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Environmental Pollution 134 (2005) 447–456
ENVIRONMENTAL POLLUTION
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Transfer of selenium from prey to predators in a simulated terrestrial food chain

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Received 28 May 2004; accepted 10 September 2004

Partitioning of selenium among tissues differs between male and female lizards.

Abstract

Little is known about the accumulation and effects of selenium in reptiles. We developed a simplified laboratory food chain where we fed commercial feed laden with seleno-D,L-methionine (30 μg/g dry mass) to crickets (*Acheta domestica*) for 5–7 d. Se-enriched crickets (~15 μg/g Se [dry mass]) were fed to juvenile male and female lizards (*Sceloporus occidentalis*) for 98 d while conspecifics were fed uncontaminated crickets. Lizards fed contaminated prey accumulated Se concentrations ranging from 9.3 (in female carcass) to 14.1 (in female gonad) μg/g compared to <1.5 μg/g in tissues of controls. Female gonad concentrations approached the highest of thresholds for reproductive toxicity in oviparous vertebrates. However, we observed no consistent effect of dietary treatment on sublethal parameters or survival. Our simplified food chain proved to be an ecologically relevant method of exposing lizards to Se, and forms the foundation for future studies on maternal transfer and teratogenicity of Se.

Keywords: Selenium; Reptiles; Lizards; Trophic transfer; Food web

1. Introduction

Trophic transfer is one of the most ecologically important modes of exposure to contaminants for predatory wildlife. For certain contaminants such as Hg and Se, dietary exposure may represent the only appreciable pathway of contaminant uptake in terrestrial predators (Ohlendorf, 2003). Thus, understanding how contaminants traverse trophic levels and where contaminant burdens are partitioned within organisms is ultimately critical for assessing health risks in high trophic level consumers.

Laboratory feeding studies are crucial for understanding the process of trophic transfer in predatory vertebrates because they allow isolation of trophic exposure from other exposure pathways, facilitate control of the dose, duration, and chemical form of contaminant exposure, and allow rigorous quantification of effects associated with exposure (Hopkins et al., 2002, 2004). Laboratory approaches used to achieve these ends vary widely, but generally fall into three categories (Fig. 1) each of which has advantages and disadvantages. At one end of the continuum, forced ingestion of contaminants (e.g., gavage) allows precise and repeatable application of target doses, but is an ecologically unrealistic technique because it fails to

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doi:10.1016/j.envpol.2004.09.010

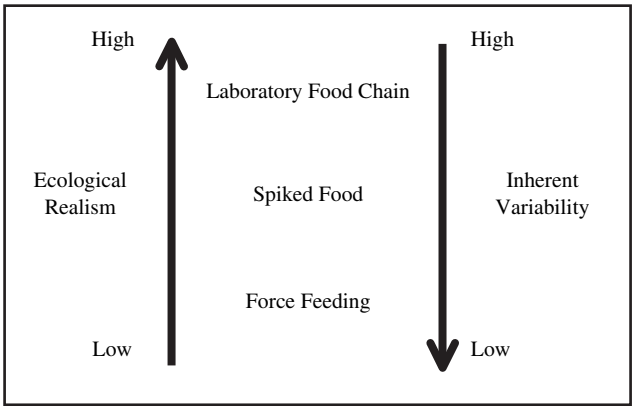

Fig. 1. Conceptual diagram outlining tradeoffs among approaches to laboratory feeding studies. Note that approaches that reduce variability tend to be less ecologically realistic, whereas more realistic approaches have greater inherent variability.

account for the voluntary feeding response of study animals. Because food consumption rates can be greatly altered in organisms exposed to contaminants (Bennet and Bennet, 1990; Hart, 1993), important responses resulting from toxicity or toxicant avoidance are not accounted for with this approach. Spiking food offers a reasonable alternative to force-feeding, but only in cases where the contaminant does not affect the palatability of food (Hopkins et al., 2004; Gregory and Wood, 1999; Ogle and Knight, 1989; Heinz and Sanderson, 1990). The most ecologically realistic approach is the laboratory food chain, where predators are exposed to contaminated prey. Prey items can be collected from known contaminated and reference sites and offered to predators in the laboratory (Hopkins et al., 2001, 2002; Rania et al., 2003; Jackson et al., 2003), but such approaches suffer from various logistical hurdles including fluctuating availability of prey items, highly variable contaminant concentrations in prey, and variable nutritional content (e.g., protein, lipid, and caloric content) of prey from contaminated and reference sites. Moreover, most contaminated field sites are polluted with multiple substances, often making the isolation of effects associated with a single contaminant difficult. However, these factors can be controlled using an alternative laboratory food chain approach, where prey items are reared on a contaminated diet prior to being offered to the predator. Although this approach has been used in aquatic systems (e.g., Besser et al., 1993), it has been infrequently adopted for terrestrial food webs.

