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Trace Metal Concentrations in Blood of the Kemp's Ridley Sea Turtle (*Lepidochelys kempii*)

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ABSTRACT. – **Trace metal concentrations were analyzed from the blood of 106 Kemp's ridley sea turtles (*Lepidochelys kempii*) captured alive off Texas and Louisiana, USA, during June–October 1994 and May–August 1995. Copper, lead, mercury, silver, and zinc concentrations were measured in wild and headstarted animals and both sexes. Overall, levels in whole blood were: copper (range = 215–1300 ng/g, mean = 524 ng/g), lead (range = 0.00–34.3 ng/g, mean = 11.0 ng/g), mercury (range = 0.50–67.3 ng/g, mean = 18.0 ng/g), silver (range = 0.042–2.74 ng/g, mean = 0.94 ng/g), and zinc (range = 3280–18,900 ng/g, mean = 7500 ng/g). None of these concentrations differed significantly among wild, headstarted, female, and male ridleys. Copper, mercury, and zinc concentrations exhibited significant positive relationships with turtle size. Female ridleys displayed a stronger positive correlation between mercury and zinc concentrations and size than did males. Trace metal blood levels were lower than tissue levels reported elsewhere for marine and freshwater turtles, other reptiles, invertebrates, fish, marine birds, and mammals. Analysis of whole blood is a safe method to monitor trace metal levels in live sea turtles but must be considered a conservative estimate of these loads when compared with potentially higher levels in organs or other tissues.**

KEY WORDS. – **Reptilia; Testudines; Cheloniidae; *Lepidochelys kempii*; sea turtle; toxicology; trace metals; copper; lead; mercury; silver; zinc; blood; Texas; Louisiana; USA**

Available information on the relationship between sea turtle mortality and marine pollution pertains primarily to plastic bag ingestion and petroleum-related toxicity. Linkages between mortality and other pollutants such as trace metals are poorly known. Short-term and long-term effects resulting from exposure of sea turtles to pollutants could theoretically increase incidence of disease and lower reproduction rate (Magnuson et al., 1990; Chang, 1996), thus compromising survival of these locally depleted species. This threat mandates that sources of sea turtle mortality other than incidental capture in commercial fisheries be better understood in order to help reverse population declines.

The purpose of this study was to investigate trace metal concentrations in the Kemp's ridley sea turtle (*Lepidochelys kempii*) to develop a baseline for understanding possible health risks posed by this potential mortality threat. Recent increases in sea turtle strandings along the Texas and Louisiana coasts have heightened concern regarding man's role in sea turtle mortality (Byles et al., 1996). Both states are centers of two major industries (chemical and oil production) whose complicity in sea turtle mortality has been identified by environmental advocacy groups. Few literature sources identify causal relationships between industrial pollution and stresses upon marine life. Frazier (1980) speculated that the decline of the Kemp's ridley may be related to contaminant levels in discharges from the Mississippi River. Also, Chesher (1975) pointed out that desalination plant discharges exhibit higher salinities and contain heavy metals such as copper which adversely affect marine life.

Longevity and high mobility may enhance sea turtle exposure to some environmental toxicants and render them ideal indicators of contamination (Meyers-Schone and Walton, 1994). However, few studies have analyzed trace metal contaminants in sea turtles, particularly Kemp's ridleys. The analysis by Lance et al. (1995) of plasma zinc concentration in Kemp's ridleys was based upon only one individual. In addition, other studies, targeting green, loggerhead, and leatherback turtles have utilized different sampling methods for contaminant analyses. Aguirre et al. (1994) utilized egg shells, while other studies analyzed egg yolks (Hillestad et al., 1974; Stoneburner et al., 1980; Sakai et al., 1995) and bone and barnacles (Witkowski and Frazier, 1982). Liver, kidney, muscle, carapace scutes, and fat also have been monitored for metal accumulation (Davenport and Wrench, 1990; Landry and Sis, 1992; Aguirre et al., 1994; Edmonds et al., 1994; Gordon et al., 1998; Storelli et al., 1998; Presti et al., 1999).

