DEVELOPMENT OF A REPTILE MODEL FOR ASSESSING ENVIRONMENTAL CONTAMINANTS

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
July, 1995

OKLAHOMA STATE UNIVERSITY

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ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. Larry G. Talent, for his friendship and many contributions to my professional and personal development. I am particularly grateful for his guidance, support, and patience during this study.

I thank my committee members Dr. S. L. Burks, Dr. C. V. Maxwell, and Dr. W. D. Warde for their advice, patience, and for service as members of my graduate committee. Dr. S. L. Burks provided constructive advice on the project design of the selenium study. Guidance and comments on the design of the diet studies were given by Dr. C. V. Maxwell. The assistance provided by Dr. Bill Warde in the project design and statistical analysis is appreciated.

Acknowledgement is due to Sarah Kimball and Elaine Stebler of the OSU Water Quality Research Laboratory for the analysis of selenium in the substrate and comments on the data analysis, respectively.

Funding was partially provided by the Department of Zoology, Oklahoma State University.

Finally, I thank my daughter, Katrina, for her inspiration throughout the project. Thanks also to my mother, Bonnie R. Rich, for her support and encouragement.

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CHAPTER I

INTRODUCTION

Assessing the risk of exposure to chemical substances that are potentially harmful is a complex task. The process involves identifying the potential hazards to exposure by collecting relevant toxicity data, determining the dose - response relationship, and estimating a safe dose. Criteria for establishing acceptable risk levels should be established using species that are representative of the group of organisms of interest. Species tested should represent the ecological, physiological, and taxonomic diversity of the organisms at risk (Urban and Cook, 1986).

Traditionally, a variety of mammal and bird species have been used for establishing safety criteria for terrestrial vertebrates. Although numerous reptile taxa are included in the list of endangered and threatened wildlife species (USFWS, 1990), reptiles are not represented among vertebrates currently tested. The assumption has been that protective criteria developed from testing various mammal and bird species would also be appropriate for other vertebrate classes, including reptiles (Urban and Cook, 1986). Extrapolating between species is difficult since even closely related species may have significantly different sensitivities (Lu, 1991; Chadwick et al., 1993; Meyers - Schone et al., 1993). The extrapolation of results across vertebrate classes is of questionable value.

Reptiles are probably not adequately protected by criteria based on mammal and bird tests for several reasons. Reptiles are ectothermic while test species traditionally used are endothermic. Also, reptiles have lower metabolic rates and therefore may be more sensitive to chemical substances that are readily metabolized and detoxified by endothermic species with higher metabolic rates (Hall, 1980). This hypothesis has been supported by several preliminary investigations in which various reptile species exhibited increased sensitivity to pesticides when compared to endothermic species (Rosata and Ferguson, 1968; Hall, 1980).

Reptiles accumulate residues of various contaminants in a variety of tissues (Ohlendorf et al., 1988; Skaare et al., 1991; Burger, 1992; Meyers - Schone et al., 1993). In some cases, residue levels reported in reptiles are higher than those reported for other vertebrates (Hall, 1980). However, in the absence of controlled laboratory testing, the significance of these levels and the impact on reptiles is not known.

Pre - registration testing of chemical toxicity to reptiles has been proposed by the EPA (Hall and Henry, 1992). However, test species and standard protocols have not been determined. Therefore, there is a need to compare sensitivities of reptiles to hazardous chemicals to the data available for other vertebrates.

The purpose of this study was to evaluate the effect of a contaminant on a reptile test species. Selection of a species requires knowledge of the factors that determine growth and good health. The study included selecting a test species and evaluating the effects of various dietary and environmental housing conditions on growth performance of the selected species. This information is prerequisite to establishing standards of care for a species in captivity. Subsequently, the influence of a test substance (selenium) on growth was evaluated in a short - term growth study. The dose - response data was compared with available data from the literature for other vertebrates.

This dissertation is composed of 8 manuscripts written in the format required for submission to scientific journals. Each manuscript is written as a complete article and does not require supporting materials, except for acknowledgements which are presented separately.

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CHAPTER II

Food Consumption, Growth, and Gross Conversion Efficiency of Western Fence Lizards (Sceloporus occidentalis) on Cricket Versus Mealworm Diets

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INTRODUCTION

Lizards are frequently maintained and propagated under artificial conditions requiring dietary manipulation. Due to the difficulty of providing "natural" diets, captive animals are often fed substitute prey items which are more readily available and can be easily maintained as a food source for captive populations. Two of the most frequently used prey items for feeding captive lizards are crickets (Acheta domestica) and mealworm larvae (Tenebrio molitor).

Although both are commonly fed to lizards, mealworm larvae are generally regarded as being less suitable for maintaining healthy lizards and crickets are often preferred (Wagner, 1980; Wynne, 1981). Lizards fed mealworms are thought to develop "intestinal clogging" and to suffer inhibited digestive function (Mattison, 1982). However, this opinion lacks supportive data. In addition, some evidence suggest that mealworm diets may even produce better growth in juvenile toads, (Bufo woodhousei) than other prey items, including crickets (Claussen and Layne, 1983).

The objective of this study was to compare consumption, growth and gross conversion efficiency of hatchling Western fence lizards (Sceloporus occidentalis) fed mealworms or crickets as single prey item diets.

MATERIALS AND METHODS

Hatchling lizards were obtained from a captive reared population of Western fence lizards originally started with lizards captured near Red Bluff, California. Each hatchling was weighed to the nearest 0.0001 g and SVL was measured to the nearest mm. Then each hatchling was paired with a sibling by sex, when possible, within 24 hours of hatching. A total of 20 individuals were used to form 10 pairs consisting of 4 male pairs, 5 female pairs, and 1 male-female pair. One member of each pair was randomly assigned to either the mealworm (M) or cricket (C) diet. The other member was assigned the remaining diet.

Lizards were housed individually in standard animal cages with inside dimensions of 28(L) X 17.5 (W) X 12.5 (H) cm. Cages of paired individuals were placed adjacent to one another and were provided continuous light and heat by a 40 watt incandescent bulb at one end. The thermal gradient in each cage ranged from about 35 degrees C beneath the light bulb to 26 degrees C at the opposite corner. Room temperature ranged between 23-27 degrees C.

All crickets and mealworms used in this study were from breeding colonies maintained at OSU. Both prey items were reared on the same commercially supplied chicken feed ration. Prey were weighed to the nearest 0.0001 g, dusted with Nekton-Rep vitamin mix and offered to the lizards each morning. Prey items were left in the cages for 8 hours, removed and reweighed. The difference was then calculated as an estimate of prey biomass consumed by each lizard. After removal of prey items, a small dish containing water and a dish containing a vitamin-mineral mix were placed in each cage and left until the next feeding.

Average prey live weights used ranged from 0.0091 to 0.0559 g for crickets and from 0.0128 to 0.0752 g for mealworm larvae. Prey sizes were increased as lizards grew.

Each lizard was weighed to the nearest 0.0001 g and SVL measured to the nearest mm at hatching and then at weekly intervals for 6 weeks. Gross conversion efficiency was determined weekly by measuring live weight consumption and live weight gain and calculating grams gained per gram consumed.

The means for weight, SVL, prey consumption, percentage body mass consumed, and gross conversion efficiency of lizards on the cricket and mealworm diets were compared by paired samples t test analysis. Cumulative average daily gain and average daily consumption were also calculated and compared between treatments. Statistical analyses were performed using SYSTAT (Wilkinson, 1989).

RESULTS

Fence lizards survived and grew on both diets. Hatchlings on the mealworm diet had significantly ($p<0.05$) greater mass after the second week of the study (Table 1). The average SVL for the mealworm fed group was greater throughout the study, however significant differences in SVL were not observed (Table 2). Hatchling average daily gain was significantly ($p<0.05$) greater for the mealworm fed group after the second week of the study (Table 3).

Average daily prey consumption was significantly ($p<0.05$) greater for hatchlings on the cricket diet after the second week of the study (Table 4). Cumulative prey consumption per gram body weight (Table 5) and weekly prey consumption as percent body weight (Table 6) were also significantly ($p<0.05$ and $p<0.001$, respectively) higher for the cricket fed group after the first week of the study. Mean gross food conversion efficiencies (g gained per g consumed) were significantly ($p<0.05$) greater for the mealworm fed group after the second week of the study (Table 7).

DISCUSSION

Growth rates of both the mealworm and cricket fed hatchlings were generally equal to or greater than those observed for *Sceloporus undulatus*, *S. graciosus*, and *S.*

occidentalis reported in other studies (Fitch, 1940; Tinkle and Ballinger, 1972; Ferguson and Brockman, 1980; Sinervo and Adolph, 1989). Therefore, the diets used in this study were capable of sustaining growth rates comparable to those reported for this species and other related species.

The greater weight growth observed for hatchlings fed the mealworm diet may be due to the higher fat content of mealworms. Mealworm larvae may contain as much as 44% fat (Dimmitt and Ruibal, 1980) whereas crickets contain only about 18% (Modzelewski and Culley, 1974). Diets with higher fat content have been associated with improved weight gain and feed efficiency (Staton and Edwards, 1987; Jorgensen, 1989).

Live weight consumption of crickets was significantly greater than that of mealworms. Western fence lizards on the cricket diet averaged weekly consumption ranging from 64 to 103% of their body mass while lizards on the mealworm diet had significantly lower consumption rates ranging from 40 to 79% of their body mass. These ranges are comparable to those reported by Boykin (1992) for the northern plateau lizard (Sceloporus undulatus elongatus).

Hatchlings fed crickets tended to eat sooner and more regularly than those offered mealworm larvae. Prey consumption differences may have been due to either differences in prey activity differences or differences in prey caloric contents. Predator responses of insectivorous lizards have been reported to vary with prey activity type and level resulting in greater consumption of more active prey types (Claussen and Layne, 1983; Gluesing, 1983; McGovern et al., 1986). The greater fat content of mealworm larvae (Dimmitt and Ruibal, 1980) may also account for prey biomass consumption differences. Hatchlings fed crickets must consume more biomass to meet the same energy requirement as hatchlings fed mealworms.

Increased biomass consumption may account for the lower gross conversion efficiency observed for hatchlings fed crickets. Higher gross conversion efficiency of mealworm diets compared to cricket diets has also been reported by Claussen and Layne (1983).

In summary, both prey items appear to support fence lizard growth. No negative effects of mealworm diets were observed during this study. Although length growth did not differ, weight growth was greater for the mealworm fed group. Further evaluation of the impact of various prey items on long term maintenance and reproduction are needed.

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Table 1. Mean mass (g) and SEM of Western fence lizards on cricket and mealworm diets. N = 10 for each diet.

	DAY						
Diet	0	7	14	21	28	35	42
Cricket	0.741 (0.021)	0.848 (0.028)	1.256 (0.043)	1.601* (0.065)	1.893* (0.083)	2.369* (0.158)	2.736* (0.236)
Mealworm	0.748 (0.024)	0.897 (0.041)	1.410 (0.075)	1.830 (0.081)	2.367 (0.143)	2.761 (0.173)	3.369 (0.246)

* Significant difference ($p<0.05$) between cricket and mealworm treatment means using paired t test.

