ELSEVIER

Environmental Pollution 115 (2001) 1–7

ENVIRONMENTAL POLLUTION

www.elsevier.com/locate/envpol

Nondestructive indices of trace element exposure in squamate reptiles

W.A. Hopkins [a,b,*], J.H. Roe [a], J.W. Snodgrass [c], B.P. Jackson [a], D.E Kling [a], C.L. Rowe [d], J.D. Congdon [a]

[a] *Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA*
[b] *Department of Biological Sciences, University of South Carolina, Columbia, South Carolina, USA*
[c] *Department of Biology, Towson University, Towson, Maryland, USA*
[d] *University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, PO Box 38, Solomons, MD 20688, USA*

Received 31 July 2000; accepted 30 December 2000

"Capsule": *Nondestructive sampling techniques, such as blood samples and tail clips, can be used to evaluate the accumulation of pollutants on reptiles.*

Abstract

Compared with birds, mammals, fish, and even amphibians, very little is known about the effects of contaminants on reptiles. Recent evidence that many reptile populations may be declining has stimulated demand for toxicological studies of reptiles as well as development of nondestructive sampling techniques useful for assessing and monitoring contaminant exposure. The current study experimentally evaluated the utility of shed skins, tail clips, and blood samples as nondestructive indices of trace element exposure in banded water snakes, *Nerodia fasciata*. For 13.5 months, snakes were either fed fish from a coal ash-contaminated site or uncontaminated food from a reference site. Snakes fed contaminated prey accumulated As, Cd, Se, Sr, and V in various organs (i.e. liver, kidney, and/or gonads). Moreover, non-parametric discriminant function analysis revealed that snakes could be placed in two groups that reliably reflected their experimental diet based upon Se, Sr, and As concentrations in tail clips, blood, and/or shed skins. We suggest that nondestructive sampling techniques, particularly analyses of blood and tail clips, may be easily applied in evaluations of contaminant exposure in the field and laboratory and may prevent excessive destructive sampling of potentially threatened reptile species.

Keywords: Reptiles; Snakes; Coal combustion; Selenium; Nondestructive indices

1. Introduction

Increased concern over the status of reptile populations (Gibbons et al., 2000) has stimulated demand for development of nondestructive sampling techniques used in assessments of contaminant exposure. Although such techniques have often been used for other vertebrates (e.g. bird feathers), similar methodologies have less frequently been employed for reptiles. In attempts to utilize nondestructive techniques, several investigators have successfully used blood and chorioallantoic membranes to examine contaminant exposure in turtles and crocodilians (Cobb et al., 1997; Cobb and Wood, 1997; De Solla et al., 1998). Few studies, however, have explored the utility of nondestructive sampling in squamates (lizards and snakes), but it has been suggested that blood, scales, and shed skins may be useful indicators in these species (Kaur, 1988; Burger, 1992; Bishop and Martinovic, 2000; Portelli and Bishop, 2000).

Recent studies indicate that reptiles, as well as other wildlife, are sublethally affected when exposed to coal combustion byproducts discharged into aquatic habitats (Lemly, 1993; Rowe et al., 1996, 1998a, b; Hopkins et al., 1997, 1998, 1999a, b, 2000a, b; Raimondo et al., 1997; Rowe, 1998). The wastes produced from coal combustion contain high concentrations of potentially toxic trace elements including As, Cd, Cu, Cr, and Se (Cherry and Guthrie, 1977; Carlson and Adriano, 1992; Rowe et al., 1996; Hopkins et al., 1998, 2000a). High trophic level predators may be particularly susceptible

* Corresponding author. Tel.: +1-803-952-7427; fax: +1-803-725-3309.
E-mail address: hopkins@srel.edu (W.A. Hopkins).

0269-7491/01/$ - see front matter
PII: S0269-7491(01)00098-7

to combustion-derived elements such as Se that are readily accumulated via trophic uptake. For example, banded water snakes (*Nerodia fasciata*) exposed to trace elements liberated from coal combustion accumulate high hepatic concentrations of Se, Cd, and As, most likely from ingesting contaminated fish and amphibians in their polluted habitat (Hopkins et al., 1999). In addition, snakes having high hepatic trace element burdens exhibit increased standard metabolic rates, an indication that they may expend considerable energy combating or resisting the deleterious effects of combustion-derived contaminants (Hopkins et al., 1999).

