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Heavy Metal Distribution in Tissues and Eggs of Chinese Alligator (*Alligator sinensis*)

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Abstract. Chinese alligator (*Alligator sinensis*) is a critically endangered species endemic to China. Concentrations of heavy metals (As, Fe, Mn, Cu, Pb, Cd, Cr, Zn, and Hg) were examined in the tissues of Chinese alligators to elucidate the background distribution of these metals in the alligator body. Generally, within the body compartments, metal concentrations were high in liver, kidney, and heart, and low in pancreas and gonad. Study of heavy metal levels in the feces and eggs of Chinese alligator suggested that Chinese alligators could reduce body burden of toxic substances by excreting them to feces and/or sequestrating them into eggs to a lesser extent. In addition, to test whether eggshell or egg membrane could be used as surrogates to measure heavy metal load in egg contents, the correlation of metal concentrations between three egg compartments was determined. Of the nine elements analyzed, concentrations of iron, copper, and zinc in the shell membrane were highly correlated with the levels in egg contents, whereas no metal was significantly correlated between eggshell and egg contents. This suggested that the shell membrane could be a useful bioindicator for Fe, Cu, and Zn contaminations in the eggs of Chinese alligator. In a comparison of metal contents in the eggs of individuals from the Anhui captive population, the wild population in Anhui Province, and those of the Changxing captive population, higher Cu, Zn, and Cd levels and a lower Pb level were found in the Changxing individuals, indicative of specific pollutants in different areas. In addition, the majority of metal elements in the muscles of Chinese alligators and American alligators are in the same ranges. As a result of the data found in the eggs of the two alligator species, the Chinese alligators may be exposed to a higher level of metal pollutants. The study provided measurement of the heavy metal distribution in the endangered Chinese alligator for the first time and could serve as the background for the monitoring of possible heavy metal contaminations in the alligator habitats.

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The Chinese alligator (*Alligator sinensis*) is endemic to China and is one of the most endangered species among the world's 23 species of crocodilians. The remaining wild individuals are now restricted to a few small, isolated areas in southern Anhui Province and the adjacent Zhejiang Province (Thorbjarnarson and Wang 1999). A survey concluded in 2002 found that the total population of this species in the wild is probably fewer than 130 and is declining at a rate of 4–6% annually. The largest population found in the wild was composed of only 9–11 individuals (Thorbjarnarson *et al.* 2002). To save this species, Anhui Xuanzhou and Zhejiang Changxing captive-bred populations were established in 1979. Releases from the two captive populations are probably the only viable option for restoring this species to the wild, and reintroduction and habitat restoration are currently being planned.

Previous studies showed that application of large amounts of chemical fertilizers and insecticides might affect the Chinese alligator (Thorbjarnarson and Wang 1999). For example, Zhou (1997) reported that in 1984 two alligators died from the effects of insecticides in a farm in Jinxian County (Anhui Provience) and alligators were found dying from eating poisoned rodents (Thorbjarnarson *et al.* 2002). So far, the survey of the heavy metal contaminations in the field where the two Chinese alligator captive-bred centers are located as well as in the habitats of the remaining wild populations out has not been carried out.

Heavy metals have been recognized as one category of environmental contaminant affecting crocodilians (Brisbin *et al.* 1998), and crocodilians could be used as the bioindicators of environment pollution (Manolis *et al.* 2002a). Many researchers have reported the presence of mercury in crocodilian species (Hord *et al.* 1990; Heaton-Jones *et al.* 1997; Elsey *et al.* 1999; Rainwater *et al.* 2002; Rumbold *et al.* 2002). Using some readily-obtained tissues, such as scutes or claws, interrelationships of mercury concentrations among a variety of tissues were also surveyed, which provided non-destructive screenings of Hg in alligator populations (Yanochko *et al.* 1997; Jagoe *et al.* 1998). However, only a limited number of studies regarding the exposure and impacts of heavy metals in internal tissues have been conducted on crocodilian species. Delany *et al.* (1988) measured 7 metals in tail muscle from 32

American alligators (*Alligator mississippiensis*) at eight Florida lakes; Jeffree *et al.* (2001) provided the baseline concentrations of 18 elements in the flesh and osteoderms of estuarine crocodiles (*Crocodylus porosus*). Burger *et al.* (2000) examined the bioaccumulation of 8 metals in different tissues of American alligators; different concentrations were detected in internal tissues, and the possibility of using skin and tail tissues as bioindicators was also assessed.