Table 2. Mean SVL (mm) and SEM of Western fence lizards on cricket and mealworm diets. N = 10 for each diet.

	DAY						
Diet	0	7	14	21	28	35	42
Cricket	27 (0.213)	29 (0.365)	32 (0.306)	36 (0.496)	38 (0.473)	40 (0.727)	42 (1.087)
Mealworm	28 (0.300)	30 (0.342)	33 (0.482)	37 (0.526)	39 (0.767)	42 (0.872)	44 (1.055)

Table 3. Mean average daily gain (g/day) and SEM of Western fence lizards on cricket and mealworm diets. N = 10 for each diet.

	DAY					
Diet	7	14	21	28	35	42
Cricket	0.015 (0.004)	0.037 (0.003)	0.041* (0.003)	0.041* (0.003)	0.047* (0.005)	0.048* (0.006)
Mealworm	0.021 (0.007)	0.047 (0.005)	0.051 (0.004)	0.058 (0.006)	0.058 (0.005)	0.062 (0.006)

* Significant difference ($p<0.05$) between cricket and mealworm treatment means using paired t test.

Table 4. Cumulative average daily prey consumption (g prey live weight consumed/day) and SEM of Western fence lizards on cricket and mealworm diets. N = 10 for each diet.

	DAY					
Diet	7	14	21	28	35	42
Cricket	0.078 (0.007)	0.130 (0.006)	0.166* (0.008)	0.187* (0.010)	0.211* (0.012)	0.226* (0.015)
Mealworm	0.072 (0.011)	0.111 (0.010)	0.128 (0.009)	0.143 (0.009)	0.149 (0.010)	0.158 (0.011)

* Significant difference ($p<0.05$) between cricket and mealworm treatment means using paired t test.

Table 5. Cumulative consumption per g body weight (g prey live weight consumed/g body weight) and SEM of Western fence lizards on cricket and mealworm diets. N = 10 for each diet.

	DAY					
Diet	7	14	21	28	35	42
Cricket	0.643	1.446*	2.169*	2.766*	3.145*	3.530*
	(0.045)	(0.036)	(0.033)	(0.054)	(0.083)	(0.104)
Mealworm	0.541	1.667	1.903	2.045	2.201	2.226
	(0.065)	(0.065)	(0.062)	(0.078)	(0.075)	(0.069)

* Significant difference (p<0.05) between cricket and mealworm treatment means using paired t test.

Table 6. Weekly consumption as % body weight (g consumed/g body weight) and SEM of Western fence lizards on cricket and mealworm diets. N = 10 for each diet.

	DAY					
Diet	7	14	21	28	35	42
Cricket	64.3 (4.525)	100.8* (2.752)	103.1* (2.659)	92.7* (3.858)	89.6* (2.852)	76.2* (3.183)
Mealworm	54.1 (6.463)	73.9 (2.536)	62.0 (1.459)	54.7 (2.060)	43.7 (1.882)	40.3 (2.464)

* Significant difference ($p<0.001$) between cricket and mealworm treatment means using paired t test.

Table 7. Mean gross conversion efficiency (g gained/g consumed) and SEM of Western fence lizards on cricket and mealworm diets. N = 10 for each diet.

	DAY					
Diet	7	14	21	28	35	42
Cricket	0.166 (0.042)	0.281 (0.011)	0.245* (0.010)	0.218* (0.007)	0.217* (0.011)	0.205* (0.012)
Mealworm	0.139 (0.156)	0.283 (0.029)	0.312 (0.021)	0.335 (0.023)	0.331 (0.020)	0.348 (0.019)

* Significant difference ($p<0.05$) between cricket and mealworm treatment means using paired t test.

CHAPTER III

Growth of Leopard Geckos (Eublepharis macularius) on Single and Combination Prey Diets

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INTRODUCTION

The leopard gecko (Eublepharis macularius) is an easily maintained, long-lived lizard species that offers potential as a laboratory reptile. Although several captive, self-sustaining populations of this species have been reported, little experimental data is available regarding basic requirements for growth and maintenance of healthy individuals.

One of the most important considerations in maintaining captive animals is diet. The natural diet of leopard geckos reportedly includes a variety of insects, scorpions, lizards, and rodents (Miller, 1980a; Nuygen, 1985). Captive geckos have been maintained succcessfully on various prey items including house crickets (Acheta domestica), neonatal mice (Mus musculus), and mealworm larvae (Tenebrio molitor) (Wagner, 1974; Thorogood and Whimster, 1978; Hingley, 1985; Nuygen, 1985). Although information on leopard gecko growth in relation to diet is anecdotal, some suggest that the optimal diet should include crickets and small mice (Miller, 1980a; Wagner, 1980) and that mealworm larvae should be offered sparingly (Miller, 1980b; Wagner, 1980). Thorogood and Whimster (1978) however, recommend feeding mealworm larvae to young geckos but only occasionally to adults.

It is apparent that leopard geckos will survive in captivity on a variety of prey items. However, no information is available on the influence of any prey item on leopard gecko growth. If the leopard gecko is to be used effectively as a lab animal, quantitative approaches to dietary studies are needed. Therefore, because house crickets and mealworms are readily available and are commonly used as food for captive lizards, we evaluated their effect on leopard gecko growth. Our study was designed to evaluate growth of leopard geckos maintained on single prey item diets, either mealworms or crickets, and on a mixed prey item diet including both prey types.

MATERIALS AND METHODS

Sixty female juvenile leopard geckos, produced by the breeding colony at Oklahoma State University, were used in this study. Geckos were housed in groups of five individuals per cage. The cages were opaque plastic animal cages with inside dimensions of 28 L x 17.5 W x 12.5 H cm. Each cage was covered with a 3 mm steel mesh lid. A 40 watt incandescent light bulb with an aluminum reflector was positioned at the corner of each cage to provide a temperature gradient of 27 - 35 degrees C within each cage. A photoperiod of LD 14:10 was provided and room temperature was maintained at 27 ± 1 degree C throughout the study.

Each individual lizard was marked by toe-clipping and randomly assigned to one of three diet groups, i.e. cricket (Acheta domestica), mealworm larvae (Tenebrio molitor), and combined cricket and mealworm diets. Food items and water were provided ad libitum. The diet of all geckos was supplemented with a vitamin - mineral mix consisting of 5% $CaPO_4$, 5% $CaCO_3$, and 90% sand with 200 units of vitamin D_3 and 100 international units of vitamin A.

Prior to the study, the geckos were maintained on a mixed prey diet of crickets and mealworm larvae offered ad libitum. At day 0 of the study, all leopard geckos were weighed to the nearest 0.1 g and snout - vent length (SVL) was determined to the nearest mm. The average juvenile weight and SVL at day 0 were 90 mm and 17.1 g, respectively. Body weight and SVL were then measured for each individual at weekly intervals for the 8 weeks of the study period.

Statistical analyses were performed using Systat (Wilkinson, 1989). Mass and average daily gain were analyzed by analysis of covariance using day 0 mass as the covariate. When F values were significant ($p<0.05$) individual treatment mean comparisons were made using linear contrasts. SVL differences were evaluated by analysis of variance.

RESULTS

There was no significant difference throughout most of the study between mean mass of juvenile leopard geckos on the cricket or mealworm diets (Table 1). However, geckos on the combined cricket and mealworm diet increased in mass significantly ($p<0.05$) more than geckos on the cricket diet at various times during the first 35 days of the study. By day 56 of the study, however, there were no significant differences ($p>0.05$) in mass between treatments.

SVL differences followed the same trend as mass (Table 2). Geckos receiving the combined diet had greater SVLs than juveniles on the single prey diets throughout the study but the difference was not stastically ($p>0.05$) significant. In addition, there was no significant difference in gecko SVL between the two single prey treatments.

Average daily gain in mass (ADG) differed significantly ($p<0.05$) occasionally among treatments at various times up to day 42 of the study (Table 3). ADG for the combined diet treatment was slightly greater than that of either single prey treatment throughout the study. However, over the entire 56 day study period the differences in ADG among diet treatments were not statistically ($p>0.05$) significant.

DISCUSSION

Juvenile leopard geckos exhibited good growth on all dietary treatments. Throughout the study those on the combined prey diet consistently weighed more than those on the single prey item treatments. However, by the end of the study, the weight differences were not significantly different between the combined prey treatment and the two single prey treatments. Length growth differences were not as apparent between treatments but would not be expected to occur as rapidly as weight differences. Longer studies may be required to more fully evaluate the effect of these treatments on length growth.

Between the two single prey treatments, average hatchling weight tended to be greater for those receiving mealworms only. However, these differences were too small to be statistically significant. The supposedly deleterious effect of feeding mealworms as single prey items was not observed in this short-term study.

Treatment differences had a rather early and pronounced effect on average daily gain (ADG). The mean ADG of those on the combined prey treatment was significantly greater ($p<0.05$) at the end of the first week but the differences diminished and were sporadic beyond that point. By the end of the study there was no significant effect on ADG due to any of the three treatments used.

In summary, our short-term study suggests that a combination of prey items is preferrable if maximal growth is desired. These findings are in agreement with those of

Vogel et al. (1986) in which juvenile _Anolis lineatopus_ fed combination prey diets gained more weight than those on single prey diets. We also conclude that mealworm larvae, even as single prey items, may not be deleterious to leopard geckos as has been previously suggested (Miller, 1980b; Wagner, 1980). However, long-term studies from hatching to maturity will be required to fully elucidate the effect of food items on leopard gecko growth. Future efforts should also be directed toward other aspects of dietary treatment such as the frequency of feedings, food consumption, and food conversion efficiency.

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Table 1. The change in mean mass (g) of juvenile leopard geckos fed crickets (C), mealworms (M), and both crickets and mealworms (CM). Treatment means within a column that have the same superscript are not significantly different ($p<0.05$). N = 20 for all means. Standard errors are in parentheses.

	Day								
Treatment	0	7	14	21	28	35	42	49	56
C	16.1^{a} (0.523)	17.8^{a} (0.541)	18.8^{a} (0.519)	20.1^{a} (0.516)	21.9^{a} (0.540)	24.1^{a} (0.583)	25.9^{a} (0.627)	25.9^{a} (0.659)	26.9^{a} (0.667)
CM	18.4^{b} (0.818)	21.1^{b} (0.852)	21.7^{a} (0.830)	24.1^{b} (0.933)	25.8^{b} (0.943)	28.2^{b} (1.073)	29.5^{a} (1.155)	29.4^{a} (1.169)	30.5^{a} (1.165)
M	16.8^{ab} (0.668)	19.1^{b} (0.770)	19.7^{a} (0.743)	21.7^{ab} (0.736)	23.2^{ab} (0.743)	25.4^{ab} (0.713)	27.1^{a} (0.674)	26.7^{a} (0.692)	27.1^{a} (0.642)

Table 2. The change in mean snout - vent length of juvenile leopard geckos fed crickets (C), mealworms (M), and both crickets and mealworms (CM). N = 20 for all means. Standard errors are in parentheses.

