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# Element Concentrations in the Flesh and Osteoderms of Estuarine Crocodiles (*Crocodylus porosus*) from the Alligator Rivers Region, Northern Australia: Biotic and Geographic Effects

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**Abstract.** The concentrations of Na, K, Ca, Mg, Ba, Sr, Fe, Al, Mn, Zn, Pb, Cu, Ni, Cr, Co, Se, U, and Ti were determined in the flesh and osteoderms of estuarine crocodiles (*Crocodylus porosus*) captured in three adjacent catchments of Kakadu National Park, within the Alligator Rivers Region of northern Australia. This study provides, for the first-time, baseline concentrations of elements in both flesh and osteoderms of wild crocodiles. Multiple linear regression analyses were used to determine the effects of total crocodile length, estimated age, gender, inferred reproductive status, physical condition, and catchment of capture on element concentrations in both tissues. The Mg concentration ($\log_{10}$) in the flesh and osteoderms of *C. porosus* significantly ($p \leq 0.001$) decreased with increasing length (1.7–5.0 m) and estimated age (5–40 years). Similarly, the Ti concentration ($\log_{10}$) in flesh significantly ($p \leq 0.01$) decreased with increasing length. In contrast, Zn and Se concentration ($\log_{10}$) in flesh significantly ($p \leq 0.001$) increased with increasing length and/or age, suggesting that these relationships are mediated by biological rather than environmental chemical factors. In flesh, Fe and Na concentrations ($\log_{10}$) significantly ($p \leq 0.05$) increased as the physical condition of *C. porosus* deteriorated. No significant ($p > 0.05$) effects of gender or inferred reproductive status on element concentrations in the flesh and osteoderms were found. The mean concentrations ($\log_{10}$) of Al, Ba, Cr, Ni, and Pb in flesh and Co, Fe, Mg, Mn, and U in the osteoderms were significantly ($p \leq 0.01$) different between catchments. The significant ($p \leq 0.05$) effects of catchment on the concentrations of various elements indicate that *C. porosus* reflects the chemistry of its environmental milieu and therefore has a certain degree of catchment fidelity, even though the catchments are adjacent to one another. Such catchment-specific signals may be useful in the determination of the provenance of itinerant crocodiles. They also point to the utility of crocodiles as long-term biomonitors of their environment.

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The Alligator Rivers Region (ARR, Figure 1) is the site of the world-heritage-listed Kakadu National Park (KNP), within which are located both the operational Ranger uranium mine and the Jabiluka mine site. Biota occurring in Magela Creek, which receives effluent from the Ranger mine site, has been a focus of previous radioecological field and experimental studies. These studies investigated natural concentrations and mechanisms of bioaccumulation of Ra-226 in the tissues of freshwater bivalves, turtles, and water lilies, which are included in the traditional diet of the Aboriginal owners of KNP (Jeffree 1988, 1991; Jeffree and Simpson 1986; Twining 1993). Martin *et al.* (1998) established baseline levels for eight natural radionuclides and their concentration factors in the edible flesh of a range of traditional Aboriginal food items from Magela Creek, including the freshwater crocodile *C. johnstoni*. Allison and Simpson (1989) established baseline concentrations for 14 elements in the tissue of the freshwater bivalve *V. angasi*, from Magela Creek and secondary catchments.

However, mine-related radionuclides and stable metals are not the only bioaccumulating contaminants that may have an impact on the resident biota of KNP. A previous study (Twining *et al.* 1999) on the Australian estuarine crocodile, *C. porosus*, indicated the animal's capacity to respond to anthropogenically enhanced Pb in its riverine environment (resulting from the use of Pb shot ammunition by the traditional owners) by accumulating this element to appreciably elevated levels in both its flesh and osteoderms. Relatively few other studies have reported on the bioaccumulation of elements in wild crocodilians (Burger *et al.* 2000), and these have focused primarily on Hg because of the human consumption of alligator flesh (Delany *et al.* 1988; Heaton-Jones *et al.* 1997; Jagoe *et al.* 1998).

The objective of this exploratory study on *C. porosus* from within the ARR of tropical northern Australia was to determine the natural concentrations of a range of elements in flesh and osteoderms; and the effects of (i) biotic factors, such as crocodile size and age, gender, and inferred reproductive status and physical condition; and also (ii) geographic effects associated with the catchment in which they were captured on element concentrations in both these tissues.

