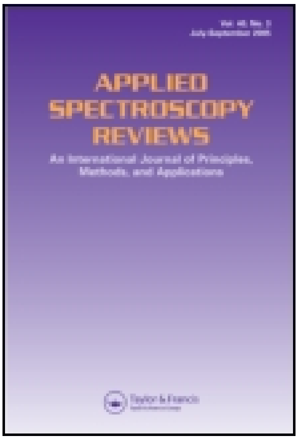

Applied Spectroscopy Reviews
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/laps20

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Accepted author version posted online: 01 Dec 2011.Version of record first published: 02 Feb 2012.

Applied Spectroscopy Reviews, 47:144–162, 2012

ISSN: 0570-4928 print / 1520-569X online
DOI: 10.1080/05704928.2011.634052

Studies of Metals in Crocodilians by Spectrochemical Methods

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Abstract: *This review covers the results of metal determinations using various spectrochemical analytical techniques in crocodilians from various geographical regions. The most widely determined metal is mercury, both inorganic and organic. However, many other metals, including the known toxic metals of lead and cadmium, have been determined. In general, more elevated levels of metals have been determined in crocodilians from potentially contaminated as opposed to more pristine areas. Significant differences in concentrations for many metals have been demonstrated in captive versus wild alligators. The part of crocodilians most widely utilized for food, tail or tail meat, has attracted the most interest, but studies on kidney and liver as well as blood have been reported. Eggs and hatchings have also been examined for several metal concentrations.*

Keywords: Metals, mercury, spectrochemical methods, crocodilians

Introduction

Crocodilians comprise a group of ancient vertebrates that currently have 23 extant species. The earliest crocodilian forms appeared on the earth approximately 200 million years ago. Alligatoroids constitute one of two lineages that diverged from the Crocodylids some 100 million years ago. In general, crocodilian species are apex predators in every environment in which they exist. Although some, such as the slender-snouted crocodile (*Mecistops cataphractus*) and the gharial (*Gavialis gangeticus*), lead piscovoris lifestyles, most are opportunistic and aggressive feeders that consume a wide variety of prey items and may potentially ingest and accumulate toxic compounds including metals that may bioconcentrate in food they eat.

In terms of geographical area, in the United States Florida and Louisiana have been the most widely sampled area, although some studies have been conducted in South Carolina. Australia, Belize, China, Costa Rica, and South Africa are other areas in the world where studies on metal concentration in crocodilians have been performed.

Though there are numerous methods for the determination of metals in samples (including crocodilians), such as electrochemical methods (voltammetry, amperometry, and coulometry) or molecular spectroscopy, spectrochemical methods are among the most widely used and accepted analytical techniques. These techniques include flame and furnace (graphite) atomic absorption spectrometry, atomic fluorescence spectrometry, inductively

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coupled plasma–optical emission spectrometry, and inductively coupled plasma–mass spectrometry. Some metals can be determined using specific variations of common spectrochemical techniques. For instance, mercury determination is often conducted using a cold vapor variation of the atomic absorption method due to increased sensitivity with a detection limit of parts per billion (ng/g) obtained. In addition, selenium, arsenic, lead, antimony, bismuth, germanium, tellurium, and tin content can be determined using hydride generation, in conjunction with traditional atomic absorption, atomic or optical emission, and inductively-coupled plasma methods. These techniques are well-documented and have been widely used (1–9).

Though direct metal determinations of tissues are desirable, spectrochemical techniques are best suited to wet chemistry methods that primarily utilize aqueous solutions. Therefore, the preparation of samples is frequently an integral part of spectrochemical methods. These digestion techniques range from classical wet chemistry through the more modern approach of microwave digestion. These techniques have been used extensively for the determination of metals (10–12).

The most widely determined metal has been mercury, both in inorganic and organic forms, although lead has also been frequently determined. However, there are a number of studies in which numerous metals have been determined.

Metal concentrations have been determined most often in the tail or tail meat, because this is the part of a crocodilian that is most widely consumed by humans. However, other studies have focused on metal concentrations in the kidneys, liver, blood, caudal scutes, various muscles and tissues, and eggs. Results are typically presented in nanograms, micrograms, or milligrams per kilogram.

Discussion

In this review, the results of metal determination in various parts of crocodilians are summarized in Table 1 and the results of several studies for mercury are presented in Table 2. The metals for which the tissues were determined, sample type (tissue, blood, liver, etc.), geographic location, species of crocodilians, and spectrochemical analytical technique with relevant information and selected results are included in Table 1.