In our study we used a simplified laboratory food chain to evaluate trophic transfer of Se from terrestrial prey to predators. We examined trophic transfer of Se from a Se-laden commercial feed to crickets (*Acheta domestica*), which were then preyed upon by juvenile western fence lizards (*Sceloporus occidentalis*). We compared Se accumulation patterns in tissues, survival, growth, and body condition of lizards between sexes and dietary treatments. We focus on Se because of its propensity to accumulate in food webs and well known teratogenicity in wildlife (Ohlendorf, 2003). Although dietary and maternal transfer of Se have been well-studied in birds (e.g., Heinz, 1996) and fish (e.g., Lemly, 1996, 1998), little is known about trophic transfer and effects of Se in reptiles or amphibians under controlled conditions (but see Hopkins et al., 2001, 2002, 2004).

2. Materials and methods

2.1. Method development and *A. domestica* exposure

Our first objective was to develop a logistically practical method of introducing Se into live prey of lizards. The house cricket (*A. domestica*) was selected as the dietary source because of their commercial availability, simple husbandry, and attractiveness as prey to lizards as well other vertebrate predators (e.g., some predatory fish and terrestrial amphibians). It is possible to simultaneously rear large numbers ($\sim$1000s) of *A. domestica* on various contaminant-laden diets, thus providing a reliable prey source for feeding studies.

For live prey to be logistically practical, it is important that the prey accumulate the contaminant of concern rapidly (e.g., $<$7 d), but without substantial effects on growth and survival. Therefore, we first conducted numerous range-finding experiments to examine the relationships among dietary Se, Se bioaccumulation, and adverse effects. *A. domestica* were reared for these pilot studies in 5 L plastic exposure chambers ($N = 12$ crickets/chamber; 6 replicates/treatment). Each chamber was placed on heat tape (Flexwatt, Inc) to maintain temperature within a target range of 28–30 °C. Chambers contained a damp sponge as a moisture source and a 10 ml glass Petri dish containing the appropriate food. Diets were formulated by grinding Iams Chunks® dog food (26% protein, 15% lipid, 4% fiber, and 4060 kcal/kg) into a fine powder, adding the appropriate amount of seleno-D,L-methionine (Sigma #S3875, St. Louis, MO, USA; Se treatments) or control amino acid solution (DL-methionine; hereafter controls) to the food matrix, and drying the food at 40 °C overnight. 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., Yamamoto et al., 1998; Fan et al., 2002). All dietary concentrations of Se were verified using an inductively coupled plasma mass spectrometer (ICP-MS; see methods below). Survival and growth (change in body mass) of *A. domestica* in each chamber were monitored daily and Se accumulation was determined at intervals leading up to 7 d.

After conducting numerous range-finding studies, we found that a diet containing 30 μg/g Se dry mass (measured concentration = 31.0 ± 1.7 μg/g; hereafter all Se concentrations are reported on a dry mass basis) for 5–7 d produced *A. domestica* whole body Se concentrations ranging from 14.8 to 16.0 μg/g with minimal effect on growth and survival. An example of Se uptake over this 7-d period in comparison to controls is presented in Fig. 2. Although Se bioaccumulation tended to be highly variable from 2 to 4 d (data not shown), asymptotic accumulation was always approached by day 5. After 7 d, exchanging the Se diet with the control diet for 24 h (depuration period; Fig. 2) suggested that most of the Se was incorporated into invertebrate tissue, rather than contained in the invertebrate gut. Importantly, adequate percent survival (control = 94% vs. Se = 82%; $P = 0.026$) and increase in mass (control = 87% vs. Se = 82%; $P = 0.580$) of *A. domestica* were maintained through 7 d using this Se exposure regime. The invertebrate Se concentrations (~15 μg/g) achieved with this protocol are within the range used in many assessments of the toxicity of Se to birds (Heinz et al., 1987; Hoffman and Heinz, 1988; Hoffman et al.,1991) and are within the range reported in terrestrial invertebrates in Se-contaminated habitats such as restored grasslands near Kesterson Reservoir (Wu et al., 1995).

Feeding *A. domestica* to lizards for prolonged amounts of time required that the invertebrates be maintained in large-scale exposures to their appropriate diet. For the lizard feeding study (described below), we exposed juvenile *A. domestica* in groups of 1000 in 50 L exposure chambers containing cardboard egg crates (for cover), two glass feeding dishes (50 ml) containing the appropriate diet, and moist sponges. Temperature was maintained in the large exposure chambers with full spectrum heat lamps (temp gradient in chambers = 25–35 °C). New exposures were initiated every 1–2 d, creating a continuous cycle of *A. domestica* that had been exposed to their appropriate diets for 5–7 d. During the course of the lizard feeding study, a subsample of *A. domestica* was removed every 2–3 d from the large exposure chambers to verify Se concentrations fed to lizards.