The accumulation of heavy metal in the crocodilian's eggs has received little attention. Metal levels in the eggs of American crocodile (*Crocodylus acutus*) and American alligator have been quantified (Ogden *et al.* 1974; Stoneburner and Kushlan 1984; Heinz *et al.* 1991). Phelps *et al.* (1986) examined heavy metals contaminations in the eggs of Nile crocodile (*Crocodylus niloticus*) sampled from several localities in Zimbabwe. Rainwater *et al.* (2002) surveyed the heavy metal concentrations in the eggs of Morelet's crocodile (*Crocodylus moreletti*) from three locations in nonalluvival lagoons. Manolis *et al.* (2002b) detected various trace element concentrations in wild saltwater crocodile eggs and revealed the habitat pollution. Ding *et al.* (2001) presented the first study on heavy metal concentrations in the egg of Chinese alligator in Anhui Province. These studies laid the foundations for further heavy metal contamination surveys for the crocodilian species.

Heavy metal distribution in internal tissues and eggs is not clear in Chinese alligators. And there are no reports, to our knowledge, of mercury pollution in this species. In this study, the concentrations of As, Fe, Mn, Cu, Pb, Cd, Cr, Zn, and Hg in various tissue types of Chinese alligator were measured. One of our objectives was to reveal the metal distribution among internal organs and elucidate the background distribution of these metals in the alligator body.

Similar to the freshwater turtle eggs (Nagle *et al.* 2001), high contaminant levels in crocodilian eggs might increase embryo mortality and decrease hatching fitness. Heavy metal concentrations in the egg may reflect long-term contamination, as some egg contents are drawn from the skeleton (Manolis *et al.* 2002b). The eggshell or shell membranes were surveyed to be used as indicators of pollutants in egg contents in some studies (Morera *et al.* 1997). Accordingly, heavy metal distributions within the three egg compartments (eggshells, shell membranes, and egg contents) of the Chinese alligator were investigated in this study. The relationships of metal concentrations within and among the three compartments were examined in order to assess the reliability of eggshell or shell membrane as substitutes for egg contents in measurement. Chinese alligators are critically endangered; intentional killing and harmful sampling of wild alligators is strictly prohibited. If the levels of metals in eggshell or shell membrane show a significant correlation with the levels in egg content, the easily collectable eggshell and shell membrane could, therefore, be used as non-invasive indicators of contaminants in alligator eggs.

Materials and Methods

Sample Collection and Preparation

The Changxing Nature Reserve and Breeding Research Center for Chinese alligator, located in Changxing County, Zhejiang Province, is centrally within the current geographical distribution range of the Chinese alligators (29° 40'∼31° 30'N, 160° 00'∼120° 00'E).

Two alligator samples (a mature male and a mature female) were collected on February 2004 after death of unidentified causes. Animals were transported to the laboratory fresh and dissected to collect tissues and organs including heart, lung, liver, stomach, kidney, intestine, tracheas, pancreas, gonad, and muscle. Ten Chinese alligator eggs from 3 different clutches (1 egg each from two different clutches and 8 eggs from a third clutch) were collected on September 2003. Eggshell, shell membrane, and egg content (yolk plus albumen) were carefully separated. Six feces samples from different alligator individuals were collected. Two species of fishes used as the feeding food of the alligators in the Changxing Breeding Center were randomly sampled. All the samples were gently washed in distilled water, left to dry at room temperature for 24 hours, and oven-dried at 60°C to constant weight, and then digested to a transparent solution with a mixture of nitric, perchloric, and sulphuric acids.

Water and sediment samples were collected from 10 breeding ponds. Five water and 5 sediment samples from different sites were collected from each pond. The water samples were then transferred to reagent bottles and acidified by a measured volume of concentrated nitric acid and were filtered through a 0.45-μm micropore membrane filter. Sediment samples were air dried and sieved through a 2-mm nylon sieve, then digested with concentrated nitric and hydrochloric acid to extract the metals.

