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Trophic and maternal transfer of selenium in brown house snakes (*Lamprophis fuliginosus*)

William A. Hopkins,[a,*] Brandon P. Staub,[a] Jennifer A. Baionno,[a] Brian P. Jackson,[a] John H. Roe,[b] and Neil B. Ford[c]

[a] *Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA*
[b] *Department of Biological Sciences, Indiana–Purdue University, Fort Wayne, IN, 46805, USA*
[c] *Department of Biology, University of Texas at Tyler, Tyler, TX, 75799, USA*

Abstract

Excessive concentrations of dietary Se are toxic to oviparous vertebrates (i.e., fish and birds) but little is known about its accumulation and effects in reptiles. We exposed female brown house snakes, *Lamprophis fuliginosus*, to 10 and 20 μg/g Se by injecting seleno-D,L-methionine into their prey items and compared the snakes to individuals receiving background levels of ~1 μg/g dietary Se. Snakes were fed meals equaling 25% of their body mass 2–3 times a month for 10 months. Snakes exposed to excessive Se accumulated significant concentrations of Se in kidney, liver, and ovarian tissue, but accumulation had no effect on female survival, food consumption, growth, or body condition. Fewer females exposed to excessive Se reproduced than females exposed to 1 μg/g Se (67% vs. 91%, respectively), but the reduction in reproductive activity was not statistically significant. Total reproductive output of females did not differ among the three dietary treatments. However, snakes exposed to 10 and 20 μg/g Se transferred significant concentrations of Se to their eggs. In the 20 μg/g treatment, maternal transfer resulted in Se concentrations in eggs that surpassed all suggested reproductive toxicity thresholds for birds and fish. Further studies are needed to more rigorously determine whether maternal transfer of Se in this snake species affects the viability of developing embryos or the health of offspring.
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Keywords: Selenium; Reptiles; Snakes; Trophic transfer; Maternal transfer

1. Introduction

Selenium can readily be transferred from one organism to another by trophic interactions and maternal transfer. Vertebrates primarily accumulate Se by digesting Se-enriched food and incorporating constituent organoselenium compounds (e.g., selenoamino acids), or Se from these compounds, into their own biomolecules (Fan et al., 2002; Reddy and Massaro, 1983; Sunde, 1984). Following Se accumulation, female oviparous vertebrates (fish and birds) readily transfer Se to developing offspring (Ohlendorf, 2003), most likely via incorporation of selenoamino acids into egg yolk proteins (Kroll and Doroshov, 1991). Upon being transferred excessively from dietary or maternal sources, some selenoamino acids (e.g., selenocystine) can be cytotoxic due to oxidative damage (Yan and Spallholz, 1993). It is also theorized that substitution of other selenoamino acids (e.g., selenomethionine) into proteins may alter their structure and therefore function (Lemly, 1998; Ohlendorf, 2003; but see Taylor et al., 1997), perhaps causing Se toxicity and teratogenesis.

Dietary and maternal transfer of Se have been well studied in birds (e.g., Heinz, 1996) and fish (e.g., Lemly, 1996), but little is known about the transfer and effects of Se in reptiles or amphibians. A limited number of field studies indicate that reptiles in Se-contaminated habitats accumulate high tissue burdens of Se (Hopkins et al., 1999; Nagle et al., 2001; Ohlendorf et al., 1988a). Although laboratory studies on reptile accumulation of Se are generally lacking, a suite of recent studies confirmed that trophic transfer is a significant route of exposure to Se (and other trace elements) for viviparous snakes (Hopkins et al., 1999, 2001, 2002; Jackson et al., 2003). Maternal transfer of Se has been documented in field-collected turtles (Nagle et al., 2001), but to our knowledge, no controlled laboratory study has

*Corresponding author. Fax: 803-725-3309.
E-mail address: hopkins@srel.edu (W.A. Hopkins).

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examined accumulation and subsequent maternal transfer of Se (or any trace element) in a reptile or amphibian (Campbell and Campbell, 2000, 2001; Linder and Grillitsch, 2000).

The current experiment was initiated to study the trophic and maternal transfer of Se in a reptile under captive conditions, enabling us to control the dose, chemical species, duration, and route of Se exposure. We fed Se-injected prey to female brown house snakes (*Lamprophis fuliginosus*) for 10 months and measured Se accumulation as well as survival, food consumption, growth, and body condition. In addition, we paired females with untreated males and quantified the number of females that reproduced, reproductive output of each female (number of clutches, total number of eggs, and total mass of combined clutches), and maternal transfer of Se to eggs.

