Journal of Wildlife Diseases, 46(4), 2010, pp. 1234–1241

HEAVY METAL AND SELENIUM CONCENTRATIONS IN LIVER TISSUE FROM WILD AMERICAN ALLIGATOR (*ALLIGATOR MISSISSIPPIENSIS*) LIVERS NEAR CHARLESTON, SOUTH CAROLINA

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ABSTRACT: Liver samples from 33 wild American alligators (*Alligator mississippiensis*) livers from the Charleston, South Carolina, area were analyzed for arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), and selenium (Se) concentrations. Alligators are top predators and are considered a good biomonitoring species for various toxins, including heavy metals. Alligators from other areas in the US have shown high concentrations of mercury and other heavy metals, but the Charleston area, which is highly industrialized, has not been investigated. We found wide variation in hepatic heavy metal and selenium concentrations among alligators. Length and sex did not show a strong relationship with any metal based on statistical analysis. However, cluster analysis revealed three groupings of alligators based on liver metal concentrations. Alligators with low Se:Hg ratios also had high concentrations of Hg. Due to the wide variation in metal concentrations among individual alligators, we postulate that individual diet and microhabitat usage could be the cause for this variation.

Key words: *Alligator mississippiensis*, American alligator, heavy metals, lead, mercury, selenium, South Carolina.

INTRODUCTION

Heavy metals and other chemicals have become major contaminants in aquatic systems worldwide. The release of heavy metals into aquatic systems has been increasing with increased industrial use of heavy metals, such as mercury (Hg) and lead (Pb), which can bioaccumulate in organisms, especially higher trophic level animals. The American alligator (*Alligator mississippiensis*) is a long-lived, top predator found throughout coastal areas in the southeastern US and is susceptible to various environmental contaminants, including those of heavy metals and organohalogen compounds (Delany et al., 1988; Brisbin et al., 1998; Guillette et al., 1999; Burger et al., 2000; Khan and Tansel, 2000). Alligator populations have recovered in most of their range following their listing on the endangered species list in the early 1970s. However, local populations of alligators can decline as a result of anthropogenic stressors such as chemical and agricultural runoff (Jennings et al., 1988). Heavy metals and other contaminants have been suggested as the cause of reproductive and developmental abnormalities in American alligators (Guillette et al., 1999). Most studies have focused on mercury and organohalogen contamination in American alligators located in Florida (Heaton-Jones et al., 1997; Guillette et al., 1999; Khan and Tansel, 2000; Rumbold et al., 2002). Heavy metals other than Hg in tissues from wild American alligator populations have been the focus of only a few studies (Lance and Elsey, 1983; Delany et al., 1988; Burger et al., 2000), although alligators have been shown to be a good, suitable monitor of localized pollution (Delany et al., 1988). The majority of research involving heavy metal concentrations found in wildlife has centered on birds, mammals, and fish (Gerstenberger and Pearson, 2002), with little attention given to reptiles, despite reptiles being essential parts of ecosystems. No studies have examined alligators in the Charleston, South Carolina, area, which is toward the northern extent of their range and a highly industrialized area. Alligators are an important part of

South Carolina's coastal ecosystem and can be considered a "keystone" predator. Decreases in alligator abundance can cause significant changes in an ecosystem (Khan and Tansel, 2000), making it a priority to examine contaminants, such as heavy metals, in this area.

Many heavy metals, such as Pb, Hg, cadmium (Cd), and arsenic (As), have no known natural metabolic function in most vertebrate animals, and high levels have negative effects on metabolism, behavior, reproduction, neurologic functions, etc. (Mulvey and Diamond, 1991; Chan, 1998). Other heavy metals (e.g., cobalt [Co], nickel [Ni], chromium [Cr]) and nonmetals such as selenium (Se) do play a role as important trace elements in organisms, but at high levels, they can also cause adverse health effects (Adriano, 1986). Sources of heavy metals vary considerably. Mercury is generally thought to be linked to atmospheric deposition and sea currents (Yanochko et al., 1997), whereas lead is probably due to a direct source such as a lead bullet, smelter sites, and other historic sources such as wheel weights and leaded gasoline (Camus et al., 1998). Other heavy metals are released into the environment from fossil fuel combustion (e.g., Cr, Co), fertilizers (Cd), and other industrial uses (Adriano, 1986).