Viera et al. (13) studied both mercury and methyl mercury ratios in *Caiman crocodilus yacare* from the Pantanal area in Brazil. Total mercury ranged from 0.02 to 0.36 μg/g, and ratios of mercury to methyl mercury were reported to be near 70%. They noted increased mercury concentrations in sites impacted with anthropogenic activities versus less industrialized areas. Mercury concentrations in crocodilian tissues from various geographic locations, primarily from *Alligator mississippiensis*, are shown in Table 2. The reported concentrations (0.02 to 5.57 μg/g) varied widely.

Campbell et al. (14) showed that the length and sex of 33 wild alligators from South Carolina showed no statistical correlation with metal concentrations for arsenic, cadmium, cobalt, chromium, mercury, nickel, lead, and selenium. They did note that cluster analysis showed three groupings of alligators based on liver concentrations had high Hg concentrations with low selenium : mercury ratios. They attributed this phenomenon to diet and micro-habitat usage.

Merchant et al. (15) investigated iron withholding as an immune mechanism in *Alligator mississipiensis* from southwest Louisiana. The work was to show that the alligator's innate immune mechanism protected it against in vivo microbial proliferation. They noted a

Table 1
Summary of metal concentrations in crocodilians

Location of research	Crocodilian species	Organs analyzed	Metals determined	Sample size	Instrumentation	Other important points	Results and comments	Reference
Pantanal area, Brazil.	Caimans (*Caiman crocodilus yacare*)	Tail muscle	Total Hg, methyl Hg, and inorganic Hg	Seventy-nine specimens from four separate locations	CVAAS	Organic Hg was calculated as difference between inorganic and total Hg; limits of detection for total Hg and MeHg were 13 and 15 μg/g, respectively	Total Hg content ranged from 0.02 to 0.36 $\mu g\ g^{-1}$; methyl Hg ratios were above 70% in most of the specimens; average total Hg concentrations ($\mu g\ g^{-1}$) in various crocodilian species from a literature search; see table 2	(13)
Charleston area, South Carolina	*Alligator mississippiensis*	Livers	As, Cd, Co, Cr, Hg, Ni, Pb, and Se	Thirty-three wild alligators	Samples were acid digested (EPA procedure) and Cd, Co, Cr, Ni, and Pb were determined by ICP-OES; Hg determined by CVAAS following EPA 7470A method; As and Se were determined by HG with EPA 7061A and 7741A methods, respectively	K-means cluster analysis was used to minimize standard deviations of means of each group; based on statistical analysis,there was no strong relationship between metal concentration and length or sex of the alligators; alligators with low Se:Hg ratio had high concentrations of Hg	Se:Hg ratio is of its molar concentration; heavy metals and Se concentrations are expressed as μg/g of the sample ($n = 33$): As, 0.243 $\pm$ 0.04; Cd, 0.489 $\pm$ 0.12; Cr, 5.66 $\pm$ 2.8; Co, 0.443 $\pm$ 0.15; Hg, 5.68 $\pm$ 1.4; Ni, 0.502 $\pm$ 0.19; Pb, 8.15 $\pm$ 3.5; Se, 3.30 $\pm$ 0.33; Se:Hg, 3.97 $\pm$ 0.8	(14)

Lake Charles, Louisiana	*Alligator mississippiensis*	Blood	Plasma Fe and serum Zn and Cu	Four juvenile captive alligators were injected with bacterial lipopolysaccharide (5 mg/kg) intraperitoneally	ICP-OES	This project was done to determine whether alligators exhibit the same innate immune mechanism to protect against in vivo bacterial infections as humans do by increasing the expression of proteins that sequester serum Fe away from bacteria; as a result, there will be reduction in plasma Fe levels during the initial phase of infection	There was a time-dependent decrease in plasma Fe levels; the plasma Fe levels reduced by5.9, 10.6, 18.6% and remained at 12% relative to untreated controls (which were injected with pyrogen-free normal saline) at 3, 6, 12, and 48 h, respectively,after injecting both groups; in contrast the serum Zn and Cu remained unchanged when compared to untreated controls	(15)
Belize and Costa Rica	*Crocodylus moreletti* for metals; *Crocodylus acutus* for metals and organochlorine pesticides	Caudal scutes	Hg, As, Cd, Cu, Pb, Zn, and organochloro pesticides (OCs): lindane, heptachlor, aldrin, DDE, DDT, dieldrin, endrin, methoxy-chlor	Twenty-five scutes were analyzed, one scute from each individual crocodile	ICP-OES; Hg by cold vapor–continuous flow injection analysis system; OCs were separated and quantified by gas chromatograph technique; column: 30 m $\times$ 0.32 mm DB-5 column, detector: ^{63}Ni electron capture detector	NIST standards were used to check the accuracy and precision of samples; detection limits for As, Cd, Cy, Pb, and Zn in water were 0.05 μg/g; detection limit for mercury was 0.5 ng/g in water; in OC pesticides analysis tetrachloro-m-xylene and decachlorobiphenyl were used as internal standards, to monitor efficiency of extraction and analysis; Florisil solid-phase extraction columns were used to separate lipids from scute extracts	Mean Hg concentrations for 9 or 10 samples ranged from 94 to 99 (Belize) and 73 to 99 (Costa Rica) ng/g	(16)