2. Materials and methods

2.1. House snake natural history

L. fuliginosus is one of the most common terrestrial colubrid snakes in Southern Africa, with a range extending across the entire sub-Saharan continent (Branch, 1998). Adult *L. fuliginosus* prey upon a wide variety of organisms including lizards, birds, and even bats, but the bulk of their diet consists of rodents (Haagner, 1987).

The ability of *L. fuliginosus* to attain reproductive size quickly and to reproduce frequently make this species a logical starting point for controlled toxicological studies on snakes. The species is oviparous, laying clutches of up to 16 eggs, but usually fewer (Haagner, 1987). In the laboratory, reproduction requires no stimulatory environmental cues (e.g., change in photoperiod or temperature) and occurs year-round. Under optimal conditions in captivity, female *L. fuliginosus* can attain reproductive size within 10 months and produce eggs as frequently as 8 times a year (Neil Ford, personal observation). In contrast, most North American colubrid species require at least 2 years to reach reproductive maturity and will only produce 1–2 clutches per year.

2.2. Snake colony and pre-exposure husbandry

Adult house snakes were obtained from the Ophidian Research Colony at the University of Texas, Tyler, on 17 February 1999. Snakes were housed individually in 11.3-L plastic containers containing aspen shavings, a hidebox, and a water bowl. Because snakes rely on behavioral thermoregulation, each container was partially placed on Flexwatt Heat tape (Flexwatt Corporation, MA, USA) to provide a thermal gradient. Snakes were housed in a temperature-controlled laboratory (target: 27.5°C; actual range; 24.4–32.5°C), with a 12:12-h (light:dark) photoperiod, located in the Animal Care Facility of the Savannah River Ecology Laboratory (SREL) near Aiken, SC.

In August 2000, adult females and males were paired and allowed to breed. Females laid eggs in moist sphagnum moss within plastic brood chambers approximately 45–60 d following copulation. Eggs were removed from brood chambers within 48 h and transferred to incubators (27°C) containing moist vermiculite. Following a 52–80 d incubation period, eggs hatched and hatchlings were removed, sexed, and measured (mass and snout–vent length [SVL]). Female hatchlings were assigned unique ID numbers and transferred to individual 1.3-L plastic bins containing aspen shavings, a hidebox, and a water dish. For approximately 6 months, snakes were trained to eat previously frozen dead mice. Female snakes that did not readily eat dead prey were excluded from the study. In total, individuals representing 16 clutches collected from eight females were used in the study.

2.3. Study design

At the beginning of the experiment, all juvenile female snakes were measured (overall mean mass = 25.93 ± 0.22 g and SVL = 40.90 ± 0.50 cm) and then arbitrarily assigned to one of three feeding treatments ($N = 11$ subadult females per treatment). Arbitrary treatment assignment was conducted by placing all females in a single container and then blindly drawing snakes from the container for each treatment. Treatments were arranged on a laboratory shelf unit in a completely randomized design. Snakes were fed thawed mice injected with either a control amino acid solution (D,L-methionine dissolved in double-distilled water) or solutions containing Se, in the form of seleno-D,L-methionine (Sigma #S3875, St. Louis, MO, USA) to create three levels ($\sim$1, 10, and 20 μg/g dry mass) of Se exposure. Seleno-D,L-methionine was chosen over other chemical forms of Se because it is believed to be one of the primary organoselenium compounds trophically transferred from prey to vertebrate predators (e.g., Fan et al., 2002). The selenium concentrations chosen are within the range used in many assessments of avian Se-toxicity (Heinz et al., 1987; Hoffman and Heinz, 1988; Hoffman et al., 1991), are comparable to concentrations fed to snakes in our previous laboratory studies (Hopkins et al., 2001, 2002), and are within the range reported in mammalian prey items in Se-contaminated habitats such as Kesterson Reservoir (Clark, 1987). To verify Se concentrations resulting from injections into prey items, a subset of whole mice ($N = 8$ amino acid-injected and 14 per Se-injected treatment group) were digested and analyzed using inductively coupled plasma mass spectrometry

(ICPMS). Actual Se concentrations (mean ± SE) in the 1, 10, and 20 μg/g diets were 1.05 ± 0.08, 12.52 ± 0.32, and 22.95 ± 0.37μg/g, respectively.