Research summarized herein differs from most previous trace metal studies in that whole blood was analyzed in lieu of other tissues. This is an initial effort in addressing concerns, generating baseline information, and developing more focused objectives for future studies involving turtles with trace metals. The depleted status of critically endangered species like the Kemp's ridley renders tissue sampling from live animals problematical, and consequently, restricted most previous trace metal assessments of stranded carcasses. Many metals accumulate in tissues such as liver and kidney as a result of blood transport to these organs. Chang (1996) found blood to be an indicator of more recent exposure to the contaminant while long-term exposure oc-

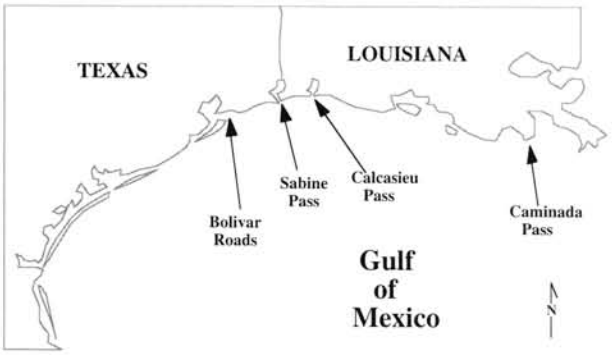

Figure 1. Sea turtle capture sites.

curs in tissues. The current research analyzed blood from a population of wild and headstarted ridleys caught and returned alive to the wild. The study measured concentrations of a suite of potentially harmful trace metals (copper, lead, mercury, silver, and zinc) in whole blood of the Kemp's ridley.

METHODS

Sea Turtle Capture. — A total of 106 Kemp's ridleys was captured alive in entanglement nets deployed at jettied beachfront habitats adjacent to Bolivar Roads, Sabine Pass, Calcasieu Pass, and Caminada Pass along the upper Texas and Louisiana coasts during June–October 1994 and May–August 1995 (Fig. 1). All ridleys were visually examined to determine whether they were wild or headstarted (if the latter, year class was determined from the type and location of the carapace scute bearing a living tag) (Landry et al., 1995). A living tag is defined as a plug of plastron tissue that is surgically embedded in a designated carapace scute denoting a particular year class. Size was measured by straight carapace length (SCL) and width (SCL) using calipers to the nearest 0.1 cm. Sex was determined by laparoscopy (Owens et al., 1978) and testosterone analysis (Coyne, 2000).

Metal Analysis. — Between 3.0 and 4.0 cc of whole blood was drawn from the cervical sinus immediately after capture (Owens and Ruiz, 1980). Each sample was placed on ice, transferred to a 5 ml cryovial, and frozen at –10°C for subsequent analysis. Silver, lead, copper, zinc, and mercury levels were measured by class-100 clean laboratory techniques (Patterson and Settle, 1976). Approximately 1 g of whole blood and 5 ml of high purity nitric acid (Q-HNO_3) were refluxed and dried in a Teflon vial at 60°C. Whole blood, instead of serum, was analyzed because trace elements adhere to blood constituents such as proteins in plasma, erythrocytes, and leukocytes (Dessauer, 1970) — as such, whole blood was considered a more reliable medium for monitoring trace metal concentrations. Upon cooling, the dried sample received 1 ml of high purity nitric acid and was again digested until dryness at 60°C and cooled. Following digestion, 2 ml of a 0.5 N high purity nitric acid solution was added to the Teflon vial.

Silver, lead, copper, and zinc determinations were conducted using a Perkin Elmer 5100 graphite furnace atomic absorption spectrophotometer (GFAAS) equipped with Zeeman background correction and L'vov platforms. The method of standard additions was used for silver and lead to correct for signal suppression by the blood matrix. For lead determinations, 5 µl of palladium-magnesium nitrate matrix modifier was used to stabilize the analyte at high temperatures during pyrolysis and control chemical interferences such as NaCl (Weltz et al., 1992).

Copper and zinc analyses required 1:100, 1:1000 dilutions, respectively, to lower the analyte signal within a suitable range (Cu: 0–10 ppb, Zn: 0–20 ppb). This large dilution precluded the blood matrix from suppressing the analyte signal significantly and, therefore, negated use of standard additions.