	Day								
Treatment	0	7	14	21	28	35	42	49	56
C	90	91	93	96	98	100	102	103	104
	(0.822)	(0.802)	(0.848)	(0.739)	(0.772)	(0.701)	(0.800)	(0.876)	(0.860)
CM	91	94	95	98	100	102	104	105	106
	(1.146)	(1.180)	(1.082)	(1.082)	(1.002)	(1.110)	(1.009)	(1.095)	(1.106)
M	90	92	94	97	98	100	102	103	104
	(0.990)	(0.974)	(0.918)	(0.806)	(0.803)	(0.788)	(0.809)	(0.707)	(0.723)

Table 3. Mean cumulative average daily gain (grams/day) of juvenile leopard geckos fed crickets (C), mealworms (M), and crickets and mealworms (CM). Treatment means within a column that have the same superscript are not significantly different ($p<0.05$). N = 20 for each treatment. Standard errors are in parentheses.

	Day							
Treatment	7	14	21	28	35	42	49	56
C	0.25^{a} (0.012)	0.19^{a} (0.012)	0.19^{a} (0.010)	0.21^{a} (0.010)	0.23^{a} (0.010)	0.24^{a} (0.009)	0.20^{a} (0.200)	0.19^{a} (0.007)
CM	0.39^{b} (0.039)	0.23^{a} (0.024)	0.27^{b} (0.022)	0.26^{b} (0.015)	0.28^{b} (0.016)	0.26^{a} (0.015)	0.22^{a} (0.224)	0.22^{a} (0.012)
M	0.33^{b} (0.025)	0.21^{a} (0.018)	0.23^{ab} (0.012)	0.23^{b} (0.022)	0.25^{ab} (0.008)	0.25^{a} (0.008)	0.20^{a} (0.203)	0.18^{a} (0.007)

Chapter IV

Food Intake, Gross Conversion Efficiency, and Growth of Leopard Geckos (Eublepharis macularius) on Cricket Versus Mealworm Diets

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INTRODUCTION

Diet is one of the most important components of successful propagation. Reptile diets are particularly difficult to manage since many species require live or freshly killed prey. Few studies have dealt with the effect of specific live prey diets on reptile growth under laboratory conditions (Allen et al., 1986; Vogel et al., 1986; Scudder-Davis and Burghardt, 1987; Xiang et al., 1993). Maintaining and propagating reptiles often requires substituting available prey items, which may be readily purchased or easily propagated, for natural prey.

Among the more frequently used prey for feeding lizards are crickets (Acheta domestica) and mealworm larvae (Tenebrio molitor). Crickets are often preferred while mealworms are generally considered less acceptable (Wagner, 1980; Wynne, 1981). Lizards fed mealworms exclusively reportedly develop "intestinal clogging" (Mattison, 1982). However, Claussen and Layne (1983) reported that juvenille toads (Bufo woodhousei) grew better on mealworm diets than on cricket diets.

The leopard gecko (Eublepharis macularius) is a species with potential as a laboratory reptile and has been successfully maintained and propagated under a variety of artificial conditions (Thorogood and Whimster, 1978; Miller, 1980a; Wagner, 1974). Most investigators recommend using mealworm larvae sparingly, if at all (Miller, 1980b; Wagner, 1980). However, the available information is anecdotal and not supported by experimental evidence. Controlled studies demonstrating the effects of different prey types on leopard gecko growth are not available.

In this study, we compare growth of gecko (Eublepharis macularius) hatchlings on cricket (Acheta domestica) or mealworm larvae (Tenebrio molitor) diets.

METHODS

Hatchling geckos were obtained from the breeding colony maintained at Oklahoma State University. All hatchlings used were from eggs incubated at 28 degrees C. Each gecko was weighed to the nearest 0.01 g and SVL measured to the nearest mm within 24 hours of hatching. A total of 40 individuals were marked by toe-clipping and were assigned to 20 pairs with one member of each pair randomly assigned to either the cricket or mealworm diet. The other member of each pair was assigned to the remaining diet. Crickets and mealworms were from colonies maintained at OSU on the same commercially supplied chicken ration.

Each gecko hatchling was housed individually in an opaque plastic animal cage with inside dimensions of 28 (L) X 17.5 (W) X 12.5 (H) cm. Each cage contained a shelter and water dish. All cages were provided with tops made of 3 mm steel mesh. A 40 watt incandescent light bulb with an aluminum reflector was placed at one corner of each cage to provide a temperature gradient of 27 - 35 degrees C. Room temperature was maintained at 27 ± 1 degree C and a LD 14:10 photoperiod was used throughout the study.

Each gecko was weighed to the nearest 0.01 g and SVL measured to the nearest mm at weekly intervals for the 6 months of the study. Prey items were weighed to the nearest 0.0001 g and offered ad libitum. Water was also available ad libitum. Prey consumption was determined weekly for each individual gecko by counting the number of prey consumed per individual and multiplying the number consumed by the average prey mass (live weight, g). Prey size was increased as lizards grew.

The means for monthly mass, SVL, prey biomass consumption, prey biomass consumption on a body weight basis, average daily gain (ADG) and gross conversion efficiency (GCE) of lizards on the cricket and mealworm diets were compared by paired samples t test analysis. All statistical analyses were performed using SYSTAT (Wilkinson, 1989).

RESULTS

Both diets supported the growth of leopard geckos. Geckos on the cricket diet had significantly ($p<0.05$) greater mass for the first 3 months of the study, but mass differences were not significant thereafter (Table 1).

Snout - vent length differences were greater for the cricket fed group throughout the study but only significantly so ($p<0.05$) during months 1 through 4 (Table 2). Although the average SVL was still greater for the cricket fed group at the end of the study, the differences were not statistically significant ($p = 0.259$).

The average daily consumption of crickets was statistically significantly greater than mealworms ($p<0.01$) thoughout the study (Table 3). When adjusted for lizard mass, (g prey consumed per g body mass) greater monthly prey consumption was still significant ($p<0.001$) for the cricket fed group (Table 4).

Leopard gecko average daily gain (g/day) was significantly ($p<0.05$) higher for the cricket fed group during the first 3 months of the study but differences were not statistically significant thereafter (Table 5).

Mean gross conversion efficiencies (GCE) differed significantly ($p<0.001$) between dietary treatments (Table 6). The mealworm fed geckos had greater GCE throughout the study.

DISCUSSION

Although diet did affect growth during our study, the mean masses at six months achieved by geckos on both treatments were greater than the maximum of 27.6 g reported from a seven month study by Allen et al. (1986).

Geckos on the cricket diet consumed significantly more biomass than those on the mealworm diet throughout the study. However, greater biomass consumption did not result in improved growth performance beyond the first three months of the study. Similar results were obtained in a previous study conducted on Western fence lizards (Sceloporus occidentalis) using mealworm and cricket diets (Rich, 1995).

The greater food intake of geckos on the cricket diet may have been due to (1) differences in response to different prey types or prey activities and (2) differences in prey item caloric values. Geckos offered crickets tended to eat sooner after hatching and to maintain consumption more regularly. In contrast, geckos on the mealworm diet were slower to begin eating and tended to consume their prey less regularly. Although individual responses varied somewhat, geckos on the cricket diet typically responded faster and more vigorously to the presence of crickets in their cages while geckos on the mealworm diet appeared less responsive to the presence of mealworms. The influence of prey activity on predator response has been reported for other insectivorous lizards which also tended to consume greater quantities of more active prey types (Gluesing, 1983; McGovern et al., 1986). Mealworms reportedly have greater caloric content per unit dry weight and also less water content than crickets. Mealworms may contain as much as 44% fat (Dimmitt and Ruibal, 1980; Xiang et al., 1993) while crickets contain less than 20% fat (Modzelewski and Culley, 1974). Therefore, greater biomass consumption of crickets would be required for the geckos to meet the same energy requirement. The increased biomass consumption may also account for the lower gross conversion efficiency of geckos fed crickets. The higher gross conversion efficiency of mealworm diets compared to cricket diets has also been reported by Claussen and Layne (1983).

In conclusion, both prey items supported leopard gecko growth. The supposedly negative effects of mealworms as a single prey item diet were not observed during this study. Future efforts are needed to evaluate the impact of various prey diets on long term maintenance and reproduction.

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Table 1. Mean mass (g) and SEM of leopard geckos on cricket and mealworm diets. N = 20 for each diet.

	MONTH						
Diet	0	1	2	3	4	5	6
Cricket	3.66 (0.124)	8.81* (0.250)	15.91* (0.336)	21.95* (0.584)	26.85 (0.693)	31.06 (0.817)	35.36 (0.935)
Mealworm	3.66 (0.124)	8.01 (0.338)	14.2 (0.493)	19.91 (0.641)	25.22 (0.846)	31.26 (0.891)	36.49 (1.076)

* Significant difference ($p<0.05$) between cricket and mealworm treatment means using paired t test.

Table 2. Mean SVL (mm) and SEM of leopard geckos on cricket and mealworm diets. N=20 for each diet.

	MONTH						
Diet	0	1	2	3	4	5	6
Cricket	54 (0.616)	72* (0.686)	85* (0.698)	94* (0.859)	101* (0.966)	107 (1.074)	113 (1.038)
Mealworm	54 (0.706)	69 (0.939)	82 (0.853)	90 (0.916)	98 (0.970)	105 (1.151)	111 (1.105)

* Significant difference ($p<0.05$) between cricket and mealworm treatment means using paired t test.

Table 3. Average daily prey consumption (g/day) and SEM of leopard geckos on cricket and mealworm diets. N=20 for each diet.

	MONTH					
Diet	1	2	3	4	5	6
Cricket	0.434* (0.022)	0.561* (0.017)	0.582* (0.021)	0.581* (0.022)	0.584* (0.021)	0.581* (0.019)
Mealworm	0.268 (0.015)	0.338 (0.013)	0.369 (0.013)	0.387 (0.014)	0.408 (0.014)	0.415 (0.014)

* Significant difference ($p<0.01$) between cricket and mealworm treatment means using paired t test.

Table 4. Mean prey consumption on a body weight basis (g prey consumed/g body weight) and SEM of leopard geckos on cricket and mealworm diets. N=20 for each diet.

	MONTH					
Diet	1	2	3	4	5	6
Cricket	1.367* (0.042)	1.208* (0.037)	0.787* (0.029)	0.600* (0.017)	0.536* (0.018)	0.447* (0.014)
Mealworm	0.935 (0.029)	0.810 (0.014)	0.606 (0.017)	0.488 (0.014)	0.441 (0.013)	0.343 (0.011)

* Significant difference ($p<0.001$) between cricket and mealworm treatment means using paired t test.

Table 5. Mean average daily gain (g/day) and SEM of leopard geckos on cricket and mealworm diets. N=20 for each diet.

