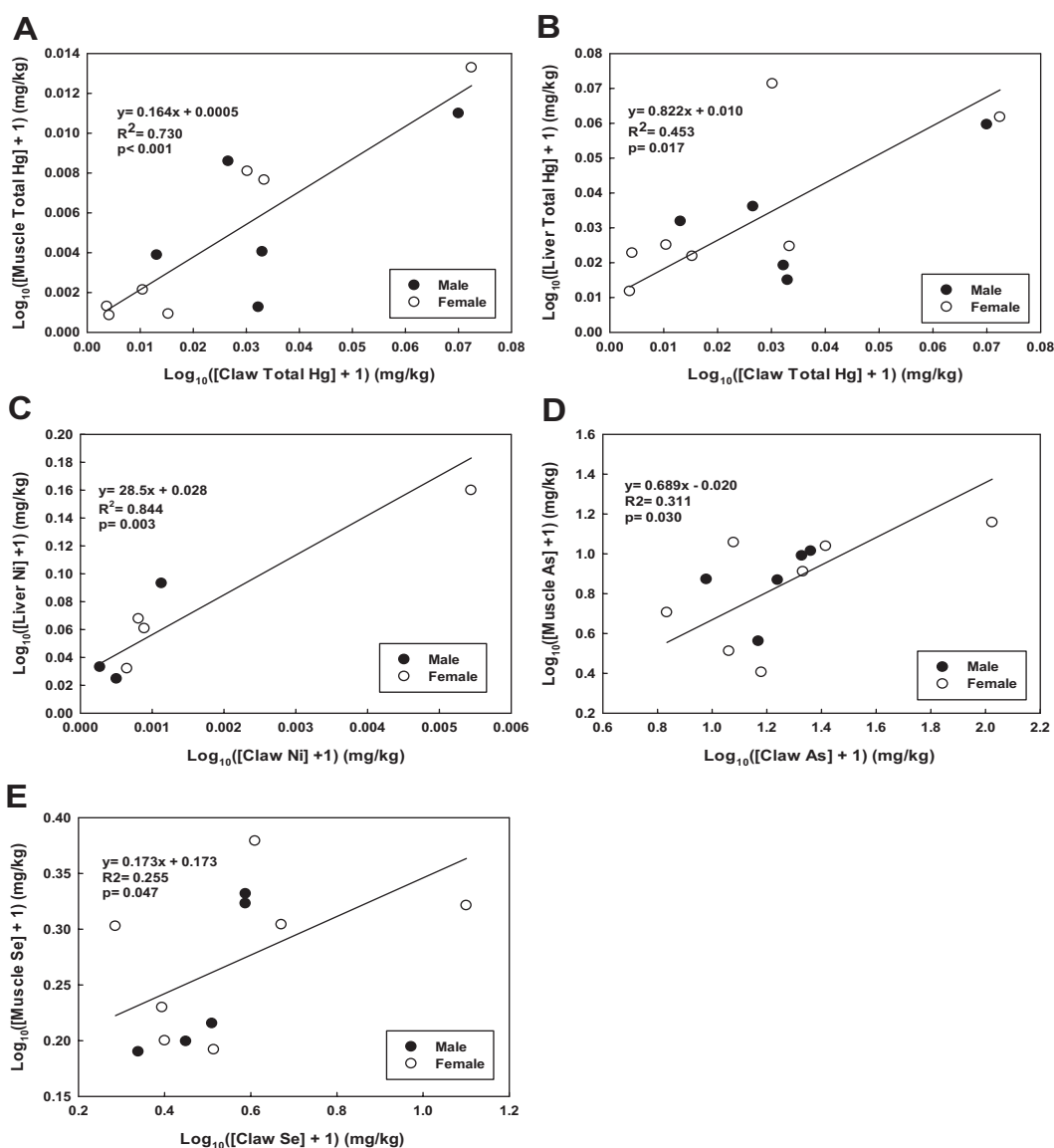


Figure 2. Regression relationships between liver or muscle tissue and claw inorganic element concentrations in green sea turtles (*Chelonia mydas*) collected from Laguna Madre at Padre Island National Seashore, Texas, USA, from 2 February to 12 February 2011 for mercury (A,B), nickel (C), arsenic (D), and selenium (E).