Mercury was monitored via an automated version of the cold vapor atomic fluorescence technique (Gill and Bruland, 1990). Analysis required a 1:4200 dilution with acidified high purity laboratory water to lower the analyte signal into a working range of 0–2 ppb.

All laboratory ware used in this work was subjected to rigorous cleaning to reduce contamination artifacts. Plasticware was soaked in a 1% laboratory detergent for at least 72 hrs, rinsed with deionized water and transferred to a 6 N HNO_3 solution, soaked at least 72 hrs, rinsed and soaked again for 72 hrs in a 6 N HCl solution. Plasticware was rinsed, dried, and stored in double ziplock bags until analysis. Method blanks, containing 1g of deionized water, accompanied each batch of 35 samples to monitor contamination. One blank contained deionized water from a heparinized syringe used in blood sampling. Detection limits for each metal were based on a signal three times the standard deviation of the method blank (Fig. 2). A certified standard reference material was analyzed with each batch of 35 samples. SRM-Dolt-1, dogfish liver, was analyzed for copper, lead, mercury, and zinc. Mean recovery and standard deviation for copper, lead, mercury, and zinc was 81 ± 2.5%, 98 ± 8.6%, 101 ± 12%, and 106 ± 6.5%, respectively. SRM-1566a, oyster tissue, was analyzed for silver and had a mean recovery of 101

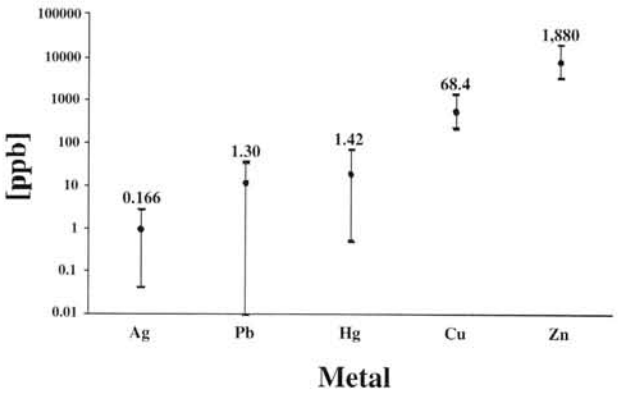

Figure 2. Range and mean of trace metal concentration in whole blood (ppb, wet weight) of all captured Kemp's ridley sea turtles. Number atop range bars represents detection limit for each metal.

Table 1. Range of trace metal concentrations (ng/g, wet weight) in whole blood of Kemp's ridley sea turtles captured during 1994 and 1995; *n* = number of captured turtles; n/d = not determined; SCL = straight carapace length.

Location	*n*	Wild/ Headstart	Sex	SCL (cm)	Silver (Ag)	Lead (Pb)	Copper (Cu)	Zinc (Zn)	Mercury (Hg)
Bolivar Roads	2	wild	female	40.6-49.1	0.43-0.49	5.0-22.7	483-616	6870-8880	14.9-17.4
Sabine Pass	24	wild	female	21.6-64.0	0.04-1.57	3.7-33.4	318-786	4370-18800	5.4-61.1
	31	wild	male	23.9-54.4	0.05-2.74	0.0-34.3	290-812	4430-13200	0.5-35.6
	1	wild	n/d	53.8	1.80	6.6	471	6830	17.2
	3	headstart	female	33.2-40.0	0.56-1.38	1.0-8.0	486-711	5880-6450	14.5-26.5
Calcasieu Pass	12	wild	female	23.6-64.6	0.43-1.63	3.9-28.2	346-1300	4040-18900	0.5-55.1
	7	wild	male	24.9-56.3	0.30-2.26	6.5-15.9	369-623	3280-7470	7.5-49.7
	21	wild	n/d	25.7-65.8	0.12-2.01	2.9-20.3	215-1150	5610-12400	6.5-67.3
	4	headstart	female	30.1-39.5	0.95-2.11	8.5-15.4	339-557	5780-8940	11.1-15.3
Caminada Pass	1	wild	female	51.9	0.86	10.7	512	7670	36.3
Overall Range	106	—	—	21.6-65.8	0.04-2.74	0.0-34.3	215-1300	3280-18900	0.5-67.3
Overall Mean	—	—	—	38.2	0.94	11.0	524	7500	18.0

± 3.6%. Repeated analyses of several samples varied by less than 10%.