(Continued on next page)

Table 1
Summary of metal concentrations in crocodilians *(Continued)*

Location of research	Crocodilian species	Organs analyzed	Metals determined	Sample size	Instrumentation	Other important points	Results and comments	Reference
China Anhui: captive and wild population Changxi: captive population	Chinese alligator (*Alligator sinensis*)	Tissues and eggs	As, Fe, Mn, Cu, Pb, Cd, Cr, Zn, and Hg	Muscle samples from two dead (one mature male and one mature female) alligators; 10 eggs from three different clutches (one egg each from two different clutches and eight eggs from the third clutch)	As, Fe, Mn, and Zn by FAAS; Cu, Pb, and Cd by FAAS but after treating the samples with diethyl-dithiocarbamate-methyl isobutyl ketone; Cr was measured by colorimetry; Hg by CVAAS	Each sample was analyzed three times and standard error was less than 5 % for each element; Mn, Pb, and Cr were significantly higher in egg shells than in egg contents and As, Fe, Zn, and Hg were higher in egg contents than shells; nonparametric Spearman's correlations were used to relate the concentrations of heavy metals within and between compartments	Concentrations (μg/g, dry weight) of heavy metals (As, Fe, Mn, Cu, Pb, Cd, Cr, Zn, and Hg) werefound in various tissues and organs; liver and kidneys were the major accumulators and pancreas and gonads accumulated the least; the concentration (μg/g, dry weight) of heavy metals in egg shells, shell membranes, and egg contents andorgans was determined	(17)
Louisiana	*Alligator mississippiensis*	Bone, liver, kidney, and yolk	Pb, Cd, and Se	Forty-four captive-reared alligators and 15 wild alligators	Microwave-assisted acid-digested samples and GFAAS	Males grow faster and reach a greater length than females; spiked reference bovine liver tissue (NIST) was tested for all three metals and consistently recovered more than 85% of each metal; lead in bone samples of captive alligators was 252,443 ± 20,462 ng/g; high Pb levels in yolk were assumed to be the probable cause for the early embryonic death in alligator eggs	Results for Pb, Cd, and Se in alligator tissues showed that Pb was higher in tissues of captive alligators than wild; Se in kidneys was 50% higher in wild than in captive alligators and Cd did not differ in wild and captive alligators; high tissue Pb levels in captive-reared alligators was probably due to the long-term consumption of nutria (*Myocastor coypus*) meat contaminated with Pb shot	(18)

Florida, Everglades	*Alligator mississippiensis*	Liver and tail muscle	Total Hg (organic and inorganic)	Twenty-eight American alligators	Total Hg by CVAAS; continuous-flow isotope-ratio mass spectrometry for the isotopic composition of samples	Samples were digested using EPA Method 245.6 (U.S. EPA, 1991); as part of quality control, DOLT-2 (National Research Council Canada Institute for National Measurement) was used as certified reference materialand recovered an average of 102% of total Hg using the same procedure and instrument	Total Hg found ranging from 0.6 to 17 mg/kg liver and in tail muscle from 0.1 to 1.8 mg/kg; alligators from Everglades National Park recorded a total Hg of about 10.4 mg/kg in liver and 1.2 mg/kg in tail muscle, which was twofold higher than alligators from basin wide locations (4.9 and 0.64 mg/kg, respectively)	(19)
Northern Belize:Laguna Seca Lagoon, Gold Button Lagoon, and Sapote Lagoon	*Crocodylus moreletti*	Nonviable eggs	Total Hg	Thirty-one eggs collected from eight different nests from three different locations	CVAAS	Limit of detection for this method was 0.05 μg/g in a 200 mg egg sample with a qualitative range 0.02-0.05 μg/g; results for Hg concentrations (ppm wet mass) in various crocodilian eggs are shown in table 11	Mean concentration of mercury was 0.11 ± 0.05 μg/g; when calculated individually the mean concentrations for Gold Button Lagoon, Sapote Lagoon, and Laguna Seca Lagoon were 0.11 ± 0.02 (n = 4), 0.02 ± 0.01 (n = 3), and 0.21 (n = 1) respectively	(33)

