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ARSENIC, CADMIUM, CHROMIUM, LEAD, MERCURY, AND SELENIUM LEVELS IN BLOOD OF FOUR SPECIES OF TURTLES FROM THE AMAZON IN BRAZIL

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Abstract

Using blood as a method of assessing metal levels in turtles may be useful for populations that are threatened or endangered or are decreasing. In this study the levels of arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), and selenium (Se) in blood of four species of turtles from the tributaries of the Rio Negro in the Amazon of Brazil were examined. The turtles included the six-tubercled Amazon (river) turtle (*Podocnemis sextuberculata*), red-headed Amazon (river) turtle (*Podocnemis erythrocephala*), big-headed Amazon (river) turtle (*Peltocephalus dumerilianus*), and matamata turtle (*Chelus fimbriatus*). Blood samples were taken from the vein in the left hind leg of each turtle. There were significant interspecific differences in the sizes of the turtles from the Rio Negro, and in concentrations of Pb, Hg, and Se; the smallest species (red-headed turtles) had the highest levels of Pb in their blood, while Se levels were highest in big-headed turtles and lowest in red-headed turtles. Hg in blood was highest in matamata, intermediate in big-headed, and lowest in the other two turtles. Even though females were significantly larger than males, there were no significant differences in metal levels as a function of gender, and the only relationship of metals to size was for Cd. Variations in metal levels among species suggest that blood may be a useful bio-indicator. Metal levels were not high enough to pose a health risk to the turtles or to consumers, such as humans.

Native peoples, tribal nations, governmental agencies, public policymakers, managers, and the public are interested in levels of contaminants in wildlife that could prove a problem for the wildlife themselves or to organisms that consume them. Environmental contamination is a global problem because of the potential adverse effects of chemicals on humans and the

environment. Contaminants enter the food chain through natural erosion, biogeochemical processes, and industrial or other anthropogenic sources. Levels of chemicals are particularly elevated in riverine and coastal ecosystems because of tributary influxes, runoff, point-source pollution, and atmospheric transport and deposition (Furness & Rainbow, 1990; Burger & Gochfeld, 2002). Many chemicals are atmospherically transported all over the world, including to relatively isolated lakes, rivers, and oceanic environments (Fitzgerald, 1989; Houghton et al., 1992; Hammerschmidt & Fitzgerald, 2006). Species that forage in aquatic environments are more vulnerable than terrestrial species because of the potential for rapid movement of contaminants in water (compared to terrestrial movement), and because chemicals are stored in bottom sediments, providing a pool for years to come.

Although there has been considerable attention devoted to determining the levels and adverse effects of metals in birds, mammals, and fish, and even for some groups of reptiles, there has been little attention devoted to turtles (Delany et al., 1988; Yanochko et al., 1997; Campbell & Campbell, 2000, 2001; Burger, 2006). Turtles are vertebrates living in aquatic habitats and have received relatively little attention with respect to metals, except for sea turtles (*Caretta caretta*; Stoneburner et al., 1980; Caurant et al., 1999; Day et al., 2005; Kampalath et al., 2006; Gardner et al., 2006; Talavera-Saenz et al., 2007), snapping turtles (*Chelydra serpentina*; Bishop et al., 1991, 1995; Bonin et al., 1995; DeSolla & Fernie, 2004; Lam et al., 2006), slider turtles (*Trachemys scripta*; Burger & Gibbons, 1998; Tryfonas et al., 2005), and diamondback terrapin (*Malaclemys terrapin*; Burger, 2002; Blanvillain et al., 2007; Bergeron et al., 2007). However, few studies determined levels of metals in blood (Day et al., 2005, 2007; Blanvillain et al., 2007; Chaffin et al., 2008), a nondestructive method of sampling metal levels, and none from freshwater turtles in South America. With the accumulation of studies on blood levels in turtles, in connection with health effects, it will be possible to assess health effects on turtles using only blood levels (Peden-Adams et al., 2003).