Parametric and non-parametric statistical tests were utilized to assess trace metal levels. These data did not satisfy required assumptions of normality and homogeneity of variance; therefore, the non-parametric chi-square test was applied to characterize differences between trace metal concentration and straight carapace length in wild, headstarted, male, and female ridleys. Log transformations were attempted to normalize the data, but proved unsuccessful. Standard deviations were not determined based on the requirements of normality. Regression analysis compared trace metal concentration with straight carapace length. Student's t-tests were used to compare slopes of regressions of concentration on straight carapace length for males and females.

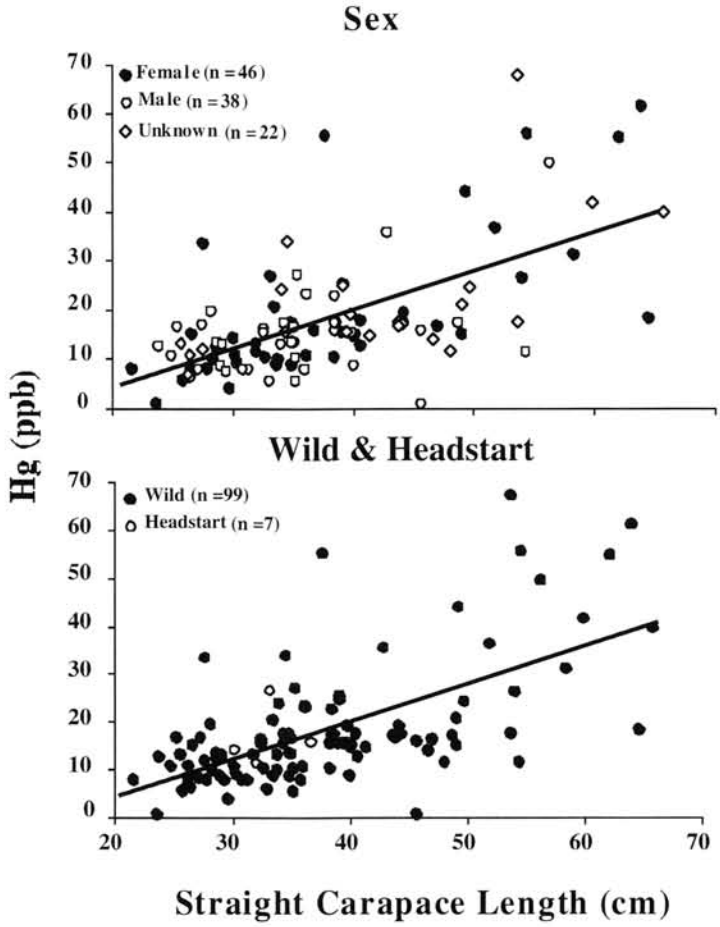

Figure 3. Regression ($y = 0.781x - 11.7$, $r^2 = 0.387$) of mercury concentration in whole blood (ppb, wet weight) versus carapace length (cm) for Kemp's ridley sea turtles captured in 1994 and 1995.

RESULTS

Turtle Demographics. — Straight carapace length of 96 wild ridleys (mean = 38.2 cm, range = 21.6–65.8 cm) did not differ significantly ($\chi^2 = 0.156$, $p < 0.693$) from that of 7 headstarted animals (mean = 35.2 cm, range = 30.1–40.0 cm) used in this study (Table 1). Sex of the sample included 38 (36%) males, 46 (43%) females, and 22 (21%) ridleys of undetermined gender (hereafter classified as unknown). All the headstarted turtles were females. Straight carapace lengths of males (mean = 35.4 cm, range = 23.9–56.3 cm) were statistically similar ($\chi^2 = 0.977$, $p < 0.323$) to those of females (mean = 38.2 cm, range = 21.6–64.6 cm).