The current study sought to determine (1) if prey items are an important source of trace element contamination for aquatic snakes and (2) whether non-destructive sampling techniques could be developed for squamates exposed to trace element contamination in the environment. To expand upon our recent fieldwork, we experimentally exposed *N. fasciata* to trace element-laden prey items from a coal ash-contaminated site to quantify accumulation of trace elements in snake organs (liver, kidney, and gonads). We sought to determine if levels detected by non-destructive techniques (i.e. blood samples, shed skins, and tail clips) were indicative of prior exposure and could serve as useful indicators of exposure in biological monitoring and assessment programs.

2. Materials and methods

2.1. Snake and prey collection

Adult and juvenile banded water snakes ($n=15$) were captured by hand and in minnow traps during spring 1998. Snakes were captured at a historically uncontaminated reference site (a series of temporary wetlands) that harbors a large number of water snakes with low trace element burdens (for site description see Hopkins et al., 1999). Snakes were housed in individual 38-l aquaria in a temperature controlled greenhouse (25±2°C) at the Aquatic Ecology Laboratory (satellite facility of the Savannah River Ecology Laboratory) near Aiken, South Carolina. Because snakes were housed in a greenhouse, they were exposed to natural photoperiod during the study. Each aquarium was equipped with a hidebox, waterbowl, and heatsource (Flexwatt heat tape, Flexwatt, Inc.).

Snakes were acclimated to greenhouse conditions for 1–3 months before initiation of experimental feeding. During the acclimation period, all snakes were fed uncontaminated prey items collected from a second reference site (an abandoned farm pond; Fire pond) that was previously found to have low sediment concentrations of trace elements (Hopkins et al., 1998). All prey items used in the study were captured in minnow traps and immediately stored frozen for future snake feedings. At the end of the acclimation period, snakes were randomly assigned to one of two feeding treatments: one treatment (control, $n=7$) continued to receive uncontaminated prey items collected from Fire Pond while the other treatment (ash, $n=8$; see description below) received prey items collected from a coal ash contaminated site. Each treatment group contained males and females across an approximately equivalent range of masses (mean initial mass (g)±S.E.: control, 62.2±16.5; ash, 61.9±16.3). Each snake was fed weekly for 13.5 months (52 total meals). Weekly food ration was 20±2% of the individual's initial body mass.

Contaminated prey items were collected from a drainage swamp downstream from two coal ash settling basins located on the Savannah River Site, South Carolina. Within the settling basins and drainage swamp, fish and amphibians contain high concentrations of trace elements such as Se and As, and possibly serve as trophic vectors of contaminants for snakes and other aquatic predators (Hopkins et al., 1999). Prey items collected from the contaminated site primarily included largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), spotted sunfish (*Lepomis punctatus*), and redbreast sunfish (*Lepomis auritus*). Largemouth bass and bluegill sunfish were also used for the control treatment, but redbreast and spotted sunfish were not available at the reference site. Instead, dollar sunfish (*Lepomis marginatus*), a centrarchid having similar trophic status as redbreast and spotted sunfish, were substituted as a third prey species for snakes in the control treatment.

2.2. Snake tissue collection

During the study, shed skins were collected from water snakes in both experimental treatments. Skins were gently rinsed to remove fecal material (if present), lyophilized, and stored for future trace element analysis.

Following 13.5 months of exposure, snakes were fasted for 5–10 days prior to termination of the study. Blood (0.5–1.0 ml) was collected from the caudal vein of each post-absorptive snake using a 26-g heparinized syringe. After blood sampling, a 2–3 cm portion of the tail was removed using a sterile razor. Snakes were then sacrificed using an overdose of Ketamine. In preparation for future trace element analysis, subsamples of liver, kidney, and gonads were removed from snakes and frozen (−20°C) along with blood, tail clips, and the final shed skin from each snake. Because frequency of skin shedding is highly variable among snakes, the final shed skin was collected opportunistically from each snake 1–9 weeks prior to termination of the study. In addition, samples of the three primary prey species ($n=4$ per species) from each site were frozen for later analysis.

2.3. Trace element analysis

Snake tissues and prey items were lyophilized and homogenized before being digested and analyzed for trace element concentrations. Approximately 100 mg of sample was used for digestion; sample masses varied because total available tissue was, on occasion, less than 100 mg. Samples were allowed to react overnight with 2.5 mls HNO_3 and 1 ml H_2O_2. Four millilitres of deionized water were added prior to microwave digestion (CEM Corp., Mathews, NC) with heating steps of 60, 75, and 95% microwave power for 10, 10, and 15 min, respectively. After digestion, samples were brought to a final volume of 25 mls with deionized water. Trace element analysis was performed by ICP-MS (Perkin Elmer, Norwalk, CT) on samples diluted 1:1 with deionized water (1:10 for tail clip samples). Calibration standards covering a range of 1–500 μg/l were prepared daily by serial dilution of NIST traceable primary standards. Certified reference materials (Dorm 2 and Tort 2, NRC, Ontario, Canada), replicates, replicate spikes and blanks were included in the digestion and analysis procedure for quality control purposes. Percent recoveries for various elements in certified reference materials and digestion spikes ranged from 92 to 103% and 93 to 102%, respectively. Percent difference in element concentrations between digestion replicates ranged from 4 to 8%.