This study examined the levels of As, Cd, Cr, Pb, Hg, and Se in blood of four species of turtles from the tributaries of the Rio Negro in the Amazon of Brazil. The turtles included the six-tubercled Amazon (river) turtle (*Podocnemis sextuberculata*), red-headed Amazon (river) turtle (*Podocnemis erythrocephala*), big-headed Amazon (river) turtle (*Peltocephalus dumerilianus*), and matamata turtle (*Chelus fimbriatus*). Of particular interest were interspecific differences, given that the species differ in overall size and have some differences in foraging behavior (Vogt, 2001), as well as geographical and size-related differences in the red-headed Amazon turtle. Because of the great concern about Hg in the Amazon (Barbarosa et al., 1995, 1997, 1998, 2001, 2003; Forsberg et al., 1995; Vogt, 2001; Belger & Forsberg, 2006) and its effect on humans and others in this ecosystem, Hg is discussed in more detail in a separate paper that deals with the muscle, liver, and carapace of some of these species (Schneider et al., 2009). Mercury in the Amazon appears to be of natural origin, as well as from damming, agricultural deforestation, and gold-mining activities (Fadini & Jardim, 2001). Data suggest that blood of turtles may be useful as a bioindicator for contamination in these ecosystems (Markert et al., 2003).

STUDY AREA AND METHODS

Under appropriate permits from the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis of Brazil (147/2006; 13347-2), blood from four species of turtles from the tributaries of the Rio Negro in the Amazon of Brazil (Figure 1) was collected, including from the six-tubercled Amazon (river) turtle (*Podocnemis sextuberculata*), red-headed Amazon (river) turtle (*Podocnemis erythrocephala*), big-headed Amazon (river) turtle (*Peltocephalus dumerilianus*), and matamata turtle (*Chelus fimbriatus*). Hereafter, they are referred to as six-tubercled, red-headed, big-headed, and matamata.

The Rio Negro Basin makes up around 10% of the Amazon basin (area = $750 \times 10^3 \text{ km}^2$; Goulding et al., 1988). Unlike many parts of the Amazon, the Rio Negro does not have any documented history of gold-mining activities along its tributaries. This Rio Negro basin has a wide range of dissolved oxygen concentrations (DOC) ranging from 3.73 to 23.4 mg/L, with pH ranging from 3.37 to 6.89 (Forsberg et al., 1995). There are seasonal periods of high and low waters (Castillo et al., 2004).

Turtle samples were collected from 9 January to 2 February 2006, during the falling water season, assuming that the metals values provided a reasonable index of the average annual values. Samples were collected from 12 tributaries located between 00°25'27" S and 02°42'45" S latitude and 60°24'43" W and 66°23'55" W. At each location, geographic coordinates were collected with a Garmin model 50 geographic positioning system (GPS). Using trammel nets, as many species as possible were collected, with a maximum of two to five individuals of each species at most sampling sites. Turtles were sexed visually by sexual dimorphism; males have a distinctive brighter color in the *Podocnemis* species, and in all species, females are larger than males and males have longer, thicker tails. Individuals that had no sexual dimorphism were classified as juveniles. Carapace lengths were measured using a 1-m ruler with 1 mm precision.

Blood samples were taken from the vein in the left hind leg of each turtle, placed in a cooler, frozen immediately thereafter, and shipped to the Environmental and Occupational Health Sciences Institute of Rutgers University in Piscataway, NJ, for analysis. All laboratory equipment and containers were washed in 10% HNO₃ solution and rinsed with deionized water prior to each use. A 0.2-g (wet weight) sample of blood was digested in 4 ml 70% Ultrex ultrapure nitric acid and 2 ml deionized water in a microwave (MD 2000 CEM), using a digestion protocol of 3 stages of 10 min each under 50, 100, and 150 lb/in² (3.5, 7, and 10.6 kg/cm²) at 70× power. Digested samples were subsequently diluted to 10 ml with deionized water. Detection limits were 0.02 for As, 0.01 ppb for Cd, 0.08 ppb for Cr, 0.15 ppb for Pb, 0.09 ppb for Mn, 0.2 ppb for Hg, and 0.7 ppb for Se. All specimens were analyzed in batches with known standards, calibration standards, and spiked specimens. Recoveries ranged from 88 to 102%. Batches with recoveries of less than 85% were reanalyzed. The coefficient of variation on replicate, spiked samples ranged up to 10%.