Heavy Metal Analysis

The resultant solutions for all the samples were diluted to a known volume with deionized water and transferred to acid-washed sample tubes. The samples were analyzed for As, Fe, Mn, Cu, Pb, Cd, Cr, Zn, and Hg. As, Fe, Mn, and Zn were directly determined by a flame atomic absorption spectrophotometer (F-AAS, Perkin Elmer). Cu, Pb, and Cd were measured by F-AAS after diethyl-dithiocarbamate-methyl isobutyl ketone treatment (Honda *et al.* 1986). The concentration of Cr for all the samples was examined by colorimetry. The concentration of Hg in sediment samples was determined by cold vapor AAS (Akagi and Nishimura 1991), while water and animal samples were measured by atomic fluorescence spectroscopic analysis. Accuracy of these analyses was controlled using a standard reference material, NIES No. 1 (Okamoto *et al.* 1978). The standard error from triplicate analysis was less than 5% for each element. Concentrations in tissues and eggs were given on a dry-weight basis.

Statistical Analysis

All statistical calculations were performed using the SPSS 11.5 software package for Windows (SPSS Inc., Microsoft Co.). Tabulated values represent mean ± SD for all the samples. A significance level of $p < 0.05$ was chosen for all statistical tests. Differences in concentrations between eggshells and egg contents, shell membranes and egg contents of Chinese alligators were determined by paired *t*-tests. The relationship between concentrations of heavy metals within and between compartments (eggshell, shell membrane, and egg contents) was assessed by nonparametric Spearman's correlations.

Results

Heavy Metal Distribution in Tissues

Concentrations of heavy metals (As, Fe, Mn, Cu, Pb, Cd, Cr, Zn, and Hg) in various tissues and organs of Chinese alligators

Table 1. Heavy metal concentrations (μg/g, dry weight) in tissues and organs of Chinese alligators

Element	As F	As M	As Mean	Fe F	Fe M	Fe Mean	Mn F	Mn M	Mn Mean	Cu F	Cu M	Cu Mean	Pb F	Pb M	Pb Mean	Cd F	Cd M	Cd Mean	Cr F	Cr M	Cr Mean	Zn F	Zn M	Zn Mean	Hg F	Hg M	Hg Mean
Heart	0.768	0.460	0.614	78.5	56.0	67.3	1.84	1.42	1.63	15.03	13.87	14.45	0.98	0.63	0.81	0.460	0.421	0.441	0.841	0.689	0.765	143.21	142.64	142.93	0.343	0.350	0.347
Lung	0.639	0.441	0.540	81.2	47.8	64.5	0.82	1.66	1.24	8.22	14.02	11.12	0.61	0.90	0.76	0.391	0.842	0.617	0.242	0.251	0.247	59.25	37.64	48.45	0.405	0.248	0.327
Liver	0.676	0.497	0.587	407.5	352.4	380.0	5.40	4.74	5.07	26.67	35.54	31.11	0.54	0.85	0.70	0.275	0.530	0.403	0.395	0.333	0.364	151.22	94.33	122.78	0.626	0.492	0.559
Stomach	0.525	0.356	0.441	48.6	57.5	53.1	0.96	1.28	1.12	11.32	15.67	13.50	0.73	0.75	0.74	0.222	0.321	0.272	0.271	0.194	0.233	61.85	67.35	64.60	0.349	0.232	0.291
Kidney	0.589	0.406	0.498	72.8	109.2	91.0	5.10	4.66	4.88	17.23	14.89	16.06	0.34	0.41	0.38	0.162	0.320	0.241	0.268	0.169	0.219	70.96	119.69	95.33	0.935	0.869	0.902
Intestine	0.327	0.250	0.289	37.5	52.5	45.0	1.67	1.52	1.60	5.12	7.45	6.29	0.51	0.47	0.49	0.177	0.086	0.132	0.102	0.095	0.099	115.54	70.37	92.96	0.409	0.389	0.399
Tracheas	0.554	0.534	0.544	55.0	35.4	45.2	2.05	1.94	2.00	4.23	5.65	4.94	0.52	0.42	0.47	0.141	0.120	0.131	0.112	0.178	0.145	51.85	81.31	66.58	0.147	0.092	0.120
Pancreas	0.176	0.126	0.151	8.7	12.8	10.8	0.58	0.67	0.63	2.74	2.91	2.83	0.23	0.26	0.25	0.038	0.036	0.037	0.147	0.142	0.145	7.46	8.94	8.20	0.080	0.042	0.061
Ov/Sp	0.595	0.078	0.337	12.1	13.1	12.6	1.86	0.42	1.14	7.81	2.65	5.23	0.37	0.21	0.29	0.101	0.038	0.070	0.142	0.135	0.139	2.11	1.42	1.77	0.085	0.032	0.059
Muscle	0.390	0.222	0.306	78.0	55.9	67.0	3.60	1.76	2.68	8.10	4.69	6.40	0.75	0.71	0.73	0.109	0.201	0.155	0.130	0.180	0.155	128.30	120.97	124.64	0.281	0.105	0.193

