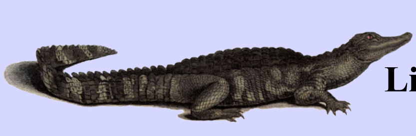
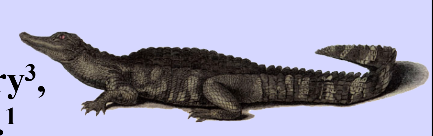
Tissue distributions of mercury in American alligators (*Alligator mississippiensis*) from the Rockefeller Wildlife Refuge, Louisiana

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ABSTRACT

American alligators (*Alligator mississippiensis*) are apex predators that can potentially biomagnify contaminants such as mercury Hg to levels exceeding those of predatory game fishes. We measured Hg concentrations of brain, gonad, kidney, liver, and cardiac, axial, and appendicular muscles of 27 wild alligators (total length: 90–268 cm) from the Rockefeller Wildlife Refuge (RWR) in Louisiana. Hg concentrations in all tissues were correlated with each other and with body size. Hg concentrations were highest in kidney (geometric mean: 2.03; 95% confidence interval: 1.41–2.93 mg Hg kg^{-1} dry mass) and liver (1.73; 1.13–2.65 mg Hg kg^{-1} dry mass) and lowest in gonad (171; 114–257 µg Hg kg^{-1} dry mass). Hg concentrations in tail muscle of RWR alligators were lower (85; 66–109 µg Hg kg^{-1} wet mass) than observed in other wild populations and were well below advisory levels for southeastern fishes. Hg concentrations in kidney, gonad, and blood were 1.8–2.2 times greater in females independent of body size. Hg concentrations differed among the five muscles examined, and all were higher and more variable in females. The highest (wet mass) tissue/blood ratios were for liver (mean = 6.39; 95% CI: 3.56–9.22) and kidney (5.52; 3.98–7.05), and the lowest were in gonad (0.41; 0.31–0.50). Tissue/blood ratios suggest that Hg levels in RWR alligators are at equilibrium, and that Hg concentrations are not increasing in edible muscle with increasing size. Hg concentrations from blood, claws, and dermal scutes were all correlated with those of internal organs, and an ANCOVA model predicting Hg in edible tail muscle from total length, together with blood, scute, and claw Hg concentrations was highly predictive ($F_{4,21}$ = 49.0; P < 0.001; R^2 = 0.90). This confirms previous observations that non-lethal sampling methods may allow monitoring Hg burdens in wild and captive populations.

INTRODUCTION

Anthropogenic releases of Hg and its concomitant increasing input into food products derived from aquatic systems is a source of increasing concern. Many important human food fishes (both freshwater and marine) are high-order predators and are known to accumulate potentially hazardous amounts of Hg. As fish stocks become depleted worldwide, protein production increasingly incorporates aquaculture and mariculture, including 'ranching' of crocodilians, turtles, and frogs that are now or formerly were frequently hunted (and, in some cases, depleted) in the wild. Despite their contribution to human diets, however, environmental Hg contamination of reptiles and amphibians consumed by humans has not been extensively investigated.

American alligators (*Alligator mississippiensis*) are capable of accumulating Hg in their tissues following dietary exposure to leves that can exceed U.S. Food & Drug Administration (FDA) limits for fishes taken for human consumption (Peters 1983). The potential effects of Hg on crocodilians, and of potential effects from consuming Hg in crocodilian meat on human health, however, are only now emerging (Hall 1980, Eisler 1987, Heinz 1996, Brisbin et al. 1998). Several studies have measured Hg in alligator tail muscle (the most commonly-consumed body part) to assess potential public health risks (e.g., Delany et al. 1988, Hord et al. 1990, Ruckel 1993, Elsey et al. 1999). Other recent studies have examined concentrations in other, non-edible tissues (e.g., Heaton-Jones et al. 1997, Yanochko et al. 1997, Jagoe et al. 1998, Burger et al. 2000). Adult alligators tend to occupy higher trophic levels than juveniles or other community inhabitants (Eisler 1987, Wolfe et al. 1998, Duvall and Barron, 2000), are non-migratory as adults, and have long (> 30 yr) lifespans. American alligators may therefore represent an ideal environmental monitor of biomagnified contaminants in southeastern US aquatic ecosystems (Yanochko et al. 1997, Khan and Tansel 2000), and potentially aid in assessing the upper limit of potential damage from Hg on aquatic species (Wren 1986).