Effects of inorganic elements and other chemical contaminants on sea turtle health are largely unknown [14,17]. However, environmental contaminants have been suggested as possible contributing factors in development of fibropapillomas among sea turtles, possibly due to immunosuppression [51]. Fibropapillomas are tumors that form on the skin, mouth, or eyes of sea turtles, often resulting in mortality. This disease tends to occur in near-shore waters and areas near large human populations that receive higher contaminant loads from anthropogenic activities [52]. In the present study, however, no fibropapilloma was observed on green sea turtles from which tissues were obtained. Nonetheless, due to the increasing prevalence of fibropapillomas and other diseases among sea turtles, the role of contaminants in sea turtle morbidity warrants further investigation [17,58]. Thus, identification of minimally invasive indicator tissues for contaminant exposure assessments in sea turtles could facilitate further investigation of the role contaminants play in the occurrence of fibropapilloma formation and other health issues.

In the present study, we observed significant relationships in arsenic, mercury, nickel, selenium, and vanadium concen-

trations between external tissues (claw or skin) and internal tissues from green sea turtles. These relationships may be used by other investigators to estimate internal tissue inorganic element concentrations when claw and skin concentrations are within the range of those observed in the present study. Day et al. [17] has similarly reported a significant relationship between mercury in loggerhead sea turtle scutes and livers ($r^2 = 0.948$, $p = 0.001$). In a study of Kemp's ridley sea turtles collected from the Gulf of Mexico, Wang [9] suggested that carapace tissue could serve as a suitable surrogate for internal tissues when monitoring mercury exposure. Hopkins et al. [47] developed models to predict total mercury in muscle and eggs of snapping turtles using blood and claws as surrogate tissues. In contrast, other studies with loggerhead, black, and Kemp's ridley sea turtles have reported no significant relationships between external and internal tissue contaminant loads [20,21,46]. Conflicting results among these studies may be due to the differences in sea turtle species life history traits or variability in age among turtles, and relationships between indicator tissues and internal tissues may change or not exist in different species or turtles of different ages.