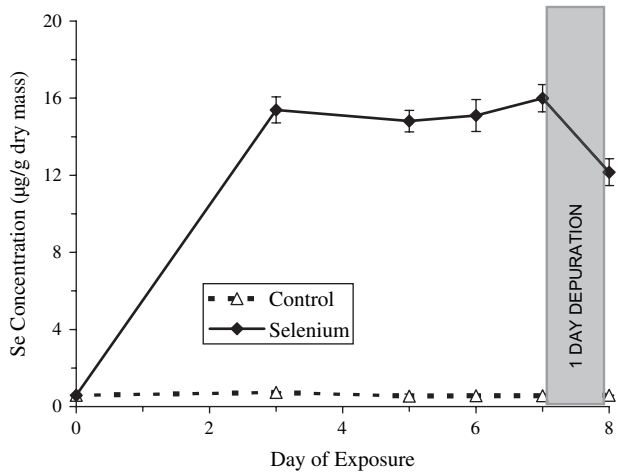

Fig. 2. Selenium accumulation (μg/g dry mass) in *Acheta domestica* over a 7-d exposure period to either 30 or <1 μg/g dietary Se as seleno-D,L-methionine (selenium and control, respectively). *A. domestica* fed the seleniferous diet were also transferred to the control diet for a 24-h depuration period. Data are presented as mean ± 1 SE.

2.2. Fence lizard natural history

Sceloporus belong to the family Phrynosomatidae, which accounts for more than one-third of lizard species in the United States. The genus is one of the most common and widespread in the United States. Some *Sceloporus* are good study organisms because their entire lifecycle is manageable in the laboratory, and a great deal is known about their ecology, physiology, performance, and life history (Bennett, 1980; Huey and Dunham, 1987; Garland et al., 1990; Sinervo and Adolph, 1989; Van Berkum et al., 1989; Sinervo and Huey, 1990; Sinervo, 1990; Sinervo et al., 1991; Sinervo and Losos, 1991; Huey et al., 1990; Angilletta et al., 2002).

S. occidentalis appears to be a particularly tractable species with attributes that predispose it to be a good model for laboratory experiments. In a recent study that examined multiple ($N = 7$) species and populations of *Sceloporus*, Talent et al. (2002) determined that *S. occidentalis* collected from the San Joaquin Valley grasslands ranked highest in a variety of attributes that make it attractive for use in captive studies. Individuals from this population are relatively large bodied (up to 20 g), making blood collection possible without harm to the animal. They thrive under captive conditions and exhibit lower mortality and higher growth rates than the other *Sceloporus* examined. Most females reach sexual maturity in 6 months under ad libitum feeding conditions in the laboratory, and lay 3–6 clutches of 8–15 eggs per year (Talent et al., 2002). For studies of Se toxicity, the San Joaquin Valley grassland lizard population is also of great environmental relevance because it occurs within regional proximity (within 100 km) of areas contaminated by seleniferous agricultural drainage that have been the focus of extensive research with other wildlife species (reviewed in Ohlendorf, 2003).

2.3. Lizard colony and pre-exposure husbandry

The original parental stock of *S. occidentalis* used in this study originated from the San Joaquin Valley grasslands population described above. On 11 Jan 2002, 128 hatchling lizards (2–3 g) representing the F1

generation, were shipped to the Savannah River Ecology Laboratory (SREL; near Aiken, SC, USA) from a breeding colony at Oklahoma State University. Upon arrival, each lizard received a unique toe clip as a permanent identifying mark. Lizards were housed in pairs in 52 × 36 × 18 cm plastic cages with screen lids arranged in a rack system. The racks were maintained in a temperature and photoperiod controlled room (12:12 h [light:dark]), located in the Animal Care Facility of SREL. Each cage contained sand, a hidebox, a water dish, a dish filled with a calcium supplement (RepCal), and a basking platform situated under a full spectrum spotlight (60 W). Because lizards rely on behavioral thermoregulation, a daytime temperature gradient (~30–42 °C) was maintained within each cage (nighttime temperature = 25 °C).

To stimulate rapid growth and sexual maturation (Talent et al., 2002), we subjected all lizards to an artificial over-wintering period. Beginning on 17 Feb 02, each lizard was fasted for 5 d prior to being placed in a perforated 15 × 15 cm plastic container containing dry sand and a water dish. Plastic containers were placed in complete darkness in an environmental chamber, where temperature was gradually dropped to 9 °C over a 10 d period. Water was refreshed and survival was assessed weekly. After 42 d in the environmental chamber, temperature was gradually increased and lizards were returned to their respective experimental cage, where they were fed uncontaminated crickets ad libitum until initiation of the experiment. The hibernation and holding period enabled us to select 60 of the most robust lizards for the study.