(Continued on next page)

Table 1
Summary of metal concentrations in crocodilians *(Continued)*

Location of research	Crocodilian species	Organs analyzed	Metals determined	Sample size	Instrumentation	Other important points	Results and comments	Reference
Three adjacent catchments within the Alligator Rivers region of northern Australia	Estuarine crocodiles (*Crocodylus porosus*)	Flesh and osteo-derms	Na, K, Ca, Mg, Ba, Sr, Fe, Al, Mn, Zn, Pb, Cu, Ni, Cr, Co, Se, U, and Ti	$N = 40$ for osteoderms and $n = 35$ for flesh	Acid-digested samples were analyzed for Al, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, Ti, U, Zn, Ag, As, Hg, Mo, Sb, Sn, Th, V, and W by ICP-MS; Na, K, Ca, Mg, and Fe by ICP-OES	Ag, As, Hg, Mo, Sb, Sn, Th, V, and W concentrations in both flesh and osteoderms were below the detection limits of the instrument; the effect of factors like total crocodile length, estimated age, gender, reproductive status, physical condition, and catchment of capture on element concentrations in both tissues was determined by using multiple linear regression analyses	Concentrations in flesh and osteoderms of estuarine crocodiles (*Crocodylus porosus*) andosteoderms (Ca, Co, Mg, and U) and for flesh (Ca, K, Mg, Na, Ni, and Pb) were important to discriminate between three catchments	(20)
Three adjacent catchments within the Alligator Rivers region of northern Australia	Estuarine crocodiles (*Crocodylus porosus*)	Flesh (tail muscle) and os-teoderms (pelvic region)	Na, K, Ca, Mg, Ba, Sr, Fe, Al, Mn, Zn, Pb, Cu, Ni, Cr, Co, Se, U, and Ti	$N = 40$ for osteoderms and $n = 35$ for flesh	Acid-digested samples were analyzed for Al, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, Ti, U, Zn, Ag, As, Hg, Mo, Sb, Sn, Th, V, and W by ICP-MS; Na, K, Ca, Mg, and Fe by ICP-OES	Ag, As, Hg, Mo, Sb, Sn, Th, V, and Wconcentrations in both flesh and osteoderms were below the detection limits of the instrument; the effect of factors like total crocodile length, estimated age, gender, reproductive status, physical condition, and catchment of capture on element concentrations in both tissues was determined by using multiple linear regression analyses; elevated Fe and Na levels in flesh were found when the animal's physical conditions deteriorated	Metal concentrations in flesh and osteoderms of estuarine crocodiles (*Crocodylus porosus*); Mg concentrations decreased significantly with increase in length (1.7 to 5 m) and age (5-40 years); Ti concentration in flesh decreased with increasing length; Zn and Se concentrations in flesh increased with increasing length and/or age	(21)

Florida Everglades	*Alligator mississippiensis*	Liver, kidneys, spleen, tail muscle, leg muscle, brain, and tail scales	Mercury (mercury bioconcentration factors)	Not presented in work	Not presented in work	Hg entering Everglades → MeHg in water column → low trophic feeders → high trophic feeders; MeHg when compared to inorganic Hg was very toxic and it biomagnifies exponentially with each successive trophic level because of its solubility; the authors expressed the extent of accumulation of mercury in alligator tissues as the BCF (the ratio of the concentration of a chemical in an organism or whole body or specific tissue to the concentration in water at equilibrium)	Mercury concentration in the alligator tissues and found that Everglades alligators showed high levels of mercury in liver and kidneys when compared to non-Everglades and farm-raised alligators; total mercury concentrations in the tissues of alligators from four regions of the southeastern United States; BCFs for juvenile alligator's liver and kidney were 39.9×10^7 and 32.9×10^7, respectively; BCFs for adult alligator liver and kidney were 10.5×10^7 and 9.34×10^7, respectively	(22)
Olifants River in the central part of the Kruger National Park.Sabi River in the southern part and Shingwedzi in the northern part of the park	Nile crocodile *Crocodylus Niloticus*	Fat, muscle, kidney, and liver	Al, Cu, Cr, Fe, Mn, Ni, Pb, Sr, and Zn	Olifants River ($n = 6$); Sabi River ($n = 2$); Shingwedzi ($n = 4$)	AAS	Blood chemical parameters such as total serum protein, albumin, chloride, urea, creatinine, blood calcium, potassium, and inorganic phosphate were also determined in this work	Concentrations of selected metals (Al, Cu, Cr, Fe, Mn, Ni, Pb, Sr, Zn) in tissues of alligators from three rivers of the Kruger National Park area	(23)