Trace Metal Analyses. — Overall range and mean values of trace metal concentrations (ng/g, wet weight) are given in Table 1 and contrasted in Fig. 2. All metals were statistically analyzed with the Pearson's correlation coefficient analysis to determine any significant relationships among metals. The analysis yielded positive significant relationships between zinc and copper ($r = 0.33$, $p < 0.00$), zinc and mercury ($r = 0.40$, $p < 0.00$), and copper and mercury ($r = 0.33$, $p < 0.00$). No other relationships were significant.

No significant concentration differences were detected among headstarted, wild, female, and male ridleys. Overall, wild ridleys displayed higher levels of lead and mercury, while headstarted turtles exhibited slightly higher concentrations of copper, silver, and zinc. Copper ($p < 0.028$), mercury ($p < 0.000$; Fig. 3), and zinc ($p < 0.000$) levels increased significantly with size for all turtles. Headstarted turtles (all females) exhibited a significant correlation ($p < 0.025$, $n = 7$) between copper concentration and size not evident for wild females ($p < 0.671$, $n = 39$). Female ridleys exhibited a more rapid increase in mercury ($p < 0.002$; Fig. 4) and zinc ($p < 0.017$) concentration with turtle size than did males.

DISCUSSION

The Kemp's ridley's endangered status in the USA creates certain regulatory problems for sampling tissues from live animals. Consequently, the present study utilized whole blood as an analytical agent to monitor trace metal concentrations in this species. Although previous sea turtle research efforts have not utilized a whole blood protocol, the present study found the sampling method safe in assessing trace metal levels, especially in live animals.

Limited pertinent literature on trace metal concentration in sea turtles rendered interpretation of our values determined from blood analysis difficult. Consequently, trace metal levels (ng/g, wet weight) in blood of the Kemp's ridley were compared to tissue levels (ng/g, wet weight) from loggerhead, green, hawksbill, and olive ridley sea turtles, freshwater turtles, other reptiles, invertebrates, fish, birds, and mammals (Orvik, 1997). Chang's (1996) description of blood as a carrier of elements to tissues such as kidney, liver, and muscle tissues suggests that organs contain higher trace metal levels than those in whole blood. Orvik's (1997) preliminary comparison of concentrations in the present study with historically reported tissue metal levels from other organisms supports this hypothesis.

This study used class-100 clean laboratory techniques which yielded lower detection levels (ppb) than most previous reports. Therefore, it is not always appropriate to compare our ridley blood concentrations reported herein with those from other analytical efforts. Furthermore, it is difficult to evaluate the accuracy of other studies, but it is important to recognize that those which did not use class-100 clean laboratory techniques may have limitations due to contamination control. As such, ridley blood levels measured herein may or may not be comparable to tissue metal levels reported elsewhere.

Copper. — Copper, an essential cofactor in blood for several metabolic pathways, is usually obtained through

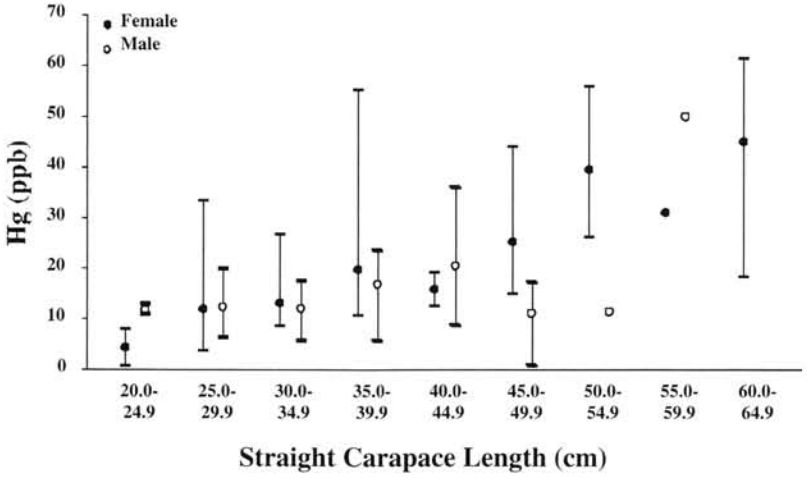

Figure 4. Range and mean mercury concentration in whole blood (ppb, wet weight) in relation to carapace length (cm) of female and male Kemp's ridley sea turtles.