2.4. Lizard feeding study

After 3 weeks of recovery from the over-wintering period, male and female lizards were randomly assigned to either a control or Se dietary treatment ($N = 15$/sex/dietary treatment). Each lizard was weighed and snout–vent length (SVL) measured prior to being introduced to individual experimental cages. Photoperiod, heat source, and cage set up were identical to that described above. The 60 cages were arranged in a completely randomized design in the rack system. Because temperature fluctuated from the bottom (cooler) to the top (warmer) of our rack system by as much as 5 °C, cage positions were rotated weekly such that each lizard spent an equivalent amount of time at each shelf elevation over the course of the study.

Critical to the success of controlled studies of trophic transfer is rigorous control over food resources. To this end, lizards were weighed every Monday morning to monitor growth and to determine their daily food ration for the upcoming week. Lizards received a ration equaling 5% of their body mass 4 d a week (Mon–Thurs), and on the fifth day (Fri) each lizard received a ration equaling 10% of its body mass. Thus, our feeding regime resulted in a weekly ration of 30% of each animal's body mass and allowed lizards to void their gut contents prior to each week's mass determination. *A. domestica* dusted with vitamins (RepCal Herptivite) were counted and weighed to the nearest 1.0 mg prior to each feeding. The following day, all remaining *A. domestica* were removed, counted, and weighed.

After 98 d on their respective diets, all lizards were measured (mass and SVL) before being euthanized by etherization. Gonads and liver were removed from each lizard, and the mass of each organ as well as the remaining carcass was recorded. A small portion of each tissue type was flash frozen in liquid nitrogen for chemical speciation, which will be reported elsewhere. The remaining portion of each tissue was frozen (−70 ° C) for future determination of trace element content. In addition, a portion of the tail (mean = 0.6 g, 74.3 mm) was removed from each carcass and frozen separately to determine whether the Se concentration of the tail could be used as a nondestructive predictor of dietary exposure history (Hopkins et al., 2001; Jackson et al., 2003).

2.5. Trace element analysis

Lizard tissues (carcass, gonads, liver, and tail tissue) were lyophilized and homogenized before being digested and analyzed for trace element concentrations. Approximately 5–210 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 (HNO_3; 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 hydrogen peroxide (H_2O_2) was added to the samples and microwaved at the same power and duration as the HNO_3 digestion. After digestion, samples were brought to a final volume of 5.0–25.0 ml (depending on tissue mass) with double-distilled water. Trace element analysis was performed by ICP-MS (Perkin Elmer, Norwalk, CT, USA) on samples diluted 1:1 with double-distilled water. External calibration standards covering a range of 1–500 μg/L were prepared daily by serial dilution of NIST traceable primary standards. Matrix-matched standard addition curves ranging from 10 to 500 μg/L (from NIST traceable primary standards) were also included in the calibration for each lizard 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 104%.

Mean instrument detection limits for Se in liver, ovaries, testes, carcass, and tail tissue varied from 0.23 to 0.56 ng/g dry mass, 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 assumptions of normality and homoscedasticity. When necessary, data were 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) and statistical significance was judged at $P < 0.05$.

A three-way ANOVA was used to test for effects of food treatment, tissue (carcass, liver, gonad), and sex on accumulation of Se in lizards; concentrations of Se were $\log_{10}$-transformed prior to analysis. Total body concentrations (μg/g) and total body burdens (μg) were estimated using individual tissue concentrations and tissue masses. Total body concentrations and burdens were then $\log_{10}$-transformed and compared among treatments and sexes using two-way ANOVAs. To determine whether sex or dietary treatment influenced the partitioning of Se among different body compartments (carcass, liver, gonad), we determined the percentage of the total body burden accounted for by each compartment. We then compared these percentages between treatments and sexes using a series of two-way ANOVAs (for each tissue type) on arcsin square root-transformed data.

To determine whether small portions of tail tissue might serve as a nondestructive index of Se exposure, we used nonparametric discriminant function analysis (Hopkins et al., 2001). 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.

We examined the effects of dietary Se exposure and sex on lizard survival, and sublethal responses (food consumption, growth, and body condition). The total % of prey items (on a mass basis) refused by lizards was compared among treatments and sexes using ANOVA on arcsin square root-transformed data. A repeated measures-ANOVA on $\log_{10}$-transformed data was used to determine the effect of food treatment and sex on changes in lizard mass over the 98-d study. Final body condition index (BCI) was calculated as $BCI = (Mass/SVL^3) \times 10^6$ (Romero and Wikelski, 2001) before being $\log_{10}$-transformed and compared among treatments and sexes using two-way ANOVA.

3. Results

Concentrations of Se in *A. domestica* sampled weekly from the large exposure chambers during the lizard experiment (Fig. 3) corroborated concentrations generated in our smaller scale studies (Fig. 2). Selenium concentrations in individual subsamples of *A. domestica* ranged from 9.7 to 23.5 μg/g, with daily means ranging from 13.5 (day 5) to 15.2 (day 7) μg/g and an overall mean of 14.7 μg/g. In contrast, *A. domestica* raised on the control diet maintained very low Se (ranging from 0.06 to 0.80 μg/g, overall mean of 0.55 μg/g; Fig. 3).