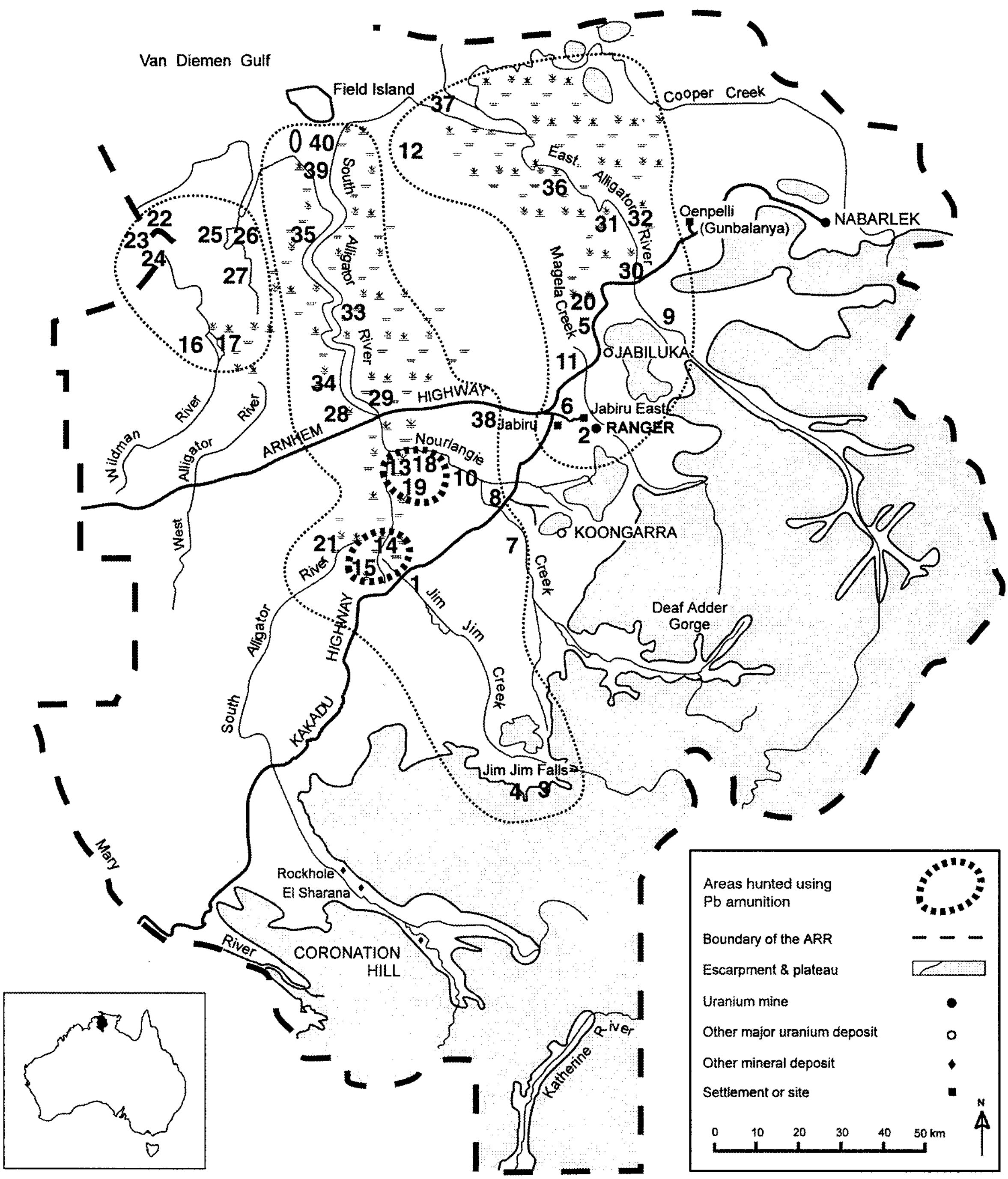


**Fig. 1.** Map of the Alligators Rivers region, encompassing Kakadu National Park. Each number represents the location at which a crocodile was captured for sampling. Habitats exposed to hunting with Pb ammunition are also shown, as are the Ranger uranium mine on the catchment of Magela Creek, East Alligator River, and the site of the Jabiluka mine

## Materials and Methods

### *Study Site*

Figure 1 shows the location of crocodiles (numbers 1–40) captured from KNP, within the ARR of tropical northern Australia. Crocodiles were caught from the three major catchments (20 in South Alligator River, 12 in East Alligator River, and 8 in Wildman/West Alligator Rivers) that flow into the Van Dieman Gulf. Waters ranged from very low ionic strength fresh waters to sea water. The location of the Ranger and Jabiluka uranium mines and two sites used by the traditional Aboriginal owners of KNP for hunting with guns employing lead shot are also shown in Figure 1. At the latter two sites, Twining *et al.* (1999) reported substantially elevated Pb levels ($p \leq 0.05$) in the flesh and osteoderms of *C. porosus*.

### *Sampling and Elemental Analysis*

Forty and 35 crocodiles were sampled for osteoderms and flesh, respectively, between November 1993 and May 1997, following capture by either box mesh trap, floating trap cage, or harpoon. For each animal, its location, capture date, several measures of size (*e.g.*, total length), and gender (21 males and 19 females) were recorded in the field (Table 1). Each animal was allocated among four ordinal scale categories of condition (Table 1).

Following the application of a local anesthetic, osteoderms were surgically removed from the pelvic region just forward of the hind leg, and flesh samples were taken from the tail. Dermastid beetles, in combination with scalpel and forceps, were used to remove tissue adhering to osteoderms. Osteoderms were then thoroughly rinsed with 0.1 M ethylenediaminetetraacetic acid (EDTA) followed by deionized water (MilliQ; 18 M$\Omega$ cm$^{-1}$ resistivity). Flesh and osteoderm samples were oven-dried (flesh 40°C and osteoderm 60°C) to a constant weight; the mean ($\pm$ SD) fresh/dry weight ratio of flesh was 3.8 $\pm$ 0.5 (n = 35). Each sample was then microwave-digested (Milestone 1200) in nitric acid (Merck, Suprapur) following a modified U.S. Environmental Protection Agency protocol (US EPA 1997). The bone and flesh digests were made up to 100 ml using deionized water ($>$ 18 M$\Omega$ cm$^{-1}$).

Digest solutions 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 using inductively coupled plasma mass spectroscopy (Hewlett Packard 4500). The latter nine elements were below the analytical detection limit of the instrument, *viz.* $<$ 0.05 $\mu$g g$^{-1}$ dry weight (DW) for flesh and osteoderm samples. The concentrations of Na, K, Ca, Mg, and Fe were measured using inductively coupled plasma atomic emission spectroscopy (Varian Liberty II). A standard reference material (Community Bureau of Reference Mussel tissue 278), sample replicates (two or three), and reagent blanks were used to evaluate analytical accuracy, precision, and limits, respectively. The mean concentrations of all elements in the standard reference material were within their certified ranges. For replicate samples, the mean percentage coefficient of variation was $<$ 10% for all elements.