	MONTH					
Diet	1	2	3	4	5	6
Cricket	0.184* (0.008)	0.219* (0.007)	0.218* (0.008)	0.207 (0.007)	0.196 (0.006)	0.189 (0.006)
Mealworm	0.155 (0.010)	0.188 (0.008)	0.193 (0.007)	0.192 (0.007)	0.197 (0.006)	0.195 (0.006)

*Significant difference ($p<0.05$) between cricket and mealworm treatment means using paired t test.

Table 6. Mean gross conversion efficiency (g gain/g consumed) and SEM of leopard geckos on cricket and mealworm diets. N=20 for each diet.

	MONTH					
Diet	1	2	3	4	5	6
Cricket	0.429* (0.008)	0.392* (0.007)	0.376* (0.007)	0.359* (0.006)	0.337* (0.006)	0.326* (0.006)
Mealworm	0.580 (0.016)	0.557 (0.009)	0.524 (0.006)	0.497 (0.006)	0.484 (0.006)	0.472 (0.005)

* Significant difference ($p<0.001$) between cricket and mealworm treatment means using paired t test.

CHAPTER V

Influence of Crowding on Leopard Gecko (Eublepharis macularius) Growth Performance

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INTRODUCTION

The choice of housing conditions for captive animals is of great importance because housing methods are known to impact the physiology of animals (Rushen and de Passille, 1992). Crowded caging conditions are generally thought to inhibit growth performance. The effects of crowding have been evaluated for a variety of captive-propagated and domestic species (McGrath et al., 1984; Adams and Craig, 1985; Gamallo et al., 1986a; Gamallo et al., 1986b; Armario et al., 1987; Harvey and Chevins, 1987; Scalera, 1992). However, few studies have been performed to determine the influence of crowding on reptiles. Bjorndahl (1986) found that grouped juvenile turtles and isolated turtles (Pseudemys nelsoni) did not exhibit differences in weight gain. McKnight and Gutzke (1993) determined that young snapping turtles (Chelydra serpentina) grew faster when in isolation. Tubbs and Ferguson (1976) found that crowding resulted in depressed growth rates and survivorship of juvenile Sceloporus undulatus garmani. However, in a later study with a different population of S. undulatus crowding had no effect (Ferguson and Brockman, 1980).

The impact of crowding on the leopard gecko (Eublepharis macularius), a native of Pakistan and the surrounding area, is not known. Leopard geckos are thought to exist in communal colonies in the wild (Miller, 1980a). In captivity, grouped housing of adults is commonly practiced with one male and several females per group (Wagner, 1974; Wagner, 1980; Bull, 1987). Although communal caging is widely practiced, some recommend individual housing (Thorogood and Whimster, 1978). Miller (1980b) reported better success with breeding leopard geckos housed in pairs than in larger groups.

Hatchling leopard geckos are more aggressive than adults and appear to be stressed by frequent interactions with conspecifics. Nuygen (1985) reported that injury and death resulted from crowded conditions and recommended individual housing of hatchlings. Miller (1980a) recommended caging hatchlings individually or in small groups with hatchlings of similar size. Similar reports of communal housing of adults and individual housing of hatchlings are available for other eublepharine geckos, particularly members of the genus Coleonyx (Benefield et. al., 1981; Cooper et al., 1985; Neitman, 1985). Although a variety of opinions can be found regarding the impact of housing conditions on leopard geckos, no supportive data are available. Our objective was to evaluate the effect of crowding on leopard gecko growth.

MATERIALS AND METHODS

Hatchling leopard geckos were obtained from a breeding colony maintained at Oklahoma State University. Individual geckos were weighed to the nearest 0.1 g and SVL was measured to the nearest mm within 24 hours of hatching and at weekly intervals thereafter throughout the six-month study period. The average initial body weight and SVL were 3.6 g and 54 mm, respectively. All hatchlings used in this study were females produced from eggs incubated at 28 degrees C. Each individual was marked by toe-clipping and randomly assigned to either individual or grouped (4 per cage) housing conditions. Geckos assigned the individual treatment were housed individually in 20 cages and those assigned the grouped treatment were housed 4 geckos per cage in 5 cages. Each treatment group contained 20 geckos.

Plastic animal cages with inside dimensions of 28 L x 17.5 W x 12.5 H cm were used for both treatments. No substrate was used in the cages. Each cage was covered with a 3 mm steel mesh lid. Cage accessories included: a 13 L x 8.5 W x 2 H cm wooden shelter placed in the center of each cage directly on the cage bottom, a 6 cm diameter x 1 cm high plastic jar lid placed on the cage bottom that was kept filled with tap water, and a 6 cm diameter x 0.8 cm high lid of a plastic petri dish was placed on one end of the cage bottom and kept filled with a vitamin-mineral mix periodically. A 40 watt incandescent light bulb was placed at one corner of each cage to provide a temperature gradient and permit thermoregulation within each cage. Room temperature was maintained at 27 ± 1 degrees C and a LD 14:10 photoperiod was provided throughout the study period.

All geckos were provided mealworm larvae (Tenebrio molitor) and water ad libitum throughout the study. In a previous study (Rich, 1995) mealworm larvae were evaluated as dietary items and were found to support leopard gecko growth. The mealworm larvae used were from an established mealworm colony at OSU that were reared on a vitamin and mineral enriched diet. Food items were checked daily to ensure that surplus food was always available to the geckos. A vitamin-mineral mix was provided ad libitum for one week per month of the study.

Treatment means for mass, SVL, and cumulative average daily gain were evaluated by t-test for statistical significance.

RESULTS

Mean mass of individually caged geckos was significantly ($p<0.01$) greater throughout the study. Significant differences in mass were apparent by the first month and were maintained throughout the 6 month study period (Table 1). Mean SVL of geckos on the individually caged treatment followed a similar trend and was also significantly ($p<0.01$) greater throughout the study (Table 2). In addition, mean cumulative average daily gain (g/day) paralleled the results for mass and SVL. Geckos individually caged had significantly ($p<0.01$) greater average daily gain by the end of the first month and maintained the difference throughout the remainder of the study (Table 3).

DISCUSSION

All individuals used in this study grew during the study period, but growth performance was consistently better among those on the individual housing treatment. Although mean weights of lizards on the grouped treatment were lower than those of lizards on the individually caged treatments, they were nevertheless, greater than those reported by Allen et al. (1986) in which a maximum of 27.6 g mass occurred in a 7 month study.

Our study did not differentiate between social and environmental stress. Social interactions as well as competition for space near the heat source may have played some role in our experiment. The heat source was on one end of each cage and it is possible that dominant geckos forced subordinate individuals to spend more time on the cool end of the cage. However, we did not observe any obvious dominance heirarchy or aggression among the grouped leopard geckos. During most of the day, all four geckos were usually under the wooden shelter in physical contact with each other. Nevertheless, the temperature of the shelter was higher near the heat source and there may have been competition for a warmer position under the shelter. Additional research is needed to determine if stress due to crowding by itself is sufficient to suppress growth in leopard geckos when all other resources are equally available to all individuals.

An appropriate housing system for laboratory animals must meet the needs of the species as well as the researcher. Recommendations are often based on experience and professional judgment because objective data are seldom available. Although guidelines are available for the most commonly used laboratory mammals (NRC, 1985), specific requirements will undoubtedly vary among species and perhaps even populations. The available recommendations for laboratory animals apply mostly to endotherms and are unlikely to be appropriate for reptiles and possibly are even detrimental to ectotherms (Pough, 1991).

Because of the adaptability of the leopard gecko to artificial environments, this species is a good reptile model for laboratory studies. Quantitative evaluation is needed to determine requirements which are not only compatible with the species but also minimize variations in experimental designs.

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Table 1. Mean mass (g), range and SEM of leopard gecko hatchlings caged individually (I) and in groups of 4 per cage (G). (N = 20 per treatment)

		MONTH						
Treatment		0	1	2	3	4	5	6
I	Mass (g)	3.7	8.0*	14.2*	19.9*	25.2*	31.3*	36.5*
	Range	2.7-4.4	5.0-11.1	9.9-18.3	14.8-26.9	18.0-35.8	23.3-40.6	24.9-44.3
	SEM	0.124	0.338	0.493	0.642	0.846	0.891	1.0761
G	Mass (g)	3.5	6.9	10.8	15.7	21.6	25.5	29.9
	Range	2.8-4.3	4.6-9.2	6.9-14.4	10.5-20.8	17.6-28.2	20.7-31.5	25.6-34.8
	SEM	0.101	0.248	0.399	0.523	0.544	0.621	0.580

*Significantly different ($p<0.01$)

Table 2. Mean SVL, range, and SEM of hatchling leopard geckos caged individually (I) and in groups of 4 individuals per cage (G). (N = 20 per treatments)

Treatment		MONTH						
		0	1	2	3	4	5	6
I	SVL (mm)	54	69*	82*	90*	98*	105*	111*
	Range	49-60	59-75	75-89	82-97	90-108	95-115	103-120
	SEM	0.706	0.939	0.853	0.916	0.970	1.151	1.105
G	SVL (mm)	54	65	76	86	95	100	106
	Range	50-57	58-72	69-82	78-95	88-102	93-108	101-111
	SEM	0.431	0.731	0.916	1.028	0.925	1.001	0.884

*Significantly different (p<0.01)

Table 3. Mean average daily gain (g/day), range, and SEM of hatchling leopard geckos caged individually (I) and caged in groups of 4 (G). (N = 20 per treatment)

		Month					
Treatment		1	2	3	4	5	6
I	Mean (g/day)	0.155*	0.188*	0.193*	0.193*	0.197*	0.195
	Range	0.082-0.241	0.130-0.260	0.128-0.276	0.134-0.287	0.145-0.264	0.132-0.242
	SEM	0.010	0.008	0.007	0.007	0.006	0.006
G	Mean (g/day)	0.122	0.132	0.146	0.162	0.157	0.157
	Range	0.052-0.187	0.074-0.187	0.091-0.201	0.126-0.217	0.124-0.197	0.133-0.185
	SEM	0.006	0.006	0.006	0.005	0.004	0.004

* Significantly different (p<0.01)

CHAPTER VI

The Influence of Sand Ingestion on Leopard Gecko (*Eublepharis macularius*) Growth Performance

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INTRODUCTION

A variety of reptile species, particularly crocodilians and lizards, commonly ingest substrate under natural conditions (Sokol, 1971). The frequency of substrate items, such as sand and pebbles in the feces or digestive tract, suggests that substrate consumption is deliberate rather than accidental in several reptile species. Stones are commonly found in the stomachs of several species of crocodilians (Davenport et al., 1990; Webb et al., 1991; Fitch-Snyder and Lance, 1993; Thorbjarnarson, 1993). In addition, several species of lizards with diverse food habits reportedly ingest substrate. Iverson (1979) found sand or other substrate in the gut of most specimens examined during an extensive study of *Cyclura carinata*. Various substrate items, including sand and small stones, were also found in stomachs of *Lacerta lepida* examined by Castilla et al. (1991). Johnson (1966) found sand and small pebbles in the stomachs of *Sceloporus undulatus*, *S.*

magister, and *Cnemidophorus tigris*. Sand was found in over 88% of the stomach contents of *Callisaurus draconoides* examined by Quijada-Mascarenas (1992). Paulissen (1987) found sand in the stomachs of most *Cnemidophorus sexlineatus* collected. The predominantly herbivorous lizard, *Cnemidophorus arbuensis*, collected from different sites also commonly had sand in their stomachs (Schall and Ressel, 1991). Sylber (1988) found gravel and sand in fecal samples of the herbivorous lizards *Sauromaulus varius* and *S. hispidus* and concluded that both species must deliberately consume substrate.