(Continued on next page)

Table 1
Summary of metal concentrations in crocodilians *(Continued)*

Location of research	Crocodilian species	Organs analyzed	Metals determined	Sample size	Instrumentation	Other important points	Results and comments	Reference
Lake Apopka, Orange Lake, and Lake Woodruff National Wildlife Refuge in Central Florida	*Alligator mississippiensis*	Liver, abdominal muscle, abdominal fat, tail muscle, tail tip, and abdominal skin	Pb, Cd, Se, Cr, Mn, Ar, Sb, and Hg	Fat ($n = 30$), liver ($n = 31$), abdominal muscle ($n = 30$), skin ($n = 29$), tail muscle ($n = 29$), tail tip ($n = 22$), regenerated tail ($n = 3$)	GFAAS except CVAAS for Hg	Instrument detection limits for metals were as follows: As $\rightarrow$ 0.2, Cd $\rightarrow$ 0.02, Cr $\rightarrow$ 0.08, Pb $\rightarrow$ 0.015, Mn $\rightarrow$ 0.09, Hg $\rightarrow$ 0.2, Se $\rightarrow$ 0.7, and Sn $\rightarrow$ 0.2 (units ng/g)	Metal concentrations in various tissues of alligators from three different lakes in Florida as shown in table 15; among all of the tissues analyzed livers accumulated high concentrations of metals except Pb, Cr, and Sn	(24)
South Louisiana	Not mentioned	Tail muscle	Hg	42	AAS	Instrument detection limit for mercury was 0.0001 ppm; Hg concentration in alligator meat in southern Louisiana was far below the FDA action level for human consumption of seafood	Hg concentration in tail muscle ranged from 0.047 to 0.386 ppm; on average it was about 0.131 ppm ($n = 42$), which was lower than Hg levelsrecorded in other southeastern states	(25)
Everglades, Florida and Par Pond reservoir in South Carolina	*Alligator mississippiensis*	Kidney, liver, muscle, and dermal scutes	Total Hg	Everglades, Florida ($n = 18$) and Par Pond reservoir ($n = 44$) in South Carolina	Tissues were analyzed for total mercury by gold-foil amalgamation using a conductivity detection technique Hg analyzer and CVAFS	Detection limits for Hg using analyzer was 5 ng and for CVAFS was 1 ng	Mean concentrations of Hg in kidney, liver, muscle, and dermal scutes of alligators from the Everglades were 36.42 ± 5.23, 41.09 ± 5.90, 5.57 ± 0.47, and 5.83 ± 1.04, respectively; mean concentrations of Hg in liver, muscle, and dermal scutes of alligators from Par Pond reservoir were 17.73 ± 2.56, 4.08 ± 0.46, and 4.58 ± 0.63, respectively	(26)

Water Conservation Area 3 of Everglades ecosystem	*Alligator mississippiensis*	Brain, cervical spinal cord, liver, paired kidneys, paired testes, paired ovaries, paired oviducts, heart, lungs, spleen, bile, tail, leg muscle, and leg scales	Total Hg (inorganic Hg and MeHg)	Twenty-four wild caught and 6 captive farm-raised alligators ($n = 30$)	Total Hg was determined using a cold vapor atomic absorption spectrophotometer with mercury hydride generator	Method detection limits were 0.01 ppm wet weight; the authors found that muscle Hg concentration in Everglades alligators exceeded state (0.50–1.50 ppm) and federal (1.00 ppm) allowances for safe human consumption	Determined whole-body mercury contamination in 30 American alligators; except in bile, mercury was consistently detected in all tissues analyzed; except in ovaries, oviduct, bile, tail scales, and leg scales all of the tissues had elevated mercury levels compared to non-Everglade alligators	(27)
Florida	*Alligator mississippiensis*	Tail muscle	Cu, Zn, Fe, Cr, Hg, Pb, As, Pt	Thirty-two American alligators	Cu, Zn, Fe, Cr, Pb, and Pt by AAS; Hg and As by hydride generation in flameless (furnace) and flame modes, respectively	Background correction with deuterium arc for furnace AAS; the detection limits for the mentioned metals using this instruments were as follows: Cu → 0.02, Zn → 0.05, Fe → 0.30, Cr → 0.02, Hg → 0.04, Pb → 0.02, Cd → 0.05, As → 0.02, and Pt → 0.50 (all in ppm)	Tail muscle of 32 American alligators to determine the concentrations of various contaminants in order to provide basic informationon the safety of alligator meat consumption in that area; determined metal concentrations in muscle samples from various lakesand of all the metals determined, mean residue concentrations were highest for Hg (0.61 ppm)	(28)

