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FUNCTIONAL RELATIONSHIPS AMONG SELENIUM CONCENTRATIONS IN THE DIET, TARGET TISSUES, AND NONDESTRUCTIVE TISSUE SAMPLES OF TWO SPECIES OF SNAKES

WILLIAM A. HOPKINS,*† JOEL W. SNODGRASS,‡ JENNIFER A. BAIONNO,† JOHN H. ROE,† BRANDON P. STAUB,† and BRIAN P. JACKSON†

†Wildlife Ecotoxicology and Physiological Ecology Program, University of Georgia, Savannah River Ecology Laboratory, P.O. Drawer E, Aiken, South Carolina 29802, USA

‡Department of Biology, Towson University, Towson, Maryland 21252, USA

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Abstract—Nondestructive sampling methods, such as removal of feathers for contaminant analysis, are desirable in ecological monitoring programs that seek to minimize the impacts of harvesting organisms. Although many reptiles are declining worldwide, nondestructive sampling techniques seldom have been employed for assessing contaminant exposure in these organisms. In this study, we examined the utility of nondestructive tissue sampling for assessing Se exposure in reptiles. We describe the functional relationships among dietary Se concentrations, target tissue Se concentrations, and Se concentrations in nondestructive tissue samples (blood and tail tissue biopsy) in two species of snakes that had been exposed to Se under very different experimental protocols. Using nonlinear regression, we found strong positive correlations ($r^2 > 0.92$) in all comparisons among Se concentrations in nondestructive tissues, diet, and target tissues. Moreover, equations describing these relationships can be used to estimate concentrations of Se in diet and target organs, from known concentrations of Se in nondestructive tissue samples. Although the current paucity of toxicity data on reptiles precludes tests of our models, we demonstrate how the equations describing these relationships might be used to make predictions about Se accumulation in target organs for risk assessment. Future studies on reptiles that examine these relationships under different Se exposure conditions, and those that document physiological responses of reptiles to various concentrations of Se, will help to refine our models and test their efficacy for predicting health risk.

Keywords—Selenium Reptiles Trophic transfer Nondestructive tissues Risk assessment

INTRODUCTION

Nondestructive sampling methods, such as the removal of feathers or hair for contaminant analysis, are desirable in ecological monitoring programs that seek to minimize the impacts of harvesting organisms. Such techniques allow investigators to obtain larger sample sizes without compromising ecological sustainability and repeatedly sample individuals over time with limited interference to the organism. However, for nondestructive sampling methods to be most useful, concentrations of contaminants in these tissues must accurately depict exposure history and concentrations of contaminants in tissue relevant to the organism's health and reproductive success (such as liver and gonads).

Although many reptile populations are declining worldwide [1], nondestructive sampling techniques seldom have been used for assessing contaminant exposure in reptiles. Previously, we developed nondestructive techniques that enable an investigator to determine whether snakes have been exposed to elevated concentrations of trace element in the environment [2,3]. Such procedures can discriminate among alternative exposure conditions relative to one another (e.g., exposed or unexposed), but they provide little information about the quantitative relationships among concentrations of contaminants in a nondestructive tissue sample and the accumulation of contaminants in tissues of concern. Ideally, the contaminant concentration of a nondestructive tissue sample could be used to make meaningful predictions about exposure conditions, body burdens, and potential health risks to the individual. However, such relationships remain unexplored in reptiles. In fact, even in birds, where much information exists on contaminant concentrations in feathers, few studies have used concentrations from tissues obtained nondestructively as a tool for estimating exposure history and predicting health risks (reviewed in [4,5]).

This study examines the potential utility of nondestructive tissue sampling for assessing Se exposure in reptiles. We focus on Se because it is a contaminant of global concern [6] and poses significant health risks to certain reptile species [7]. We describe the relationships among Se concentrations in diet, target organs, and nondestructive tissue samples (blood and tail tissue biopsy) in two species of snakes. To further link concentrations of Se in nondestructive tissues to ecological risk, we also describe the relationship between nondestructive tissue samples and Se content of eggs produced by one of the snake species. Furthermore, we demonstrate how the equations describing these relationships might be used to estimate Se exposure history and accumulation in tissues of concern.

MATERIALS AND METHODS

Exposure conditions

Tissues used for this study were collected from banded water snakes (*Nerodia fasciata*) and brown house snakes (*Lamprophis fuliginosus*) fed Se-contaminated prey items under captive experimental conditions. Details regarding experimental exposures and Se accumulation in target organs from the two studies are presented in previous publications [2,3,

* To whom correspondence may be addressed (hopkins@srel.edu).

8–10]. A brief synopsis of pertinent details from each study follows.

In the first study [9,10], juvenile male and female *N. fasciata* were fed fish collected from either a reference site, a contaminated wetland downstream from a coal-burning plant, or from both sites on alternating weeks. Prey items from the contaminated wetland contained elevated concentrations of Se compared to prey items from the reference site [2]. Thus, the treatments resulted in three levels of dietary Se exposure: Mean monthly Se concentrations in diet were 0.99, 11.36, or 22.70 parts per million (ppm) dry mass [9]. Exposing the snakes to the two contaminated diets for two years resulted in significantly elevated tissue concentrations of Se, exceeding those known to be toxic to other vertebrates [11,12]. Chemical speciation of Se in one fish species (*Micropterus salmoides*) fed to the snakes was determined using size exclusion and ion exchange chromatography inductively coupled plasma mass spectrometry. These analyses showed that Se occurred in a variety of forms, including high–molecular weight seleno-proteins and a variety of low–molecular weight compounds [13].

In the second study [8], juvenile female *L. fuliginosus* were fed mice injected with either a control amino acid solution (D,L-methionine dissolved in double deionized water) or solutions containing Se in the form of seleno-D,L-methionine. Three levels of Se in the diets were used for the exposures: Approximately 1 ppm, 10 ppm, and 20 ppm dry mass. Seleno-D,L-methionine was used in the study because it is thought to be one of the primary organic forms trophically transferred from prey to vertebrate predators [14]. Snakes were maintained on their respective diets for ten months, during which time a variety of physiological endpoints were monitored but no adverse physiological effects were observed [8]. However, exposing the snakes to Se for ten months resulted in accumulation of Se in target organs and maternal transfer of Se to eggs at levels that exceeded established toxicity thresholds for birds and fish [11,12].

At the end of each experiment, a blood sample was collected from the caudal vein of each snake using a 26-gauge heparinized syringe. After blood sampling, a 2- to 3-cm portion of the tail was removed using a sterile razor and the sample was then rinsed with deionized water. Snakes were dissected and organs removed. Blood, tail biopsy, and organs were then frozen (−70°C) for future analysis of trace elements.