In captivity, lizards are commonly observed ingesting substrate material (Brodsky, 1969; Demeter, 1976; Wagner, 1980). The leopard gecko (*Eublepharis macularius*), a terrestrial species which inhabits arid rock and sand terrain in Pakistan and the surrounding area (Thorogood and Whimster, 1978) is known to regularly ingest substrate in captivity (Wagner, 1974; Miller, 1980; Nuygen, 1985; Pugsley et. al., 1985).

The benefits of ingesting substrate have not been determined but lithophagy and geophagy must be natural for many reptiles and presumably is beneficial in some way. Under natural conditions, ingesting substrate may be a means of acquiring additional minerals and trace elements that are deficient in dietary items (Robbins, 1983). In addition, anecdotal reports suggest that lithophagy may help in the mechanical breakdown of food items and, therefore, increase digestive efficiency. The purpose of our study was to determine if lithophagy affected food consumption, gross conversion efficiency, and growth of captive leopard geckos.

MATERIALS AND METHODS

Leopard geckos were obtained from the breeding colony at Oklahoma State University. All geckos used were females produced from eggs incubated at 28 degrees C. Each individual was marked by toe-clipping and caged in groups of five within one week of hatching. Plastic animal cages with inside dimensions of 28 L x 17.5 W x 12.5 H cm were used for all animals. Each cage had a 3 mm steel mesh lid and was provided a 40 watt incandescent light bulb at the corner to permit thermoregulation. A temperature gradient of 27-35 degrees C was maintained within the cage. The room temperature was maintained at 27 ± 1 degrees C and a LD 14:10 photoperiod was provided.

All leopard geckos were maintained under identical conditions until they were 42 days old. They were provided with a shelter, water, and vitamin and mineral supplemented food, i.e. crickets, _Acheta domestica_, and mealworms, _Tenebrio molitor_, for the first 28 days but only mealworms thereafter. Leopard gecko growth performance on mealworm larvae had previously been evaluated and was determined to not differ significantly from growth on cricket diets (Rich, 1995). On day 42, hatchlings were assigned to pairs based on SVL and mass with one member of each pair serving as the control (0 sand) and the other assigned to the sand treatment. Twenty - nine lizards were assigned to each treatment and individually caged as described above. Mealworms and water were provided ad libitum to all geckos. Individuals assigned the sand treatment were offered sand ad libitum in a 6 cm diameter x 1 cm high plastic jar lid whereas controls did not have sand available.

At the beginning of the study and at weekly intervals throughout the 28 day study, SVL and mass was determined for all geckos. Food consumption was determined by recording the number of mealworms consumed daily and multiplying the number of mealworms consumed by the average weight of mealworms offered during that interval. Gross conversion efficiency of the individual leopard geckos was determined weekly by dividing the increase in mass by the mass of food consumed. Sand consumption was determined by daily monitoring and measuring the amount of sand consumed per individual lizard. Food consumption, growth, and gross conversion efficiency differences between treatment groups were analyzed by t-test for significant differences.

RESULTS

Lizards on both treatments grew throughout the study period. Lizard mass varied with treatment and was significantly ($p \leq 0.05$) lower for the sand treatment group by day 7 and remained so throughout the study (Table 1).

Differences in snout-vent lengths followed the same trend as mass and were significantly ($p \leq 0.05$) lower for the sand treatment group by day 7 and remained lower at all intervals measured thereafter (Table 2). Average daily gain (ADG) of the lizards on the sand treatment were also significantly ($p \leq 0.05$) lower throughout the study period (Table 3).

Mean weekly mealworm consumption was significantly ($p \leq 0.05$) greater for the control group for the first two weeks of the study. However, no statistically significant differences in mealworm consumption were apparent between the control and sand treatments during weeks 3 or 4 of the study (Table 4). Cumulative average daily mealworm consumption was significantly ($p \leq 0.01$) lower for geckos on the sand treatment throughout the study (Table 5). When adjusted to body mass, average daily cumulative mealworm consumption remained significantly ($p \leq 0.01$) lower for the sand treatment group (Table 6).

For the sand treatment group, mean weekly sand consumption ranged from 5.336 g during week 1 to 5.685 g sand during week 4 (Table 7). On a body weight basis, sand consumption decreased over the study period from a maximum mean value of 0.427 g sand consumed per g body weight during week 1 to 0.365 g sand consumed per g body weight during week 4. During the same period, mean weekly mealworm consumption per g body weight increased from 0.071 g mealworms per g body weight during the first week to 0.101 g mealworms per g body weight during the fourth week (Table 7).

Although growth and mealworm consumption differed significantly due to sand consumption, gross conversion efficiencies (grams gained per gram mealworm consumed) were not significantly different ($p>0.05$) at any time during the study (Table 8).

The average daily intake of sand by lizards receiving the sand treatment was 0.793 grams. Average daily sand consumption per gram body weight was 0.052 g sand per g body weight over the 28 day period.

DISCUSSION

All leopard geckos used in this study grew during the study period, but growth was consistently lower for those that ingested sand. No adverse effects, other than reduced growth, were observed in this study. However, other investigators (Wagner, 1974; Nuygen, 1985; Pugsley et. al., 1985) have reported deaths in leopard geckos due to ingestion of substrate resulting in gut impactation. None of the geckos in our study developed any problems related to gut impaction.

The effect of sand ingestion on growth was inhibitory to both mass and length growth. Growth differences were significant between treatments early in the study and persisted throughout the length of the study. Lizards on the sand treatment averaged ingesting approximately 3.5 to 6 times more grams of sand than grams of mealworms on a weekly basis. Mealworm consumption on a weekly basis was significantly lower for lizards receiving sand during the first two weeks of the study but not during the last two weeks. This was due to both an increase in mealworm consumption by the sand group and a decrease in mealworm consumption by the control group. Although average daily mealworm consumption per gram body weight was higher for the control group, it decreased with time while increasing for lizards on the sand treatment. Over the study period, sand consumption decreased while mealworm consumption increased for the group receiving sand. A similar trend was observed for growth rates. Although average daily gain was significantly higher for the control group, it tended to decrease for the control group while simultaneously increasing for the sand group. Therefore, differences in growth were due to reduced mealworm consumption by the sand treatment group.

Although the long-term effect of substrate ingestion on leopard geckos may differ from our short-term study, our study does suggest that ingestion of substrate probably does not increase the digestive efficiency of this gecko. Gross conversion efficiency of food did not differ between geckos ingesting sand and those without sand. However, the geckos used in our study were parasite free and it is possible that wild lizards, which are usually infected with intestinal parasites, could receive some benefits from ingesting substrate if it reduced the effects of parasites. In addition, geophagia is probably a means of acquiring minerals (Robbins, 1983).

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Table 1. Increase in mass of leopard geckos ingesting sand compared to geckos ingesting no sand. N = 29 for each treatment.

	Week				
Treatment	0	1	2	3	4
Control	11.6 (0.337)	13.1* (0.170)	14.5* (0.416)	15.6* (0.456)	17.0* (0.505)
Sand	11.7 (0.339)	12.5 (0.346)	13.6 (0.342)	14.6 (0.386)	15.4 (0.427)

Significant differences ($p<0.05$) between treatments within the same time period are identified with an *.

Table 2. Mean snout-vent length (mm) and standard error of the mean, in parentheses, of leopard geckos. Significant differences ($p<0.05$) between treatments within the same time period are identified with an *. N = 29 for each treatment.

	Week				
Treatment	0	1	2	3	4
Control	77	80*	82*	84*	86*
	(0.655)	(0.652)	(0.818)	(0.733)	(0.748)
Sand	77	79	81	82	83
	(0.608)	(0.559)	(0.643)	(0.630)	(0.627)

Table 3. Mean average daily gain (g/day) and standard error of the mean, in parentheses,of leopard geckos. Significant differences ($p<0.05$) between treatments within the same time period are identified with an *. N = 29 for each treatment.

	Week			
Treatment	1	2	3	4
Control	0.212*	0.206*	0.189*	0.192*
	(0.019)	(0.011)	(0.010)	(0.010)
Sand	0.112	0.131	0.136	0.131
	(0.019)	(0.013)	(0.011)	(0.011)

Table 4. Mean weekly mealworm consumption (live weight, g) and standard error of the mean, in parentheses, of leopard geckos. Significant differences ($p<0.05$) between treatments within the same time period are identified with *. N = 29 for each treatment.

	Week			
Treatment	1	2	3	4
Control	2.430*	2.268*	2.013	1.814
	(0.165)	(0.134)	(0.109)	(0.151)
Sand	0.877	1.626	1.773	1.595
	(0.138)	(0.115)	(0.115)	(0.158)

Table 5. Mean cumulative average daily mealworm consumption (live weight, g/day) and standard error of the mean, in parentheses, of leopard geckos. Significant differences ($p<0.01$) between treatments within the same time period are identified with *. N = 29 for each treatment.

	Week			
Treatment	1	2	3	4
Control	0.347*	0.336*	0.320*	0.304*
	(0.024)	(0.017)	(0.014)	(0.014)
Sand	0.125	0.179	0.204	0.210
	(0.020)	(0.015)	(0.012)	(0.012)

Table 6. Mean cumulative average daily mealworm consumption per g body mass (live weight, g/day) and standard error of the mean, in parentheses, of leopard geckos. Significant differences (p<0.01) between treatments within the same time period are identified with *. N = 29 for each treatment.

	Week			
Treatment	1	2	3	4
Control	0.026*	0.023*	0.020*	0.018*
	(0.001)	(0.001)	(0.001)	(0.001)
Sand	0.010	0.013	0.014	0.014
	(0.002)	(0.001)	(0.001)	(0.001)

Table 7. Mean weekly consumption of sand/(g) and mealworms (live weight, g) and standard error of the mean, in parentheses, by geckos on the sand treatment. N = 29 for all means.

	Week			
	1	2	3	4
Sand (g)	5.336 (0.268)	5.568 (0.414)	5.685 (0.293)	5.610 (0.324)
Sand per g body mass	0.427 (0.019)	0.415 (0.034)	0.391 (0.019)	0.365 (0.020)
Mealworm (g)	0.877 (0.138)	1.626 (0.115)	1.773 (0.115)	1.595 (0.158)
Mealworm per g body mass	0.071 (0.011)	0.121 (0.008)	0.121 (0.007)	0.101 (0.009)

Table 8. Mean leopard gecko cumulative assimilation efficiency (g gain per g consumed), and standard error of the mean in parentheses. N = 29 for each treatment.