To determine the extent of contamination and the distribution of Hg in various body organs and tissue compartments in a population with no obvious point source of Hg exposure, we measured Hg concentrations and tissue/blood ratios of wild alligators in the Rockefeller Wildlife Refuge (RWR). We examined correlations between Hg in internal organs and non-lethal samples of blood, claws, and dermal tail scutes to determine whether these might be used to reliably estimate Hg levels in edible skeletal muscle, and whether these measurements would justify the additional sampling and analytical effort.

METHODS

Sample collection

The alligators were captured on the night 20 June 2002 within Unit 13 of the RWR, a 320-km^2 coastal marsh located in eastern Cameron and western Vermilion Parishes in SW Louisiana, USA. The following day, the alligators were euthanized, their total length measured, and dissected. We sampled liver, kidney, gonad, brain, whole blood, cardiac muscle, two appendicular muscles of the front and rear legs (*anconeus* and *flexor tibialis internus*), two axial muscles of the jaw (*pterygoideus internus*) and tail (*longissimus dorsi*: part of the group of tail muscles that are most commonly eaten by humans). We also sampled claws and dermal tail scutes.

Hg Analyses

Samples were stored in separate, sterile polyethylene bags and frozen at –10 °C until testing. Samples were weighed, freeze dried, reweighed to determine moisture content, and homogenized. Hg levels were measured on a Milestone DMA80 Direct Hg Analyzer, following EPA Method 7473. Replicates and tissue standards (DORM-2 dogfish muscle, DOLT-1 dogfish liver, and TORT-2 defatted lobster hepatopancreas, National Research Council of Canada, Ottawa, Canada) were run together with each set of samples. Based on a 250-mg sample and a mean blank of 0.5 ng Hg, the method detection limit (MDL) was estimated as 20 µg kg^{-1}.

Statistical analyses

Dry mass Hg concentrations were used for all between-organ comparisons except for whole blood and blood/tissue ratios. All data were tested for normality and homoscedasticity and log-transformed as necessary to meet the assumptions for parametric statistical analyses. Power-function regression models of total body length and tissue Hg concentrations were used to determine potential allometric increases in Hg levels. Similarly, differences in tissue Hg concentrations between sexes were examined using ANCOVA models with mass as a covariate and sex as a factor, because alligators are sexually dimorphic with respect to size. Separate ANOVAs were used to compare Hg concentrations among the muscle tissues examined (cardiac, jaw, tail, front leg, and rear leg). Pearson's product-moment correlations (r) were used to examine Hg relationships concentrations among tissues examined.

We also evaluated the utility of non-lethal measurements (measurements of total length and determinations of sex, alone and in combination with Hg in whole blood, claws, and scutes) in predicting Hg concentrations in edible tail muscle. For these analyses, we designated a "Reduced" regression model of total length and sex (the most easily-determined and least-invasive variables) on Hg concentration in tail muscle, and compared this model with the fits of "Full" models that included combinations of what would be nonlethal tissue samples. Significant improvements in predicting tail muscle Hg concentrations were determined using F tests of the multiple-partial correlation coefficients (R^2) of the "Full" and "Reduced" models, i.e, whether the addition of combinations of tissues significantly improves the prediction of muscle Hg (Kleinbaum et al. 1988).

RESULTS

Table 1. Hg concentrations of alligators from the Rockefeller Wildlife Refuge. Concentrations of Hg (mg kg^{-1}) are dry masses for all tissues except for whole blood.