We focused on two nondestructive tissues, blood and tail biopsy, that show promise as nonlethal means of exposure assessment based on previous findings [2,3]. The suitability of these two tissues may vary depending upon the aims of specific studies. Whereas concentrations of Se in blood represent current exposure and Se mobilized from tissues, the tail biopsy likely provides a composite archive of Se incorporated into multiple tissue matrices including blood, muscle, bone, and skin. It also should be noted that, because snakes from both experiments were raised in a clean laboratory environment and trophic transfer was the only route of Se exposure, contamination of the tail biopsy with external Se is highly unlikely. However, future studies that apply these techniques under field situations will require more rigorous sample preparation to remove surface contamination that could influence concentrations of contaminants in tail biopsies.

Trace element analysis

Detailed descriptions of the analyses of snake organs and eggs are provided in Hopkins et al. [8,9]. Tail and blood samples were analyzed using similar methods to Hopkins et al. [2]. Briefly, snake tail tissues were lyophilized and homogenized before being digested and analyzed for Se. Blood samples were thawed, but not lyophilized before digestion and analysis. Approximately 40 to 90 mg and 100 to 225 mg of tail tissue (*N. fasciata* and *L. fuliginosus*, respectively) were used for digestion; sample masses varied because of differences in tail morphology between species. Approximately 220 to 260 mg of blood was used for digestion and analysis. Nitric acid (2.5–5.0 ml) was added to samples before digestion in a microwave (CEM, Matthews, NC, USA) with heating steps of 60, 60, 70, and 80% microwave power for 10, 10, 15, and 20 min, respectively. After digestion with HNO_3, 0.5 to 1.0 ml of H_2O_2 was added to the tail tissue samples only, and microwaved at the same power and duration as the HNO_3 digestion. After digestion, tail tissue samples were brought to a final volume of 10 or 25 ml (depending on mass of the tissue) with double-deionized water. Blood samples were brought to a final volume of 10 ml with double-deionized water. Trace element analysis was performed by inductively coupled plasma mass spectrometry (Perkin-Elmer, Norwalk, CT, USA) on samples diluted 1:1 with double-deionized water. External calibration standards covering a range of 1 to 500 μg/L were prepared daily by serial dilution of traceable primary standards (National Institute of Standards and Technology). Matrix-matched standard addition curves ranging from 10 to 500 μg/L (also from the National Institute of Standards and Technology traceable primary standards) were included in the calibration for each snake tissue analyzed. Certified reference material (Tort 2; National Research Council, Ottawa, ON, Canada) and blanks were included in the digestion and analysis procedure for quality-control purposes. Mean percent recovery for Se in certified reference materials was 107%. Mean detection limits for Se for the blood and tail tissues were 0.261 and 0.276 μg/g, respectively.

Statistical analyses

The goals of our statistical analyses were to produce simple models for the estimation of Se concentrations in diet and target tissues (i.e., tissues of importance for toxicity: Gonads, kidneys, liver, and eggs) based on concentrations of Se in tissues collected in a nondestructive fashion (tail biopsy and blood). In these analyses, we considered diet to be an independent variable that affected nondestructive tissue concentrations directly. We considered relationships among nondestructive tissue concentrations of Se and target tissue concentrations of Se to be correlative in nature (i.e., no clear cause-and-effect relationship). Because we viewed Se concentrations in these two sets of tissues as having no cause-and-effect relationship and, therefore, both subject to error, the slopes estimated from regression analyses are biased and model II regression is recommended [15]. However, approaches to model II regression do not allow prediction or calculation of prediction intervals, whereas our goal was to provide such predictive equations. Therefore, we also used regression to describe target organ concentration as a function of nondestructive tissue sample concentrations. Because preliminary analyses indicated little difference between slopes estimated using a principal axis approach (recommended as least biased given our data structure [16]) and those estimated using simple linear regression, bias associated with the use of regression appeared to have minimal effect on the slope estimates.

We used nonlinear regression to describe relationships among concentrations of Se in diet and nondestructive tissue,

Table 1. Nonlinear regression coefficients describing the effect of diet on Se concentrations in nondestructive tissue samples (i.e., tail biopsies and blood) from *Lamprophis fuliginosus* ($n = 15$) and *Nerodia fasciata* ($n = 29$) exposed to diets containing various levels of Se. Intercepts and slopes are estimated using nonlinear regression of $\log_{10}(x + 1)$ transformed data. In all cases, the intercepts and slopes differed significantly from zero ($p < 0.001$). The r^2 is the coefficient of determination. The general model is $\log_{10}(y + 1) = \alpha + \beta^{\log_{10}(x+1)}$ where y is tail biopsy or blood sample concentration and x is diet concentration. Combined models are fit with data from both species of snakes ($n = 44$). CI = Confidence intervals

Species	Nondestructive tissue	Intercept	95% CI for intercept		Intercept skewness	Slope	95% CI for slope		Slope skewness	r^2
			Lower	Upper			Lower	Upper		
L. fuliginosus	Tail biopsy	−0.9295	−1.0356	−0.8234	0.0125	1.8052	1.7158	1.8947	−0.0327	0.991
	Blood	−0.9693	−1.0195	−0.9190	0.0126	1.2929	1.2467	1.3390	−0.0264	0.986
N. fasciata	Tail biopsy	−0.9180	−0.9573	−0.8786	0.0060	1.6378	1.6034	1.6721	−0.0152	0.995
	Blood	−0.9246	−0.9618	−0.8874	0.0099	1.2843	1.2498	1.3188	−0.0212	0.984
Combined	Tail biopsy	−0.9219	−0.9888	−0.8549	0.0096	1.6959	1.6382	1.7536	−0.0246	0.982
	Blood	−0.9392	−0.9696	−0.9087	0.0083	1.2866	1.2584	1.3148	−0.0175	0.983

and nondestructive tissues and target tissues. After describing the effect of dietary Se concentrations on nondestructive tissue concentrations, we provided inverse prediction equations and 95% prediction intervals for each nondestructive tissue type and snake species. Nonlinear regression was chosen because heterogeneity in variance required transformation of the data, but transformations failed to both stabilize variance and produce linear relationships for all models (as indicated by examination of residual plots). Therefore, we applied a $\log_{10}(x + 1)$ transformation to all data to stabilize variance (and avoid modeling negative numbers), then used the nonlinear procedure (PROC NLIN) of SAS (SAS Institute, Ver 8.1; Cary, NC, USA) to fit the model:

$$\log_{10}(y + 1) = \alpha + \beta^{\log_{10}(x+1)} \quad (1)$$

where x is either diet, tail biopsy, or blood concentration, y is organ, tail biopsy, or blood concentration, and α and β are estimated parameters, which can be referred to as the intercept and slope of the nonlinear model, respectively. The inverse prediction equation for this model is:

$$10^{\log_\beta(\log_{10}(y+1)-\alpha)} - 1 = x \quad (2)$$

We used the Gauss-Newton method of minimizing the sums-of-squares error, with initial starting values of α and β set at -0.5 and 1.2 based on inspection of raw data plots and preliminary calculations. We used F tests to test the null hypothesis that the estimated slope of each equation did not differ from zero. We used t tests to test for differences ($\alpha = 0.05$) in slopes and intercepts between species following the procedures outlined in Zar [17]. Because these inferential tests assume that the standard errors and confidence intervals produced by nonlinear methods have properties similar to those produced by least-squares linear regression methods, we calculated Hougaard's measure of skewness to assess this assumption for each parameter estimate. When the absolute value of Hougaard's skewness is less than 0.25, the parameter estimates are expected to have properties similar to linear estimators. Standard errors from these nonlinear models can be used in inferential tests and to produce approximate confidence intervals [18].

RESULTS

For both snake species, nonlinear regression indicated strong, significant ($p < 0.001$) relationships among dietary Se concentrations and concentrations of Se in nondestructive tissue samples (Table 1 and Fig. 1). Skewness values indicated near-linear relationships between dietary Se concentrations and concentrations in nondestructive samples (Table 1 and Fig. 1). The slope of the relationship between diet and tail biopsy concentration differed significantly between species ($p = 0.002$). In contrast, the slope of the relationship between diet and blood concentrations of Se did not differ significantly between species ($p = 0.397$), but the intercepts did ($p = 0.019$); the intercept for *L. fuliginosus* was lower than *N. fasciata* (Table 1).

Relationships among Se concentrations in nondestructive tissues and concentrations in target tissues of both species also were strong and significant ($p < 0.001$; Table 2, Figs. 2 and 3). In general, skewness values were low for all parameter estimates in models relating tail biopsy concentrations to organ concentrations. For models describing the relationship between tail biopsy and gonads, the model slope did not differ significantly between species ($p = 0.462$; Fig. 4), but the intercept did ($p < 0.001$). For the models relating kidney and liver concentrations to tail biopsy concentrations, the slope of the models differed significantly between species ($p = 0.041$ and 0.008, respectively). In general, intercepts for models relating blood concentrations to organ concentration were similar to those for tail biopsy models, but slopes for the blood models were approximately three times greater than slopes for the tail biopsy models (Table 2). Skewness values for slopes of models relating organ to blood concentrations were relatively high for *L. fuliginosus* (Table 2). For models examining the relationship between blood and gonads and between blood and kidney, there was a significant difference between species in the slope of the models ($p < 0.001$ and $p = 0.001$, respectively; Fig. 4). The differences between species in the models relating liver concentrations to blood concentrations were not significant ($p = 0.084$ and 0.859 for the slope and intercept, respectively).

Selenium concentrations in tail biopsies and blood also showed strong, significant relationships with concentrations in eggs from *L. fuliginosus* (Table 2, Fig. 5). Again, skewness-values were low with the exception of the slope from the blood–egg concentration model; however, even for this model, the absolute skewness-value was <0.25.

Although the models described above differed significantly between species in most cases, other investigators may want to apply our results or test our models using other species exposed to Se under a variety of environmental conditions. Therefore, we also provide models for data from both experiments combined (Tables 1 and 2, Figs. 1–3). These models all had skewness values close to zero and were significant ($p < 0.001$). Moreover, they differ little from models for individual species (Figs. 1–3).

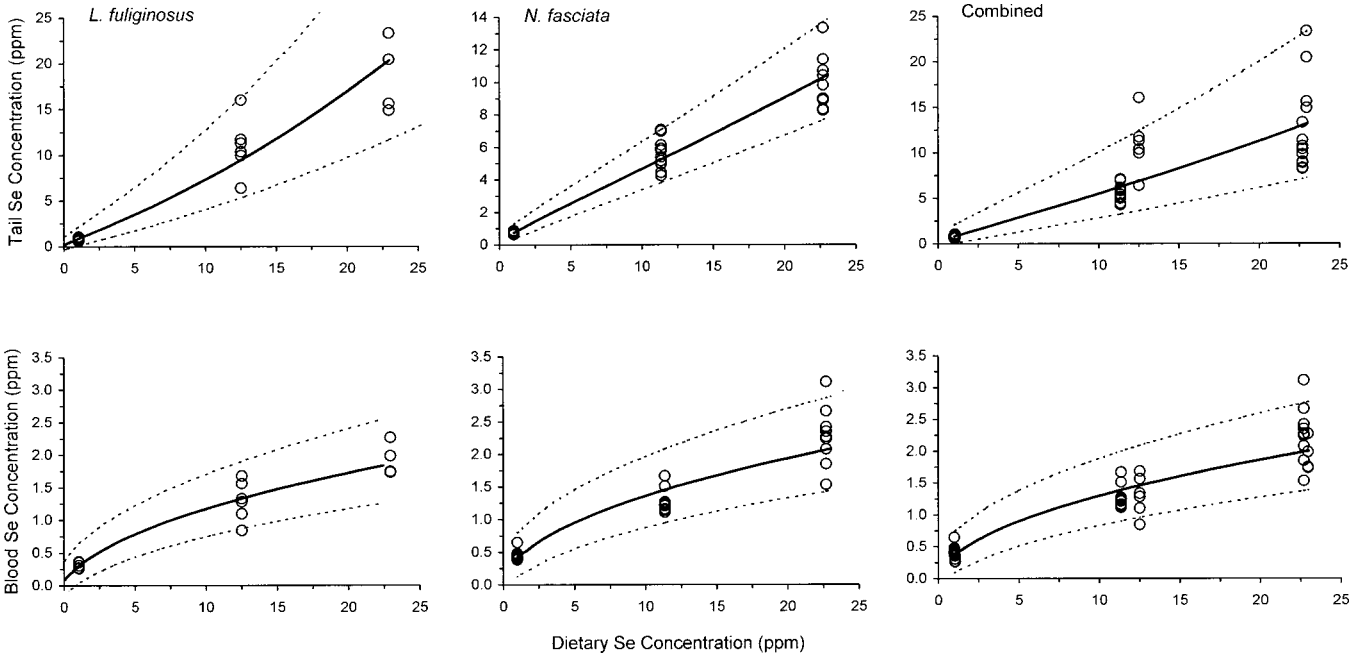

Fig. 1. Inverse predictions (solid line) of concentrations of Se in the diet of two snake species from concentrations in tail biopsies and blood samples. Nonlinear regression model of $\log_{10}(x + 1)$ transformed data was used to estimate model parameters. Dashed lines are approximate 95% prediction limits. Points are the original data used in the regression analyses. Dietary and tail biopsy concentrations are expressed on a dry mass basis; blood is expressed as parts per million (ppm) wet mass. Note that the scale of the *y* axis differs among graphs.