	Week			
Treatment	1	2	3	4
Control	0.572	0.620	0.590	0.635
	(0.065)	(0.026)	(0.023)	(0.020)
Sand	0.887	0.608	0.642	0.591
	(0.157)	(0.140)	(0.035)	(0.049)

CHAPTER VII

The Effect of Excessive Selenium on a Reptile Species (<u>Eublepharis macularius</u>): A Short-Term Chronic Test on Juvenile Leopard Gecko Growth

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INTRODUCTION

Ecological risk assessment for animals traditionally uses mammal, avian, fish, and invertebrate species as representatives of biota in a larger system. Although preliminary investigations (Culley and Applegate, 1967; Rosata and Ferguson, 1968; Bauerle et al., 1975; Stafford et al., 1976; Ryan et al., 1986; Skaare et al., 1991; Burger, 1992; Hebert et al., 1993; Meyers - Schone et al., 1993) suggest that reptiles may serve as good indicators of environmental contamination, the class Reptilia is not represented by any species currently used to evaluate the impact of environmental contaminants. The assumption made by regulatory agencies has been that criteria developed to protect birds and mammals also affords protection to reptiles (Urban and Cook, 1986).

Due to their unique physiology, reptiles may not be adequately protected by criteria established from tests on other vertebrates. Because reptiles are ectothermic, their metabolism is slower and less flexible than that of endothermic mammals and birds (Bennett and Dawson, 1976; Smith, 1976; Gleeson, 1979; Else and Hulbert, 1981; Coulson, 1984; Andrews and Pough, 1985). Thus, reptiles may be more susceptible than other terrestrial vertebrates to environmental contaminants because metabolic rates affect the elimination and detoxification of toxic substances. Although there is a paucity of information on the effects of toxicants on reptiles (Herald, 1949; Munro, 1949; Hall, 1980; Hall and Clark, 1982; Bishop et al., 1991; Hall and Henry, 1992; Struger et al., 1993; Bishop et al., 1994; Lambert, 1994), Rosata and Ferguson (1968) found that, on a body weight basis, reptiles fed endrin-resistant mosquitofish were more susceptible to lower doses of endrin than fish or avian species used in their study. Therefore, there is reason to doubt that reptiles are protected by criteria developed for birds and mammals. The paucity of empirical data on the effects of contaminants on reptiles demonstrates a need to identify a reptile model for environmental risk assessment and use a test substance to initiate evaluating the sensitivity of the model.

The leopard gecko (_Eublepharis_ _macularius_), a native of Pakistan and the surrounding area, is a species of lizard with apparent potential as a reptile model for ecological risk assessment. Several features of the leopard gecko make this a practical species to work with. It adapts well to captive propagation and is easily maintained in artificial environments similar to those for maintaining and rearing laboratory animals (Wagner, 1974; Thorogood and Whimster, 1978; Miller, 1980; Wagner, 1980; Hingley, 1985; Nuygen, 1985). Another attribute of the leopard geckos is that sex of hatchlings is determined by egg incubation temperature (Wagner, 1980; Viets et al., 1993), permitting better control of the experimental design. In addition, leopard geckos regularly seek out and consume substrate material (Rich, 1995) and, therefore, would ingest any soil contaminant present in their natural habitat. Substrate consumption has also been observed in several other lizard and reptile species and represents an exposure route encountered under natural conditions (Johnson, 1966; Sokol, 1971; Kramer, 1973; Iverson, 1979; Sylber, 1988; Castilla et al., 1991; Quijada-Mascarenas, 1992; Beyer et al., 1994).

Selenium was selected as a test substance because it occurs naturally in the environment (Cooper and Glover, 1974) and because the toxic effects of selenium on wildlife have generated much concern (Eisler, 1985; Clark, 1987; Ohlendorf, 1989; Ohlendorf et al., 1989; Williams et al., 1989; Burger et al., 1992; Clark et al., 1992; Leonzio et al., 1992; Lonzarich et al., 1992; Nakamoto and Hassler, 1992; Benson et al., 1993; Goede, 1993; Guitart et al., 1994). Although a naturally occurring trace element, selenium is considered a priority pollutant (Wilber, 1983; Engberg and Sylvester, 1993).

Experimental verification of the uptake and toxicity of selenium has been well documented for a number of amphibian, avian, fish, and mammal species (Ort and Latshaw, 1978; Browne and Dumont, 1980; Julius et al., 1983; Nash et al., 1983; Beems and van Beek, 1985; Birt et al., 1986; USFWS, 1986; Heinz et al., 1987; Gillespie et al., 1988; Fairbrother and Fowles, 1990; Besser et al., 1993; Heinz, 1993; Heinz and Fitzgerald, 1993; Rousseaux et al., 1993; Chen et al., 1994). Lance et al (1983) found that captive alligator egg quality decreased as a consequence of consuming higher levels of selenium in fish diets. No other comparable experimental data, however, are available for the effects of selenium on reptiles (Peterson and Nebeker, 1992).

The recent confirmation of high tissue levels of selenium in snakes from selenium contaminated sites demonstrates that reptiles can accumulate excessive levels of selenium (Ohlendorf et al., 1988). Selenium has also been found in tissues of Pine snake hatchlings (Burger, 1992), but, experimental evaluation of the effects of selenium on reptiles is lacking.

The purpose of our study was to determine the effect of selenium on reptile growth in a short-term chronic test using the leopard gecko as a reptile model.

MATERIALS AND METHODS

A selenium spiked sand substrate was selected as the method of exposing leopard geckos to selenium. The substrate was prepared by dissolving sodium selenite (Sigma) in ASTM Type 1 reagent grade water and saturating fine sand with the solution to attain the following concentrations of sodium selenite in the dry sand substrate: 0.1, 1, 10, 25, and 50 ppm. Selenium as selenites is the predominant form of selenium in many seleniferous soils (Berrow and Ure, 1989; Wahl et al., 1994). These concentrations were based on a preliminary range finding study. The control sand mix was prepared by saturating the sand with an equivalent volume of ASTM Type 1 reagent grade water. The saturated sand mixes were then oven dried at 60 degrees C for 48 hours. To ensure homogeneity in the final mix, the dried mix was then further mixed by tumbling for 2 hours in an evaporative film drier.

The leopard geckos used for the study were obtained as hatchlings from the breeding colony at Oklahoma State University. All individuals used were females produced from eggs incubated at 28 degrees C. The geckos were housed in plastic animal cages with inside dimensions of 28 L x 17.5 W x 12.5 H cm. Each cage was covered with a 3 mm steel mesh lid and a 40 watt incandescent light bulb was positioned at the corner of each cage to permit thermoregulation across a temperature gradient of 27 - 35 degrees C within the cage. The room temperature was maintained at 27 ± 1 degrees C and a LD 14:10 photoperiod was provided.

Hatchling geckos were acclimated to laboratory conditions for six weeks prior to being used in the study. During this period, hatchlings were offered crickets, mealworms, and a vitamin - mineral mix in sand which the geckos readily consumed. After 28 days, only mealworms were fed because they could be placed in a container and maintained separate from the experimental substrates whereas crickets could not. Mealworm larvae have been shown previously (Rich, 1995) to support leopard gecko growth. At day 42, a randomized complete block design was used with geckos randomly assigned to treatments within blocks. Each block consisted of six cages placed at the same height on a rack to compensate for thermal variations within the room. Only individuals that demonstrated sand and mealworm consumption by day 42 were assigned to treatments.

At the beginning of the study hatchling body mass was determined to the nearest 0.1 g and snout - vent length (SVL) was determined to the nearest mm. Thereafter, each gecko was measured at weekly intervals for the following 28 days. Food consumption was determined by recording the number of mealworms consumed daily and multiplying the number of mealworms consumed by the average weight of mealworms offered during that interval. Gross conversion efficiency (GCE) of the individual leopard geckos was determined weekly by dividing the increase in mass by the mass of food consumed. Selenium consumption was determined by daily monitoring and measuring the amount of selenium - sand mix consumed per individual lizard and calculating actual selenium consumed.

Food consumption, growth, and gross conversion efficiency differences between treatment groups were analyzed by analysis of variance ($p<0.05$). Individual mean comparisons were tested for significant differences ($p<0.05$) between the control and selenium treatments by Dunnett's test. Average daily selenium intake by hatchlings on selenium treatments were compared by Tukey's method of multiple comparisons ($p<0.05$). Pearson correlations were used to determine relationships between growth and selenium ingested. Statistical analyses were performed using TOXSTAT (Gulley et al., 1989).

RESULTS

Average daily selenium intake varied with the Se concentration in the sand substrate (Table 1). Selenium consumption varied directly with the concentration of the sand mixes from 0.1 to 10 ppm. However, selenium consumption of geckos receiving selenium sand mixes of 25 and 50 ppm was less than expected due to reduced sand consumption. Nevertheless, daily selenium consumption within a treatment group was fairly consistent throughout the 28 day study period.

Hatchling mass varied as a function of treatment as early as day 7 and those receiving the 25 and 50 ppm selenium substrate grew significantly less than those on the control treatment. By day 28, hatchling mass at all levels of selenium treatment were less than those of the controls but the significance levels remained the same as on day 7 (Table 2).

Differences in snout - vent length were not detected as early as mass differences but were apparent by day 14 when geckos receiving 25 and 50 ppm selenium substrates had significantly less snout - vent lengths than those on the control treatment (Table 3).

Differences in average daily gain (ADG) were similar to trends in mass and SVL. By day 7 leopard geckos on the 25 and 50 ppm selenium substrates had significantly less average daily gain than controls. By day 28, mean ADG's at all levels of selenium treatment were lower than those of the control group but the differences were significant only at the 10 ppm and higher levels of selenium treatment (Table 4). Growth, as ADG, was negatively correlated to the calculated amount of selenium ingested (Pearson product - moment correlation coefficient -0.817, $p \leq 0.05$).

Growth differences of geckos on the different selenium substrates corresponded to differences in food consumption. Mealworm consumption was significantly lower for hatchlings receiving the 25 and 50 ppm Se treatments by day 7 and by the end of the study period, average daily food consumption at other levels of selenium treatment were lower than the control but the differences were not significant (Table 5).

Gross conversion efficiency followed the same trend as food consumption. GCE for geckos on the 25 and 50 ppm selenium treatments were significantly lower than controls throughout the study. However, geckos on all other selenium treatments did not convert food to biomass significantly differently than controls (Table 6).