Crocodile age was estimated (Table 1) as the mean of several relationships with head length or snout length, previously established for *C. porosus* in northern Australia (Webb *et al.* 1978). The total lengths of sampled crocodiles ranged from 1.7 to 5.0 m, which represents a calculated age span of 5–40 years. Sexually mature females may remobilize elements from the osteoderms during vitellogenesis, possibly changing their concentrations, although such an effect was not observed for Pb in *C. porosus* (Twining *et al.* 1999). Significant differences ($p \leq 0.05$) between human males and females in elemental bone concentrations have been reported (Yoshinaga *et al.* 1989). To evaluate this potential effect, crocodiles were separated both by gender and also inferred reproductive status into two categories, (1) females likely to be sexually mature ($>$ 2.3 m total length and about 12 years old), and (2) females likely to be sexually immature ($<$ 2.3 m total length) plus both sexually immature and mature males.

### *Statistical Analysis*

*Characterization of Element Concentrations:* Frequency distributions of the concentrations of each element measured in both flesh and osteoderms were initially evaluated for departures from normality using a Shapiro-Wilk test (Sokal and Rohlf 1995). The tests showed that 11 out of the 28 distributions were significantly ($p \leq 0.05$) different from normality, all being negatively skewed. Accordingly, data from these distributions were $\log_{10}$-transformed to approximate normality. Mean values and their 95% confidence intervals were calculated and back-transformed to determine arithmetic element concentrations, taking into account any bias (Newman 1993). Arithmetic mean values and their 95% confidence intervals were also calculated for all other sets of element concentrations whose frequency distributions did not deviate significantly ($p > 0.05$) from normality.

A previous study (Twining *et al.* 1999) on Pb concentrations in the flesh and osteoderms of *C. porosus* showed significantly ($p \leq 0.05$) elevated concentrations in five crocodiles sampled from two habitats (Figure 1) exposed to anthropogenically enhanced Pb. As a result, flesh and osteoderm samples from these crocodiles were excluded in calculation of mean Pb concentrations and also for subsequent multiple linear regression analyses.

*Predictors of Element Concentrations:* Linear regression analyses were used to determine whether element concentrations in flesh and osteoderm samples were significantly ($p \leq 0.05$) related to the following biotic and geographic variables: (1) total length, (2) estimated age, (3) gender, (4) inferred reproductive status, (5) physical condition, and (6) the catchment in which they were captured. The inclusion of these potential predictors were relevant, based on the findings of Delany *et al.* (1988) and Heaton-Jones *et al.* (1997), who found that Cu and Hg concentrations in the tissues of alligators can vary with geographical region and with individual size and also that mature females may possibly remove contaminants from their tissues when eggs are laid (Hall 1980). Therefore, it was important to use a statistical procedure that would identify both these potential biotic and geographical effects on element concentrations in flesh and osteoderms, that could be simultaneously present in *C. porosus*.

The concentrations (arithmetic or $\log_{10}$-transformed) of each element were linearly regressed against the following combinations of predictors, (1) total length with gender, inferred reproductive status, physical condition, and catchment; and (2) estimated age with gender, inferred reproductive status, physical condition, and catchment. Total length and estimated age were both used as predictors of element concentrations because, although age was estimated from length, the relationship was not linear (Webb *et al.* 1978). Gender and inferred reproductive status were entered as dummy variables where "female" and "sexually mature female," respectively, were used as the reference group. Catchment was entered as a polytomous dummy variable (Hardy 1993) where the reference group was the combined Wildman/West Alligator River catchment.

Where catchment was found to be a significant ($p \leq 0.05$) predictor of element concentration, an adjusted Bonferroni (Dunn-Sidák) procedure was used to test for differences in the mean element concentrations between the three catchments. In cases where total length and/or estimated age were also significant ($p \leq 0.05$) copredictors, the mean element concentrations were adjusted using analysis of covariance (Sokal and Rohlf 1995).

Physical condition, being an ordinal scale variable, was only regarded as a significant predictor at the more stringent level of $p \leq 0.025$. A "backward elimination" stepwise procedure was used to generate a series of linear regression models, where the independent variable with a nonsignificant ($p > 0.05$) t value of lowest absolute value was eliminated from each consecutive regression until a regression whose F ratio was significant ($p \leq 0.05$) remained, and it contained only independent variables with significant ($p \leq 0.05$) t values (Sokal and Rohlf 1995).

## Results

### *Element Concentrations in Flesh and Osteoderms*

The arithmetic mean concentrations of elements that were above their limits of detection in flesh (n = 35) and osteoderm (n = 40) samples are given in Table 2. The 95% confidence limits and ranges of concentrations are also shown for each of

**Table 1.** Sample number, location and date, total length, gender, estimated age and condition index for each crocodile