Nonparametric Wilcoxon chi-square tests were used to examine differences among islands, and Duncan multiple-range tests were performed to distinguish significant differences between species, using log-transformed data (SAS, 1995). Both arithmetic and geometric

means are given to facilitate comparisons with other studies. Kendall tau correlations were used to examine the relationship among metals and among size measurements and metal levels. A probability level of .05 was accepted for significance, but we present all values below 0.1 to allow the readers to assess the significance themselves.

RESULTS

There were significant interspecific differences in the sizes of the turtles from the Rio Negro, and in concentrations of Pb, Hg, and Se (Table 1). Even though red-headed turtles were the smallest turtles, they had the highest levels of Pb in their blood. Se levels were highest in big-headed turtles, and lowest in red-headed turtles (Table 1). Hg in blood was highest in matamata turtles, intermediate in big-headed turtles, and lowest in the other two turtles.

There were significant size differences as a function of collection location for red-headed turtles (the only one with a sufficient sample size to examine this variable), but these differences were not great, except for turtles collected in the Itu and Negro tributaries (Table 2). Only Pb concentrations differed, and the differences were very small. A lack of locational differences, however, no doubt relates to the small sample sizes from the different tributaries, and needs to be considered tentative only.

Even though females were significantly larger than males, there were no significant differences in metal levels as a function of gender (Table 3). Similarly, although carapace length and weight were significantly correlated for red-headed turtles ($r^2 = .90$), there were almost no significant correlations among As, Cd, Pb, and Se, or among these metals and size, except for Cd. Cd levels were inversely correlated with length ($r^2 = -.26$) and weight ($r^2 = -.27$).

DISCUSSION

Interspecific Differences

In this study, there were interspecific differences in metal levels only for Pb, Hg, and Se, even though there were size-related differences among species. Pb levels were highest in red-headed turtles, Hg was highest in matamata, and Se levels were highest in big-headed turtles and matamata. Thus, the same species did not have the highest levels for all metals. Although Hg and Se were lowest in the redheaded turtle, this species had the highest levels of Pb. In many groups of closely related species, contaminant levels are higher in those organisms that are higher on the trophic scale and that are larger, but in this study, the size relationship held only for Hg. Size and trophic-level relationships have not been examined for turtles or other reptiles in South America; this represents the first comparative study of four species of turtles from the Amazon.

It was expected that there would be significant interspecific differences in metals levels. Although all chelonian species of the Podocne-mididae family are opportunistic omnivores, the percentage of animal matter varies. However, only Pb, Hg, and Se differed significantly among species. Hg levels were lowest in redheaded turtles, perhaps because they are

exclusively herbivorous (Mittermeier & Wilson, 1974; Vogt, 2001), and the aquatic plants and fruits this turtle eats are typically low in Hg. For redheaded turtles, the species with the largest sample size, there was no correlation between size and Hg levels. This suggests that this species may be eliminating Hg from the body, and not accumulating Hg with time as the species grows.

Risk to Human Consumers

Turtles are an important food source for people living in the Rio Negro tributary system (Vogt, 2001). Of particular interest was whether there were interspecific differences that might provide information for individuals who eat turtles, and whether there were geographical and size-related differences in the red-headed Amazon turtle, the most commonly caught species. Because of the great concern about Hg in the Amazon (Forsberg et al., 1995; Vogt 2001; Belger & Forsberg, 2006), information on its occurrence was of particular interest (Schneider et al., 2009). Although Hg in the Amazon appears to be of natural origin, as well as from damming, agricultural deforestation, and gold-mining activities (Fadini & Jardim, 2001), it nonetheless could pose a risk to humans who consume turtles. The levels of Hg in blood (averages ranged from 0.02 to 0.16 ppm, this study) and muscle (averages ranged from 0.01 to 0.07 ppm in red-headed turtles, Schneider et al., 2009) were well below the level considered to pose a risk for human consumers (0.3 ppm, Burger & Gochfeld, 2007).

Use of Metals in Blood as a Bioindicator

The variation in metal levels among species indicates that metals in blood can be a useful bioindicator of exposure. However, significantly more work is required to correlate metal levels in blood with other tissues before blood levels can be used to accurately reflect the levels in kidney and liver (indicative of health of the turtles) and in tissue (indicative of possible risk to consumers of turtles, such as humans). This study provides data useful in this endeavor.