The 10-month feeding study was divided into two phases, a 6-month growth phase and a 4-month reproductive phase. Over the entire study, snakes were weighed weekly, and SVL was determined every 2 months. During the first six months of the experiment, each snake received three meals a month, each ration equaling 25% of the individual snake's body mass. During the 4-month reproductive period, feeding frequency was reduced to twice a month due to reproductive activity (see below). Thus, all snakes were offered a total of 26 meals during the study.

After 6 months, female snakes were paired with mature male snakes that had not been previously exposed to elevated Se. Over this reproductive phase of the study, females had the opportunity to copulate with four different males per month, resulting in a total of 16 opportunities to copulate. Each breeding opportunity lasted 24 h, ample time for receptive females to respond to males (Walker and Ford, 1996).

Upon commencement of breeding, brood chambers (containing moist sphagnum moss) within each female's housing container were checked for eggs every 24–48 h. After oviposition, females were weighed and their SVL was determined. Clutches were removed from brood chambers and each egg was measured (length and mass). The viability of all eggs was determined by visual examination. A thin, brown membrane surrounds inviable eggs, whereas viable eggs have a chalky, white shell. All inviable eggs were immediately frozen (−70°C) for trace element analysis. Viable eggs were transferred to incubators containing moist vermiculite and maintained at 27°C. In cases where all or most of a clutch was viable, one egg was randomly selected at the time of oviposition and frozen (−70°C) for trace element analysis.

Eggs were checked weekly during the incubation period. Upon hatching, snakes were measured (mass and SVL) and their sex was determined. After being examined for morphological abnormalities, all hatchlings were sacrificed and frozen (−70°C) for trace element analysis.

2.4. Tissue collection

Adult female snakes ingested their final rations in September 2002. After digesting this meal, a subset of snakes ($N = 4$–6 per treatment) were euthanized using an overdose of anesthesia-grade ether. Final mass and SVL were determined for each snake prior to dissection. Gonads, kidney, and liver were removed from each snake. A small portion of each organ was preserved for histological analysis, which will be reported elsewhere. The remaining portion of each organ was frozen (−70°C) for future determination of trace element content.

2.5. Trace element analysis

Snake tissues (adult organs, eggs, and whole hatchlings) were lyophilized and homogenized before being digested and analyzed for trace element concentrations. Egg contents and shells were separated and only egg contents were digested and analyzed. A total of 117 eggs and 39 hatchlings were analyzed for Se content. Approximately 60–250 mg of sample was used for digestion; sample masses varied because large differences existed in dry masses among tissue types. Trace metal grade nitric acid (2.5–5.0 mL) was added to samples before digestion in a microwave (MDS 2000, CEM Corporation, 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, 0.5–1.0 mL of trace-metal-grade H_2O_2 was added to the samples and microwaved at the same power and duration as for the HNO_3 digestion. After digestion, samples were brought to a final volume of 10 or 25 mL (depending on tissue mass) with double-deionized water. Trace element analysis was performed by ICP-MS (Perkin Elmer, Norwalk, CT, USA) on samples diluted 1:1 with double-deionized water. External calibration standards covering a range of 1–500μg/L were prepared daily by serial dilution of NIST traceable primary standards. Matrix-matched standard addition curves ranging from 10 to 500μg/L (from NIST traceable primary standards) were also included in the calibration for each snake tissue analyzed. Certified reference material (Tort 2; NRC, Ottawa, 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 105.1%.

Detection limits (ng/g dry mass) for Se varied depending on the tissue being analyzed. Mean instrument detection limits for Se in liver, kidney, and ovaries were 0.231, 0.198, and 0.338 ng/g, respectively. Mean instrument detection limits of Se in eggs and hatchlings were 0.233 and 0.305 ng/g, respectively. All Se concentrations are presented on a dry mass basis.

2.6. Statistical analysis

Prior to all comparisons using analysis of variance (ANOVA), we tested the assumptions of normality and homoscedasticity. When necessary, data were $\log_{10}$ transformed to more closely adhere to assumptions of the statistical model. All statistical tests were conducted using SAS (SAS version 8.1, SAS Institute, Cary, NC, USA).

Two-way ANOVA was used to test for effects of food treatment and organ on accumulation of Se; concentrations of Se were $\log_{10}$ transformed prior to analysis.

We examined the effects of dietary Se exposure on female survival, sublethal responses (food consumption, growth, and body condition), and reproduction. Because survival rates were high in all treatments, we used Fisher's exact test to compare survival among feeding treatments. The total number and total mass of prey items refused by snakes was compared among treatments using multivariate analysis of variance (MANOVA) on $\log_{10}$ transformed data. Repeated measures ANOVAs on $\log_{10}$ transformed data were used to determine the effect of food treatment on changes in snake mass and SVL over the first 6 months of the study. Snake growth over the last 4 months of the study was not compared among treatments because body size of most individuals fluctuated dramatically due to reproductive activity.