Lizards fed Se-contaminated prey accumulated significant concentrations of Se (Diet: $F = 3490.11$, $P < 0.001$), but Se accumulation was dependent on tissue (Diet × Tissue: $F = 15.65$, $P < 0.001$) and influenced by sex (Tissue × Sex: $F = 9.29$, $P < 0.001$; Fig. 4). In carcass, control males and females had similar Se concentrations but Se-exposed males had higher carcass concentrations than Se-exposed females (Diet: $P < 0.001$; Diet × Sex: $P = 0.021$). In liver, males tended to have slightly higher Se concentrations than females regardless of dietary treatment (Diet: $P < 0.001$; Sex: $P = 0.045$). In contrast, males had lower Se concentrations in gonads than females in both dietary treatments (Diet: $P < 0.001$; Sex: $P = 0.006$). Reconstruction of whole body Se concentration (μg/g) and

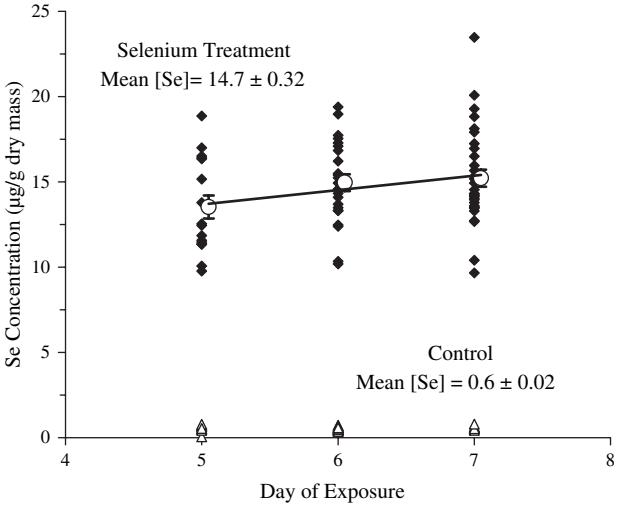

Fig. 3. Selenium accumulation (μg/g dry mass) in *Acheta domestica* after 5–7 d exposure period to either 30 or <1 μg/g dietary Se as seleno-D,L-methionine (selenium and control, respectively). Closed diamonds (selenium) and open triangles (control) represent individual subsamples retrieved every 2–3 d over the course of the 98 d trophic transfer experiment on lizards. Open circles represent mean ± 1 SE of Se-fed invertebrates at day 5–7. Overall mean of all subsamples for the two dietary treatments are presented as text in the figure.

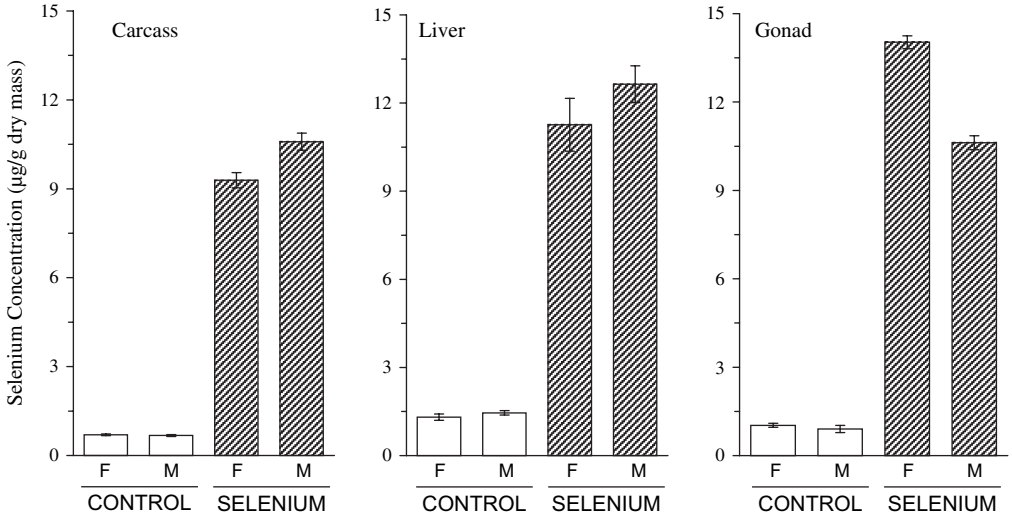

Fig. 4. Mean selenium accumulation in organs (μg/g dry mass) of female (F) and male (M) fence lizards (*Sceloporus occidentalis*) fed *Acheta domestica* containing different concentrations of Se (<1 and 15 μg/g dry mass; control and selenium, respectively) for 98 d. Error bars represent ±1 SE.

whole body burden (μg) indicated that both differed by dietary treatment ($P < 0.001$ in both cases) but not by sex ($P > 0.196$ in both cases; Fig. 5). On a percent of total body burden basis, partitioning of Se among tissues differed by sex and dietary treatment (Fig. 6). Both sexes partitioned 2–5% of their Se burden into liver (Sex: $P = 0.826$), but controls partitioned about twice as much of their Se burden in hepatic tissue compared to lizards fed Se (Diet: $P < 0.001$). Regardless of dietary treatment, females partitioned approximately 31–34% of their Se burden into ovarian tissue, whereas males only partitioned ~1% into their testes (Diet: $P = 0.605$; Sex: $P < 0.001$; Fig. 6). The remaining ~95% and 65% of the Se body burden was partitioned in the carcass of males and females, respectively (Diet: $P = 0.533$; Sex: $P < 0.001$; Fig. 6).