| Sample number[a] | Location | Sample date | Total length (mm)[b] | Gender | Estimated age (years)[c] | Condition index[d] |
|---|---|---|---|---|---|---|
| 1 | Jim Jim Creek (Patonga Billabong) | 12/3/96 | 2456 | Male | 8.5 | 2 |
| 2 | Retention Pond 1 (Ranger uranium mine) | 27/3/96 | 2125 | Female | 8.0 | 2 |
| 3 | Jim Jim Falls | 5/5/96 | 4070 | Male | 21 | 3 |
| 4 | Twin Falls | 23/5/96 | 2890 | Male | 11 | 2 |
| 5* | Magela Creek (Jabiluka Billabong) | 27/8/95 | 4990 | Male | 40 | 3 |
| 6* | Barallil Creek | 9/3/95 | 4400 | Male | 27 | 4 |
| 7 | Nourlangie Creek (Muriella Park) | 24/6/96 | 3020 | Female | 20 | 2 |
| 8 | Nourlangie Creek (Bardulba Point) | 4/7/96 | 4710 | Male | 34 | 1 |
| 9 | East Alligator River (Rockholes) | 26/7/96 | 4145 | Male | 22 | 1 |
| 10 | Nourlangie Creek (Mungung Area) | 31/7/96 | 4025 | Male | 20 | 2 |
| 11* | Magela Creek (Island Billabong) | 10/11/93 | 3670 | Male | 17 | 2 |
| 12* | South Alligator River (Killuppa Springs) | 9/11/93 | 4000 | Male | 20 | 2 |
| 13* | South Alligator River (Goose Camp) | 20/11/93 | 3780 | Male | 18 | 1 |
| 14 | South Alligator River (Home Billabong) | 20/8/96 | 2925 | Male | 12 | 2.5 |
| 15 | South Alligator River (Home Billabong) | 28/8/96 | 3100 | Male | 13 | 2 |
| 16 | Wildman River (Four Mile Hole) | l18/9/96 | 3840 | Male | 19 | 2.5 |
| 17 | Wildman River (Four Mile Hole) | 21/9/96 | 3130 | Female | 23 | 2 |
| 18 | South Alligator River (Goose Camp) | 5/10/96 | 2950 | Female | 19 | 1 |
| 19 | South Alligator River (Goose Camp) | 8/10/96 | 2800 | Male | 10 | 2 |
| 20 | Magela Creek (Jabiluka Billabong) | 29/11/96 | 2730 | Female | 15 | 2 |
| 21 | South Alligator River (Red Lily Billabong) | 16/10/96 | 3870 | Male | 19 | 2 |
| 22 | Wildman River | 3/12/96 | 1680 | Female | 5.0 | 2 |
| 23 | Wildman River | 3/12/96 | 1800 | Female | 5.0 | 2 |
| 24 | Wildman River | 3/12/96 | 2700 | Female | 15 | 2 |
| 25 | West Alligator River | 7/12/96 | 2598 | Female | 14 | 2 |
| 26 | West Alligator River | 7/12/96 | 3300 | Male | 14 | 2 |
| 27 | West Alligator River | 8/12/96 | 2300 | Female | 10 | 1 |
| 28 | South Alligator River | 11/12/96 | 2820 | Female | 17 | 2 |
| 29 | South Alligator River (bridge) | 11/12/96 | 2820 | Female | 17 | 2 |
| 30 | East Alligator River | 1812/96 | 2770 | Female | 16 | 2 |
| 31 | East Alligator River | 18/12/96 | 2910 | Female | 19 | 1.5 |
| 32 | East Alligator River | 18/12/96 | 2590 | Female | 14 | 2 |
| 33 | South Alligator River (Munmalary area) | 13/1/97 | 2690 | Male | 11 | 2 |
| 34 | South Alligator River (Kapalga) | 14/1/97 | 2725 | Female | 15 | 2 |
| 35 | South Alligator River | 13/2/97 | 3135 | Male | 12 | 2.5 |
| 36 | Magela Creek confluence | 19/2/97 | 3110 | female | 24 | 1 |
| 37 | East Alligator River mouth | 24/97 | 2435 | Female | 11 | 2 |
| 38 | Nanamhu Creek | 4/5/97 | 2520 | Male | 8.0 | 2 |
| 39 | South Alligator River (Midnight point) | 14/5/97 | 2515 | Male | 8.0 | 2 |
| 40 | South Alligator River (Brook Creek) | 14/5/97 | 2350 | Female | 10 | 2 |

[a] Flesh was not sampled from the five crocodiles marked with an asterisk(*)
[b] Several other measures of animal size (*e.g.*, head and snout length) are reported by Twining *et al*. (1998)
[c] Age was estimated as the mean of several relationships with head length or snout length previously established for *C. porosus* (Webb *et al*. 1983)
[d] An arbitrary condition scale: 1, very good health; 2, good health; 3, poor health and 4, very poor health [see Twining *et al*. (1998) for more detail]

these elements. Among the alkaline-earth metals, the mean Ca concentration in the osteoderms is more than three orders of magnitude higher than that of the flesh, whereas the mean osteodermal Mg concentration is about a factor of four higher than that in flesh. The mean Ba concentration is more than a factor of 40 higher in the osteoderm than in flesh. Sr could not be detected in flesh, but had a mean concentration of 318 $\mu g\ g^{-1}$ (dry weight) in the osteoderm.

The concentration ranges of these alkaline-earth metals in both the flesh and osteoderm samples varied by factors of between two and eight with the exception of Ba in osteoderm, which varied over a factor of 75. Other elements that varied over ranges of about one to two orders of magnitude of concentration in osteoderm were Mn (71) and Fe (16). In flesh, Fe concentrations varied by a factor of 23 but the other non alkaline-earth metals varied by less than a factor of 10 in both flesh and osteoderm.

## *Predictors of Element Concentrations*

Table 3 shows the results of multiple linear regression analyses where element concentration in flesh ($\log_{10}$ or arithmetic) was regressed against sets of predictors for each of 15 elements. The following predictors or copredictors were significant ($p \leq 0.05$):