F = female; M = male; Ov/Sp = ovary/spermary.

are given in Table 1. All the elements were detected in all of the samples analyzed. In general, concentrations were higher in liver, kidney, and heart, and lower in pancreas and gonad for Chinese alligators. Liver and kidney had a higher accumulation of heavy metals, which was similar to the reports from America alligators, marine turtles, and many other animals (Burger *et al.* 2000; Storelli and Marcotrigiano 2003; Ikemoto *et al.* 2004).

The highest concentrations of Fe, Mn, and Cu were found in the liver for both female and male individuals. The highest rate of Hg was found in the kidney, which was dissimilar to some reptiles where the highest concentration of Hg was generally found in the liver (Yanochko *et al.* 1997; Jagoe *et al.* 1998; Storelli *et al.* 1998; Godley *et al.* 1999; Sakai *et al.* 1995, 2000). The highest levels of As, Pb, Cd, and Zn differed between female and male individuals. Overall, in 63.3% of measurements, the female samples had higher heavy metal concentrations than the males, while 36.7% cases were the opposite.

Heavy Metal Distribution in Eggs

Heavy metal concentrations in eggshell, shell membrane, and egg contents are given in Table 2. All the elements were detected in the three egg compartments. Levels were significantly higher in the eggshells than in the egg contents for Mn, Pb, and Cr, whereas the concentrations were significantly higher in the egg contents for As, Fe, Zn, and Hg, with no difference for Cu and Cd between the two compartments. Except for As, Cu, and Cd, concentrations of other elements were significantly (1.6–6.1 times) greater in shell membranes than in egg contents. In contrast, the levels of As and Cd in egg contents were higher than in shell membranes, with Cu showing no significant difference.

Significant negative correlation was found between Fe and Pb in the eggshells, while significant positive correlation was deduced between Cu and Pb in the shell membranes. Further, significant positive correlations existed in the egg contents among concentrations of As and Cu, Pb and Cr (Table 3).

One of our objectives was to determine if eggshells or shell membranes could be used as bioindicators of the metal level in eggs of the Chinese alligators. Of the nine elements analyzed in the three compartments, Fe, Cu, and Zn showed significant correlations between shell membranes and egg contents. Two other metals (As and Hg) showed a significant correlation between eggshells and shell membranes. No metal showed a significant correlation between eggshells and egg contents (Table 3).

Heavy Metal Concentrations in the Alligator Feces and in the Diet of the Alligators

The metal concentrations in two species of feeding fish were measured and the heavy metal concentrations of 6 fecal samples were also analyzed to investigate possible ways of heavy metal excretion. The concentrations of 9 heavy metals (As, Fe, Mn, Cu, Pb, Cd, Cr, Zn, and Hg) in the alligator feces and in the diet of the Chinese alligators in the Changxing Captive-

Table 2. Heavy metals (μg/g, dry weight) in the eggshells (ES), shell membranes (SM), and egg contents (EC) from Chinese alligators