Concentrations of Se in tail tissue were much higher in animals exposed to excessive Se (7.6 ± 0.22 μg/g Se) compared to controls (0.5 ± 0.02 μg/g Se). Posterior probability error rates from nonparametric discriminant function analysis indicated that analysis of tail tissue was powerful for predicting exposure to Se, with <0.001% chance of misclassification. Cross-validation tests based on Se concentration in tail tissue revealed that all 60 individuals were properly classified by dietary exposure history (i.e., the analysis enabled us to accurately discriminate between treatments 100% of the time based upon tail concentration).

Despite the accumulation of Se in tissues, lizards in both treatments appeared healthy. All 60 lizards survived until the end of the study and reached sexual maturity (based on secondary sexual characteristics and gross gonad morphology). Mass of food refused by lizards was low in both sexes and treatments, with mean % refusal ranging from 7.3 ± 1.19% to 8.3 ± 0.65% across sexes and treatments (for both sex and diet,

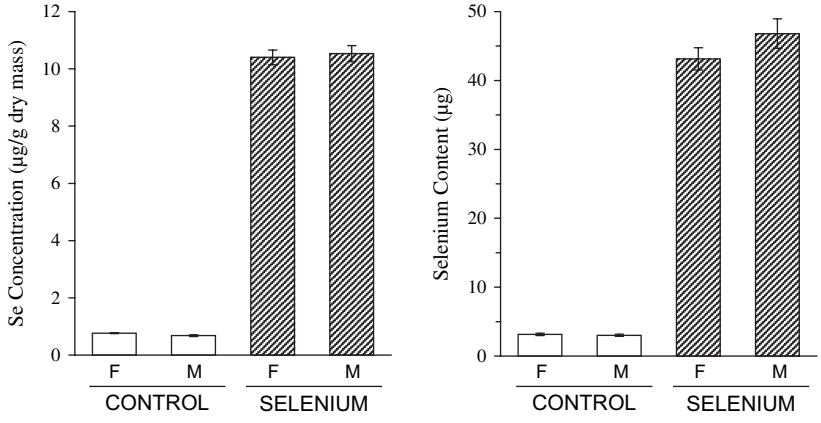

Fig. 5. Mean whole body selenium concentration (μg/g dry mass) and content (μg) of female (F) and male (M) fence lizards (*Sceloporus occidentalis*) fed *Acheta domestica* containing different concentrations of Se (<1 and 15 μg/g dry mass; control and selenium, respectively) for 98 d. Error bars represent ±1 SE. Concentrations and content were estimated by reconstructing whole bodies using the concentrations and masses of individual body components.

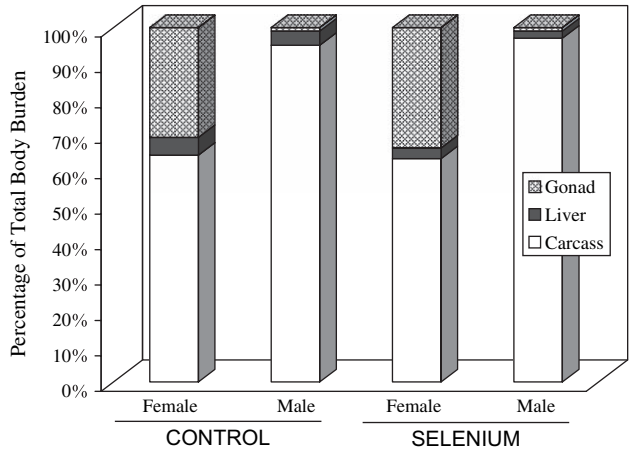

Fig. 6. Influence of sex and dietary Se content on Se partitioning in *Sceloporus occidentalis* expressed as a percentage of the total Se body burden.

$P > 0.721$). Male lizards attained greater mass than females ($P = 0.014$), but growth was not influenced by the amount of Se in the diet ($P = 0.696$; Fig. 7). Body condition was influenced by dietary Se, but this effect was dependent upon sex (Diet × Sex, $P = 0.029$). Females exposed to excessive Se had increased BCI compared to controls, whereas the opposite was true for males (Fig. 8).