2.4. Statistical analyses

Trace element concentrations in prey items were log transformed prior to statistical analysis to meet assumptions of normality and homoscedasticity. Transformed concentrations were then compared using a full factorial two-way analysis of variance (ANOVA), with site and species as main effects in the model. ANOVA models were used to estimate least squared (LS) means for each site to control for differences in species. LS means were then backtransformed to generate geometric LS means.

Because several trace elements were below detection limits in snake tissues, data could not be transformed to meet assumptions of parametric statistical tests. Therefore, nonparametric tests were used to compare trace element concentrations among snake tissues and feeding treatments. For statistical analyses, we replaced values that were below detection limits with half of the element detection limit for that tissue. We compared median organ trace element concentrations for overall treatment effects as well as individual organ concentrations between treatments using a series of Wilcoxon rank-sum tests. Because these comparisons are not independent of one another, a sequential Bonferroni correction was utilized to adjust critical values downward (minimum $P = 0.05/24 = 0.002$) to maintain the experiment-wide error rate at $P \leqslant 0.05$ (Rice, 1989).

To determine whether our nondestructive tissue samples (i.e. tail clips, blood, and shed skins) allowed accurate detection of prior dietary exposure, we used nonparametric discriminant function analysis. We used the nearest neighbor option of the DISCRIM procedure of SAS with three nearest neighbors to estimate densities (i.e. estimated distributions) for each dietary treatment (SAS/STAT, 1990). To evaluate the performance of the classification criteria we used cross-validation procedures. Posterior probability error-rate estimates from cross validation were used as an indication of the accuracy of our classification criteria.

3. Results

Least squared geometric means and ranges of trace element concentrations in prey from each site are presented in Table 1. There was a significant main effect of

Table 1
Geometric least squared means and ranges of trace element concentrations (ug/g dry mass) in whole bodies of fish from an ash contaminated site (ash) and a reference site (control)[a]

Trace element	Geometric LS mean (range)		Main effect of site (P)
	Ash	Control	
As	1.281 (0.347–2.637)	0.155 (0.115–0.332)	< 0.001
Cd	0.227 (0.103–0.697)	0.051 (0.019–0.129)	< 0.001
Cr	0.590 (0.438–0.931)	0.592 (0.389–1.088)	0.960
Cu	2.398 (1.240–6.169)	1.738 (1.208–2.454)	0.025
Ni	0.447 (0.242–0.902)	0.411 (0.233–0.793)	0.526
Se	22.700 (15.960–40.801)	0.994 (0.622–1.321)	< 0.001
Sr	274.992 (220.727–362.650)	128.085 (104.038–184.044)	< 0.001
Zn	135.137 (111.993–171.046)	139.692 (96.852–187.906)	0.582
V	1.786 (0.347–7.281)	0.163 (0.045–0.463)	< 0.001

[a] Values represent means and ranges of concentrations found in three fish species from each site ($n = 4$/species/site) that were used as prey items in snake dietary study. Detection limits (ug/g dry mass) for each element are as follows: As 0.003; Cd 0.002; Cr 0.014; Cu 0.005; Ni 0.004; Se 0.040; Sr 0.001; Zn 0.014; V 0.003.

prey species on whole body prey concentrations of As, Cr, Ni, V, and Se ($P \leqslant 0.030$ in all cases). Site of prey capture was a significant main effect for As, Cu, V, Cd, Se, and Sr ($P \leqslant 0.025$ in all cases); for each of these elements, fish from the ash-contaminated site contained significantly higher body burdens of trace elements (Table 1).

Only trace elements significantly elevated in prey items from the polluted site are reported for snake tissues. Thus, we do not report Ni, Cr, or Zn in snake tissues since snakes were not exposed to elevated concentrations of these elements in their experimental diet. Because we used non-parametric comparisons, descriptive statistics for snake tissue concentrations are presented as medians and ranges (Tables 2 and 3).