We used ANCOVA to test for effects of food treatment on the relationship between mass and SVL at 6 months (i.e., snake body condition), with SVL included as a covariate in the model. Before analysis, snake mass and SVL were $\log_{10}$ transformed to increase linearity. Our comparisons using ANCOVA are analogous to traditional comparisons of power functions among food treatments (Ricker, 1979).

To investigate the effects of Se treatment on reproduction, we compared the number of females who reproduced and reproductive output (number of clutches, total number of eggs, and total mass of combined clutches) of females among treatments. A Fisher's exact test was used to compare the number of females in each treatment that laid eggs during the study. No reproductive output characteristics were related to snake body size (in all cases $r^2 < 0.01$, $P > 0.60$), probably due to the small range in body size of snakes in the study. Therefore, all reproductive characteristics were compared using statistical models independent of female body size. The numbers of clutches laid by females were compared among treatments using a Fisher's exact test. For each female, we summed all eggs and the mass of all eggs produced to determine total reproductive output during the 4-month reproductive phase of the study. Total reproductive output of females was compared among treatments using MANOVA on $\log_{10}$ transformed data.

To investigate the effects of dietary treatment on maternal transfer of Se to eggs, we compared Se concentrations in eggs among treatments using one way ANOVA on $\log_{10}$ transformed data. Before comparisons of egg Se content were made among treatments, we first confirmed that Se concentrations of viable and inviable eggs were similar (ANOVA comparisons of viable and inviable eggs within dietary treatments, $P > 0.20$; Table 1). Thus, Se content of inviable eggs accurately reflected maternal transfer of Se to viable eggs. For instances where only one egg was analyzed per female (because all eggs appeared viable at oviposition), the concentration of Se in that single egg was used in the statistical model. However, for most females, multiple eggs were analyzed for Se content. Because Se concentrations of individual eggs from a female were not independent of one another, we used the mean Se concentration of all eggs analyzed for a female in the statistical model. To ensure that there was no difference between Se content of first and second clutches, we compared Se concentrations among females who produced two clutches with repeated-measures ANOVA. Because only 49% of the eggs laid during the study were viable at oviposition, we did not statistically compare hatching success, hatchling size, or hatchling body burdens of Se among feeding treatments.

Table 1
Reproductive traits of female *L. fuliginosus* fed diets containing varying levels of Se (1–20 μg/g dry mass)

Reproductive parameter	Treatment		
	1 μg/g	10 μg/g	20 μg/g
%Reproductive females	91	60	73
Reproductive output per female:			
Total # clutches	1.18±0.18	0.90±0.28	1.18±0.26
Total # eggs	7.55±2.77	3.00±1.07	5.18±1.71
Mass of combined clutches (g)	51.04±12.99	31.76±11.16	42.62±11.40
Individual clutch characteristics:			
#Eggs/clutch	6.38±1.22	3.33±0.41	4.38±0.67
Clutch mass (g)	43.18±4.69	35.29±3.59	36.07±3.83
Egg characteristics:			
Egg length (mm)	37.68±1.69	57.69±2.98	47.10±1.85
Egg mass (g)	6.76±0.35	10.59±0.84	8.23±0.35
% viable at oviposition	60	0	59
% viable that hatched	76	N/A	76
Se content (μg/g dry mass)	0.92±0.02	12.03±0.33	22.65±0.49
Se content of viable eggs	0.88±0.04	N/A	22.57±1.00
Se content of inviable eggs	0.94±0.03	12.03±0.33	22.70±0.55

Note: With the exception of parameters expressed on a percentage basis, all data are presented as mean ±1SE.

3. Results

Snakes fed Se-contaminated prey accumulated significant concentrations of Se ($F_{2,33} = 191.08$, $P < 0.001$),