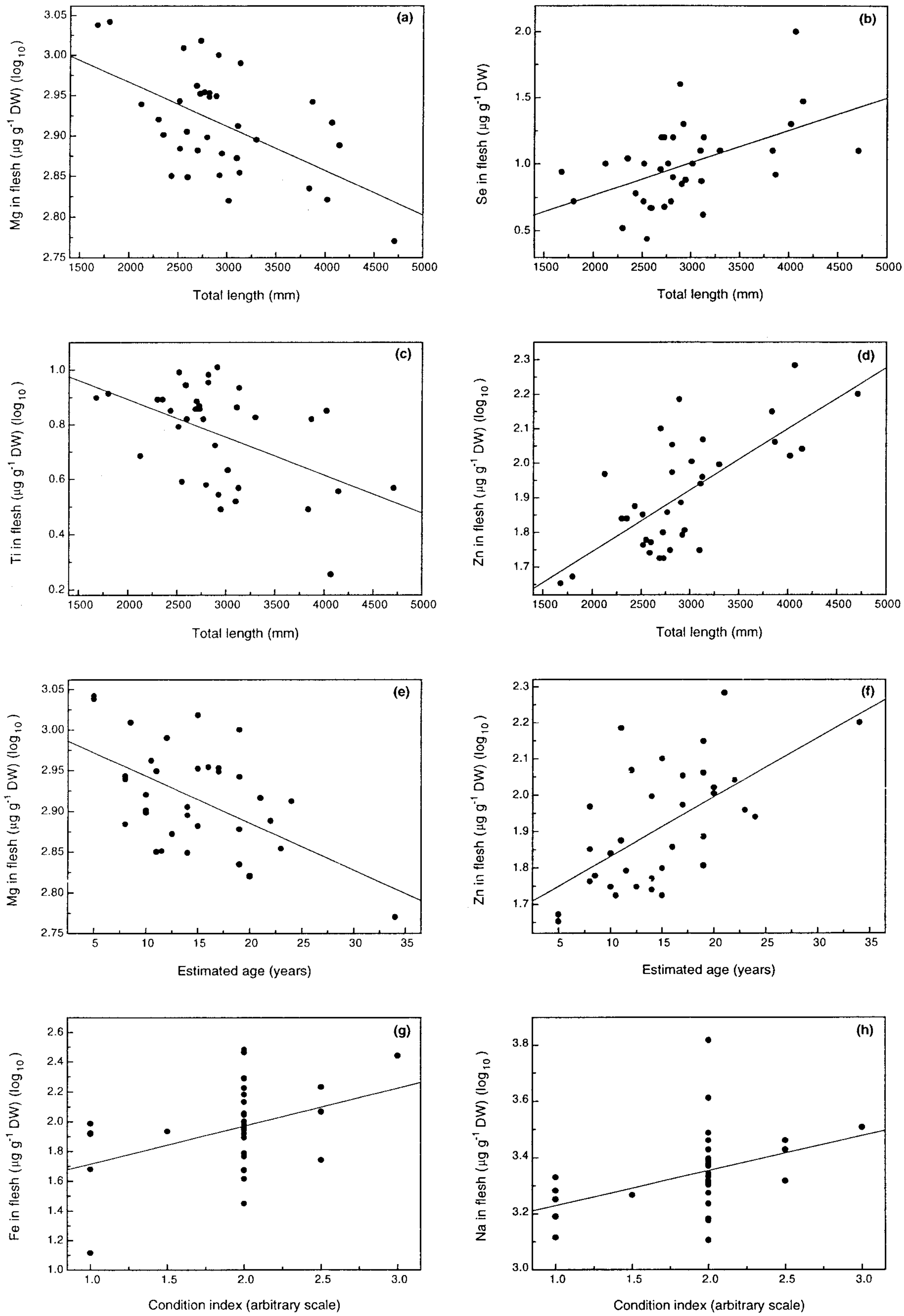


**Fig. 2.** Linear regressions of element concentrations ($\log_{10}$ and arithmetic) in flesh and osteoderms of *C. porosus* plotted against biotic measures

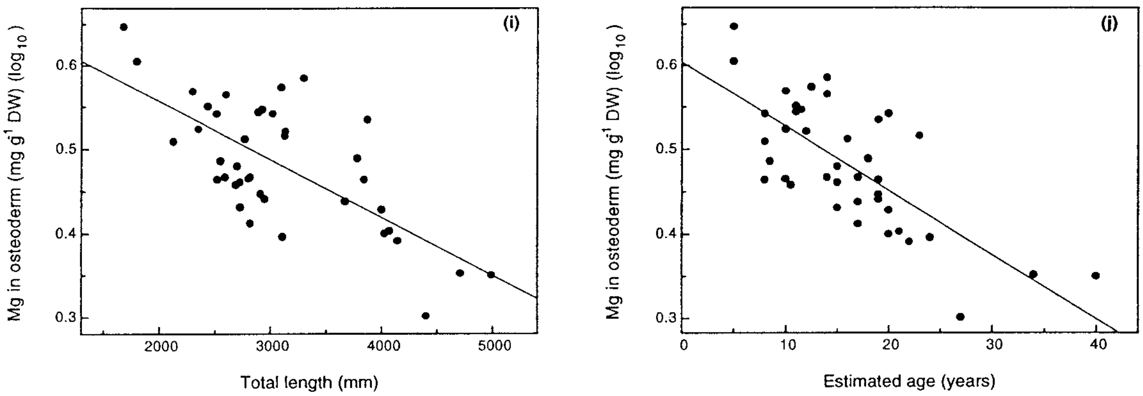


**Fig. 2.** Continued

**Table 2.** Element concentrations (μg·g$^{-1}$ dry weight) in the flesh and osteoderms of *C. porosus*

| | Flesh[a] | | | Osteoderm[b] | | |
|---|---|---|---|---|---|---|
| Element | Mean | 95% Cl | Range | Mean | 95% Cl | Range |
| Na | 2200[c] | 1960–2560 | 1280–6560 | <0.05[f] | — | — |
| K | 10.7 | 10.1–11.3 | 7.99–15.7 | <0.05[f] | — | — |
| Ca | 202[c] | 181–237 | 106–660 | 219000 | 215,000–227,000 | 191,000–242,000 |
| Mg | 823[c] | 782–868 | 588–1100 | 3080 | 2910–3250 | 2000–4440 |
| Ba | 0.554[c] | 0.501–0.611 | 0.270–0.920 | 24.3[c] | 18.3–32.9 | 1.90–143 |
| Sr | <0.05[f] | — | — | 318[c] | 279–372 | 121–923 |
| Fe | 88.7[c] | 71.5–111 | 13–303 | 6.52[c] | 5.24–8.22 | 1.33–21.2 |
| Al | 89.9 | 79.9–99.9 | 26–132 | <0.05[f] | — | — |
| Mn | 0.706[c] | 0.635–0.795 | 0.350–1.30 | 2.63[c] | 2.54–2.77 | 0.190–13.4 |
| Zn | 81.4[c] | 73.1–95.7 | 45.0–192 | 5.23[c] | 4.63–5.90 | 1.73–9.57 |
| Pb | 0.308[c,d] | 0.280–0.342 | 0.120–0.450 | 3.00[c,e] | 2.50–3.68 | 1.2–9.4 |
| Cu | 1.14 | 1.00–1.28 | 0.340–2.02 | 4.68[c] | 3.98–5.64 | 2.20–13.1 |
| Ni | 0.507[c] | 0.443–0.571 | 0.140–0850 | 4.20 | 3.72–4.68 | 1.16–7.22 |
| Cr | 0.413[c] | 0.352–0.494 | 0.120–1.02 | 0.219 | 0.193–0.245 | 0.07–0.39 |
| Co | <0.01[f] | — | — | 0.344 | 0.319–-365 | 0.220–0.478 |
| Se | 0.993 | 0.886–1.10 | 0.440–2.00 | <0.01[f] | — | — |
| U | <0.01[f] | — | — | 0.035[c] | 0.032–0.039 | 0.018–0.075 |
| Ti | 6.22[c] | 5.45–7.03 | 1.80–10.2 | <0.01[f] | — | — |