DISCUSSION

Comparisons to other reptiles

This study provides the first description of functional relationships among contaminant concentrations in diet, target tissues, and nondestructive tissue samples in reptiles. We found strong, positive correlations in all comparisons of Se concentrations in diet to various snake tissues. Moreover, equations produced from these relationships can be used to estimate concentrations of Se in diet and target organs from known concentrations of Se in nondestructive tissue samples. These findings are unique to the reptile toxicology literature, where the only other studies to evaluate the use of nondestructive tissues as estimators of exposure to inorganic contaminants have focused on discriminating between alternative exposure scenarios (exposed vs unexposed; [2,3]). In fact, we know of only one other study that has used nondestructive tissue samples to estimate Se concentrations in the diet or target organs of any vertebrate [19]. Although our study is unique, several other investigations have used similar principles to examine the utility of chorioallantoic membranes (CAMs) as nondestructive predictors of embryonic exposure to persistent organic pollutants in turtles and crocodilians [20]. Chorioallantoic membranes are highly vascularized extra-embryonic membranes that typically are discarded with the eggshell in oviparous vertebrates. Total polychlorinated biphenyls in CAMs from American alligators (*Alligator mississippiensis*) nesting in a contaminated area in South Carolina, USA, correlated positively with egg contents [21]. Similarly, concentrations of polychlorinated biphenyls in CAMs were used to estimate concentrations in eggs of loggerhead sea turtles (*Caretta caretta*) [22]. These studies provide evidence that CAMs may be useful indicators of concentrations of organic contaminants maternally transferred to the egg. Whether concentrations in CAMs are useful estimators of maternal diet, maternal organ burdens, or reproductive success remains unknown. Construction of models to estimate these parameters probably will require laboratory testing on more tractable reptile species and/or controlled incubation of turtle and crocodilian eggs collected from the field.

Comparisons to other vertebrates

Because the toxicological literature on reptiles is sparse compared to the literature on other vertebrates [23], it is useful to compare our approach to techniques adopted for other wildlife. Most notably, many studies have assessed the utility of CAMs and feathers as nondestructive indicators of contaminant exposure in birds [4,5,20]. Similar to the previously mentioned studies on reptiles, CAMs have been used to estimate concentrations of organochlorines in eggs of herons and stilts ([20]; see also Pastor et al. [24]), but we know of only one study that used CAMs for estimating egg concentrations of inorganic contaminants (i.e., Hg in mallard eggs [25]). In contrast, bird feathers primarily have been used for monitoring exposure to inorganic contaminants, particularly Hg [4]. Positive correlations have been documented between metal concentrations in feathers and other tissues sampled from the same individuals [26–28], female feather and egg concentrations [29,30], female and chick feather concentrations [31], and chick feathers and eggs collected from the same nests [32]. However, other analyses have revealed contradictory relationships [27,33,34].

Although many studies on birds have reported significant correlations between contaminant concentrations in feathers and tissues of concern, few have provided mathematical descriptions of their functional relationships for predictive purposes. To date, most studies that have attempted to use feathers to estimate contaminant concentrations in other tissues primarily have relied upon conversion ratios (e.g., [feather Hg]:[liver Hg]; [4,27]; see also Yamamoto et al. [19]). However, as pointed out by Thompson et al. [35] and Becker et al.

Table 2. Nonlinear regression coefficients describing the relationships among Se concentrations in nondestructive tissue samples (i.e., tail biopsies and blood) and target tissues from *Lamprophis fuliginosus* ($n = 15$) and *Nerodia fasciata* ($n = 29$) exposed to diets containing various levels of Se. Intercepts and slopes are estimated using nonlinear regression of $\log_{10}(x + 1)$ transformed data. In all cases, the intercepts and slopes differed significantly from zero ($p < 0.001$). The r^2 is the coefficient of determination. The general model is $\log_{10}(y + 1) = \alpha + \beta^{\log_{10}(x + 1)}$ where y is tail biopsy or blood sample concentration and x is organ concentration. Combined models for gonads, kidney, and liver are fit with data from both snakes ($n = 44$); eggs were not included in the overall model because eggs were available only from *L. fuliginosus*. CI = Confidence intervals

Species	Nondestructive tissue	Target tissue	Intercept	95% CI for intercept		Intercept skewness	Slope	95% CI for slope		Slope skewness	r^2
				Lower	Upper			Lower	Upper		
L. fuliginosus	Tail biopsy	Gonads	−0.6024	−0.7318	−0.4730	0.0161	1.6693	1.5417	1.7969	−0.0413	0.989
		Kidney	−0.4712	−0.5634	−0.3789	0.0120	1.7094	1.6223	1.7964	−0.0283	0.996
		Liver	−0.6927	−0.8574	−0.5279	0.0215	1.7085	1.5531	1.8640	−0.0505	0.979
		Eggs	−0.8759	−0.9756	−0.7762	0.0096	1.8755	1.7821	1.9689	−0.0262	0.993
L. fuliginosus	Blood	Gonads	−0.6231	−0.8036	−0.4427	0.0300	4.2514	2.9942	5.5086	0.1898	0.980
		Kidney	−0.4691	−0.6161	−0.3222	0.0269	4.4757	3.4473	5.5041	0.1501	0.989
		Liver	−0.6949	−0.8926	−0.4972	0.0360	4.5042	3.116	5.8924	0.2014	0.971
		Eggs	−0.9070	−1.0441	−0.7699	0.0170	5.8240	4.7416	6.9064	0.1208	0.987
N. fasciata	Tail biopsy	Gonads	−0.5011	−0.5470	−0.4552	0.0093	1.7183	1.6566	1.7801	−0.0087	0.997
		Kidney	−0.4326	−0.4849	−0.3803	0.0094	1.8386	1.7680	1.9091	−0.0089	0.996
		Liver	−0.6189	−0.6695	−0.5682	0.0083	1.9425	1.8741	2.0109	−0.0080	0.996
N. fasciata	Blood	Gonads	−0.5152	−0.5900	−0.4404	0.0248	3.1037	2.7300	3.4774	0.0638	0.991
		Kidney	−0.4419	−0.5303	−0.3534	0.0263	3.5325	3.0595	4.0055	0.0717	0.990
		Liver	−0.6248	−0.7193	−0.5303	0.0256	3.9316	3.3973	4.4659	0.0734	0.986
Combined	Tail Biopsy	Gonads	−0.5078	−0.5659	−0.4498	0.0123	1.6682	1.5985	1.7380	−0.0189	0.991
		Kidney	−0.4091	−0.4670	−0.3511	0.0113	1.7334	1.6664	1.8004	−0.0182	0.993
		Liver	−0.5770	−0.6700	−0.4840	0.0178	1.7508	1.6435	1.8582	−0.0288	0.976
Combined	Blood	Gonads	−0.5426	−0.6187	−0.4665	0.0221	3.3505	2.9381	3.7628	0.0717	0.985
		Kidney	−0.4391	−0.5197	−0.3586	0.0221	3.7308	3.2683	4.1933	0.0728	0.987
		Liver	−0.6456	−0.7314	−0.5599	0.0219	4.0828	3.5663	4.5993	0.0747	0.981