				Ratios of elements	
Element	Eggshells (ES)	Shell membranes (SM)	Egg contents	ES/EC	SM/EC
As	0.262 ± 0.081 (0.125–0.356)	0.167 ± 0.061 (0.103–0.274)	0.474 ± 0.022 (0.446–0.501)	0.6*	0.4*
Fe	45.88 ± 4.61 (39.1–52. 3)	117.95 ± 17.08 (93.8–133.9)	63.58 ±8.36 (53.7–70.4)	0.7*	1.9*
Mn	14.21 ± 2.34 (9.28–16.73)	10.32 ± 1.44 (8.80–12.61)	1.68 ± 0.40 (1.22–2.05)	8.5*	6.1*
Cu	43.46 ± 6.89 (32.26–50.82)	32.71 ± 5.35 (24.69–38.80)	33.33 ±2.33 (29.56–35.21)	1.3	1.0
Pb	1.16 ± 0.17 (0.94–1.52)	1.27 ± 0.22 (0.94–1.62)	0.80 ± 0.11 (0.71–0.98)	1.5*	1.6*
Cd	0.230 ± 0.058 (0.150–0.347)	0.088 ± 0.014 (0.070–0.107)	0.172 ± 0.056 (0.122–0.261)	1.3	0.5*
Cr	0.314 ± 0.040 (0.256–0.370)	0.319 ± 0.057 (0.175–0.387)	0.098 ± 0.005 (0.091–0.104)	3.2*	3.3*
Zn	8.66 ± 2.06 (6.22–11.56)	93.96 ± 20.05 (67.25–128.0)	58.87 ± 8.31 (53.20–72.72)	0.1*	1.6*
Hg	0.057 ± 0.014 (0.043–0.083)	0.175 ± 0.025 (0.145–0.213)	0.111 ± 0.010 (0.098–0.124)	0.5	1.6*

The values represent the mean ± SD (range).
*Significant differences by paired *t*-test ($p < 0.05$).

Table 3. Correlations of heavy metal concentrations in the eggshell (ES), shell membrane (SM), and egg contents (EC) of Chinese alligators

Eggshell (ES)	Fe–Pb (−0.646)*	
Shell membrane (SM)	Cu–Pb (0.827)**	
Egg content (EC)	As–Cu (1.000)**	Pb–Cr (1.000)**
As (ES–SM) (0.815)**		Fe (SM–EC) (0.975)**
Hg (ES–SM) (−0.656)*		Zn (SM–EC) (−0.900)*
		Cu (SM–EC) (−0.900)*

The values in parentheses are Spearman's correlation coefficient.
*$p < 0.05$.
**$p < 0.01$.

Table 4. Concentrations of heavy metals (μg/g, dry weight) in the alligator feces and in the diet of the alligators in Changxing captive Breeding Center

Element	As	Fe	Mn	Cu	Pb	Cd	Cr	Zn	Hg
Food	0.156 ± 0.080	19.45 ± 6.29	8.26 ± 1.47	6.15 ± 1.14	1.05 ± 0.35	0.496 ± 0.021	0.067 ± 0.013	33.33 ± 1.00	0.102 ± 0.006
Feces	1.515 ± 0.414	940.9 ± 415.9	157.1 ± 136.7	15.15 ± 3.15	6.10 ± 0.90	3.269 ± 1.095	3.937 ± 0.365	263.70 ± 33.46	0.456 ± 0.241

Two species of fish were used as the diet of Chinese alligators.

bred Center are presented in Table 4. Concentrations of heavy metals in fecal samples were relatively higher than the diet fishes examined (Table 4).

Heavy Metal Concentration in Water and Sediments

The concentrations (mean ± SD) of 9 heavy metals (As, Fe, Mn, Cu, Pb, Cd, Cr, Zn, and Hg) in water and sediment samples from the Changxing Nature Reserve and Breeding Research Center are presented in Table 5. All the surveyed heavy metals were present in these samples. Results showed Fe ranked at the highest concentrations (126 ± 23 μg/L in water; 10,700 ± 800 μg/g in sediment), while As showed the lowest level in water (0.770 ± 0.396 μg/L) and Hg exhibited the lowest level in sediment (0.084 ± 0.008 μg/g).

Discussion

Heavy Metal Uptake and Elimination

Environmental contaminants such as metals, organochlorines, and other persistent chemicals would be expected to exert their toxic and teratogenic effects on crocodilians (Woodward *et al.* 1993; Guillette *et al.* 1994, 1996). A variety of heavy metals

Table 5. Heavy metal concentrations in water (μg/L) and sediments (μg/g) sampled from Chinese alligator breeding ponds of the Changxing Nature Reserve and Breeding Research Center