dietary sources (Chang, 1996). Kemp's ridleys inhabiting shallow Gulf waters forage predominantly on blue crabs (Shaver, 1991; Werner, 1994), whose blood pigment hemocyanin typically contains a high percentage of copper (Lance et al., 1983). Overall mean copper concentration (524 ng/g, wet weight) in ridley blood was much lower than that in edible blue crab tissue (9220 ng/g, wet weight; Brooks et al., 1992) analyzed from Galveston Bay. Marine species from Sabine Pass, Calcasieu Pass, and Galveston Bay are more likely to be exposed to similar pollution sources because of their proximity in location and related chemical and oil production industries. Ridleys' whole blood copper levels were similar to plasma levels of alligators (600–770 ng/g, wet weight; Lance et al., 1983), another crab predator.

The significant positive relationship detected between ridley copper concentration and size may be due to bias from headstarted animals fed a pelletized food during captivity. Headstarted ridleys exhibited increasing copper concentration with size, a trend not evident among wild animals. Pelletized food fed to headstarted animals prior to their release into the wild was recently analyzed, which indicated that it contains approximately 800 ng/g wet weight of copper (Orvik, unpubl. data).

The mean copper concentration reported in this study was lower than that detected for green and loggerhead sea turtle eggs (1480–6000 ng/g, wet weight; Hillestad et al., 1974; Sakai et al., 1995), kidney (1100–10,500 ng/g, wet weight; Landry and Sis, 1992; Aguirre et al., 1994) and liver (1300–189,000 ng/g, wet weight; Landry and Sis, 1992; Aguirre et al., 1994). Slightly higher copper levels reported for loggerheads by these workers suggest this element bioconcentrates within organs, and since both species forage primarily on portunid crabs, they may exhibit similar trends in trace metal dynamics (Magnuson et al., 1990).

Lead. — Lead is a biologically toxic nonessential element subject to bioaccumulation, and absorbed into the circulatory system and distributed to kidney, liver, and bone. Highest lead concentrations are typically found in the skeleton of vertebrates (Chang, 1996). Overall, mean blood lead concentration (11 ng/g, wet weight) was similar to that of ridley food sources (blue crabs, 23 ng/g, and edible fish tissue, 16 ng/g, wet weight) reported from Galveston Bay (Brooks et al., 1992). These similarities suggest that ridleys blood may reflect the lead levels in prey species. Moreover, wild ridleys contained higher blood lead levels than did headstarted animals, a pattern possibly due to the former's