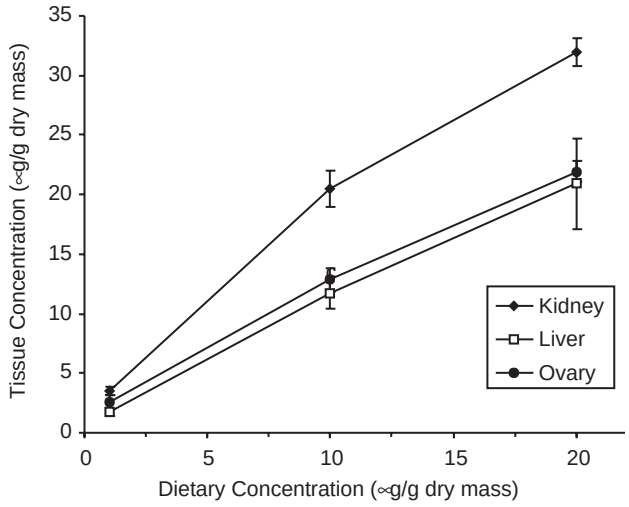

Fig. 1. Mean trace element accumulation in organs (μg/g dry mass) of female brown house snakes (*L. fuliginosus*) fed rodents containing different dietary concentrations of Se (1, 10, and 20 μg/g dry mass) for 10 months. Error bars represent ±1SE.

but Se accumulation differed among target organs ($F_{2,33} = 13.50$, $P < 0.001$, Fig. 1). Selenium accumulation in all organs was related to dose and was highest in kidneys (Fig. 1).

Survivorship did not differ among experimental treatments ($P = 0.99$). Only one snake died during the 10-month experiment; an individual from the 10 μg/g dietary treatment stopped eating and died 7 days before the end of the study. The individual that died was not included in any other statistical analyses. Overall, prey refusal seldom occurred among feeding treatments; of the 26 meals offered to snakes, approximately 2 meals were refused per snake (all treatments combined). The number and mass of prey refused did not vary significantly ($P = 0.532$) among treatments (mean number of meals refused: 1 μg/g = 1.36 ± 0.65, 10 μg/g Se = 2.20 ± 1.33, 20 μg/g Se = 2.73 ± 1.05; mean mass (g) of prey refused: 1 μg/g = 44.40 ± 18.57, 10 μg/g Se = 67.46 ± 23.31, 20μg/g Se = 91.27 ± 27.63).

Over the 6 month growth phase of the study, all snakes exhibited positive growth (mass: $F_{6,174} = 1508.12$, $P < 0.001$; SVL: $F_{3,87} = 777.77$, $P < 0.001$), but dietary treatment had no effect on changes in mass ($F_{2,29} = 0.07$, $P = 0.930$) or SVL ($F_{2,29} = 1.52$, $P = 0.236$; Fig. 2). Snout–vent length and mass were positively correlated ($F_{1,26} = 10.62$, $P = 0.003$); however, there was no significant effect of treatment on the relationship between mass and SVL (i.e., snake body condition; $F_{2,26} = 1.31$, $P = 0.288$).

Whereas 91% of females in the 1 μg/g treatment laid eggs at least once during the study, only 60 and 73% of females in the 10 and 20 μg/g Se treatments (respectively) produced eggs (Table 1); however, the number of females reproducing did not differ significantly among treatments ($P = 0.274$). The number of clutches produced ranged from 0 to 2 clutches per female and did not differ among feeding treatments ($P = 0.330$). Total reproductive output of females (total number of eggs and combined mass of clutches) during the study was also similar among treatments (Pillai's Trace = 0.081; $F_{4,58} = 0.61$, $P = 0.655$), but on average, females in the 1 μg/g treatment produced 82% more eggs (equaling 36% greater clutch mass) than females exposed to excessive Se (Table 1). Of the females who produced clutches in each treatment, the number (2–14 eggs/clutch) and size of eggs in each clutch were highly variable. The original snake population from the Ophidian Research Colony had been intentionally established with females with a variety of clutch and egg size characteristics, which underpins the variation observed. For descriptive purposes, we present a variety of clutch and egg characteristics in Table 1.