There was an overall effect of feeding treatment on accumulation of As, Cd, Se, Sr, and V; in all cases, snakes fed the contaminated diet accumulated significantly higher element concentrations compared to controls (Table 2). Within specific organs, Se concentrations were significantly elevated in liver, kidney, and gonads (Table 2). In addition, As concentrations were significantly elevated in liver and kidney and Cd concentrations were significantly elevated in kidney (Table 2). Although small sample sizes prohibited statistical comparison between sexes within each treatment, visual inspection of the data revealed no conspicuous sex-specific differences in trace element concentrations in any target organs. Despite significant accumulation of trace elements by snakes fed contaminated food, all snakes appeared healthy and showed no visible symptoms of toxicity. Snakes in both treatments continued to eat and grow throughout the study (final mean mass (g)±S.E.: control, 136.3±29.7; ash, 169.6±40.7).

Medians and ranges of nondestructive tissue samples and posterior probability error-rates from discriminant function analysis are presented in Table 3. Of the six elements that were statistically compared, nondestructive sampling of tissues appeared to be useful for detecting dietary exposure to Se, As, and Sr (Table 3). For Se, there was a very low probability of misclassification ($<0.10\%$) for all tissues. However, for As and Sr there was variation in posterior probability error-rates among tissues (Table 3). For As, tail clips were clearly most informative. For Sr, posterior probability error-rates were high for shed skins but lower for both blood and tail clips.

Table 2
Trace element concentrations (ug/g dry mass) in target organs of banded water snakes, *Nerodia fasciata*, fed prey items from contaminated (ash) and uncontaminated (control) sites[a]

Organ	Element	Median (range)		*P*
		Ash	Control	
Gonads	As	0.15 (BDL–0.33)	BDL (BDL–0.08)	0.008
	Cd	BDL (BDL)	BDL (BDL–0.04)	0.350
	Cu	7.55 (BDL–8.58)	6.28 (4.31–7.63)	0.772
	Se	15.34 (11.09–18.33)	3.40 (2.47–3.97)	0.001*
	Sr	1.98 (1.13–3.27)	0.88 (0.35–1.95)	0.009
	V	BDL (BDL–1.87)	BDL (BDL–0.99)	0.414
Kidney	As	0.35 (0.25–0.74)	0.03 (BDL–0.07)	0.001*
	Cd	0.44 (0.14–0.57)	0.06 (0.02–0.15)	0.002*
	Cu	7.78 (5.72–11.85)	7.45 (5.03–26.07)	0.772
	Se	23.20 (14.92–26.84)	3.41 (2.14–4.24)	0.001*
	Sr	2.10 (1.72–2.82)	1.00 (0.81–2.18)	0.006
	V	0.25 (0.06–0.90)	BDL (BDL–0.25)	0.009
Liver	As	0.86 (0.39–2.06)	BDL (BDL)	<0.001*
	Cd	1.07 (0.70–2.11)	0.18 (0.09–0.80)	0.004
	Cu	35.07 (19.00–54.44)	17.73 (14.68–65.90)	0.183
	Se	22.63 (13.46–27.53)	2.37 (2.04–2.95)	0.001*
	Sr	0.90 (0.68–1.79)	0.46 (0.29–1.02)	0.018
	V	1.38 (0.99–3.29)	BDL (BDL–1.50)	0.012
Overall	As	0.35 (BDL–2.06)	BDL (BDL–0.08)	0.001*
	Cd	0.44 (BDL–2.11)	0.06 (BDL–0.80)	0.002*
	Cu	8.07 (BDL–54.44)	7.63 (4.31–65.9)	0.685
	Se	18.14 (11.09–27.53)	2.90 (2.04–4.24)	0.001*
	Sr	1.89 (0.68–3.27)	0.88 (0.29–2.18)	0.003*
	V	0.63 (BDL–3.29)	BDL (BDL–1.50)	0.003*

[a] Values are expressed as medians and ranges. BDL = Below detection limits. Detection limits (ug/g dry mass) for each element (in gonad, kidney, and liver, respectively) are as follows: As, 0.009,0.007, 0.007; Cd, 0.005, 0.004, 0.004; Cu, 0.017, 0.014, 0.013; Se, 0.134, 0.110, 0.102; Sr, 0.005, 0.004, 0.004; V, 0.011, 0.009, 0.009. Detection limits are calculated based on the instrument detection limits and are converted to a dry mass basis using the average sample mass used for a particular sample matrix. Asterisks identify statistically significant differences after the Bonferroni correction.

4. Discussion

4.1. Accumulation

Our experimental results demonstrate that prey items are an important source of trace element contamination for high trophic level predators such as water snakes. When overall tissue concentrations were considered, water snakes fed contaminated prey items accumulated significant quantities of As, Cd, Se, Sr, and V. Within specific organs, snakes fed contaminated prey exhibited significant elevations of As, Se, and Cd, the same three elements that were previously determined to be elevated in snakes naturally occurring in the polluted site (Hopkins et al., 1999). In addition, there were clear trends suggesting accumulation of Sr and V in specific organs of snakes fed contaminated prey, but these trends were not statistically significant.