(Continued on next page)

Table 1
Summary of metal concentrations in crocodilians *(Continued)*

Location of research	Crocodilian species	Organs analyzed	Metals determined	Sample size	Instrumentation	Other important points	Results and comments	Reference
Eggs were collected from three sites in South Carolina, ha drainage swamp (ABS) which is the sink for effluents coming from a coal-burning power plant, downstream from the power plant along Beaver Dam Creek (BDC), and reference pond	*Alligator mississippiensis*	Eggs and hatchling	Se	Total seven clutches: three clutches from ABC site and clutch size were 30, 35, and 29; two from BDC site and clutch size were 44 and 46; two from reference sites and clutch size were 31 and 41, respectively	ICP-MS	Certified reference material was analyzed using the same instrument as samples and the mean percentage recovery of Se was 106%; instrument detection limits of Se for eggs, hatchlings, and CAMs were 0.155, 0.596, and 0.139 ppm, respectively	Se concentration in eggs and hatchlings collected at ABS site (2-ha drainage swamp) and BCD were higher (2.1–7.8 ppm) than that of the reference site (1.4–2.3 ppm); hatchlings of the ABC site and BDC site had lower viability (30–54%) than the reference site (67–74%); within the compartments of the egg, chorioallantoic membraneshowed a high concentration of Se showing that this membrane can be used asan nondestructive index of Se exposure for embryos	(29)
Everglades aquatic food webs, in Florida	*Alligator mississippiensis*, the great egret (*Egretta alba*), and raccoon (*Procyon lotor varius*)	None	Methyl Hg	Not mentioned	Monte Carlo simulations (using probability distribution functions, ranges of MeHg concentrations in aquatic biota, and prey selection at each trophic group and each exposure area were used to determine the methyl mercury exposure in piscivorous wildlife in Everglade aquatic food webs	Reported no observed effect (NOEC) and lowest observed effect (LOEC) concentrations obtainedfrom long-term laboratory studies were used to calculate toxicity reference values (TRVs) for dietary MeHg	Using Monte Carlo simulations the authors assessed the probability distributions of risk estimates for piscivorous wildlife in Everglade aquatic food webs; if the exposure concentrations exceededTRVs for dietary MeHg it was considered to be at risk for wildlife; in this way they found that alligators in the south central Everglades had excess of 100% chronic risk thresholds of Hg	(34)

Lynd River, northeastern Australia	Freshwater crocodiles, *Crocodylus johnstoni*	Osteoderms (dermal bones)	Ca, Na, Mg, Sr, Fe, K, Zn, Ba, Mn, Ni, Cu, Pb, U, Cd, Co, As, Cr, Hg, Mo, Sb, Se, Sn, and Th	Thirty freshwater crocodiles, *Crocodylus johnstoni*	Zn, Ba, Mn, Ni, Cu, Pb, U, Cd, Co, As, Cr, Hg, Mo, Sb, Se, Sn, and Th in acid-digested samples by ICP-MS and the last eight metals were far below the analytical detection limit of the instrument; that is, less than 0.01 ppm; Na, K, Ca, Mg, Sr, and Fe in ICP-OES	Linear regression analyses were used to predict the effect of the following variables: snout-vent length, age, gender, and reproductive status on osteoderm metal concentrations	Determined concentration of mentioned metals in the osteoderms of 30 freshwater crocodiles, (*Crocodylus johnstoni*); except for Na and K, age, size, and osteoderm Ca were significant predictors of the osteoderm metal concentrations; both size ($r^2 = 0.52$–0.92) and age ($r^2 = 0.52$–0.84) were inversely related to osteoderm metal concentrations and osteoderm calcium ($r^2 = 0.67$–0.92) was positively related toosteoderm metal concentrations	(35)
Alligator femurs were obtained from Rockefeller Wildlife Refuge in Louisiana	*Alligator mississippiensis*	Femurs	Pb, Ca, and Sr	Seven femurs, of which four were from captive farm-reared alligators and three were from wild alligators	Laser ablation ICP-MS	The frequency of laser used was 5 Hz and laser pulse energy was 0.6 mJ; Ca was used as an internal standard	Femurs of both captive and wild alligators for three metals with high Pb concentration in captive alligators and high Sr concentration in wild alligators	(30)

(Continued on next page)