					Muscle							
						Axial		Appendicular				
	Liver	Kidney	Gonad	Brain	Cardiac	Jaw	Tail	Front Leg	Rear Leg	Blood	Scute	Claw
Geo. mean	1.733	2.033	0.171	0.217	0.365	0.434	0.376	0.311	0.335	0.093	0.250	0.598
95% CI (low)	1.133	1.409	0.257	0.167	0.284	0.338	0.293	0.244	0.263	0.070	0.160	0.450
95% CI (high)	2.651	2.932	0.114	0.281	0.468	0.559	0.481	0.398	0.428	0.123	0.390	0.795

Table 2. Estimates of Pearson correlation coefficients (r) and associated P-values among log-transformed tissue Hg concentrations and total lengths (TL, cm). Correlations are for dry tissues except for whole blood. For all comparisons, n = 27, except for blood vs. gonad (n = 25), and blood or gonad vs. other tissues (n = 26).

					Muscle								
						Axial		Appendicular					
	Liver	Kidney	Gonad	Brain	Cardiac	Jaw	Tail	Front Leg	Rear Leg	Blood	Scute	Claw	TL (cm)
Liver		0.877	0.819	0.829	0.833	0.823	0.833	0.791	0.797	0.650	0.613	0.622	0.749
Kidney	<0.0001		0.897	0.879	0.871	0.885	0.893	0.838	0.858	0.759	0.587	0.689	0.811
Gonad	<0.0001	<0.0001		0.887	0.874	0.886	0.902	0.856	0.871	0.761	0.656	0.681	0.675
Brain	<0.0001	<0.0001	<0.0001		0.960	0.944	0.949	0.925	0.936	0.784	0.806	0.798	0.704
Heart	<0.0001	<0.0001	<0.0001	<0.0001		0.983	0.981	0.958	0.976	0.830	0.773	0.741	0.650
Jaw	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.991	0.951	0.981	0.842	0.740	0.743	0.667
Tail	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.991	0.987	0.828	0.748	0.750	0.658
Front Leg	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.960	0.791	0.801	0.616	0.657
Rear Leg	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.854	0.750	0.727	0.625
Blood	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.577	0.589	0.492
Scute	0.0005	0.0010	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0016		0.577	0.453
Claw	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0012	<0.0001		0.408
TL (cm)	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.0001	0.0003	0.0098	0.0168	0.0336	

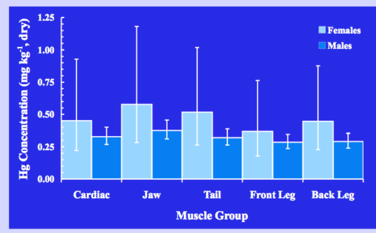
Table 3. Results of ANCOVAs predicting Hg in alligator tissues as a function of sex and total length (TL). The model used for these comparisons was:

$$\log \text{Hg concentration} = \beta_0 + \beta_1 \text{ sex} + \beta_2 \log \text{TL}$$

Except for muscle, no ANCOVA comparisons showed significant sex × log TL interactions. Tests of β_1 that differed significantly from zero are listed, together with the ratio of the geometric mean Hg concentrations of females/males.

Tissue	*df*	*F*	*P*	Female/Male
Kidney	1,24	11.13	0.003	2.23
Gonad	1,24	8.75	0.007	2.15
Tail muscle	1,24	5.11	0.033	1.65
Whole blood	1,23	4.69	0.041	1.79

Figure 1. Hg concentrations (mg kg^{-1}) in muscle groups of American alligators from RWR. Repeated-measures ANCOVA analysis of the ranked data, with sex and muscle group as factors and TL as a covariate showed significant differences in both the slope (P = 0.0008) and intercept (P = 0.0128) between sexes, as well as by muscle group (P << 0.0001). For the overall model, $F_{7,127}$ = 41.16; P << 0.0001; R^2 = 0.69.