[a] $n = 35$
[b] $n = 40$
[c] Geometric mean values
[d] Mean value for animals not exposed to Pb pollution ($n = 31$); the mean value for lead-exposed animals was 0.54 (0.44–0.64, 95% Cl) μg·g$^{-1}$ dry weight[3] ($n = 4$)
[e] Mean value for animals not exposed to Pb pollution ($n = 35$); the mean value for lead-exposed animals was 24.0 (14–39, 95% Cl) μg·g$^{-1}$ dry weight[3] ($n = 5$)
[f] Below the analytical detection limits of the instruments.

1. *Length and age.* Mg and Ti concentrations decreased, whereas Ba, Zn, and Se increased with increasing length and/or age. The simple regression relationships are shown in Figure 2.
2. *Condition.* Fe and Na increased ($p \leq 0.025$) in concentration ($\log_{10}$) as the condition of the crocodile deteriorated, *i.e.*, as its condition score increased (Figure 2).
3. *Catchment.* Ba concentration was significantly related to catchment, as a copredictor with length.

Neither gender nor inferred reproductive status was a significant ($p > 0.05$) predictor of element concentration in flesh.

Table 3 shows the results of the regression analyses where element concentration ($\log_{10}$) in osteoderm was regressed against sets of predictors, for each of 13 elements. The following predictors or copredictors were significant ($p \leq 0.05$):

1. *Length and age.* Mg concentration declined as length and age increased (Figures 2 i and j), whereas Co increased with length.
2. *Catchment.* Co, Fe, Mg, Mn, and U concentrations were related to catchment.

Condition, gender, and inferred reproductive status were not

**Table 3.** Results of simple or stepwise multiple linear regression analyses where biotic and geographic variables are used to predict element concentrations ($\mu g \cdot g^{-1}$ dry weight) in the flesh and osteoderm of *C. porosus*[a]

| Element | Predictor(s) | $r^2$ |
|---|---|---|
| *Flesh*[b] | | |
| Al | Catchment | 0.29*** |
| Ba | Catchment/total length (+) | 0.23/0.33** |
| Cr | Catchment | 0.34*** |
| Fe | Condition index (+) | 0.17* |
| Mg | Estimated age (−) | 0.29*** |
| | Total length (−) | 0.30*** |
| Na | Condition index (+) | 0.17* |
| Ni | Catchment | 0.19** |
| Pb[c] | Catchment | 0.25** |
| Se | Total length (+) | 0.26** |
| Ti | Total length (−) | 0.25** |
| Zn | Estimated age (+) | 0.37*** |
| | Total length (+) | 0.51*** |
| *Osteoderm*[d] | | |
| Co | Catchment/total length (+) | 0.22/0.33** |
| Fe | Catchment | 0.21** |
| Mg | Estimated age (−)/catchment | 0.55/0.66*** |
| | Total length (−) | 0.49*** |
| Mn | Catchment | 0.55/0.32*** |
| U | Catchment | 0.42*** |

[a] For stepwise multiple linear regression analyses the $r^2$ values are cumulative. $***P \leq 0.001$; $**0.001 < P \leq 0.01$; $*0.01 < P \leq 0.05$ (or 0.025 for condition index). (+) indicates a positive linear regression and (−) a negative linear regression
[b] $n = 35$
[c] *a priori* Pb-contaminated samples ($n = 4$ for flesh and $n = 5$ for osteoderms) were excluded from the analyses
[d] $n = 40$

significant ($p > 0.025$; $p > 0.05$) predictors of the concentration of any element in the osteoderm.

With regard to the significant ($p \leq 0.05$) effects of catchment on metal concentrations, Table 4 shows the results of an adjusted Bonferroni (Dunn-Sidák) procedure or covariance analysis to determine the significant differences in their mean concentrations between each pair of catchments.

For flesh, mean concentrations of Al, Ba, Ni, and Pb were significantly ($p \leq 0.05$) higher in crocodiles from the Wildman/West Alligator River catchment relative to one or both of the other two catchments. The mean concentration of Cr however was highest in the South Alligator River catchment. The Wildman/West Alligator River samples were all taken from crocodiles captured in closer proximity to the river mouths, and hence in greater salinity relative to those from the other two catchments (Figure 1). To evaluate this potential effect crocodiles from the other two catchments were partitioned into those occurring within the range of salinities characteristic of the Wildman/West Alligator River catchment, based on measured salinity values (M. Lawton, unpublished). The mean elemental concentrations in the flesh were subsequently compared using one-way analysis of variance (see Materials and Methods). These analyses confirmed the results given in Table 4, and, hence, the postulated salinity effect could not explain the elevated levels of these elements in the flesh of crocodiles captured from the Wildman/West Alligator River catchment.