Table 1
Summary of metal concentrations in crocodilians *(Continued)*

Location of research	Crocodilian species	Organs analyzed	Metals determined	Sample size	Instrumentation	Other important points	Results and comments	Reference
Lake Griffin, Florida	*Alligator mississippiensis*	Liver and kidney tissues	Cu, Fe, Zn, Rb, and Se	Sixteen samples	Particle-induced X-ray emission spectrometry (PIXE)	Tandem accelerator was used to generate a proton beam of 2.5 MeV; to avoid overheating and charge buildup on the sample the total beam intensity was maintained below 35 nA	In this study statistically significant data were not obtained but this study developed a method (using PIXE to analyze a wide range of elements in animal tissue) that has a scopeto generate high-resolution elemental analyses in the near future	(31)
Holly Beach and Rockefeller Wildlife Center in southwest Louisiana	Not given	Blood samples	Sodium, potassium, and chlorine	Eleven blood samples from alligators from Holly Beach and 201 from alligators from Rockefeller Wildlife Refuge			Collected blood samples from alligators on Holly Beach after the beach was affected by Hurricane Rita; Na, K, Cl-, osmolality, and corticosterone levels in blood were determined and all of these blood parameters were elevated	(32)
Australia	Estuarine crocodiles (*Crocodylus porosus*)	Blood samples	Pb	Six juvenile crocodiles	ICP-MS	Pb shots were retained in alligator stomach for about 20 weeks and 13–30% of lead shot was eroded	Four alligators were fed with known concentrations of lead shot and blood Pb concentration over a period of 5–20 weeks after dosing was observed; during the first week blood lead levels increased by an order of magnitude and attained steady-state equilibrium within 5–20 weeks after exposure	(36)

Florida Everglades; Okefenokee National Wildlife Refuge, Georgia; Savannah River Site (SVS), South Carolina; and central Florida	*Alligator mississippiensis*	Blood, brain, liver, kidney, muscle, bone, fat, spleen, claws, and dermal scutes	Total Hg	Florida Everglades ($n = 18$); Okefenokee National Wildlife Refuge, Georgia ($n = 9$); Savannah River Site, South Carolina ($n = 49$); and central Florida ($n = 21$)	CVAAS		Total Hg concentration in the tissues provided the following observations: Everglades alligators, even though most of them were juvenile, showed the highest concentration of total mercury (liver 41.0, kidney 36.4, muscle 5.6 mg Hg/kg dry weight); total Hg concentrations in alligators from central Florida, Okefenokee, and SVS site were provided respectively as follows (mg Hg/kg dry weight): Liver, 14.6, 4.3, 14.9; kidney, 12.6, 4.8, N/A; and muscle, 1.8, 0.8, 4.8	(37)
Southwest Lousiana	*Alligator mississippiensi*	Tail meat	Cd, Cu, Fe, Pb, and Zn		ICP-OES, sample preparation by microwave digestion	Cd and Pb not detected	Results were not considered toxic	(38)

CVAAS, Cold vapor atomic absorption spectrometry; EPA, Environmental Protection Agency; NIST, National Institute of Standards and Technology; ICP-OES, inductively coupled plasma–optical emission spectrometry; FAAS, flame atomic absorption spectrometry; GFAAS, graphite furnace atomic absorption spectrometry; CVAFS, cold vapor atomic fluorescence spectrometry; ICP-MS, inductively coupled plasma-mass spectrometry; HG, hydride generation; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichlorethylene; OC, organochlorine; BCF, bioconcentration factors; CAM, chloriallantoic membrane.

Table 2

Average total mercury concentrations (μg/g) observed in the literature for different crocodilian species

Study area	Number of samples	Total Hg content	Species	Reference
Paraguay River, Brazil	79	0.05 ± 0.01 (ww)	*Caiman c. yacare*	(13)
B. Gomes, Brazil		0.27 ± 0.08 (ww)	*Caiman c. yacare*	
Cuiaba, Brazil		0.14 ± 0.04 (ww)	*Caiman c. yacare*	
Nhecolandia, Brazil		0.12 ± 0.10 (ww)	*Caiman c. yacare*	
Everglades	28	0.63 ± 0.54 (ww)	*Alligator mississippiensis*	(19)
Everglades	18	5.57 ± 0.47 (dw)	*Alligator mississippiensis*	
Florida	31	0.06 ± 0.02 (ww)	*Alligator mississippiensis*	(24)
Louisiana	42	0.047 ± 0.386 (ww)	*Alligator mississippiensis*	(25)
Everglades, Florida	18	1.17 ± 0.36 (dw)	*Alligator mississippiensis*	(26)
Florida	32	0.04 to 0.61 (ww)	*Alligator mississippiensis*	(28)
Australia	75	<0.01 (dw)	*Crocodylus porosus*	(35)
Florida	21	1.85 ± 0.35 (dw)	*Alligator mississippiensis*	(37)
Okefenokee	9	0.08 ± 0.12 (dw)	*Alligator mississippiensis*	
South Carolina	49	4.83 ± 0.88 (dw)	*Alligator mississippiensis*	

time-dependent decrease in iron blood plasma after injection of bacterial lipopolysaccharide but none for serum zinc and copper.