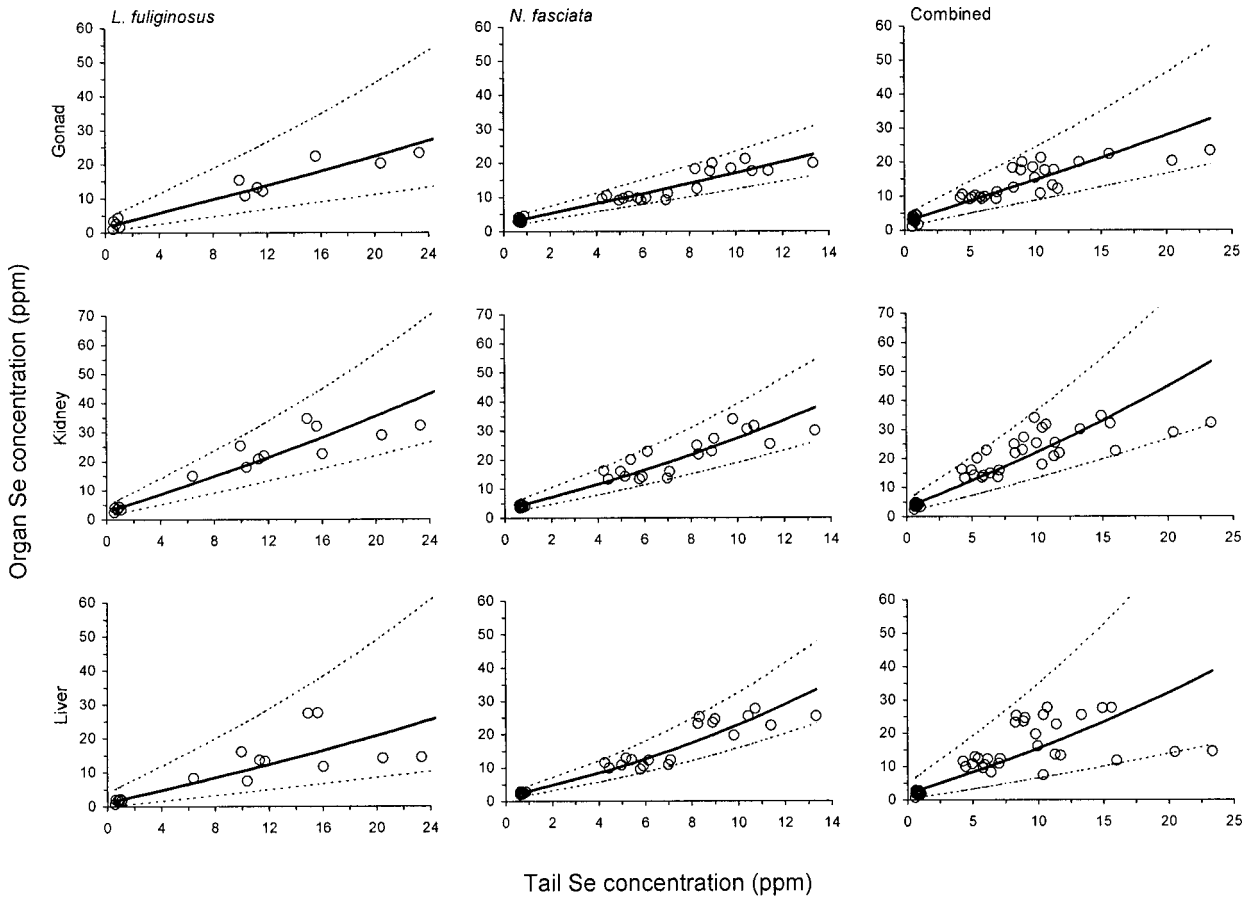

Fig. 2. Predictions (solid line) of concentrations of Se in organs from concentrations in tail biopsies of two snake species. Dashed lines are approximate 95% prediction limits. Points are the original data used in the regression analyses expressed on a dry mass basis. Nonlinear regression of $\log_{10}(x + 1)$ transformed data was used to build the models.