In osteoderms there was no obvious prominence of one catchment having repeatedly higher concentrations of elements relative to the others. Whereas Mn concentration was higher ($p \leq 0.05$) in samples from the Wildman/West Alligator River catchment relative to the South Alligator River catchment, Fe was significantly ($p \leq 0.05$) elevated in the East Alligator River catchment samples relative to those from the Wildman/West Alligator River catchment. U is the only element that shows significantly ($p \leq 0.05$) different means between each pair of catchments, being highest in the East Alligator River catchment, that contains the Ranger and Jabiluka uranium mine sites (Figure 1), and lowest in the Wildman/West Alligator River catchment.

## Discussion

With regard to element concentrations measured in the flesh of other crocodilian species, Table 5 compares mean concentrations of nine elements measured in the American alligator (*A. mississippiensis*) with those determined in *C. porosus* in this study. Fe, Zn, Cu, Pb, Se, and Mn concentrations are very comparable, only varying by about a factor of two or less between species, but Cd and Hg are respectively at least one to two orders of magnitude less in *C. porosus* compared to *A. mississippiensis*. Concentrations of various elements in flesh and bone of *C. porosus* are also comparable to those measured in sea turtles (Witkowski and Frazier 1982; Caurant *et al.* 1999) and bony fish (Currey *et al.* 1992; Emara *et al.* 1993) from uncontaminated sites.

Significant ($p \leq 0.05$) linear relationships were established for several elements in both flesh and osteoderm with increasing size and age (Table 3, Figure 2), which are indicative of biologically mediated influences. By way of contrast, it is unlikely that these relationships reflect consistently increasing or decreasing environmental concentrations of these elements. With respect to these notional biologically mediated patterns (Figure 2), the following similar patterns have been reported for other long-lived vertebrates in both soft and calcified tissues, *e.g.*,

1. Hg concentration in tissues increase with increasing size in some populations of American alligator (*A. mississippiensis*), but not others (Yanochko *et al.* 1997; Heaton-Jones *et al.* 1997; Jagoe *et al.* 1998; Burger *et al.* 2000);
2. Zn and Na concentrations in annual laminations of dugong tusk increase with the age at which the lamination was constructed (Edmonds *et al.* 1997); and
3. Al, Fe, Pb, and Zn linearly increase in human bone with increasing age, whereas Mg declines with age (Yoshinaga *et al.* 1989).

In this study we have analyzed the whole osteoderm for its Mg concentration, to show its significant ($p \leq 0.05$) decline in concentration with increasing size and age of crocodile. Previously we had used secondary ion mass spectrometry to measure Pb in individual ostoedermal laminations to indicate historical patterns of exposure to anthropogenic Pb, relative to a control (Twining *et al.* 1999). If the osteodermal laminations do operate as a sink for deposited Mg, then the rate of decline in Mg

**Table 4.** Mean (± 95% CI) element concentrations ($\mu g \cdot g^{-1}$ dry weight) in the flesh and osteoderm of *C. porosus* between catchments[a]

| Element | Wildman/West Alligator River catchment | South Alligator River catchment | East Alligator River catchment |
|---|---|---|---|
| *Flesh*[b] | | | |
| Al | $115^{b}$ (102–128) | $87^{a}$ (74–100) | $70^{a}$ (47–93) |
| Ba[c] | $0.75^{b}$ (0.63–0.91) | $0.51^{a}$ (0.44–0.59) | $0.56^{ab}$ (0.47–0.66) |
| Cr | $0.35^{ab}$ (0.21–0.58) | $0.54^{b}$ (0.49–0.66) | $0.26^{a}$ (0.20–0.34) |
| Ni | $0.63^{b}$ (0.55–0.71) | $0.46^{a}$ (0.38–0.54) | $0.37^{ab}$ (0.23–0.60) |
| Pb[d] | $0.38^{b}$ (0.33–0.44) | $0.29^{a}$ (0.26–0.32) | $0.27^{ab}$ (0.20–0.34) |
| *Osteoderm*[e] | | | |
| Co[c] | $0.39^{ab}$ (0.32–0.48) | $0.30^{a}$ (0.28–0.33) | $0.37^{b}$ (0.34–0.40) |
| Fe | $3.9^{a}$ (2.0–5.5) | $6.8^{ab}$ (4.6–10) | $9.6^{b}$ (7.6–14) |
| Mg[c] | $3500^{b}$ (3200–3900) | $3000^{ab}$ (2800–3200) | $2900^{a}$ (2500–3100) |
| Mn | $7.5^{b}$ (5.2–11) | $1.6^{a}$ (1.0–2.5) | $3.0^{ab}$ (1.5–6.0) |
| U | $0.026^{a}$ (0.023–0.029) | $0.034^{b}$ (0.030–0.038) | $0.049^{c}$ (0.040–0.058) |

[a] Based on linear regression analysis where catchment was found to be a significant ($P \leq 0.05$) predictor of element concentration. Differences in mean values were tested using an adjusted Bonferroni (Dunn-Sidák) procedure. Mean values depicted with an *a* are significantly ($P \leq 0.05$) different to those depicted by *b* or *c*. Similarly, mean values depicted with *b* are significantly ($P \leq 0.05$) different to those depicted by *c*. Mean values depicted with the same letter (e.g., *ab*) are not significantly ($P \leq 0.05$) different from one another. $Log_{10}$ transformed element concentrations were back-transformed to determine the arithmetic element concentrations, taking into account any bias (Newman 1993)
[b] $n = 35$ (19 from the South Alligator River, 8 from the East Alligator River and 8 from the Wildman/West Alligator Rivers)
[c] Mean values adjusted for total length and/or estimated age (see Table 3) using analysis of covariance
[d] *a priori* Pb-contaminated samples ($n = 4$) were excluded from the analyses
[e] $n = 40$ (20 from the South Alligator River, 12 from the East Alligator River and 8 from the Wildman/West Alligator Rivers)