Rainwater et al. (16) investigated the presence of several metals, including cadmium, copper, lead, zinc, and mercury, as well as organochlorine pesticides in caudal scutes from crocodiles from Central America (Belize and Costa Rica). Mercury, cadmium, copper, lead, and zinc were found in all 25 samples.

Studies on arsenic, iron, manganese, copper, lead, cadmium, chromium, lead, zinc, and mercury in feces and eggs of Chinese alligator were performed by Xu et al. (17). Of the nine metals investigated, they showed that there was a high correlation between metal contents of shell membrane and egg content.

Lance et al. (18) found that captive ($n = 44$) over wild ($n = 15$) alligators had higher lead concentrations, there were no significant differences in cadmium concentrations, and selenium was 50% higher in wild compared to captive. Bone, liver, kidney, and egg yolk were analyzed. The elevated levels of lead were attributed to the alligator eating nutria meat containing lead shot.

Rumbold et al. (19) studied mercury (organic and inorganic) in 28 alligators from the Florida Everglades and found ranges from 0.6 to 17 mg/kg in the tail as well as the total mercury in liver of around 1.2 mg/kg. Further work on crocodiles, in particular eggs from Belize, showed mean mercury concentrations of $0.11 + 0.05$ μg/g[20]. Several studies were performed on estuarine crocodiles (*Crocodylus porosus*; tail and pelvic area) from regions in northern Australia (20, 21) and some general conclusions were reported, including that concentrations of magnesium decreased significantly with increase in length (1.7 to 5 m) and age (5–40 years) in the flesh and osteoderms of estuarine crocodiles, titanium concentration in flesh decreased with increasing length, and zinc and selenium concentrations in flesh increased with increasing length and/or age. Khan and Tansel (22) showed bioaccumulation for mercury particularly in the liver and kidneys of the Florida Everglades alligators compared to alligators from other areas.

Swanepoel et al. (23) correlated several parameters (such as total serum protein albumin, chloride, urea, creatine, blood calcium, potassium, and inorganic phosphate) with several metals in Nile crocodiles (*Crocodylus niloticus*) from Kruger National Park in South Africa. Burger et al. (24) showed the bioaccumulation of several metals in alligators from three lakes in central Florida and concluded that the liver showed increased (more than expected) concentrations of lead, chromium, and tin. Elsey et al. (25) determined mercury in edible tail meat from alligators from Louisiana and found that the levels were far below Food and Drug Administration (FDA) action levels for seafood. Several studies (26, 27) determined elevated concentrations levels of mercury in parts of alligators in the Everglades of Florida but also in South Carolina. Further work on assessing the safety of alligator tail meat from Florida showed a mean concentration level of 0.621 ppm mercury (28). Roe et al. (29) studied selenium concentrations in alligator eggs and hatchlings in the neighborhood of a power plant in South Carolina and noted increased levels ranging from 2.1 to 7.9 ppm compared to a reference (and presumably more pristine area) of 1.4–2.3 ppm. The topical analytical technique of laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) was used to determine lead, calcium, and strontium in both wild and captive alligators (30), with lead concentrations in femurs high in captive alligators. Particle-induced X-ray emission spectrometry (PIXE) was primarily investigated to show its potential for future high-resolution metal analyses (31). Lance et al. (32) determined sodium, potassium, and chlorine in blood samples from alligators from a beach in southwest Louisiana shortly after a hurricane impacted this area. The authors noted elevated levels of these metals and the results were attributed to increased stress from the hurricane.

Conclusion

Not surprisingly, elevated concentrations of metals were often found in crocodilians in areas where there was a high probability of pollution generated from industrialized and anthropogenic sources. However, bioaccumulation of metals has also been found in non-industrialized areas. Mercury has been the most widely determined metal, in both the inorganic or organic forms. Clearly there is a continued need to determine and monitor metals in crocodilians. Several of the studies reported in this review could be used as a baseline for future studies. Due to their low sensitivity and specificity, spectrochemical methods will continue to provide the means for metal determination in crocodilians.

Acknowledgments

Joseph Sneddon acknowledges, with thanks, the support of a 2010–2011 McNeese State University Alumina Award.

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