In this study, we analyzed wild alligator livers from the Charleston, South Carolina, area for As, Cd, Co, Cr, Hg, Ni, Pb, and Se concentrations. We hypothesized that larger alligators and alligators living closer to the city of Charleston would have higher concentrations of several of these contaminants. We also reasoned that determining where alligators within the Charleston area contain high levels of heavy metals and selenium would help predict the locations of potentially contaminated aquatic systems.

MATERIALS AND METHODS

Thirty-three wild American alligators were captured in and around the city of Charleston, South Carolina (Berkeley, Charleston, Colleton, Clarendon counties) between April and September 2008 (Fig. 1). Most of the alligators were captured within the South Carolina coastal watershed (n=30), and a few alligators were captured in the Edisto (n=2) and Lake Marion (n=1) watersheds. Alligators were captured using a variety of techniques by professional animal trappers. All alligators were deemed nuisance alligators or legally hunted alligators and were captured with proper permits via South Carolina Department of Natural Resources (SC Code of Laws, Title 50 Chapter 15: SECTION 50-15-65: Alligator hunting, control and management). Alligators were captured from as far inland as Lake Marion to barrier islands off the coast of the Charleston area (Fig. 1). Alligators were euthanized by cervical dislocation shortly after capture, and liver samples were immediately extracted by opening the ventral side of the alligators with a scalpel down the midline of the belly to expose organs and liver. Liver samples were then placed in a cooler and frozen for future analysis. Liver has been shown to correlate with other tissues (e.g., tail muscle) for many metals (Burger et al., 2000) and is a good general indicator of environmental contamination of heavy metals. Alligators were sexed during dissection and ranged from 152 to 336 cm in total length.

Frozen liver samples were shipped to Waters Agricultural Laboratories, Inc. (Camilla, Georgia, USA) for analysis. Metals were extracted from preweighed liver samples using an acid digestion and following standard (US Environmental Protection Agency [EPA]) methods. Cadmium, Co, Cr, Ni, and Pb were measured using a Thermo ICP analyzer Model 35–60 following the EPA 6010B method. Mercury was measured using a PSA 10.033/10.044 Millennium Excalibur following the EPA 7470A method. Arsenic and Se were measured using a PSA 10.025 Millennium Merlin and EPA 7061A and 7741A methods, respectively.

Alligator liver metal data were incorporated into a database, and k-means cluster analysis was used to group alligators into three clusters (SAS JMP, 2006). K-means cluster analysis uses a specified number of cluster seed points and repetitively groups data to minimize standard deviations of means for each group. Furthermore, principal component analysis was used to determine variables that accounted for the greatest amount of variance within the data set. In addition, metal concentrations in male and female alligators were compared using a two-tailed t-test (α=0.05) and the concentrations for each metal were correlated with alligator length.