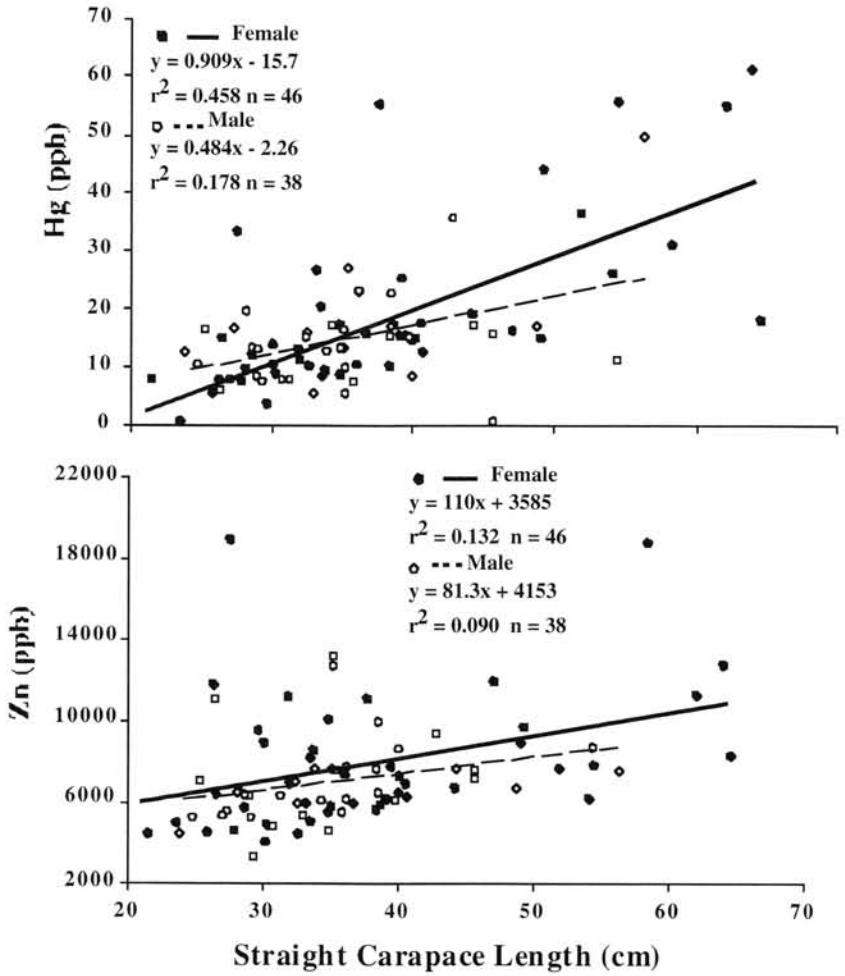

Figure 5. Regression of mercury and zinc concentration in whole blood (ppb, wet weight) and carapace length (cm) for female and male Kemp's ridley sea turtles captured in 1994 and 1995.

additional years of exposure to wild food sources and waterborne lead in the Gulf.

Ridley blood lead levels were lower than plasma lead levels (280–2514 ng/g, wet weight) found in snapping turtles (*Chelydra serpentina*) from three different locations in the midwest (Overmann and Krajicek, 1995). These authors also indicated that there were positive correlations found between liver and blood, liver and carapace, and bone and carapace of the snapping turtles. Lead concentrations in ridley blood also were lower than those reported in loggerhead and green sea turtle eggs, kidney, and liver (Hillestad et al., 1974; Landry and Sis, 1992; Aquirre et al., 1994; Storelli et al., 1998). Witkowski and Frazier (1982) found lead bone concentrations in an unidentified sea turtle species (39,000–110,000 ng/g, dry weight) a magnitude or more higher than those reported herein for blood, thereby suggesting accumulation in calcified tissues of sea turtles. Likewise, Hulse et al. (1980) found that certain birds, laughing gulls (*Larus atricilla*) and cattle egrets (*Bubulcus ibis*), exhibited highest concentrations in their bones.

Mercury. — Mercury is a nonessential metal and is seriously toxic to marine life. Most mercury exposure results from consumption of fish or other marine life whereby it is transported by the blood to the kidney, liver, and brain (Chang, 1996). Mercury measurements in this study were for total mercury (both inorganic and organic monomethylmercury); however, only methylmercury markedly bioaccumulates up the food chain (Mason et al., 1996). Methylmercury has also been found to have a relatively long half-life. Blood analysis may reflect long-term mercury uptake provided that the animal is repeatedly exposed to the dietary resource (Chang, 1996). Most studies have utilized total mercury analysis rather than methylmercury analysis because it is the most common method for determining mercury in organisms and it creates a standard for comparisons between laboratories (Bloom, 1991).

Kemp's ridley blood mercury concentration increased with turtle size. A similar relationship between mercury accumulation and age/size has been found in other marine species, including bony fish (Wiener and Spry, 1996) and sharks (Marchovecchio et al., 1991). Sharks prey on demersal crustaceans and fish which tend to accumulate toxic levels of methylmercury (Mason et al., 1996). Ridleys' preference for crabs and fish may explain the high blood-borne mercury concentration trends. Overall mean blood mercury level (18 ng/g, wet weight) was similar to that in edible tissue from oysters (20 ng/g, wet weight) and blue crabs (40 ng/g, wet weight), but lower than that in fish (110 ng/g, wet weight) in Galveston Bay (Brooks et al., 1992).

Whole blood mercury concentration in female and male ridleys increased with turtle size. Female ridleys exhibited more rapid increase in mercury concentration with size than did males, suggesting that the former forage on different food sources or in different habitats. Reports by Hillestad et al. (1974) and Stoneburner et al. (1980) that gravid loggerhead turtles sequester trace metal burdens to their eggs suggest females may seasonally accommodate higher trace metal levels than do males.