The high concentrations of elements accumulated by snakes fed contaminated food indicates that snakes feeding within the coal ash-contaminated site may experience significant health risks. For example, Se concentrations in prey ingested by snakes in our laboratory study are more than seven times the dietary toxic effects threshold determined for predatory fish (Lemly, 1996). Although tissue accumulation threshold

Table 3
Trace element concentrations in tissues of water snakes, *Nerodia fasciata*, fed contaminated (ash) and uncontaminated (control) diets for 13.5 months[a]

Element	Tissue type							
	Shed skin median (range)		Posterior error rate	Blood median (range)		Posterior error rate	Tail clip median (range)		Posterior error rate
	Ash	Control		Ash	Control		Ash	Control	
As	4.00 (1.88–6.25)	0.28 (0.14–0.47)	0.045	0.03 (0.02–0.03)	0.01 (0–0.01)	0.018	0.34 (0.22–0.58)	<0.01 (BDL–0.04)	<0.001
Cd	0.28 (0.10–0.76)	0.17 (0.11–0.31)	0.259	<0.01 (BDL–<0.01)	<0.01 (BDL–<0.01)	0.441	0.04 (0.02–0.74)	0.02 (0.01–0.04)	0.294
Cu	82.48 (7.88–172.90)	103.78 (9.44–246.46)	0.267	0.54 (0.42–0.68)	0.65 (0.53–5.22)	0.265	2.27 (1.06–6.79)	1.95 (1.42–3.08)	0.231
Se	26.14 (22.85–34.31)	2.11 (1.41–3.21)	<0.001	1.61 (1.14–2.42)	0.35 (0.27–0.38)	<0.001	7.74 (6.84–9.07)	0.04 (BDL–0.64)	<0.001
Sr	6.51 (4.21–10.25)	5.14 (1.57–19.64)	0.235	0.42 (0.34–0.51)	0.2 (0.12–0.25)	0.025	238.00 (184.9–320.73)	102.64 (69.85–149.2)	0.040
V	0.47 (0.12–1.35)	0.05 (BDL–0.32)	0.146	0.01 (BDL–0.02)	<0.01 (BDL–0.02)	0.374	BDL	BDL	N/A

[a] Element concentrations in blood are presented as mg/L and concentrations in shed skins and tail clips are reported as ug/g dry mass. Posterior error rates indicate the likelihood of misclassification of snakes into treatments based upon element concentration in specific tissues. BDL = Below detection limits. Detection limits for each element (in shed skin, blood, and tail clip, respectively) are as follows: As, 0.006, 0.002, 0.006; Cd, 0.003, 0.001, 0.003; Cu, 0.012, 0.005, 0.011; Se, 0.091, 0.035, 0.088; Sr, 0.002, 0.001, 0.003; V, 0.008, 0.003, 0.007. Detection limits in blood are presented as mg/l and detection limits in shed skins and tail clips are reported as ug/g dry mass.

limits are not yet known for snakes, concentrations of Se accumulated in snake liver were twice the hepatic concentration thresholds associated with reproductive failure in fish (Lemly, 1996).

Comparisons of hepatic trace element concentrations between our current feeding study and previous field-sampling reveal that snakes exposed in the laboratory accumulated As and Se levels that were only a fraction of what was accumulated by snakes in the field. Snakes naturally inhabiting the polluted site had mean hepatic burdens of both As and Se exceeding 140 μg/g dry mass (Hopkins et al., 1999b), whereas median liver concentrations of As and Se in snakes fed contaminated prey were 0.86 and 22.63 μg/g, respectively. The simplest explanation for differences between our field and laboratory studies is that snakes collected during the field surveys had been exposed for a longer period of time and therefore accumulated higher concentrations of trace elements. However, other factors could also contribute to the observed differences. Banded water snakes undergo an ontogenetic shift in dietary preferences; smaller snakes tend to feed predominately on fish whereas larger snakes, such as those used in our laboratory and field studies, tend to rely more heavily on amphibians as prey (Mushinski et al., 1982). Our previous work indicates that amphibians at the site have much higher body burdens of trace elements than fish (Hopkins et al., 1998, 1999b, 2000a; Rowe et al., 1996) and even contain elevations of additional trace elements not elevated in fish from the site (e.g. Cr; Rowe et al., 1996; Hopkins et al., 2000a). Thus, larger snakes would predominately ingest amphibians in the field and be exposed to more diverse contaminants at higher concentrations compared with snakes fed only fish in the laboratory. Such a scenario is further complicated if snakes accumulate additional contaminants from water or sediments in the field. Regardless of the source of differences between field and laboratory results, it is clear that snakes exposed under more complex field conditions face greater health risks than snakes in our conservative laboratory exposures.