4. Discussion

4.1. Trophic transfer

Our study demonstrated that lizards accumulate Se from ingesting seleniferous prey, ultimately resulting in potentially hazardous tissue concentrations. Few previous studies have experimentally examined trophic

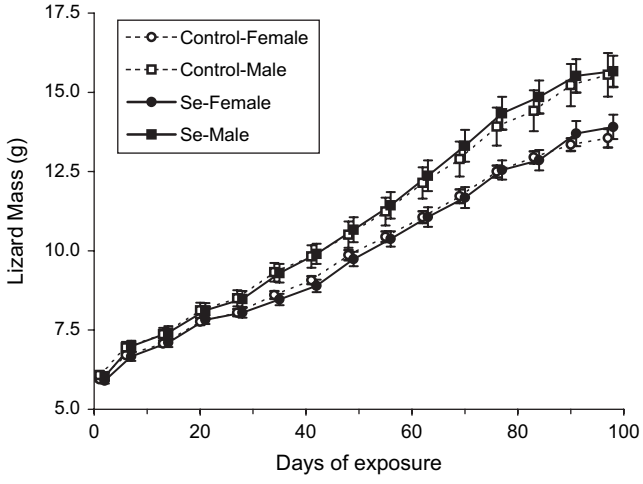

Fig. 7. Change in mean mass of male and female fence lizards (*Sceloporus occidentalis*) fed *Acheta domestica* containing different concentrations of Se (<1 and 15 μg/g dry mass; control and selenium, respectively) for 98 d. Error bars represent ±1 SE.

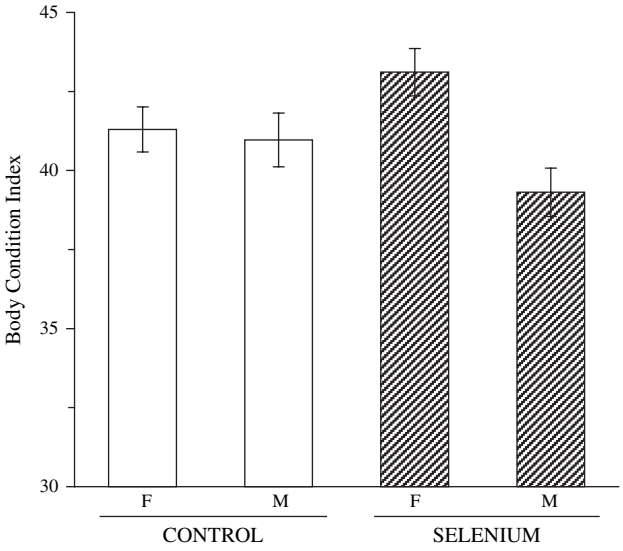

Fig. 8. Final body condition index of male and female fence lizards (*Sceloporus occidentalis*) fed *Acheta domestica* containing different concentrations of Se (<1 and 15 μg/g dry mass; control and selenium, respectively) for 98 d. Data are presented as mean ± 1 SE.

uptake of contaminants in squamate reptiles (but see Hopkins et al., 2001, 2002, 2004), and our study is the first to document trophic transfer of a trace element in a lizard using a laboratory-based food chain.

Lizards fed seleniferous *A. domestica* accumulated Se concentrations in liver and gonads that approached levels of toxicological concern. Concentrations of Se in livers (~12 μg/g) of lizards from the Se dietary treatment equaled those known to be reproductively toxic to both fish and birds (Lemly, 1996; Heinz, 1996). Although maternal transfer was not evaluated in this study, females exposed to Se accumulated Se concentrations in reproductive tissue that were equivalent to dietary concentrations. 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). Trophic transfer resulted in Se concentrations in lizard ovarian tissue that exceeded reproductive toxicity thresholds for fish ovaries (10 μg/g dry mass; Lemly, 1996). Although toxicity thresholds for Se in bird eggs and ovaries are debated (Fairbrother et al., 1999; Adams et al., 2003; Ohlendorf, 2003), concentrations of Se in ovarian tissue of lizards approached even the highest recommended thresholds for avian reproductive impairment (EC 50 of 12–15 μg/g dry mass for embryo viability; Adams et al., 2003).

Partitioning of Se among tissues was particularly interesting when Se content was considered as a percentage of the total body burden. Regardless of dietary exposure, sexually mature females partition a much greater proportion of their total Se burden to ovaries than males do to testes. This relationship is a result of the combined influence of Se gonad concentration and

the mass of the gonads; females had higher gonadal Se concentration as well as gonad mass compared to males. The differential partitioning of Se between sexes in the control treatment suggests that ovarian tissue may contain selenoproteins not present (or in greater concentration) in testicular tissue.