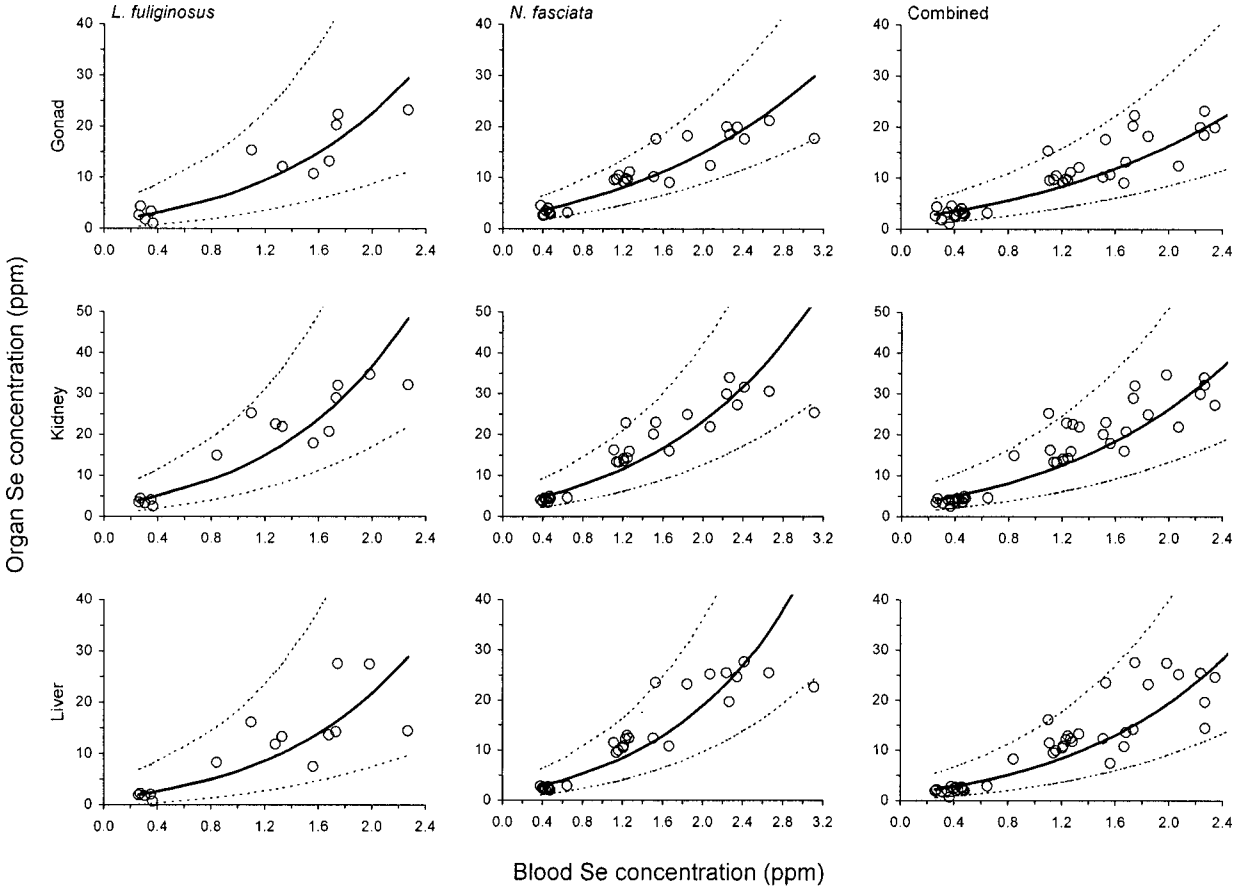

Fig. 3. Predictions (solid line) of concentrations of Se in organs from concentrations in blood samples from two snake species, *Lamprophis fuliginosus* and *Nerodia fasciata*. Dashed lines are approximate 95% prediction limits. Points are the original data used in the regression analyses. Organ concentrations are expressed on a dry mass basis; blood is expressed as parts per million (ppm) wet mass. Nonlinear regression of $\log_{10}(x + 1)$ transformed data was used to build the models.

[36,37], such ratios should be used cautiously for various reasons. Although ratios may be useful in some instances, they require the assumption that the relationship between feather and organ concentration is linear over a wide range of concentrations. Our data clearly illustrate that the relationships among tissue concentrations in snakes can be nonlinear (e.g., Fig. 3). Thus, compared to the equations we provide, conversion ratios would have produced less accurate estimates of target organ concentrations of Se in our study. Future studies that describe the functional relationships between nondestructive and target tissues, rather than isometric ratios, could enhance greatly the value of nondestructive tissue samples as predictive tools.

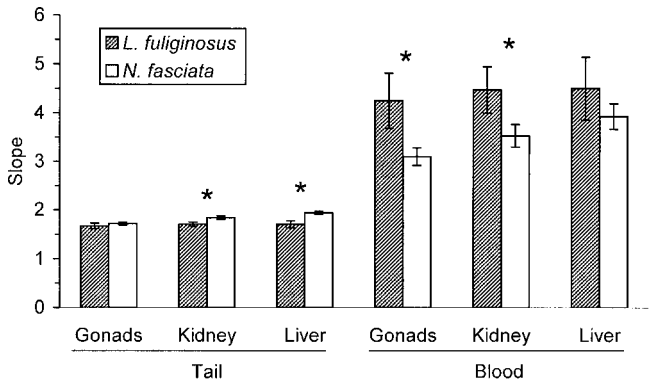

Fig. 4. Comparison of the slopes from regression models describing the relationships among nondestructive tissue samples (tail biopsy and blood) and target organs in two species of snakes. Asterisks indicate significant differences in slope between species (*Lamprophis fuliginosus* and *Nerodia fasciata*).

Applications for risk assessment

Ultimately, nondestructive tissue samples will be most useful if they can be used to estimate concentrations in target tissues and to predict health risks to the individual. Surprisingly few studies have evaluated the relationship between concentrations of contaminants in nondestructive tissue samples and adverse biological effects. Bowerman et al. [38] used feathers from bald eagles to demonstrate that neither Hg nor Se influenced reproductive productivity and nesting success. Similarly, Thompson et al. [26] demonstrated the lack of a relationship between Hg concentrations in feathers of seabirds and their breeding performance and survival. Based on experimental dose-response studies, Scheuhammer [39] estimated that growing piscivorous birds with Hg concentrations in feathers exceeding 20 μg/g are at risk of reproductive impairment. Clearly, more studies are needed that examine relationships between nondestructive tissue concentrations and meaningful biological effects (e.g., survival, growth, and reproduction).

In studies aimed at determining risks, toxicity thresholds based on published tissue values offer a practical alternative to quantifying biological effects. If organ concentrations can be estimated from nondestructive tissues and toxicity thresh-

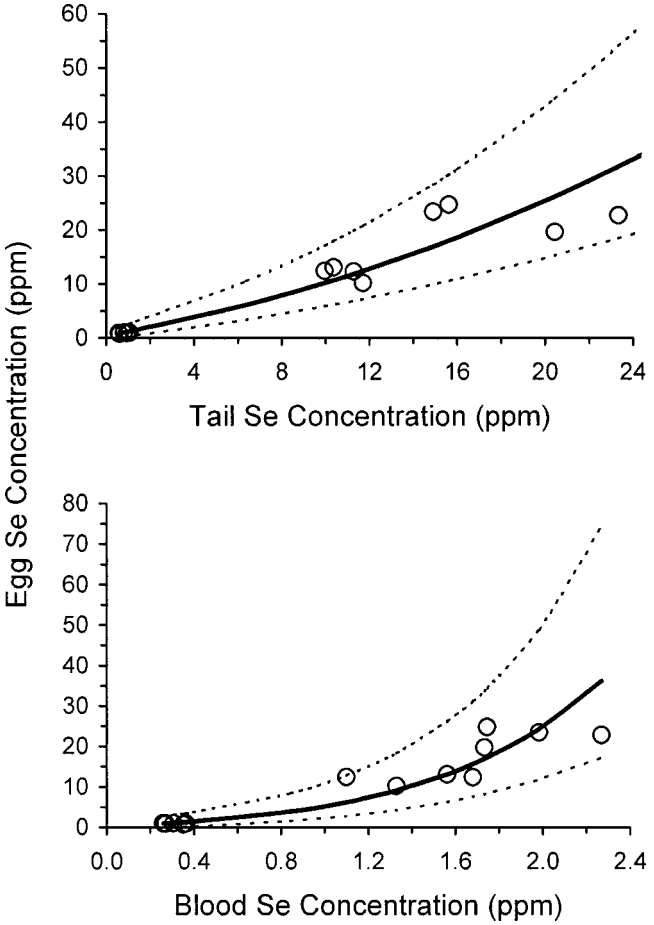

Fig. 5. Predictions (solid line) of concentrations of Se in eggs from concentrations in tail biopsies and blood of house snakes. Dashed lines are approximate 95% prediction limits. Points are the original data used in the regression analyses. Eggs and tail biopsy concentrations are expressed on a dry mass basis but blood is expressed as parts per million (ppm) wet mass. Nonlinear regression of $\log_{10}(x + 1)$ transformed data was used to build the models.