The overall mean blood mercury level in ridley blood was lower than that reported for loggerhead, green, hawksbill, and olive ridley sea turtle kidney (49–247 ng/g, wet weight; Landry and Sis, 1992; Sakai et al., 1995; Gordon et al., 1998) and liver (52–378 ng/g, wet weight; Landry and Sis, 1992; Sakai et al., 1995; Gordon et al., 1998), but relatively similar to that for loggerhead and green sea turtle eggs (8–90 ng/g, wet weight; Hillestad et al., 1974; Sakai et al., 1995). The overall mean mercury blood levels were also lower than in black sea turtle (*Chelonia mydas agassizii*) carapacial keratinized scutes (0–308 ng/g, wet weight; Presti et al., 1999).

Silver. — Silver is receiving more scientific attention because of its high toxicity at low concentrations and elevated bioaccumulation trends (Luoma et al., 1995), especially in the liver of marine mammals (Becker et al., 1995; Mackey et al., 1996). Ridley blood silver levels were a magnitude or more lower than tissue levels reported for marine mammals (5930–110,000, wet weight) and somewhat reduced from those in oysters (281 ng/g, wet weight) and blue crabs (172 ng/g, wet weight) (Brooks et al., 1992; Becker et al., 1995). Conversely, Brooks et al. (1992) found edible fish tissue exhibited non-detectable levels (i.e., presumably lower than the ridley blood values).

Zinc. — Zinc, like copper, is an essential metal in the blood for several metabolic pathways (Chang, 1996). The ridley mean blood zinc value of 7500 ng/g, wet weight, was lower than that contained in blue crab tissue (33,800 ng/g, wet weight), but higher than that in fish (4580 ng/g, wet weight) from Galveston Bay (Brooks et al., 1992). Increased blood zinc concentration with turtle size suggested that ridleys accumulate this element. As with mercury, females exhibited more rapid increases in zinc levels with turtle size.

The mean zinc concentration reported in this study was similar to kidney and liver tissue levels (12,500–45,000 ng/g, wet weight) detected for green sea turtles (Aquirre et al., 1994) but lower than that in loggerheads (Landry and Sis, 1992; Sakai et al., 1995). Blood zinc levels also were similar to tissue zinc concentrations in snapping turtles (kidney, 8800–10,500 ng/g, fat, 5600 ng/g, wet weight; Albers et al., 1986) and alligators (muscle, 14,200–36,000 ng/g, wet weight; Delany et al., 1988). This study found that whole blood zinc levels (3280–18,900 ng/g; $n = 106$) were much higher than the plasma zinc level (840 ng/g) found in one Kemp's ridley and other sea turtle species (370–2220 ng/g, wet weight, $n = 15$; Lance et al., 1995). These disparities in zinc levels between sea turtles may result from differences in utilizing whole blood versus plasma. Lance et al. (1995) also found that plasma zinc levels of sea turtles were much lower than levels found in other turtles and tortoises.

Research Needs. — The present study indicates that blood analysis is a safe and effective method to monitor trace metal levels in living sea turtles. Nevertheless, evidence for a direct causal relationship between metal uptake and accumulation in the blood is yet to be established. A synoptic

trace metal analysis of blood and selected tissues (kidney, liver, fat, keratinized tissues, eggs) from the Kemp's ridley is needed to fully understand this relationship. This analysis will require that additional permits and sampling procedures be developed before tissues prerequisite to the proposed comparisons can be obtained. Permits to biopsy skin, carapace, and/or internal tissues in conjunction with blood sampling of live turtles are an urgent need in understanding blood/tissue transport and accumulation relationships. Once these relationships are established, future trace metal studies of sea turtles may rely solely on minimally invasive blood sampling techniques as a safe and effective protocol for assessing exposure to trace metals.

Until the difficulties of sampling tissue from a live endangered sea turtle species are resolved, research efforts may include synoptic analysis of coagulated blood and tissue from stranded, cold-stunned, fresh-dead, or Code 1 condition turtles. These turtles are a good surrogate for live counterparts because they typically do not exhibit the decomposition and related analytical difficulties associated with rotting carcasses. Researchers armed with proper permits could also monitor long-term blood and tissue concentrations of captive ridleys fed food sources with known trace metal levels to examine metal uptake and bioaccumulation.

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