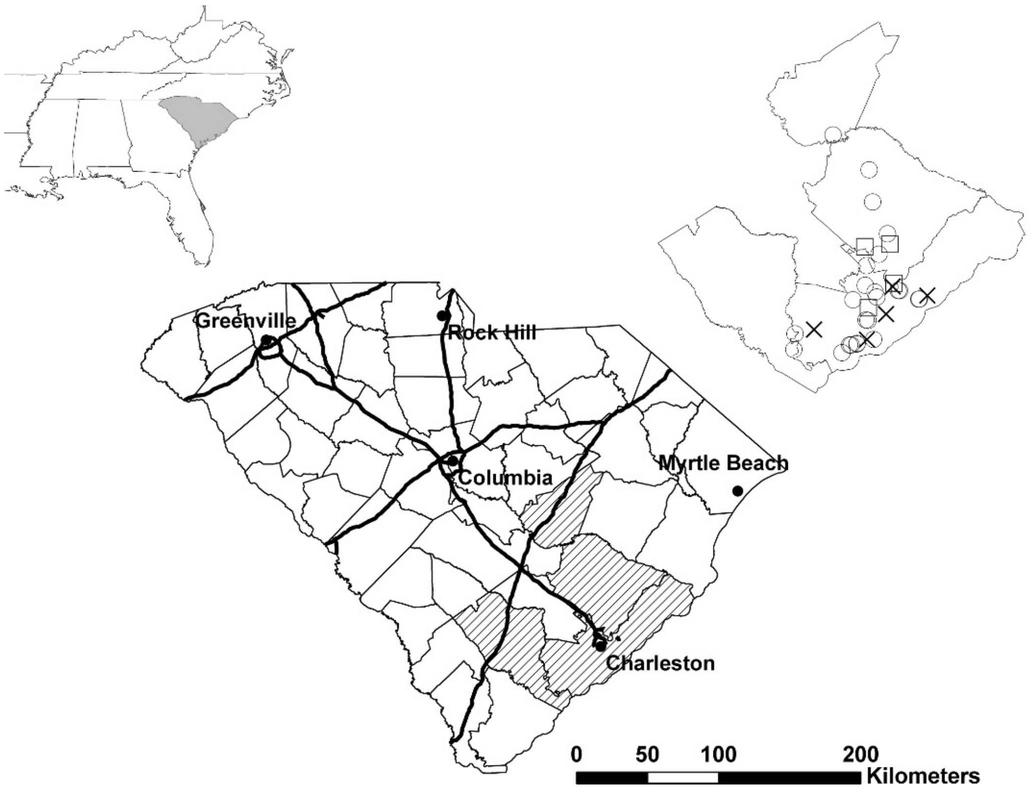

FIGURE 1. Approximate locations of capture sites of American alligators in Berkeley, Charleston, Colleton, and Clarendon counties, South Carolina, between April and September 2008. □=Cluster 1, ×=Cluster 2, ○=Cluster 3.

RESULTS

Heavy metal and selenium concentrations varied greatly among individual alligators. However, cluster analysis revealed three groupings of alligators (Fig. 2). Cluster one was characterized by high concentrations of Hg and Se; cluster 2 had relatively high concentrations of As, Co, and Ni (Table 1). Overall, Co and Ni were found in low concentrations in all alligators. Cluster 3, which contained most of the alligators sampled, had relatively high concentrations of Cr. Three alligators had Cr concentrations above 30 μg/g. Lead was found in very high concentrations (>25 μg/g) in three alligators and moderately high concentrations (12–15 μg/g) in five. Only one alligator had an As concentration >1.0 μg/g. Four alligators had Hg concentrations >10 μg/g. The majority (91%) of molar Se:Hg ratios were well above 1:1, but three alligators (9%) were <1 (Table 1). With the exception of Cd, which was weakly correlated, length of alligators and the various heavy metal concentrations were not correlated. No significant differences in heavy metal concentrations were detected between sexes.

A plot of principal components 1 (PC1) and 2 (PC2) shows the orientation of metal eigenvectors as well as separation of the clusters (Fig. 2). PC1 represented 33.5% of the variance of the data set, and PC2 represented 29.4%. Lead, As, Ni, and Co were positively correlated with both PC1 and PC2. Cadmium, Se, and Hg were positively correlated with PC1 and negatively correlated with PC2. Chromium is the only metal that was negatively corre-

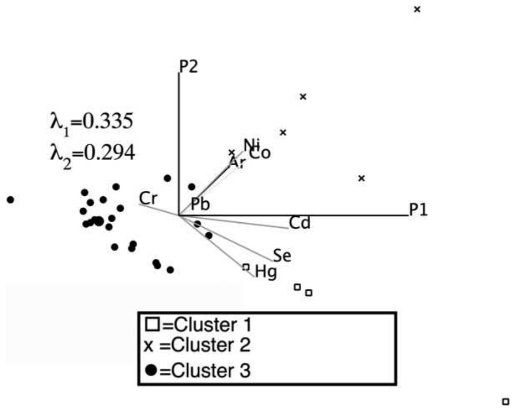

FIGURE 2. Scatter plot of principal components 1 (P1) and 2 (P2) showing correlations with individual metals. Eigenvalues (λ) for P1 and P2 are 0.335 and 0.294 and account for 33.5% and 29.4% of the data set's variance, respectively. Plot symbols noted on the scatter plot correspond to clusters determined by k-means cluster analysis of all metals measured.

lated with PC1 but was positively correlated with PC2.

DISCUSSION

Our data show large variation in metal concentrations among individual alligators. Despite the variation, American alligators did appear to be a useful upper-trophic level biomonitor for heavy metals and selenium. However, a larger sample size (>30) is needed because a small sample size could give misleading or faulty results due to this wide variance in metal concentrations.