4.2. Nondestructive tissue samples

The current study illustrates the potential utility of nondestructive tissue sampling techniques for determining whether water snakes have a history of exposure to As, Se, and Sr. All three tissue-sampling techniques were particularly reliable for indicating previous exposure to Se (all <0.10% chance of error), an element readily incorporated into tissues of predators following trophic exposure. In contrast to Se, there was considerable variation among tissue sampling techniques in their reliability for determining exposure to As and Sr. Tail clips were the best predictors of As exposure (<0.10% chance of error) with blood and shed skins providing

reasonable predictive capabilities (1.8 and 4.5% chance of error, respectively). Although shed skins were not reliable predictors of Sr exposure, blood or tail clips could be useful nondestructive indicators for this element (2.5 and 4.0% chance of error, respectively). Future studies that evaluate varying levels of trace element exposure and how resulting target organ concentrations correlate with skin, blood, and tail clip concentrations would be valuable in determining whether nondestructive samples can indicate relative degree of exposure.

Each of the tissues sampled nondestructively has advantages and disadvantages for practical application in future studies. For example, because the collection of shed skins is non-intrusive, this tissue may be a useful tool for monitoring exposure over time in highly controlled laboratory studies. However, investigators have little control over the timing and frequency of skin shedding for each individual; thus, uniform sampling intervals cannot be scheduled. Moreover, shed skins are not as easily obtained from animals captured in the field. Shed skin collection from field-captured snakes requires that individuals captured be returned for a holding period in the laboratory, during which time they would require provision of prey from their site of capture. Thus, monitoring efforts using shed-skins would have limited practicality for field investigations.

In contrast to shed skins, blood and tail clips have great potential for use in the field as well as the laboratory. Despite the increased intrusiveness of these techniques compared with shed skins, blood and tail clips have the advantage of predicting exposure to another contaminant (i.e. Sr) in addition to Se and As. Because tail clips are a composite sample of multiple tissue types (e.g. bone, connective tissue, blood, skin, etc.), investigators may be able to detect contaminants that may not be present in blood or skin alone. Collecting blood and tail clips are both relatively easy procedures, although tail clips require less time and technical expertise. Small tail clips may not be detrimental to the organism, but caution should be exercised since the tail has critical functions (e.g. breeding, climbing, balance, and lipid storage) in some squamate species. In addition, the number of repeated applications of tail clipping is obviously limited on the same individuals. Therefore, under conditions where only one or two samples per individual would be taken over an extended period of time, such as in a field study, tail clips may be the most widely applicable and informative technique. In contrast, blood samples can be taken at more frequent intervals without having long-term effects on study animals and may be most applicable under conditions where repeated samples are desired.

4.3. Utility of squamates in ecotoxicology

Despite the fact that many squamate reptiles are more logistically practical than turtles and crocodilians for use in experimental evaluations of toxicological issues, they remain grossly understudied by ecotoxicologists (Hopkins, 2000). To date, the majority of ecotoxicological evaluations on reptiles have focussed on alligators and turtles, with the largest effort specifically targeting snapping turtles (*Chelydra serpentina*). Although long-lived species of reptiles such as turtles and alligators are well suited for evaluations of chronic exposure to contaminants in the field, they are less tractable for controlled laboratory experiments. In contrast, many squamates grow relatively quickly, reach sexual maturity in 1–3 years, and are easily maintained in captivity. The current study, in conjunction with others (Fleet et al., 1972; Bauerle et al., 1975; Janssen et al., 1976; Stafford et al., 1976; Ohlendorf et al., 1988; Hopkins et al., 1999; Lambert, 1999), demonstrates the potential utility of squamates in not only field studies, but also laboratory manipulations.

Aquatic and semi-aquatic snakes (e.g. genera *Nerodia* and *Thamnophis*) are among the most common reptiles found in the southeastern USA (Mushinski et al., 1982) and may be well suited for certain ecotoxicological evaluations. Unlike most other squamates, aquatic snakes inhabit virtually all types of freshwater habitats and may therefore be useful species for monitoring contamination in the aquatic landscape. The trophic status of aquatic snakes (i.e. carnivorous diet) makes them particularly useful in evaluating sites contaminated with compounds that are transferred via trophic mechanisms. Moreover, the site fidelity of aquatic snakes, relative to many mammalian or avian carnivores, presents opportunities to compare contaminant accumulation in individuals inhabiting polluted and reference sites within a narrow geographical area (Hopkins, 2000).