In recent years, some debate has emerged as to whether Se biomagnifies in the food chain (e.g., Wu, 2004). The current study, in conjunction with our previous work (Hopkins et al., 2001, 2002, 2004), indicates that although Se is readily bioaccumulated in high trophic level reptiles, it does not biomagnify under the exposure conditions we examined. On a dry mass basis, a diet containing 30 μg/g Se as seleno-D,L-methionine fed to *A. domestica* for 5–7 d resulted in invertebrate Se concentrations of ~15 μg/g. Subsequent trophic transfer to lizards for 98 d resulted in whole body Se concentrations of 10.5 μg/g. Although our previous work did not estimate whole body concentrations of Se in snakes, target organ concentrations of Se were generally ≤ Se concentrations in the snakes' diet (Hopkins et al., 2001, 2002, 2004). Our field work indicates that snakes accumulate much higher target organ tissue concentrations of Se (~150 μg/g dry mass; Hopkins et al., 1999) than in the laboratory (24 μg/g dry mass; Hopkins et al., 2002). We initially hypothesized that these field-captured snakes primarily fed on amphibians and fish with whole body Se concentrations reaching 25 μg/g dry mass (Hopkins et al., 1999). However, more recent work at our field site indicates that some potential prey items have much higher whole body concentrations of Se. For example, metamorphic *Rana sphenocephala* and adult *Gastrophryne carolinensis* contained up to 57 and 100 μg/g Se, respectively (unpublished data). Unfortunately, the precise diet and exposure history of field-captured snakes remains unknown, further illustrating the value of our laboratory-based food chain approach as a complement to field studies.

4.2. Nondestructive tissue sampling

The results of this study further indicate that small amounts of tissue can be collected from reptiles as nondestructive indices of Se exposure. Selenium concentrations in the tails of lizards exposed to excessive Se were an order of magnitude higher than controls, and were used to statistically classify all 60 experimental animals by their Se exposure history. This is the first use of such a procedure for examining Se exposure in a lizard, but we have previously demonstrated that similar techniques can be performed by sampling blood, shed skins, and tail tissue from semiaquatic and terrestrial snakes (Hopkins et al., 2001, in press; Jackson et al., 2003). We also recently developed mathematical equations describing the functional relationships among Se concentrations in the diet, target tissues (e.g., liver, gonads), and tail tissue of snakes (Hopkins et al., in press). Using Se concentrations in the tail and target tissues of lizards from the current study, we can demonstrate that these nonlinear equations have strong predictive value for squamates exposed to Se. For example, the models predict that a squamate reptile with a Se concentration of 7.6 μg/g in the tail (the mean concentration in lizard tails) should have 12.5 and 11.7 μg/g Se in the liver and gonad tissue, respectively. Actual mean Se concentrations in liver and gonad tissue were 12.0 and 12.3 μg/g, respectively. Given that the actual values were within 0.6 μg/g of the predicted Se concentration, this initial validation suggests that our models hold much promise and warrant further study.

4.3. Survival and sublethal effects

Despite the elevated concentrations of Se accumulated by lizards in our study, no adverse biological effects were documented. Lizards with high Se tissue burdens survived to the end of the study and exhibited normal food consumption and growth. Body condition was influenced by Se exposure, but the effect was the opposite for males and females making the biological significance of the finding unclear. Overall, our results were consistent with three previous laboratory studies on snakes exposed to excessive dietary Se that exhibited normal survival, food consumption, growth, and body condition compared to controls (Hopkins et al., 2001, 2002, 2004). However, these snakes did exhibit histological abnormalities (Rania et al., 2003). In general, our findings were also consistent with most studies on birds that indicate dietary organoselenium concentrations >40 μg/g (dry mass) are required to adversely affect growth and survival (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 d of exposure (Ogle and Knight, 1989).

Wildlife exposed to excessive Se can sometimes appear outwardly healthy, but adverse effects of Se may manifest as reproductive impairment (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. Based on the Se concentrations found in ovarian tissue of lizards exposed to Se, future studies

are needed to examine the reproductive consequences of maternal transfer in this species.

5. Conclusions

Over the last few years, environmental concerns over Se toxicity in wildlife have re-emerged and become a topic of substantial debate. Unfortunately, amphibians and reptiles have received little attention from scientists with regard to Se toxicology (Ohlendorf, 2003), despite the fact that many herpetofauna occur in regions where Se pollution is a known environmental problem. In particular, studies are needed that provide insight into the accumulation patterns and responses of herpetofauna relative to other more commonly studied vertebrate wildlife (e.g., fish and birds; Hopkins, 2000). As illustrated herein, laboratory food chain approaches are powerful in this regard because rigorous quantification of effects can be achieved in relation to known exposure history. Such studies will help to determine whether current regulatory criteria are protective of herpetofauna, which is particularly important since amphibian and reptile populations appear to be declining around the world at alarming rates (Gibbons et al., 2000).

Acknowledgement

Justin Congdon and Tony Mills provided advice or technical assistance during portions of the study. This research was supported by the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, U.S. Department of Energy through the Financial Assistant Award no. DE-FC09-96SR18546 to the University of Georgia Research Foundation and through an award by the Long Range Research Initiative of the American Chemistry Council to Oklahoma State University.

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