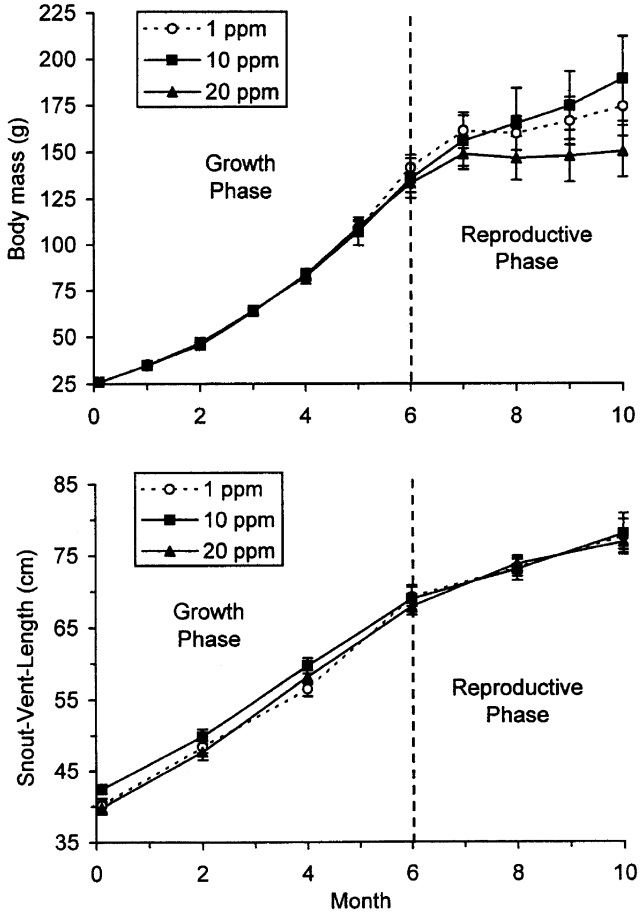

Fig. 2. Change in mean mass and snout-vent length of female brown house snakes (*L. fuliginosus*) fed rodents containing different dietary concentrations of Se (1, 10, and 20 μg/g dry mass) over the 6-month growth phase of the study. Error bars represent ±1SE.

Dietary treatment had a significant effect on the amount of Se transferred from females to their eggs ($F_{2,21} = 2,087.02$, $P < 0.001$), with maternal transfer of Se being dependent upon maternal dose (Table 1, Fig. 3). There was no difference in Se transfer between

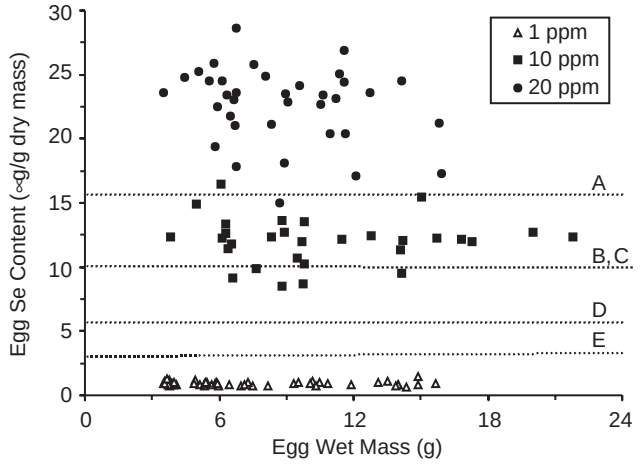

Fig. 3. Concentrations of Se in individual eggs resulting from maternal transfer from female snakes (*L. fuliginosus*) ingesting rodents containing different dietary concentrations of Se (1, 10, and 20 μg/g dry mass). Horizontal lines with corresponding letters represent toxic effects thresholds for reproductive toxicity presented by (A) Fairbrother et al. (1999), (B) Heinz (1996), (C) Lemly (1996), (D) Skorupa (1998), and (E) Skorupa and Ohlendorf (1991). A total of 116 eggs are presented ($N = 51$, 30, and 36 eggs in the 1, 10, and 20 μg/g Se treatments, respectively).

Table 2
Characteristics of hatchling *L. fuliginosus* from mothers fed diets containing 1 or 20 μg/g (dry mass) selenium

Hatchling characteristic	Treatment	
	1 μg/g	20 μg/g
SVL (cm)	20.32±0.16	21.82±0.40
Mass (g)	4.30±0.09	5.55±0.18
Sex ratio (M/F)	16/16	13/8
Se content (μg/g dry mass)	0.98±0.02	24.25±0.49

Note: No hatchling characteristics are presented for the 10 μg/g dietary treatment due to 100% inviability of eggs at oviposition for that treatment (see Table 1). SVL, mass, and Se content are presented as Mean ±1SE.

first and second clutches in females who produced two egg clutches ($F_{1,8} = 1.65$, $P = 0.235$).

During the reproductive phase of the study, the thermostat in the animal care facility failed to control our target temperature of 27.5°C. Instead, temperatures remained close to, and sometimes exceeded 30°C. *L. fuliginosus* is sensitive to these higher temperatures during egg formation; at temperatures above 29°C, viability of eggs decreases substantially (W. Hopkins, Personal observation; J. Hiduke, Personal communication). Unfortunately, 87 of 170 eggs (51%) were inviable at oviposition and no eggs from females in the 10 μg/g group were viable at oviposition (Table 1). The thermostat failure made it impossible to distinguish inviability attributable to elevated temperature from effects of dietary treatment. Because of these complications, we do not statistically compare egg viability, hatching success, and hatchling traits among treatments. Instead, we report descriptive statistics pertaining to egg viability and hatching in Table 1, as well as hatchling traits (SVL, mass, sex) and Se content of hatchlings in Table 2.