Ideally, future exposure assessments could be performed on snakes from contaminated sites using nondestructive techniques similar to the methods employed in the current investigation. Such techniques are not only important from a conservation standpoint, but also enable investigators to repeatedly sample the same individuals over an extended period of time. Water snakes can be easily equipped with radiotransmitters for tracking in the field (Fitch, 1987; Keck, 1998) or simply marked using passive integrated transponder (PIT) tags (Keck, 1994; Mills et al., 1995) for future identification upon recapture. Theoretically, ecotoxicologists could therefore document the progression of contaminant exposure over time, or even document the recovery of individuals after conditions at a site improve. Future studies that evaluate snakes in the field based upon nondestructive indices will not only be an important next step in ecotoxicology, but will also set new precedents by applying a conservation-minded approach to the sampling of reptiles.

Acknowledgements

We thank Brandon Staub and Whit Gibbons for their insightful comments on the manuscript. The project was supported by US Department of Energy Financial Assistance Award Number DE-FC09-96SR18546 to the University of Georgia Research Foundation.

References

Bauerle, B., Spencer, D.L., Wheeler, W., 1975. The use of snakes as pollution indicator species. Copeia 1975, 366–368.

Bishop, C.A., Martinovic, B., 2000. Guidelines and procedures for field investigations using amphibians and reptiles. In: Sparling, D.W., Linder, G., Bishop, C. (Eds.), Ecotoxicology of Amphibians and Reptiles. SETAC Press, Pensacola, FL, pp. 697–726.

Burger, J., 1992. Trace element levels in pine snake hatchlings: tissue and temporal differences. Archives of Environmental Contamination and Toxicology 22, 209–215.

Carlson, C.L., Adriano, D.C., 1993. Environmental impacts of coal combustion residues. Journal of Environmental Quality 22 (2), 227–247.

Cherry, D.S., Guthrie, R.K., 1977. Toxic metals in surface waters from coal ash. Water Resources Bulletin 13 (6), 1227–1235.

Cobb, G.P., Wood, P.D., O'Quinn, M., 1997. Polychlorinated biphenyls in eggs and chorioallantoic membranes of American alligators (*Alligator mississippiensis*) from coastal South Carolina. Environmental Toxicology and Chemistry 16 (7), 1456–1462.

Cobb, G.P., Wood, P.D., 1997. PCB concentrations in eggs and chorioallantoic membranes of loggerhead sea turtles (*Caretta caretta*) from the Cape Romain Wildlife Refuge. Chemosphere 34 (3), 539–549.

De Solla, S.R., Bishop, C.A., Brooks, R.J., Van der Kraak, G., 1998. Organochlorine contamination, sex hormones and sexual morphology of common snapping turtles (*Chelydra serpentina serpentina*). Environmental Health Perspectives 105 (5), 1–7.

Fitch, H.S., 1987. Collecting and life-history techniques. In: Siegel, R.A., Collins, J.T., Novak, S.S. (Eds.), Snakes: Ecology and Evolutionary Biology. MacMillan, NY, pp. 143–164.

Fleet, R.R., Clark Jr., D.R., Plapp Jr., F.W., 1972. Residues of DDT and dieldrin in snakes from two Texas agro-systems. Bioscience 22, 664–665.

Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., Tuberville, T., Metts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S., Winne, C.T., 2000. The global decline of reptiles, déjà vu amphibians. Bioscience 50 (8), 653–666.

Hopkins, W.A., 2000. Reptile toxicology: challenges and opportunities on the last frontier in vertebrate ecotoxicology. Environmental Toxicology and Chemistry 19 (10), 2391–2393.

Hopkins, W.A., Mendonça, M.T., Congdon, J.D., 1997. Increased circulating testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion wastes. General and Comparative Endocrinology 108, 237–246.

Hopkins, W.A., Mendonça, M.T., Rowe, C.L., Congdon, J.D., 1998. Elevated trace element concentrations in southern toads, *Bufo terrestris*, exposed to coal combustion wastes. Archives of Environmental Contamination and Toxicology 35, 325–329.

Hopkins, W.A., Mendonça, M.T., Congdon, J.D., 1999a. Responsiveness of the hypothalamo-pituitary-interrenal axis in an amphibian (*Bufo terrestris*) exposed to coal combustion wastes. Comparative Biochemistry and Physiology 122, 191–196.