Tissue	Geo. Mean	95% CI	Relative ratio
Liver	6.39	3.56–9.22	1
Kidney	5.52	3.98–7.05	0.863
Gonad	0.41	0.31–0.50	0.064
Brain	0.49	0.40–0.59	0.077
Cardiac muscle	0.73	0.61–0.85	0.114
Jaw muscle	1.15	0.98–1.33	0.180
Tail muscle	0.98	0.81–1.15	0.153
Front leg muscle	0.80	0.65–0.95	0.125
Rear leg muscle	0.83	0.70–0.95	0.129

Table 4. Soft tissue/blood Hg concentration ratios of American alligators from RWR. Ratios are calculated for wet masses of tissue to whole blood (n = 26, one alligator was not bled).

Table 5. Regression model predictions of (dry) Hg concentrations in tail muscle. Prediction of Hg concentrations from a Reduced Model, i.e, log TL and Sex regressed on tail muscle Hg concentration were compared with Full Models that included non-lethal tissue samples. For each model, underlined variables are the most predictive.

Independent Variables	*F*	*df*	*P*	R^2
Reduced Model: log TL, Sex	13.69	2,24	0.0001	0.533
Full Model:				
log TL, Sex, log Claw, log Scute, log Blood, Sex × log Blood	56.74	6,19	<0.0001	0.947
log TL, Sex, log Claw, Sex × log Claw	40.63	4,22	<0.0001	0.881
log TL, Sex, log Claw, log Scute, Sex × log Claw	40.15	5,21	<0.0001	0.905
log TL, Sex, log Blood, Sex × log Blood	36.49	4,21	<0.0001	0.874
log TL, Sex, log Scute, Sex × log Scute	26.05	4,22	<0.0001	0.826

DISCUSSION

Hg concentrations of the nine female and 18 male alligators (total length: 90–269 cm) were well above the MDL in all tissues tested, and were highest in kidney and liver and lowest in brain and gonad (Table 1). Hg concentrations were positively correlated among all nearly tissues and all tissue concentrations were with total length (TL, Table 2). Notably, blood, claws and scutes were well correlated with all Hg concentrations from other tissues. The slopes of the power functions of TL and tissue Hg concentrations were all statistically indistinguishable from a value of 1, except for liver (t = 3.590, P = 0.0015), kidney (t = 4.361, P = 0.0002), and gonad (t = 2.736, P = 0.0118), which exceeded a slope of 1. ANCOVAs of sex and body length regressed on Hg concentrations showed that the intercepts (but not slopes) of the body length-Hg relationships were greater in females for kidney, gonad, tail muscle and blood (Table 3). None of the other tissues differed between sexes.

Geometric mean concentrations of Hg in all muscles were higher in females than in males (Fig. 1). These numbers should be interpreted cautiously, however, as these data remained very heteroscedastic (females showing a substantially greater variance than males) even after log transformation, and ANCOVA comparisons of Hg in muscle tissues as a function of size and sex using log-transformed data were similarly not possible. Comparisons of muscle tissues were therefore conducted on ranks. Concentrations in the five muscles differed significantly ($F_{4,127}$ = 8.060; P < 0.0001), the slope (Sex: $F_{1,127}$ = 6.340; P = 0.0128) and intercept (Sex × TL: $F_{1,127}$ = 11.77; P = 0.0008) factors were significantly different. This suggests that even when corrected for size, Hg concentrations in the muscles of females exceed those of males.

Soft tissue/blood ratios were greatest in liver and kidney and lowest in gonad and brain (Table 4). Tissue/blood ratios did not differ significantly among muscle groups, and Hg concentrations in all muscles were slightly lower than blood levels. The greater Hg concentrations in liver and kidney, coupled with the lower muscle concentrations suggests that these alligators are feeding on organisms (e.g., predatory fishes) that are at effectively higher trophic levels than the alligators are themselves (i.e., RWR alligators are not apex predators).

In all cases, Full Models incorporating Hg concentrations of even one non-lethal tissue sample (whole blood, claw, or dermal tail scute) were much more predictive (all P-values <0.0001) of Hg in edible tail muscle than TL and sex alone (Table 5). Of the three tissues, claw measurements were the best single predictor, and were most likely to remain significant in combination with other tissues. Whole blood was least predictive of tail muscle Hg.

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