The varying concentrations of heavy metals and selenium found within individual alligators, as well as the lack of statistical relationships between length and gender, suggest that alligator liver heavy metal concentrations are a result of individual diet or localized pollution sources instead of an ecosystem-wide pollution with heavy metals. The few alligators with very high concentrations of Pb could have ingested lead bullet–contaminated organisms. Camus et al. (1998) found that lead poisoning due to accidental ingestion of lead bullet fragments from nutria (*Myocastor coypus*) was a leading cause of disease in captive-reared, juvenile alligators. Seltzer et al. (2006) also found high levels of Pb in femurs of alligators and attributed this to incidental exposure of Pb from diet. Wild alligators with high Pb concentrations

TABLE 1. Mean (±SE) length, various heavy metals, and molar ratio of Se:Hg in alligators from the Charleston, South Carolina, area based on sex and cluster analysis. Correlation values based on length and *P*-values comparing sex with the various heavy metals are found in the last two columns.

Variable[a]	Total	Male[b]	Female	Cluster 1	Cluster 2	Cluster 3	Male: female[c]	Correlation with length[d]
n	33	17	14	4	5	24		
Length	234.4 (9.2)	257.6 (13.9)	206.7 (7.5)	276.2 (35.0)	198.1 (26.9)	235.1 (55.2)		
As	0.243 (0.04)	0.187 (0.02)	0.318 (0.08)	0.23 (0.02)	0.45 (0.21)	0.20 (0.02)	0.125	−0.107
Cd	0.489 (0.12)	0.562 (0.20)	0.389 (0.12)	1.9 (0.21)	1.1 (0.12)	0.13 (0.06)	0.359	0.397
Cr	5.66 (2.8)	4.42 (2.4)	7.35 (5.9)	0.01 (0.003)	3.9 (0.42)	7.0 (3.9)	0.677	−0.332
Co	0.443 (0.15)	0.319 (0.19)	0.610 (0.25)	0.02 (0.008)	2.2 (0.97)	0.15 (0.03)	0.347	0.242
Hg	5.68 (1.4)	7.32 (2.4)	3.44 (0.82)	21.6 (6.9)	2.4 (1.4)	3.7 (0.6)	0.103	0.194
Ni	0.502 (0.19)	0.206 (0.09)	0.904 (0.4)	0.01 (0.006)	2.8 (1.2)	0.11 (0.02)	0.074	−0.006
Pb	8.15 (3.5)	5.52 (2.2)	11.7 (7.8)	13.2 (6.6)	12.6 (5.6)	7.7 (1.6)	0.455	0.074
Se	3.30 (0.33)	3.44 (0.56)	3.10 (0.29)	6.5 (1.5)	3.7 (0.53)	2.7 (0.25)	0.466	0.204
Se:Hg	3.97 (0.8)	3.64 (1.2)	4.42 (1.0)				0.134	−0.09

[a] As = arsenic, Cd = cadmium, Cr = chromium, Co = cobalt, Hg = mercury, Ni = nickel, Pb = lead, Se = selenium, Se:Hg of molar concentrations. Heavy metal and Se concentrations are μg/g.

[b] The sex was unknown for two of the alligators sampled.

[c] Values are *P*-values for a two-tailed *t*-test comparing male and female alligators.

[d] Values are r^2 for correlations between length and variable.

could feed in an area that humans consistently hunt or fish and recently may have fed on an animal that contained a lead bullet or fishing weights. Numerous waterfowl and other birds are known to ingest lead and succumb to lead poisoning (Scheuhammer and Norris, 1996); these birds could serve as food for alligators. Other possible sources of lead are smelter sites and, historically, leaded gasoline and wheel weights. However, most alligators did not contain high concentrations of Pb, which indicates a direct source (e.g., lead shot) rather than an ecosystem-wide input of lead from industry or historic sources.

Numerous alligators were found to have relatively high concentrations (compared to other alligators) of Cd, Se, and As. Banded water snakes (*Nerodia fasciata*) with high levels of As, Se, and Cd, have been found living near coal combustion plants and have been associated with prey items that contain high concentrations of these contaminants in their tissues (Hopkins et al., 1999). These snakes exhibited higher metabolic rates, which could result in less energy expended on growth and reproduction. Arsenic exposure has been shown to cause decreased growth (Eisler, 1994), and Se and Cd cause reproductive problems (Hew et al., 1993; Lemly, 1993). Although some alligators had higher concentrations of As and Cd than other alligators in this study, very high concentrations were not found. This may indicate that these metals do not readily bioaccumulate in alligators or that these metals are not present in high concentrations in the environment in the Charleston area. Almli et al. (2005) also found low concentrations of Cd and As in crocodile liver tissue.