Methodological Considerations

Finally, we offer a few comments on methodological issues with work with contaminant levels in biota. One of the major difficulties with contaminant studies is the lack of uniformity in the tissue samples collected, as well as the metals examined, making comparisons among species and geographical regions difficult. Further, while often whole organisms are collected, making analysis of individual tissues possible, there is a trend toward sampling methods that are nondestructive and, where possible, noninvasive. For example, feathers and hair can be collected noninvasively and repeatedly from the same individuals. The problem is more difficult with amphibians and reptiles that do not have feathers or hair, although a sample can be taken from the carapace of turtles. While the collection of blood is invasive and requires careful handling of both the turtles and the specimen itself, it still can provide information without unduly harming the individual, and is useful for threatened or endangered species and for repeated sampling of the same individuals.

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FIGURE 1. Map showing the locations where turtles were collected from tributaries of the Rio Negro basin of the Amazon in Brazil.

TABLE 1

Metal Levels (ppb, wet weight) (ng/g) in Blood of Turtles Collected from Brazil. Given are Arithmetic Means $\pm$ SE (Geometric Means Below) with Kruskal-Wallis Chi Square Values and p Values. Duncan Values are Given in Parenthesis

	Red-headed Amazon river turtle n = 39	Six-tubercled Amazon river turtle n = 12	Big-headed Amazon river turtle n = 5	Matamata n = 4	X ² (p)
Weight (g)	930 $\pm$ 102	a $\pm$	285 $\pm$ 41.0	5450 $\pm$ 524	15.6 (0.0004)
Shell Length (cm)	20.7 $\pm$ 1.0	23.1 $\pm$ 1.3	31.8 $\pm$ 0.9	35.3 $\pm$ 1.2	22.5 (<0.0001)
arsenic	3.80 $\pm$ 1.10 0.70 (A)	3.50 $\pm$ 1.20 1.10 (A)	1.30 $\pm$ 0.70 0.40 (A)	4.90 $\pm$ 4.10 1.20 (A)	0.8 (NS)
cadmium	0.12 $\pm$ 0.07 0.02 (A)	0.01 $\pm$ 0.00 0.01 (A)	0.01 $\pm$ 0.00 0.01 (A)	0.01 $\pm$ 0.00 0.01 (A)	2.9 (NS)
chromium	5.49 $\pm$ 1.05 1.96 (A)	6.61 $\pm$ 2.18 2.39 (A)	4.69 $\pm$ 1.85 1.92 (A)	4.09 $\pm$ 1.63 1.53 (A)	0.1 (NS)
lead	25.4 $\pm$ 3.10 12.3 (A)	9.10 $\pm$ 2.90 1.50 (B)	13.4 $\pm$ 3.30 12.1 (B)	16.1 $\pm$ 7.90 10.0 (B)	10.2 (0.02)
mercury	2.00 $\pm$ 0.40 1.10 (C)	7.30 $\pm$ 1.20 6.10 (C)	28.2 $\pm$ 5.20 25.5 (B)	158 $\pm$ 14.5 156 (A)	37.6 (<0.0001)
selenium	164 $\pm$ 28.3 120 (C)	276 $\pm$ 76.4 155 (B,C)	538 $\pm$ 170 388 (A)	463 $\pm$ 66.5 448 (A,B)	12.0 (0.007)

^a weights not taken.

TABLE 2

Metal levels (ppb, wet weight)(ng/g) in Blood of Red-headed Amazon River Turtle by Location (All Turtles). Given are Arithmetic Means $\pm$ SE (Geometric Means Below) with Kruskal-Wallis Chi Square Values and p Values. Duncan Values are Given in Parenthesis