Element	Water (μg/L)	Sediment (μg/g)
As	0.770 ± 0.396	4.96 ± 0.46
Fe	126 ± 23	10700 ± 800
Mn	12.14 ± 2.11	385.9 ± 75.97
Cu	6.34 ± 0.82	16.15 ± 3.49
Pb	4.45 ± 0.70	34.99 ± 5.01
Cd	4.50 ± 0.94	0.27 ± 0.04
Cr	5.93 ± 0.32	55.46 ± 5.15
Zn	12.78 ± 1.00	48.1 ± 8.58
Hg	1.04 ± 0.30	0.084 ± 0.008

have been identified in water and sediments in the Changxing Nature Reserve and Breeding Research Center (Table 5), and the concentrations of these metals might be site-specific and variable. Our data showed that the environmental metals have been accumulated to various amounts in the tissues and eggs of the alligators (Table 1 and Table 2), with low levels of toxic heavy metals detectable in this captive population. Furthermore, Table 1 showed that large inter-organ variations in metal accumulations existed in the Chinese alligator body. Due to lack of adequate information on metal metabolism in Chinese alligators, it was difficult to establish a general pattern of progressive relationship between heavy metals in water and in the Chinese alligator body. The exact tolerable levels of various heavy metals in water and sediments for Chinese alligators need further investigations.

In addition to water and sediments containing metals, metals were also detected in alligator food items. How the Chinese alligators cope with these metals is an interesting issue. The metal concentrations in the two feeding species of fish and 6 feces samples were compared to gain a physiological pathway of heavy metal excretion in the alligators. Concentrations of heavy metals in fecal samples were higher compared to those in the diet fish and body compartments (Table 5). For example, the concentration of Cr in feces was about 58 times higher than in the food taken. Compared to the concentrations of heavy metal in the eggs, feces also showed higher concentrations for those elements (except Cu) (Tables 2 and 5). As for the body organs, only Cu and Hg in liver and kidney showed higher accumulations than in the feces (Tables 1 and 5). Thus, we confirmed that Chinese alligators could transfer toxic metals such as Cd, Cr, Hg, Pb, and Zn to the feces and excrete them.

Metal Concentration in Chinese Alligator Eggs

Heavy metal in eggs may affect the development and hatchling of offsprings and, thus, is an important aspect in determining the physiological and ecological effects of metal contaminations. However, there are currently only a few studies that have reported heavy metal contamination in crocodilian eggs (Campbell 2003; Ogden *et al.* 1974; Stoneburner and Kushlan 1984; Heinz *et al.* 1991; Phelps *et al.* 1986; Rainwater *et al.* 2002; Ding *et al.* 2001).

In this study, we found that heavy metals were present in eggshell, shell membrane, and egg contents (Table 2). Diverse animal species have been reported to excrete heavy metals in egg compartments, such as female birds in their egg contents (Burger and Gochfeld 1991) and eggshells (Burger 1994). The high concentrations of many types of metals in the egg compartments found in this study may suggest that female alligators also use the eggs as a mean of reducing body burden of toxic substances through maternal transfer to eggs. Similar situations were reported in other reptiles, including loggerhead turtles (*Caretta caretta*), green turtles (*Chelonia mydas*), and slider turtles (*Trachemys scripta*) (Sakai *et al.* 1995, 2000; Burger and Gibbons 1998).

Concentrations of Cu, Zn, Cd, and Pb in a single infertile Chinese alligator egg from Anhui Chinese alligator Breeding Center (Anhui) and from the wild in Anhui Province (wild) were quantified by Ding *et al.* (2001). Comparisons between Anhui Province (Ding *et al.*'s study) and the Changxing Center (current study) can, therefore, be made. Cu, Zn, and Cd were higher and Pb was much lower in the eggs of the Changxing population than the Anhui Center and the wild alligators (Table 6). Pollution is one of the threats to the remaining wild Chinese alligator populations (Thorbjarnarson *et al.* 1999). The higher Cu, Zn, and Cd levels and the lower Pb levels in the eggs from the Changxing individuals are possibly indicative of specific pollutants in different areas, namely, higher Cu, Zn, and Cd content in the Changxing area, and heavier Pb pollution in the Anhui Captive Center and the surrounding wild. Unfortunately, the data by Ding *et al.*'s study was very much limited due to the small sampling. Another comparison that can be drawn is between the American alligators from Florida, USA, and the Chinese alligators. It has been reported that the eggs of American alligators showed no contamination of As, Cd, and Hg (Heinz *et al.* 1991). This implied that the critically endangered Chinese alligators experience a more hazardous environment and this should be a factor to consider in the reintroduction program.