We found total mercury in some alligators (e.g., those in cluster 1) to be high relative to other alligators, and this may be reflective of local environmental factors. Alligators are considered a very good biomonitor of mercury due to their high trophic status and ability to bioaccumulate Hg in tissue (Khan and Tansel, 2000). Methylmercury is the most toxic form of mercury and tends to bioaccumulate readily; however, all forms of mercury can persist in aquatic environments and are toxic to organisms (Clarkson, 1992; Heaton-Jones et al., 1997). Long-range atmospheric deposition of inorganic mercury is thought to be the common way for mercury to enter aquatic systems and food chains (Khan and Tansel, 2000). Wetlands can enhance methylation of Hg, where it is absorbed by sediments and organic matter (St. Louis et al., 1994). Because total Hg concentration differed greatly among individual alligators, industrial discharges may be a more important source of mercury near the Charleston area. Alligators that exhibited relatively higher concentrations of Hg also showed higher concentrations of selenium. Selenium is thought to be an important component of detoxification of methyl-Hg (Martoja and Berry, 1980; Chen et al., 2006). Positive relationships between Hg and Se have been documented in crocodiles (Almli et al., 2005). Molar Se:Hg ratios observed in liver have been shown to vary in different organisms (Dietz et al., 2000). Alligators with a molar Se:Hg ratio near or below 1 also had high Hg concentrations, which is consistent with results found previously for some mammals and fish (Koeman et al., 1975; Luten et al., 1980). Other animals, such as birds, have not shown a clear relationship with respect to the molar Se:Hg ratio (Hutton 1981; Sepulveda et al., 1998). Cobalt and Ni were also found in low concentrations in all alligators, which suggests that these metals are in low abundance, or alligators do not bioaccumulate these metals.

We expected larger (older) alligators to contain higher concentrations of metals. A positive correlation between body size and age of American alligators until adulthood does exist (Khan and Tansel, 2000). Positive body size relationships with heavy metal concentrations have been seen in fish (Wiener et al., 1990), and lizards (Marquez-Ferrando et al., 2008). However, our data did not show a relationship between size or sex and heavy metal concentration. Food habits should also differ between larger and smaller alligators, putting larger alligators more at risk for heavy metal contamination due to the probability of feeding on larger prey items. Almli et al. (2005) did not find a relationship between body length of crocodiles (*Crocodylus niloticus*) and Hg and Pb concentrations, and Jagoe et al. (1998) did not always find a correlation between American alligator length and Hg concentration. Female alligators usually remain in a small area, whereas male alligators have a much larger home range, especially during the breeding season (Levy, 1991). Female alligators, if inhabiting contaminated water, would probably be more at risk for heavy metal accumulation. Given that our data did not show this trend, we infer that individual diet probably plays a crucial role in metal accumulation in alligators and may not necessarily depend on long-term bioaccumulation. We caution, however, that our sample size may not have been large enough to detect size or sex differences.

The data from this study indicate varying concentrations of heavy metals and selenium from wild alligator livers near Charleston, South Carolina, suggesting isolated heavy metal exposure. Estuaries and coastal systems are very dynamic and complex, which cause considerable variation in trace metals from site to site (Benoit et al., 1994). Anthropogenic activities, river inputs, and various biogeochemical cycling may influence metal concentrations greatly (Munksgaard and Parry, 2001). Most likely, these contaminants are coming from multiple sources, both point and nonpoint. According to the EPA, over 500 facilities in the Charleston area have been issued permits to discharge water or air that may contain various contaminants. It has not been confirmed whether these heavy metals and selenium concentrations are higher than their concentrations in alligators living in pristine environments. The lack of knowledge on the effects of the majority of heavy metals and other contaminants on reptiles makes it difficult to speculate about the biologic significance of each metal or the persistence of synergistic effects. Future research should focus on the specific effects of contaminants and the sources of contaminants in areas, such as the Charleston, South Carolina, area, that do not exhibit uniform distribution of contaminants.

ACKNOWLEDGMENTS

We thank Ron Russell from Gator Getter Consultants and Neil Alexander from Critter Control for donating their time and resources in gathering liver samples. This research was funded by a Shorter College research grant.

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Submitted for publication 12 September 2009.
Accepted 13 April 2010.