DISCUSSION

The sensitivity of several mammal and avian species to sodium selenite has been reported by a number of investigators and provides comparative data for this study. Decreased food consumption and growth have been reported for Japanese quail (Coturnix japonica), domestic chicks (Gallus gallus), mallard ducklings (Anas platyrhynchos), and Black-crowned night-heron hatchlings (Nycticorax nycticorax) receiving 6, 4, 20, and 10 ppm Se, respectively, in their diets or in drinking water (El-Begearmi et al, 1977; Nash et al., 1983; Heinz et al., 1988; and Smith et al., 1988). Similar results have been found in experiments using several mammal species including hamsters (Mesocricetus auratus) and rats (Rattus norvegicus), (Julius et al., 1983; Beems and van Beek, 1985). However, most studies report the selenium level offered in food and water and provide no data on the amount of selenium actually consumed. Results from such studies are difficult to interpret because the amount of Se ingested that caused an effect is unknown.

The observed differences in weight gain of leopard geckos at the 25 and 50 ppm Se levels were probably due mainly to significant decreases in food consumption and gross conversion efficiency. However, the causes of the decrease in ADG at the 10 ppm Se level is not clear because these lizards did not have significant decreases in food consumption or GCE. The average daily selenium intake at 10 ppm Se was significantly higher than at 0.1 and 1 ppm Se (Table 1). This suggests that the observed decrease in ADG may have been related to some toxic effect other than depressed food consumption or GCE.

In our study, food consumption was observed independently of selenium intake. Both food and the selenium spiked sand mix were available ad libitum so that individual hatchlings were free to select either. This permitted the daily monitoring and evaluation of food consumption and selenium ingestion separately. Lithophagy has been documented for a variety of reptile species, as well as in other gecko colonies (Sokol, 1971; Wagner, 1980; Beyer et al., 1994). Because our colony of leopard geckos readily consume sand, the administration of the test substance mixed with sand probably represents a realistic exposure route likely to be encountered by lizards in the wild as well as a convenient method of administering and monitoring the ingestion of a test substance.

The calculated average daily selenium intake by leopard geckos that significantly reduced average daily gain in mass by the end of our experiment was 0.204 mg Se/kg body weight/day at the 10 ppm treatment level. Beems and van Beek (1985) reported a no-observable-effect level of 0.7 mg Se/kg body weight/day while 1.4 mg Se/kg body weight/day (as sodium selenite) decreased food intake and weight gain in a 6 week growth study using hamsters. In a 20 day study using domestic chickens, Nash et al. (1983) found that an average daily selenium intake (as sodium selenite) at or above 0.939 mg/kg body weight produced significant reductions in feed intake and weight growth. Therefore, leopard gecko growth appears to be more sensitive to selenium than the birds or mammals tested to date.

The greater sensitivity of the leopard gecko to selenium suggests that reptiles may be more susceptible than other terrestrial vertebrates to some toxicants. Therefore, there is a need for a comparative data base for the effect of environmental contaminants on reptiles.

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Table 1. Calculated average daily selenium consumption (mg Se/kg body weight/day) by leopard geckos on different Selenium treated substrates. N = 21 for all means.

	Average daily selenium consumption (Mean ± S.D.)			
Na_2SeO_3 Treatment	Day 7	Day 14	Day 21	Day 28
0.1 ppm	0.002 ± 0.0^{a}	0.002 ± 0.0^{a}	0.002 ± 0.0^{a}	0.002 ± 0.0^{a}
1 ppm	0.024 ± 0.0^{a}	0.023 ± 0.0^{a}	0.022 ± 0.0^{a}	0.022 ± 0.0^{a}
10 ppm	0.221 ± 0.1^{b}	0.221 ± 0.1^{b}	0.214 ± 0.1^{b}	0.204 ± 0.0^{b}
25 ppm	0.600 ± 0.2^{c}	0.527 ± 0.1^{c}	0.466 ± 0.1^{c}	0.430 ± 0.1^{c}
50 ppm	0.922 ± 0.2^{d}	0.684 ± 0.1^{d}	0.586 ± 0.1^{d}	0.531 ± 0.1^{d}

Column means with the same letter are not significantly different ($p \le 0.05$) using Tukey comparison of means.

Table 2. Leopard gecko growth in mass (g) in relation to different selenium treated substrates and selenium consumption. N = 21 for all means.

Na_2SeO_3 treatment	Average daily Selenium Consumption	Mass (Mean ± S.D.)				
		Day 0	Day 7	Day 14	Day 21	Day 28
Control	0	13.2 ± 1.7	14.7 ± 1.9	16.1 ± 2.0	17.4 ± 2.2	18.6 ± 2.3
0.1 ppm	0.002 ± 0.0	13.1 ± 1.8	14.8 ± 2.3	15.8 ± 2.4	17.4 ± 2.4	18.3 ± 2.4
1 ppm	0.022 ± 0.0	13.3 ± 2.2	15.0 ± 2.5	16.1 ± 2.7	17.2 ± 3.3	18.1 ± 3.3
10 ppm	0.204 ± 0.0	13.6 ± 1.7	15.1 ± 2.2	15.9 ± 2.2	17.0 ± 2.2	17.6 ± 2.2
25 ppm	0.430 ± 0.1	13.0 ± 1.7	13.0 ± 1.8*	12.7 ± 2.1*	13.1 ± 2.3*	13.6 ± 2.7*
50 ppm	0.531 ± 0.1	12.6 ± 1.5	12.1 ± 1.7*	11.4 ± 1.8*	11.1 ± 1.8*	10.9 ± 1.6*

* Significant difference ($p \leq 0.05$) between control and treatment means using Dunnett's Test.

Table 3. Leopard gecko growth in snout-vent length (mm) is relation to different selenium treated substrates and selenium consumption. N = 21 for all means.

Na_2SeO_3 treatment	Average daily Selenium Consumption	Snout - vent length (Mean ± S.D.)				
		Day 0	Day 7	Day 14	Day 21	Day 28
Control	0	80 ± 3.2	83 ± 3.5	86 ± 3.4	87 ± 2.7	89 ± 3.4
0.1 ppm	0.002 ± 0.0	80 ± 4.2	82 ± 4.1	85 ± 4.6	86 ± 4.6	86 ± 4.4
1 ppm	0.022 ± 0.0	80 ± 3.9	82 ± 4.3	84 ± 5.1	86 ± 4.9	87 ± 5.3
10 ppm	0.204 ± 0.0	81 ± 3.6	84 ± 3.9	86 ± 4.3	87 ± 4.2	88 ± 4.2
25 ppm	0.430 ± 0.1	80 ± 3.0	81 ± 3.4	82 ± 3.6*	83 ± 4.1*	84 ± 4.1*
50 ppm	0.531 ± 0.1	80 ± 3.0	81 ± 2.6	82 ± 2.9*	82 ± 2.8*	83 ± 2.9*

* Significant difference ($p \leq 0.05$) between control and treatment means using Dunnett's Test.

Table 4. Leopard gecko growth in average daily gain (g/day) in relation to different selenium treated substrates and selenium consumption. N = 21 for all means.

Na_2SeO_3 treatment	Selenium consumption	Average Daily Gain (Mean ± S.D.) Day 7	Day 14	Day 21	Day 28
Control	0	0.22 ± 0.1	0.21 ± 0.1	0.20 ± 0.1	0.20 ± 0.1
0.1 ppm	0.002 ± 0.0	0.23 ± 0.1	0.19 ± 0.1	0.20 ± 0.1	0.19 ± 0.0
1 ppm	0.022 ± 0.0	0.24 ± 0.1	0.20 ± 0.1	0.19 ± 0.1	0.17 ± 0.1
10 ppm	0.204 ± 0.0	0.22 ± 0.2	0.17 ± 0.1	0.16 ± 0.1	0.14 ± 0.1*
25 ppm	0.430 ± 0.1	-0.01 ± 0.1*	-0.02 ± 0.1*	0.003 ± 0.1*	0.02 ± 0.1*
50 ppm	0.531 ± 0.1	-0.08 ± 0.1*	-0.09 ± 0.1*	-0.07 ± 0.0*	-0.06 ± 0.0*

* Significant difference ($p \leq 0.05$) between control and treatment mean within a column using Dunnett's Test.

Table 5. Leopard gecko average daily mealworm consumption (live weight, g) in relation to different selenium treated substrates and selenium consumption. N = 21 for all means.

Na_2SeO_3 treatment	Average Daily Selenium consumption	Average Daily Mealworm Consumption (Mean ± S.D.)			
		Day 7	Day 14	Day 21	Day 28
Control	0	0.408 ± 0.1	0.391 ± 0.1	0.378 ± 0.1	0.361 ± 0.1
0.1 ppm	0.002 ± 0.0	0.403 ± 0.2	0.361 ± 0.1	0.362 ± 0.1	0.351 ± 0.1
1 ppm	0.022 ± 0.0	0.396 ± 0.2	0.369 ± 0.1	0.347 ± 0.1	0.342 ± 0.1
10 ppm	0.204 ± 0.0	0.402 ± 0.2	0.354 ± 0.1	0.339 ± 0.1	0.315 ± 0.1
25 ppm	0.430 ± 0.1	0.152 ± 0.1*	0.131 ± 0.1*	0.135 ± 0.1*	0.143 ± 0.1*
50 ppm	0.531 ± 0.1	0.076 ± 0.1*	0.048 ± 0.1*	0.044 ± 0.1*	0.046 ± 0.0*

* Significant difference ($p \leq 0.05$) between control and treatment mean within a column using Dunnett's Test.

Table 6. Leopard gecko gross conversion efficiency (g gain/g consumed) in relation to different selenium substrates and selenium consumption. N = 21 for all means.

Na_2SeO_3 treatment	Average Daily Selenium consumption	Gross Conversion Efficiency (Mean ± S.D.)			
		Day 7	Day 14	Day 21	Day 28
Control	0	0.53 ± 0.2	0.53 ± 0.1	0.53 ± 0.1	0.54 ± 0.1
0.1 ppm	0.002 ± 0.0	0.55 ± 0.2	0.51 ± 0.2	0.55 ± 0.1	0.53 ± 0.1
1 ppm	0.022 ± 0.0	0.59 ± 0.3	0.52 ± 0.2	0.51 ± 0.2	0.49 ± 0.1
10 ppm	0.204 ± 0.0	0.50 ± 0.4	0.45 ± 0.2	0.47 ± 0.1	0.45 ± 0.1
25 ppm	0.430 ± 0.1	-1.11 ± 2.2*	-1.54 ± 3.3*	-1.19 ± 3.3*	-1.15 ± 4.2*
50 ppm	0.531 ± 0.1	-2.27 ± 3.1*	-5.00 ± 7.2*	-3.71 ± 4.7*	-2.59 ± 3.2*

* Significant difference ($p \leq 0.05$) between control and treatment mean within a column using Dunnett's Test.