4. Discussion

The current study demonstrated that snakes accumulate Se from ingesting seleniferous prey, ultimately resulting in maternal transfer of potentially toxic quantities of Se to their offspring. Few previous studies have experimentally examined trophic uptake of contaminants in squamate reptiles (but see Hopkins et al., 2001, 2002), and our study is the first to document maternal transfer of an environmental contaminant in a reptile under controlled laboratory conditions.

4.1. Trophic transfer, survival, and sublethal effects

Snakes accumulated Se in a dose-dependent manner, and accumulation was higher in kidney than in liver or ovarian tissue. Although toxicity thresholds for Se in kidney tissue are not available for oviparous vertebrates, concentrations of Se in livers of snakes from the 20 μg/g dietary treatment exceeded levels known to cause deleterious sublethal responses in birds (Heinz, 1996). Moreover, concentrations of Se in livers of snakes from both the 10 and 20 μg/g dietary treatments equaled or exceeded levels known to be reproductively toxic to both fish and birds (Lemly, 1996; Heinz, 1996). Partitioning of higher concentrations of Se in to kidney compared to other organs is consistent with findings of a recent study on aquatic snakes fed seleniferous prey (Hopkins et al., 2002), but contrasts with findings from studies on birds. Most avian species exposed to excessive Se partition similar or higher concentrations of Se in liver tissue compared to kidney (Moksnes, 1983; Ohlendorf et al., 1988b; Sorenson, 1986). The observed differences in Se partitioning in snakes compared to birds could have important implications for target organ toxicity and are worthy of further investigation.

Despite the elevated concentrations of Se accumulated by snakes in our study, no adverse biological effects were documented. Snakes with high Se tissue burdens survived to the end of the study and exhibited normal food consumption, growth, and body condition. These findings were consistent with two previous laboratory studies on aquatic snakes exposed to excessive dietary Se that exhibited normal survival, food consumption, growth, and body condition compared to unexposed conspecifics (Hopkins et al., 2001, 2002; but see Rania et al., 2003 for description of histological abnormalities). In general, our findings were also consistent with most studies on birds that indicate

dietary organoselenium concentrations >40 μg/g are required to adversely affect growth and survival (reviewed in Ohlendorf, 2003). In contrast, there is some evidence that deleterious sublethal effects (i.e., effects not related to reproductive impairment) are expressed by fish exposed to concentrations similar to those used in our study. For example, fathead minnows (*Pimephales promelas*) exposed to diets containing 20 μg/g Se (as a mixture of selenite, selenate, and selenomethionine) exhibited reduced growth after 56 days of exposure (Ogle and Knight, 1989).

Previous investigations suggest that wildlife exposed to excessive Se can sometimes appear outwardly healthy, but adverse effects of Se manifest as reproductive impairment (e.g., Lemly, 1999). In both birds and fish, reproductive impairment due to Se toxicity generally occurs at lower dietary exposure levels than the levels required to affect growth and survival (Lemly, 1996; Ohlendorf, 2003). Thus, measurements of reproductive parameters may ultimately be the most sensitive indices of Se effects on wildlife.

4.2. Reproductive effects and maternal transfer

Selenium accumulation had no effect on reproductive output of females, but some statistically insignificant trends are worthy of further investigation. On the average, female snakes that accumulated Se from their prey were less likely to reproduce than snakes exposed to 1 μg/g Se (67% vs 91%, respectively). Similarly, total number of eggs and total mass of clutches produced by females in the 1 μg/g treatment were 20–150% greater than in Se-treated snakes. However, high variability in reproductive output among females made it difficult to discern meaningful trends from natural variability.

Although reproductive output of females was not significantly affected by dietary treatment, females exposed to Se transferred significant amounts of Se to their eggs. Maternal transfer of Se increased with dietary exposure and in both Se treatments, Se concentrations of eggs were nearly equivalent to dietary concentrations (Table 1). Similar relationships (i.e., 1:1 or 1:>1) between dietary Se content and deposition of Se in eggs have been described in a wide variety of bird species (Ohlendorf, 2003). In both the 10 and 20 μg/g Se treatments, maternal transfer resulted in Se concentrations in snake eggs that exceeded reproductive toxicity thresholds for fish (10 μg/g dry mass; Lemly, 1996). Although toxicity thresholds for Se in bird eggs are debated, concentrations of Se in eggs of snakes from the 20 μg/g Se treatment exceeded even the highest thresholds for avian reproductive impairment (Fig. 3). At egg concentrations of Se≥16 μg/g, most bird species experience developmental malformations and decreased hatching success (Fairbrother et al., 1999).