For some endangered species such as Chinese alligator, deliberate killing and harmful sampling are strictly prohibited. Thus, non-lethal monitoring and non-destructive sampling methods for heavy metal estimations are needed. In this study, eggshell and shell membrane were investigated as indicators for monitoring heavy metal levels in Chinese alligator eggs.

We determined if there was a correlation between metal levels among the three egg compartments to test the utility of eggshell and/or shell membrane as non-invasive bioindicators of heavy metal levels in the entire alligator egg. After hatching, the Chorioallantoic membranes (CAMs) are usually retained within the eggshell and can be used for chemical residue analysis. CAMs have been used successfully to examine contaminant exposure and predict chemical concentrations in multiple species of birds and reptiles (Pepper *et al.* 2004). As shown in Table 3, Fe, Cu, and Zn showed significant correlations between shell membrane and egg contents, while no metal was significantly correlated between eggshell and egg contents, suggesting that shell membranes could be used as a bioindicator of the Chinese alligator egg for the three metals: Fe, Cu, and Zn.

Comparisons with American Alligator

The data from the present study indicated various levels of heavy metal accumulation in tissues and eggs of Chinese

Table 6. Mean concentrations (μg/g, dry weight) of metals in infertile Chinese alligator eggs from Changxing Captive Breeding Center (Changxing), Anhui Captive Breeding Center (Anhui), and the wild population in Anhui Province (Wild)

Element	Changxing[a]	Anhui[b]	Wild[c]
Cu			
Eggshells	43.46	3.67	2.21
Shell membranes	32.71	20.03	7.88
Egg contents	33.33	(2.24, 2.54)	(1.38, 2.22)
Zn			
Eggshells	8.66	37.55	24.45
Shell membranes	93.96	45.47	10.70
Egg contents	58.87	(10.27, 7.08)	(4.53, 6.22)
Cd			
Eggshells	0.230	3.414	1.846
Shell membranes	0.088	0.234	0.180
Egg contents	0.172	(0.100, 0.095)	(0.024, 0.056)
Pb			
Eggshells	1.16	26.41	14.69
Shell membranes	1.27	3.33	1.813
Egg contents	0.80	(1.17, 1.02)	(0.204, 0.730)

[a] Abstracted from Table 2. Ten eggs from Changxing Captive Breeding Center were used for metal detection.
[b] Data from Ding *et al.* (2001). One egg from Anhui Captive Breeding Center and one egg from the wild (Anhui Province) were used for metal detection.
(Numbers in Parentheses): indicated the metal concentration in (albumen, yolk).

Table 7. Heavy metal concentrations (μg/g, dry weight) in the muscle of Chinese alligator and the American alligator

Element	Chinese alligator (*Alligator sinensis*)[a]	American alligator (*Alligator mississippiensis*)[b]	
	Mean	Mean	Range
Fe	67.0	45	17–86
Cu	6.40	2.3	1.1–23
Pb	0.73	0.31	0.15–0.46
Cr	0.155	0.21	0.11–0.42
Cd	0.155	0.11	0.038–0.23
Zn	124.64	90	54–137
Hg	0.193	1.16	0.15–2.3

[a] Abstracted from Table 1.
[b] Data from Delany *et al.* (1988). Original data (reported as μg/g, wet weight) were multiplied by a factor of 3.8 (the wet/dry weight ratio calculated by Jeffree *et al.* 2001) for comparison.

alligator, suggesting heavy metal exposure in this captive population. To evaluate the extent of heavy metal contamination in this population, we compared the mean concentrations of several elements to those of the American alligators (Table 7). As shown in Table 7, similar concentrations of heavy metals were present in the muscles of the two species. The Concentrations of the majority of the metals in the Chinese alligators were within the ranges of those detected in American alligators, except the element Pb, which showed a higher concentration in the Chinese alligators.

This study provides documentation of metal distribution in captive Chinese alligators and an impetus for more in-depth studies on exposure and response of this critically endangered species to environmental contaminants for possible heavy metal contamination control for this critically endangered species. However, a paucity of information on the effects of heavy metals on crocodilians makes it difficult to speculate on the biological significance of these concentrations (Stoneburner and Kushlan 1984). Whether the heavy metal concentrations in the alligator tissues were within the normal range has not been clear so far. Controlled laboratory studies and further investigations concerning heavy metal effects on alligators are warranted.

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