olds are available for the taxa under study (or for closely related taxa), reasonable risk assessments might be achieved. For fish and birds, such thresholds exist for various taxa (although some are heavily debated). To date, no toxicity thresholds exist for reptiles. Because Se dose-response relationships in reptiles have not been established, we can only speculate about the utility of our equations for predicting health risks to snakes. For example, based on the equations describing the relationship between nondestructive samples and eggs in *L. fuliginosus* (Table 2, Fig. 5) we estimate that individuals of this species having more than 14.3 ppm Se (dry mass) in a tail biopsy or 1.7 ppm Se (wet mass) in a blood sample would exceed the highest toxicity threshold established for eggs of other oviparous vertebrates (i.e., bird egg concentration of 16 ppm dry mass; [40–42]) and be at risk of reduced reproductive success.

The question remains as to whether the functional relationships reported here may prove useful in field situations, where animals are exposed to Se under more variable exposure conditions. The strong correlations we found in Se concentrations among tissue types probably in part are due to the highly controlled nature of our experimental approach. However, we suggest that the functional relationships reported here show promise as predictive tools, especially because the conditions of our two experiments were very different, yet produced largely similar results. In addition to the fundamental differences in physiological, ecological, and life history traits between study species, exposure to Se differed between studies both in chemical form and duration. Moreover, *N. fasciata* was exposed to a mixture of inorganic contaminants including As, Se, Sr, and Cd (possibly influencing toxicokinetics of Se), whereas *L. fuliginosus* was exposed only to Se. Although such differences may account for the variation in slopes of the functional relationships between species, the difference between slopes was small relative to the magnitude of the slopes (3–12% of the maximum slope for tail models and 12–26% for blood models; Fig. 4).

The next step towards improving these models may be the generation of a set of refined models that combine the variance attributable to additional species- and exposure-differences. Clearly, development of individual models for every reptile species of concern under a broad range of exposure conditions is not feasible and defeats the purpose of developing predictive models for risk assessment in the first place. As an initial step towards achieving this objective, we combined the data from both experiments to produce an additional set of models that serve as a compromise between the individual models. Until further data are available, our combined models provide investigators with a conservative estimate of the relationships among Se concentrations in diet, target organs, and nondestructive tissue samples in snakes. For example, based on our combined model describing the relationship between Se concentrations in nondestructive samples and snake liver, we estimate that snakes that have >7.3 ppm Se (dry mass) in a tail biopsy or >1.6 ppm Se (wet mass) in a blood sample would exceed liver toxicity thresholds recommended for other oviparous vertebrates (e.g., fish liver concentration of 12 ppm dry mass; [12]) and be at risk of reduced reproductive success.

CONCLUSION

The nondestructive techniques described here offer a potential alternative to sacrificing animals for assessments of contaminant exposure and risk, but future work is required before such models can be applied with confidence. Until more species under a range of exposure scenarios are included in the models, caution should be exercised when applying these models to animals with extremely high nondestructive tissue concentrations of Se because the differences in slopes observed between species suggest that relationships may diverge at higher Se concentrations. Moreover, because responses of surrogate species (e.g., birds and fish) may not accurately depict risk to reptiles due to inherent differences in their ecology, physiology, and life history [23], the utility of our equations for predicting risk will be realized only when controlled dose-response studies are conducted with reptiles. Future studies on reptiles that examine relationships among Se concentrations in target organs and nondestructive tissues will help refine the models presented here and assess their utility for real-world applications.