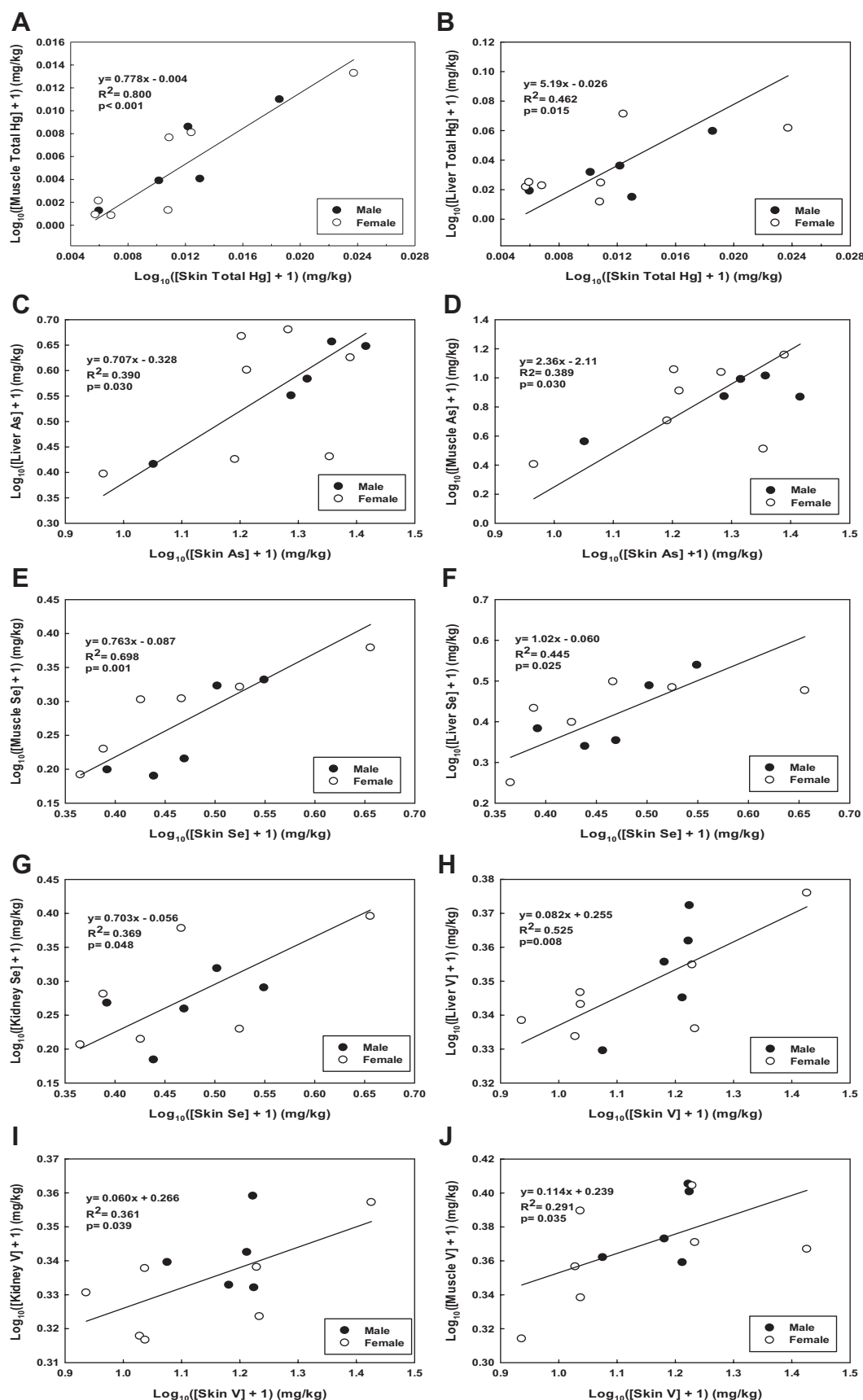


Figure 3. Regression relationships between liver, kidney, or muscle tissue and skin biopsy inorganic element concentrations in green sea turtles (*Chelonia mydas*) collected from Laguna Madre at Padre Island National Seashore, Texas, USA, from 2 February to 12 February 2011 for mercury (A,B), arsenic (C,D), selenium (E–G), and vanadium (H–J).

In the present study, concentrations of many inorganic elements in external tissues (i.e., claw and skin biopsies) were substantially higher than those quantified in internal tissues. Both the claw and epidermis are largely comprised of keratin [17], which has abundant sulfhydryl functional groups to which some inorganic elements may bind [59]. Some inorganic elements found in keratinized tissues often have limited mobility due to strong bonds formed with sulfhydryl groups and, as such, are isolated from metabolic activity resulting in accumulation in keratinized tissues. Accumulation in keratin-rich tissues may therefore reflect long-term exposure [32,48]. Distribution of inorganic elements to skin and claw tissues indicates that these tissues serve as excretion pathways for inorganic elements as has been observed for corn snake skin sheds (*Elaphe guttata*) [60]. Other highly keratinized and bony tissues of the carapace and plastron may also act to sequester inorganic elements in turtles.

In conclusion, positive relationships between internal and external tissue inorganic element concentrations indicate that claw clippings may serve as an indicator tissue for exposure to mercury, nickel, arsenic, and selenium in green sea turtles, whereas skin biopsies may be indicative of mercury, arsenic, selenium, and vanadium exposure. Therefore, claw clippings and skin biopsies may be used to estimate inorganic element body burdens among green sea turtles from the Gulf of Mexico. However, it is clear that inorganic element concentrations in green sea turtles vary in response to age, diet, and geographic region. Additional studies evaluating the effects of these factors on external and internal tissue inorganic element concentrations would be useful. Refinement and further validation of these and other minimally invasive, nonlethal sampling techniques may ultimately serve to better define concentrations of inorganic elements in internal tissues of species whose threatened and endangered status make meaningful contaminant monitoring difficult. Improved definition of the breadth of inorganic element concentrations would allow for anticipation of potential health effects as well as improved understanding of exposure pathways through correlations with elemental concentrations in ambient sediment, water, and prey/forage species.

SUPPLEMENTAL DATA

Table S1. (12 KB DOC).

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