**Table 5.** Element concentrations ($\mu g \cdot g^{-1}$ dry weight) in the flesh of *C. porosus* and the alligator, *A. mississippiensis*

| | Crocodile (*C. porosus*)[a] | | Alligator (*A. mississippiensis*)[b,c] | |
|---|---|---|---|---|
| Element | Mean | Range | Mean | Range |
| Fe | 89 | 13–300 | 45 | 17–86 |
| Zn | 81 | 45–190 | 90 | 54–137 |
| Cu | 1.1 | 0.34–2.0 | 2.3 | 1.1–23 |
| Pb | 0.31 | 0.12–0.45 | 0.31 | 0.15–0.46 |
| Cr | 0.41 | 0.12–1.0 | 0.21 | 0.11–0.42 |
| | | | 0.18[d] | — |
| Se | 0.99 | 0.44–2.00 | 0.40[d] | — |
| Mn | 0.71 | 0.35–1.30 | 0.95[d] | — |
| Cd | <0.01[e] | — | 0.11 | 0.038–0.23 |
| | | | 0.038[d] | — |
| Hg | <0.01[e] | — | 1.16 | 0.15–2.3 |
| | | | 0.16[d] | — |
| | | | | 0.18–1.47[c,f] |
| | | | | 0.80–5.57[g] |

[a] Abstracted from Table 2
[b] Data from Delaney *et al.* (1998), $n = 24$
[c] Original data (reported as $\mu g \cdot g^{-1}$ wet weight) were multiplied by a factor of 3.8 (the wet: dry weight ratio calculated in the present study) for comparison
[d] Burger *et al.* (2000)
[e] Below the analytical detection limits of the instruments
[f] Elsey *et al.* (1999) and references therein
[g] Jagoe *et al.* (1998). Includes contaminated sites

concentration observed in the total osteoderm of *C. porosus*, with increasing size and age, may be even more pronounced by its measurement in their individual annual laminations.

This study did not detect any effect of either gender or inferred reproductive status on the concentration of any investigated element in either osteoderm or flesh. Previous studies on Hg levels in flesh of *A. mississippiensis* found no differences between the sexes (Yanachko *et al.* 1997; Elsey *et al.* 1999); however, Burger *et al.* (2000) found females had more Hg in fat and less Sn in tail tissue than males.

Differences in mean element concentrations in *C. porosus* between catchments of the ARR (Table 4), independent of the influence of biotic factors (Table 2), point to the crocodiles' responses to chemical environmental variables. Similarly, previous studies of Ra-226 bioaccumulation in the tissue of freshwater bivalves (*V. angasi*) from the ARR had shown significant ($p \leq 0.05$) differences between populations in Magela Creek, that occurs within the East Alligator River catchment (Figure 1) (Jeffree 1988). These results were interpreted as reflecting different ratios of Ra-226: Ca concentrations in water, as supported experimentally for Ra-226 (Jeffree and Simpson 1986) and other Ca analogues (Markich and Jeffree 1994). With regard to the capacity of another aquatic reptile to reflect the chemistry of its aquatic environment, previous experimental studies showed that the snapping turtle *Elseya dentata* from Magela Creek accumulated Ra-226 and radio-calcium directly from the aquatic medium via its cloaca and buccopharynx, where Ca and Mg water concentrations also affected its rate of accumulation (Jeffree 1991; Jeffree and Jones 1992).

With respect to *C. porosus* used in this study, it is difficult at this stage to further interpret its catchment-specific elemental concentrations in relation to those of its environmental milieu because of insufficient water and sediment chemistry data for each catchment; however, there are reported differences between the catchments in their underlying geology (Needham 1988) that could be expected to influence their water and sediment chemistries.

Location-specific differences in tissue concentrations have been previously reported in crocodilians. Out of eight metals detected by Delany *et al.* (1988) in tail muscle of similar sized

*A. mississippiensis* sampled from eight lakes in Florida, Hg and Cu showed significant differences between sites in their concentrations. Heaton-Jones *et al.* (1997) investigated Hg levels in various tissues of *A. mississippiensis* in Florida and found very high levels in wild individuals from the Everglades, compared to other geographical regions and control specimens fed a low-Hg diet under captive conditions. Burger *et al.* (2000) found levels of Pb, Hg, and Cd in tissues of *A. mississippiensis* that varied significantly between sampled lakes in central Florida. The significant ($p \leq 0.05$) effects on mean element concentrations in flesh and osteoderm (Table 4) of the catchment of capture of *C. porosus*, point to the following.

*C. porosus* is responsive to variable elemental signals in its environmental milieu, and most crocodiles investigated must exhibit enough catchment fidelity to reflect these differential element signatures within catchments, even though these catchments are in close proximity and individuals were captured over river lengths of up to 100 km. Such an interpretation accords with the high site residency that has been demonstrated in larger *C. porusus* and also *C. johnstoni* (Webb *et al.* 1983; Walsh and Whitehead 1993; Tucker *et al.* 1997). For smaller individuals, a detailed study showed that 57% of juvenile *C. porusus* (2–4-year-olds) were found within 10 km of the site where they were originally caught as hatchlings; however, some juveniles travelled up to 81 km in 1 year (Webb and Messel 1978), but could also travel in the opposite direction in the following year.

Because such catchment-specific signals based on individual element concentrations are readily identifiable (despite their waters of capture ranging from fresh to estuarine), they may be useful in determination of provenance for itinerant crocodiles and the degree to which crocodiles do move between catchments. Multivariate elemental signatures may indicate even greater catchment specificity for individual crocodiles. These results also suggest the utility of *C. porosus* as a long-term biomonitor of its aquatic environment.

In conclusion, our analyses have established baseline concentrations of a range of elements in *C. porosus* from throughout the KNP in tissues that can be readily sampled from extant individuals over a wide size range. Against these established concentrations enhanced levels could be detected in the future. The identification of significant ($p \leq 0.05$) relationships between elemental concentrations and biotic and geographic parameters will permit a more accurate interpretation of any changes in elemental concentrations that may be detected in the future. However, our ability to interpret the underlying factors that determine these relationships is currently limited.

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