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REFERENCES

1. Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville T, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, Winne CT. 2000. The global decline of reptiles, déjà vu amphibians. *BioScience* 50:653–666.
2. Hopkins WA, Roe JH, Snodgrass JW, Jackson BP, Kling DE, Rowe CL, Congdon JD. 2001. Nondestructive indices of trace element exposure in squamate reptiles. *Environ Pollut* 115:1–7.
3. Jackson BP, Hopkins WA, Baionno JA. 2003. Laser Ablation-ICP-MS analysis of microdissected tissue: A conservation-minded approach to assessing contaminant exposure. *Environ Sci Technol* 37:2511–2515.
4. Burger J. 1993. Metals in avian feathers: Bioindicators of environmental pollution. *Rev Environ Toxicol* 5:203–311.
5. Burger J, Gochfeld M. 1997. Risk, mercury levels, and birds: Relating adverse laboratory effects to field biomonitoring. *Environ Res* 75:160–172.
6. Lemly DA. 2004. Aquatic selenium pollution is a global environmental safety issue. *Ecotoxicol Environ Saf* 59:44–56.
7. Hopkins WA, Rowe CL, Congdon JD. 1999. Increased maintenance costs and trace element concentrations in banded water snakes, *Nerodia fasciata*, exposed to coal combustion wastes. *Environ Toxicol Chem* 18:1258–1263.
8. Hopkins WA, Staub BP, Baionno JA, Jackson BP, Roe JH, Ford NB. 2004. Trophic and maternal transfer of selenium in brown house snakes (*Lamprophis fuliginosus*). *Ecotoxicol Environ Saf* 58:285–293.
9. Hopkins WA, Roe JH, Snodgrass JW, Staub BP, Jackson BP, Congdon JD. 2002. Trace element accumulation and effects of chronic dietary exposure on banded water snakes (*Nerodia fasciata*). *Environ Toxicol Chem* 21:906–913.
10. Rania LC, Hopkins WA, O'Neil L, Hasse S, Roe JH, Sever DM. 2003. Liver histopathology of the southern water snake, *Nerodia fasciata*, following chronic exposure to trace element–contaminated prey from a coal ash disposal site. *J Herpetol* 37:219–226.
11. Heinz GH. 1996. Selenium in birds. In Beyer WN, Heinz GH, Redmon-Norwood AW, eds, *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. Lewis, Boca Raton, FL, USA, pp 427–445.
12. Lemly AD. 1996. Selenium in aquatic organisms. In Beyer WN, Heinz GH, Redmon-Norwood AW, eds, *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. Lewis, Boca Raton, FL, USA, pp 427–445.
13. Jackson BP, Shaw-Allen P, Hopkins WA, Bertsch PM. 2002. Trace element speciation in largemouth bass (*Micropterus salmoides*) from a fly ash settling basin by liquid chromatography-ICP-MS. *Anal Bioanal Chem* 374:203–211.
14. Fan TWM, Teh SJ, Hinton DE, Higashi RM. 2002. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium laden drainage waters in California. *Aquat Toxicol* 57:65–84.
15. Sokal RR, Rohlf FJ. 1995. *Biometry: The Principles and Practices of Statistics in Biological Research*, 3rd ed. W.H. Freeman, New York, NY, USA.
16. Rayner JMV. 1985. Linear relations in biomechanics: The statistics of scaling functions. *J Zool (Lond) Ser A* 206:415–439.
17. Zar JH. 1996. *Biostatistical Analysis*, 3rd ed. Prentice Hall, Upper Saddle River, NJ, USA.
18. Ratkowsky D. 1990. *Handbook of Nonlinear Regression*. Marcel Dekker, New York, NY, USA.
19. Yamamoto JT, Santolo GM, Wilson BW. 1998. Selenium accumulation in captive American kestrels (*Falco sparverius*) fed selenomethionine and naturally incorporated selenium. *Environ Toxicol Chem* 17:2494–2497.
20. Cobb GP, Bargar TA, Pepper CB, Norman DM, Houlis PD, Anderson TA. 2003. Using chorioallantoic membranes for nonlethal assessment of persistent organic pollutant exposure and effect in oviparous wildlife. *Ecotoxicology* 12:31–45.
21. Cobb GP, Wood PD, O'Quinn M. 1997. Polychlorinated biphenyls in eggs and chorioallantoic membranes of American alligators (*Alligator mississippiensis*) from coastal South Carolina. *Environ Toxicol Chem* 16:1456–1462.
22. Cobb GP, Wood PD. 1997. PCB concentrations in eggs and chorioallantoic membranes of loggerhead sea turtles (*Caretta caretta*) from the Cape Romain National Wildlife Refuge. *Chemosphere* 34:539–549.
23. Hopkins WA. 2000. Reptile toxicology: Opportunities and challenges on the last frontier of vertebrate ecotoxicology. *Environ Toxicol Chem* 19:2391–2393.
24. Pastor D, Ruiz X, Jover L, Albaigés JA. 1996. The use of chorioallantoic membranes as predictors of egg organochlorine burden. *Environ Toxicol Chem* 15:167–171.
25. Heinz GH, Hoffman DJ. 2003. Predicting mercury in mallard ducklings from mercury in chorioallantoic membranes. *Bull Environ Contam Toxicol* 70:1242–1246.
26. Thompson DR, Hamer KC, Furness RW. 1991. Mercury accumulation in great skuas (*Catharacta skua*) of known age and sex, its effects upon breeding, and survival. *J Appl Ecol* 28:672–684.
27. Lewis SA, Becker PH, Furness RW. 1993. Mercury levels in eggs, tissues, and feathers of herring gulls *Larus argentatus* from the German Wadden Sea coast. *Environ Pollut* 80:293–299.
28. Spalding MG, Frederick PC, McGill HC, Bouton SN, McDowell LR. 2000. Methylmercury accumulation in tissues and its effects on growth and appetite in captive great egrets. *J Wildl Dis* 36: 411–422.
29. Burger J, Gochfeld M. 1991. Cadmium and lead in common terns (Aves: *Sterna hirundo*): Relationship between levels in parents and eggs. *Environ Monit Assess* 16:253–258.
30. Burger J, Gochfeld M. 1996. Heavy metal and selenium levels in Franklin's gull (*Larus pipixcan*) parents and their eggs. *Arch Environ Contam Toxicol* 30:487–491.
31. Scheuhammer AM, Atchison CM, Wong AHK, Evers DC. 1998. Mercury exposure in breeding common loons (*Gavia immer*) in central Ontario, Canada. *Environ Toxicol Chem* 17:191–196.
32. Rumbold DG, Niemczyk SL, Fink LE, Chadrasekhar T, Harkanson B, Laine KA. 2001. Mercury in eggs and feathers of great egrets (*Ardea albus*) from the Florida Everglades. *Arch Environ Contam Toxicol* 41:501–507.
33. Hughes KD, Ewins PJ, Clark KE. 1997. A comparison of mercury levels in feathers and eggs of osprey (*Pandion haliaetus*) in the North American Great Lakes. *Arch Environ Contam Toxicol* 33: 441–452.
34. Caldwell CA, Arnold MA, Gould WR. 1999. Mercury distributions in blood, tissues, and feathers of double-crested comorant nestlings from arid-lands reservoirs in south central New Mexico. *Arch Environ Contam Toxicol* 36:456–461.
35. Thompson DR, Stewart FM, Furness RW. 1990. Using seabirds to monitor mercury in marine environments: The validity of conversion ratios for tissue comparisons. *Mar Pollut Bull* 21:339–342.
36. Becker PH, Furness RW, Henning D. 1993. Mercury dynamics in young common tern (*Sterna hirundo*) chicks from a polluted environment. *Ecotoxicology* 2:33–40.
37. Becker PH, Furness RW, Henning D. 1993. The value of chick feather to assess spatial and interspecific variation in the mercury contamination of seabirds. *Environ Monit Assess* 28:255–262.
38. Bowerman WW, Evans ED, Giesy JP, Postupalsky S. 1994. Using feathers to assess risk of mercury and selenium to bald eagle reproduction in the Great Lakes region. *Arch Environ Contam Toxicol* 27:294–298.
39. Scheuhammer AM. 1991. Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. *Environ Pollut* 71:329–375.
40. Fairbrother A, Brix KV, Toll JE, McKay S, Adams WJ. 1999. Egg selenium concentrations as predictors of avian toxicity. *Hum Ecol Risk Assess* 5:1229–1253.
41. Adams WJ, Brix KV, Edwards M, Tear LM, DeForest DK, Fairbrother A. 2003. Analysis of field and laboratory data to derive selenium toxicity thresholds for birds. *Environ Toxicol Chem* 22: 2020–2029.
42. Ohlendorf HM. 2003. Ecotoxicology of selenium. In Hoffman DJ, Rattner BA, Burton GA, Cairns J, eds, *Handbook of Ecotoxicology*. Lewis, Boca Raton, FL, USA, pp 465–500.