Hopkins, W.A., Rowe, C.L., Congdon, J.D., 1999b. Elevated trace element concentrations and standard metabolic rate in banded water snakes, *Nerodia fasciata*, exposed to coal combustion wastes. Environmental Toxicology and Chemistry 18 (6), 1258–1263.

Hopkins, W.A., Congdon, J.D., Ray, J., 2000a. Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal burning power plant. Environmental Toxicology and Chemistry 19 (4), 862–868.

Hopkins, W.A., Snodgrass, J.W., Roe, J.H., Jackson, B.P., Gariboldi, J.C., Congdon, J.D., 2000b. Detrimental effects associated with trace element uptake in lake chubsuckers (*Erimyzon sucetta*) exposed to polluted sediments. Archives of Environmental Contamination and Toxicology 39, 193–199.

Janssen, H.E., Dennis, R., DeShaw, J.R., 1976. Aquatic snakes as composite samples for organochlorine pesticide residues. In: Proceedings International Conference on Environmental Sensing and Assessment, Vol. 1, Paper 13–3; 14–19 Sept. 1975; Las Vegas, Nevada; Institute of Electrical and Electronic engineers, Incorporated. Catalogue #75-CH 1004-1 ICESA, New York, pp. 1–5.

Kaur, S., 1988. Lead in the scales of cobras and wall lizards from rural and urban areas of Punjab, India. Science of the Total Environment 77 (2/3), 289–290.

Keck, M.B., 1994. Test for detrimental effects of PIT tags in neonatal snakes. Copeia 1994 (1), 226–228.

Keck, M.B., 1998. Habitat use by semi-aquatic snakes at ponds on a reclaimed strip mine. Southwestern Naturalist 43 (1), 13–19.

Lambert, M.R.K., 1999. Lizards as bioindicators. Biologist 46 (1), 12–16.

Lemly, A.D., 1993. Teratogenic effects of selenium in natural populations of freshwater fish. Ecotoxicology and Environmental Safety 26, 181–204.

Lemly, A.D., 1996. Selenium in aquatic organisms. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publishers, Boca Raton, FL, pp. 427–445.

Mills, M.S., Hudson, C.J., Berna, H.J., 1995. Spatial ecology and movements of the brown water snake (*Nerodia taxispilota*). Herpetologica 51 (4), 412–423.

Mushinski, H.R., Hebrard, J.J., Vodopich, D.S., 1982. Ontogeny of water snake foraging ecology. Ecology 63 (6), 1624–1629.

Ohlendorf, H.M., Hothem, R.L., Aldrich, T.W., 1988. Bioaccumulation of selenium by snakes and frogs in the San Joaquin Valley, California. Copeia 1988, 704–710.

Portelli, M.J., Bishop, C.A., 2000. Ecotoxicology of organic contaminants in reptiles: a review of the concentrations and effects of organic contaminants in reptiles. In: Sparling, D.W., Linder, G., Bishop, C. (Eds.), Ecotoxicology of Amphibians and Reptiles. SETAC Press, Pensacola, FL, pp. 495–544.

Raimondo, S.M., Rowe, C.L., Congdon, J.D., 1998. Exposure to coal ash impacts swimming behavior and predator avoidance in larval bullfrogs (*Rana catesbeiana*). Journal of Herpetology 32, 289–292.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.

Rowe, C.L., Kinney, O.M., Fiori, A.P., Congdon, J.D., 1996. Oral deformities in tadpoles (*Rana catesbeiana*) associated with coal ash deposition: Effects on grazing ability and growth. Freshwater Biology 36, 723–730.

Rowe, C.L., Kinney, O.M., Congdon, J.D., 1998a. Oral deformities of the bullfrog (*Rana catesbeiana*) caused by conditions in a polluted habitat. Copeia 1998 (1), 244–246.

Rowe, C.L., Kinney, O.M., Nagle, R.D., Congdon, J.D., 1998b. Elevated maintenance costs in an anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during the embryonic and early larval periods. Physiological Zoology 71, 27–35.

Rowe, C.L., 1998. Elevated standard metabolic rate in a freshwater shrimp (*Palaemonetes paludosus*) exposed to trace element-rich coal combustion waste. Comparative Biochemistry and Physiology 121 (A), 299–304.

SAS Institute Inc., 1990. SAS/STAT Users Guide, Version 6, 4th Edition, Cary, North Carolina.

Stafford, D.P., Plapp, F.W., Fleet, R.R., 1976. Snakes as indicators of environmental contamination; relation of detoxification enzymes and pesticide residues to species occurrence in three aquatic ecosystems. Archives of Environmental Contamination and Toxicology 5, 15–27.