Although unfavorable temperature conditions in our animal care facility (see methods) prevented us from ascertaining whether dietary treatment affected egg viability or hatching success, some important information pertaining to effects of maternal transfer was collected from this initial study. Of the 43 and 29 viable eggs that were incubated in the 1 μg/g and 20 μg/g dietary treatments (respectively), 76% of eggs hatched in each treatment. Moreover, all 21 hatchlings from the 20 μg/g treatment appeared morphologically normal despite having mean whole body concentrations of Se>24 μg/g (Table 2). Similar concentrations in bird and fish hatchlings typically result in a variety of conspicuous developmental malformations (Ohlendorf, 2003). Future studies are needed to determine whether maternal transfer of similar concentrations of Se decreases the overall reproductive success of snakes and the health of their offspring.

4.3. Considerations for future research

The method of Se administration developed for this study was a significant step towards future evaluations of trace element toxicity in snakes. Previous laboratory exposures of fish and birds to Se have primarily relied upon mixing dissolved Se into a commercial feed (e.g., Heinz and Sanderson, 1990; Ogle and Knight, 1989). A common response of organisms in these studies is decreased food consumption, but the cause of diminished feeding remains unclear. Because stress can decrease appetite (Gregory and Woods, 1999), food avoidance could be a physiological response to ingesting Se-contaminated food. Alternatively, if fish and birds can detect Se in their food, food avoidance might result from unpalatability (Ogle and Knight, 1989), or conditioned aversion associated with illness after ingestion of seleniferous food (Heinz and Sanderson, 1990). Regardless of the reason for avoidance of seleniferous food, decreased food consumption clearly makes discerning direct effects of Se from effects of nutritional deficits difficult. Because snakes should not detect contaminants injected into prey items, our technique will enable investigators to distinguish between toxicological responses to dietary Se and issues related to food palatability. Indeed, food avoidance did not occur in our study, indicating that the Se concentrations administered did not affect the appetite of *L. fuliginosus*. Our technique has also recently been effectively adopted for administering Hg to snakes (Bazar et al., 2002) and shows promise for future ecotoxicological studies.

Our study also provides insight into complications associated with addressing toxicological effects of contaminants on reproductive parameters in some snake species. Although *L. fuliginosus* appeared to be a logical starting point for toxicological testing, high variation in reproductive output among females made it difficult to

discern responses to Se administration from variability in our colony. Such variation may be overcome in future studies using larger sample sizes or by using genetic strains of *L. fuliginosus* with less variation in reproductive traits, although the former is clearly constrained by logistical considerations. Alternatively, other species such as the corn snake (*Elaphe guttata*) are good candidates for toxicological studies because of their simple husbandry, but do not reproduce frequently like *L. fuliginosus*.

5. Conclusions

Over the last few years, environmental concerns over Se toxicity in wildlife have re-emerged and become a topic of substantial debate. Recent volumes in the literature (e.g., *Human and Ecological Risk Assessment*, 1999, Vol. 5, No. 6; *Aquatic Toxicology*, 2002, Vol. 57, No. 1) and sessions at national meetings (e.g., 1999 and 2002 meetings of the Society of Environmental Toxicology and Chemistry) concentrated on toxicity thresholds and whether current regulatory criteria are sufficient to protect wildlife. Unfortunately, these debates have focussed on birds and fish since studies on Se accumulation, maternal transfer, and toxicity have been conducted almost entirely on these organisms. Amphibians and reptiles have received little attention from scientists with regard to Se toxicology (Ohlendorf, 2003).

Our study clearly demonstrated that *L. fuliginosus*, like many bird and fish species studied to date, has the capacity to accumulate Se from Se-laden prey items and to transfer potentially toxic quantities of Se to their developing offspring. If current or revised regulatory criteria for Se are to be deemed protective of wildlife, generalizations for all wildlife species cannot be based solely on criteria for fish and birds (Hopkins, 2000). Thus, the scientific community must provide regulators with fundamental information on Se accumulation and toxicity in understudied organisms such as amphibians and reptiles. Such information is particularly important since amphibian and reptile populations appear to be declining around the world at alarming rates (e.g., Gibbons et al., 2000).

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