River	n	Weight	Shell Length	Arsenic	Cadmium	Chromium	Lead	Mercury	Selenium
Maricé	2	1800 $\pm$ 20.0 1800 (A)	27.0 $\pm$ 0.00 27.0 (A)	6.85 $\pm$ 1.95 6.57 (A)	0.01 $\pm$ 0.00 0.01 (A)	6.45 $\pm$ 3.55 5.39 (A)	37.0 $\pm$ 10.0 35.6 (A,B)	0.53 $\pm$ 0.43 0.31 (A)	170 $\pm$ 30.0 167 (A)
Tea & Enuexi	8	696 $\pm$ 17.1 695 (B)	20.6 $\pm$ 0.30 20.6 (A,B)	4.86 $\pm$ 1.59 2.04 (A)	0.01 $\pm$ 0.00 0.01 (A)	9.90 $\pm$ 4.06 5.04 (A)	23.4 $\pm$ 5.03 11.6 (A,B,C)	1.36 $\pm$ 0.49 0.61 (A)	89.9 $\pm$ 10.6 85.7 (A)
Ayuanã & Urubaxi	7	1343 $\pm$ 250 1207 (A,B)	24.8 $\pm$ 1.50 24.6 (A)	0.80 $\pm$ 0.47 0.25 (A)	0.01 $\pm$ 0.00 0.01 (A)	4.90 $\pm$ 1.93 1.88 (A)	18.8 $\pm$ 6.7 9.40 (B,C)	2.65 $\pm$ 0.76 1.64 (A)	335 $\pm$ 138 182 (A)
Daraha & Mabahá	4	903 $\pm$ 492 50.3 (B)	19.7 $\pm$ 3.60 18.6 (A,B)	3.68 $\pm$ 2.33 0.82 (A)	0.15 $\pm$ 0.14 0.03 (A)	5.14 $\pm$ 2.50 1.71 (A)	8.61 $\pm$ 5.62 1.62 (C)	1.32 $\pm$ 0.65 0.67 (A)	102 $\pm$ 23.1 92.7 (A)
Itu & Negro	8	479 $\pm$ 213 72.7 (B)	14.6 $\pm$ 2.79 12.7 (B)	1.91 $\pm$ 1.08 0.43 (A)	0.43 $\pm$ 0.32 0.03 (A)	4.69 $\pm$ 1.59 1.59 (A)	26.9 $\pm$ 7.9 12.6 (A,B,C)	3.39 $\pm$ 1.74 1.56 (A)	160 $\pm$ 28.9 143 (A)
Quimicuri	4	843 $\pm$ 330 527 (B)	19.3 $\pm$ 3.20 18.3 (A,B)	9.33 $\pm$ 9.23 0.44 (A)	0.01 $\pm$ 0.00 0.01 (A)	2.64 $\pm$ 1.34 1.37 (A)	17.5 $\pm$ 5.68 14.3 (B,C)	1.98 $\pm$ 0.23 1.93 (A)	80.3 $\pm$ 22.3 64.4 (A)
Jufaris & Caurés	6	1152 $\pm$ 173 1095 (A,B)	23.5 $\pm$ 0.94 23.4 (A)	3.52 $\pm$ 2.17 0.47 (A)	0.10 $\pm$ 0.08 0.03 (A)	3.17 $\pm$ 1.58 0.78 (A)	46.3 $\pm$ 7.26 43.7 (A)	1.20 $\pm$ 0.10 1.18 (A)	164 $\pm$ 29.1 149 (A)
$\chi^2(p)$		14.9 (0.02)	14.2 (0.03)	6.9 (ns)	6.6 (ns)	3.3 (ns)	12.6 (0.05)	6.9 (ns)	8.0 (ns)

TABLE 3

Metal levels (ppb, wet Weight)(ng/g) in Blood of Adult Red-Headed Amazon River Turtle by Gender. Given are Arithmetic Means $\pm$ SE (Geometric Means Below) with Kruskal-Wallis Chi Square Values and p Values

	Male	Female	X ² (p)
	n = 14	n = 17	
Weight	813 $\pm$ 39.2	1412 $\pm$ 142	5.4 (0.02)
Shell Lengh	21.3 $\pm$ 0.34	24.9 $\pm$ 0.8	8.9 (0.003)
arsenic	2.89 $\pm$ 1.16 0.56	2.94 $\pm$ 0.84 0.77	0.1 (NS)
cadmium	0.05 $\pm$ 0.04 0.02	0.01 $\pm$ 0 0.01	2.5 (NS)
chromium	8.38 $\pm$ 2.45 3.76	4.35 $\pm$ 1.06 1.82	1.8 (NS)
lead	25.7 $\pm$ 4.24 20.5	26.6 $\pm$ 4.87 10.6	0.03 (NS)
mercury	1.47 $\pm$ 0.35 0.78	1.81 $\pm$ 0.35 1.11	0.5 (NS)
selenium	187 $\pm$ 59 120	155 $\pm$ 42.5 119	0.01 (NS)