CHAPTER VIII

The Influence of Cricket Versus Mealworm Diets on Leopard Gecko (*Eublepharis macularius*) Molting

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INTRODUCTION

Skin shedding in reptiles is influenced by several factors. Traditionally, emphasis has been placed on endogenous (primarily hormonal) factors (Licht and Jones, 1967; Maderson and Licht, 1967; Maderson et al., 1970; Maderson and Chiu, 1981; Chandola-Saklani and Kar, 1990). Other factors which effect molting include growth (Fitch, 1940; Cliburn, 1976; Semlitsch, 1978; Macartney et al., 1990), food ingestion (Cliburn, 1976; Spellerberg, 1982), reproductive state (Kar and Chandola-Saklani, 1985; Bauwens et al., 1989), temperature (Semlitsch, 1978), and general metabolic status (Chiu and Maderson, 1980). Maderson (1984) proposed that shedding is controlled by an interaction between endogenous and exogenous factors. Although the effects of a number of factors on molt are known, controlled experiments on the influence of diet are lacking.

The energy loss due to shedding may be significant and is known to range from 3-11% of ingested energy for the few species analyzed (Smith, 1976). Cutaneous water loss is also high during shedding (Zucker and Maderson, 1980). Consequently, few species are known to shed frequently. Species that shed regularly and frequently probably do so to maintain certain epidermal specializations (Lillywhite and Maderson, 1982; Maderson, 1984).

The leopard gecko, Eublepharis macularius, is a long lived, xeric adapted species which molts regularly. The first molt usually occurs within one week of hatching and molting continues regularly thereafter at three to four week intervals (Thorogood and Whimster, 1978). The leopard gecko relies on regular shedding to maintain skin-derived chemicals used to determine sex of conspecifics (Mason and Gutzke, 1990). Therefore, the leopard gecko may serve as an appropriate experimental subject for evaluating the effect of various factors on molting.

The objective of this study was to determine the effect of cricket (Acheta domestica) versus mealworm (Tenebrio molitor) diets on leopard gecko molting.

METHODS

Hatchling geckos were obtained from the breeding colony at Oklahoma State University. All hatchlings were from eggs incubated at 28 degrees C. Initial mass and SVL measurements were taken on each gecko within 24 hours after hatching. Each individual was marked permanently by toe - clipping and paired with another gecko of similar mass. All geckos were marked dorsally with a black permanent magic marker to permit easy detection of molt. Each individual was observed daily and weighed, measured, and remarked within 24 hours after molting.

A total of 40 geckos were assigned to 20 pairs with one member of the pair randomly assigned to either the cricket or mealworm diet. The other member of a pair was assigned to the remaining diet. Crickets and mealworms were from colonies maintained at Oklahoma State University on the same commercially prepared ration. Prey items and water were offered ad libitum to the geckos. A vitamin - mineral mix was provided ad libitum for one week per month of the study. Prey consumption was determined for each individual gecko by counting the number of prey consumed and multiplying the number consumed by the average prey mass (live weight, g). Prey size was increased throughout the study as lizards grew.

After initial measurements, marking, and treatment assignments were made, each gecko was housed individually in an opague plastic animal cage with inside dimensions of 28 L X 17.5 W X 12.5 H cm. Each cage contained a shelter and water dish. All cages were provided with tops made of 3 mm steel mesh. A thermal gradient of 27 - 35 degrees C was provided within each cage by a 40 watt incandescent light bulb with an aluminum reflector positioned at one corner. The room temperature was maintained at 27 ± 1 degree C and an LD 14:10 photoperiod was used throughout the study.

The means for age, mass and SVL at molts 1 - 7 for lizards on cricket and mealworm diets were compared by paired samples t tests. Growth (mm/day, g/day) and food consumption (live prey mass, g/day) treatment means for the 6 month period of study were also compared by paired samples t tests. All statistical analyses were performed using SYSTAT (Wilkinson, 1989).

RESULTS

At the termination of the 6 month study, mean mass and SVL for geckos on the cricket diet (35.36 g and 113 mm, respectivly) and the mealworm diet (36.49 g and 111 mm, respectivly) were not significantly different. In addition, cumulative average daily growth in mass and SVL of geckos did not differ significantly with either diet over the 6 month period observed, although hatchlings fed crickets ingested significantly ($p<0.01$) more live mass than the mealworm fed group. However, hatchlings on the mealworm diet had a signifantly greater total number of molts during the 6 months (Table 1).

Positive significant ($p<0.01$) correlations were found between consumption and growth (cumulative average daily gain, g/day) for both treatments (Pearson product-moment correlation coefficient = 0.860, cricket diet, and = 0.941, mealworm diet). However, significant correlations between growth and the number of molts were not found for either treatment.

Nevertheless, diet did affect the molt pattern of leopard geckos. Hatchlings did not differ significantly in age at their first five molts. However, the group fed mealworms molted at significantly ($p<0.05$) younger ages than the group fed crickets at molts 6 and 7 (Table 2). In addition, from molt 3 through 7, geckos on the mealworm diet molted at a smaller mass ($p<0.01$) than the geckos fed crickets (Table 3). Similarly, geckos fed mealworms had significantly smaller snout-vent lengths at molts 3 through 7 (Table 4).

DISCUSSION

All individuals used in this study grew well during the six months observed, regardless of dietary treatment. Mean masses of geckos on both diets were greater than those reported by Allen et al. (1986).

Because average daily gain in mass and SVL did not differ between diet treatments, the observed differences in molting frequency are apparently not due to differences in growth. Significant correlations between the number of molts and growth were not found. This observation is in agreement with that of Maderson and Licht (1967) who found no correlations between growth and molt frequency of Anolis carolinensis fed equal amounts of mealworm larvae. Our results suggest the greater frequency of molts observed for the mealworm fed group may have been due to differences in either prey biomass ingestion or prey nutrient composition.

Mealworm larvae contain approximately twice as much lipid content as crickets (Modzelewski and Culley, 1974; Dimmitt and Ruibal, 1980; Xiang et al., 1993). Therefore, increased biomass consumption by the cricket fed group may be required to meet the same energy requirement.

Cliburn (1976), in a study of the Black Pine Snake (Pituophis melanoleucus lodingi), reported that molt frequency increased with prey consumption. However, in our study, increased prey biomass consumption by the geckos on the cricket diet did not result in increased growth or molt frequency. Significant differences in prey intake may have been due to the lower energy content of crickets. As a result, less energy may have been allocated for molting by the cricket fed group. Alternatively, since lipids are significant components of the integument of this species (Mason and Gutzke, 1990), and mealworm larvae have greater lipid composition than crickets, the greater frequency of molts by the geckos fed mealworms may have been due to ingesting more lipids. Although this study does not permit further elucidation, it does suggest the need for future studies to further evaluate the influence of food consumption rate on molting frequency and to distinguish the effects of various dietary nutrients on molting.

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Table 1. The effect of diet on mean leopard gecko growth (mm/day, g/day), prey consumption (g/day), and total number of molts during a six-month study. Standard error of the means are in parentheses. N = 20 for each diet.

	Gecko Growth/day		Food Consumption	Total Number Molts
Diet	(g/day)	(mm/day)	(g/day)	
Cricket	0.189 (0.006)	0.350 (0.011)	0.581* (0.019)	9.5* (0.267)
Mealworm	0.195 (0.006)	0.345 (0.011)	0.415 (0.014)	11.1 (0.228)

*Significant difference ($p<0.01$) between cricket and mealworm treatment means using paired t test.

Table 2. The effect of diet on mean leopard gecko age (days) at molts 1-7. Standard error of the means are in parenthesis. N = 20 for each diet.

	MOLT						
Diet	1	2	3	4	5	6	7
Cricket	4	19	33	47	64	85*	105*
	(0.930)	(1.358)	(1.526)	(1.894)	(2.610)	(3.480)	(4.811)
Mealworm	4	20	33	45	59	75	90
	(1.110)	(1.375)	(1.331)	(1.610)	(1.638)	(2.485)	(2.718)

* Significant difference ($p < 0.05$) between cricket and mealworm treatment means using paired t test.

Table 3. The effect of diet on mean leopard gecko mass (g) at molts 1-7. Standard error of the means are in parenthesis. N = 20 for each diet.

	MOLT						
Diet	1	2	3	4	5	6	7
Cricket	3.97	6.44	10.17*	13.66*	17.81*	22.05*	25.75*
	(0.162)	(0.347)	(0.447)	(0.505)	(0.729)	(1.037)	(1.157)
Mealworm	3.92	5.87	8.82	11.57	14.53	17.87	20.69
	(0.173)	(0.257)	(0.397)	(0.447)	(0.572)	(0.588)	(0.679)

* Significant difference ($p < 0.01$) between cricket and mealworm treatment means using paired t test.

Table 4. The effect of diet on mean leopard gecko SVL (mm) at molts 1-7. Standard error of the means are in parenthesis. N = 20 for each diet.

	MOLT						
Diet	1	2	3	4	5	6	7
Cricket	57 (0.833)	65 (1.098)	74* (1.177)	81* (1.087)	88* (1.312)	94* (1.384)	99* (1.627)
Mealworm	57 (0.805)	64 (0.783)	71 (0.887)	76 (0.964)	83 (0.972)	87 (1.077)	92 (1.163)

* Significant difference ($p < 0.01$) between cricket and mealworm treatment means using paired t test.

CHAPTER IX

The Influence of Crowding on Leopard Gecko (Eublepharis macularius) Molting

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INTRODUCTION

The influence of stress associated with maintenance of animals in laboratory environments is an issue of growing concern (Chiszar et al., 1993; Kreger, 1993). Stress due to crowding is known to impact physiology (Rushen and de Passille, 1992) and the effects of stress due to crowding have been evaluated for a variety of captive-propagated and domestic species (McGrath et al., 1984; Adams and Craig, 1985; Gamallo et al., 1986; Scalera, 1992; Armario et al., 1987). Endocrine effects are often examined as indicators of distress. Individual whiptail lizards (Cnemidophorus uniparens) did not differ in circulating corticosterone levels whether housed singly or in groups (Grassman and Crews, 1990). However, Dauphin-Villemant and Xavier (1987) observed that Lacerta vivipara housed individually had increased corticosterone levels. In crocadilians, stress due to crowded conditions has been shown to increase plasma corticosterone (Elsey et al., 1990) and decrease plasma calcium (Morpurgo et al., 1992). However, few studies have been performed to determine the influence of stress due to crowding on other parameters.

The effect of stress due to housing conditions on growth are known for relatively few ectotherms. Bjorndahl (1986) reported no difference in weight gain due to crowding in juvenile turtles (Pseudemys nelsoni). However, McKnight and Gutzke (1993) determined that young snapping turtles (Chelydra serpentina) grew faster when reared in isolation. In addition, captive reared juvenile alligators (Alligator mississipiensis) grew better when maintained at lower stocking densities (Elsey et al., 1990). Tubbs and Ferguson (1976) found that crowding depressed growth rates and survivorship of juvenile eastern fence lizards (Sceloporus undulatus garmani). However, in a later study crowding had no effect on a different population of S. undulatus and sagebrush lizards (S. graciosus), (Ferguson and Brockman, 1980). Therefore, the influence of crowding on growth performance varies between species and care must be given when extrapolating effects to different species.

Skin shedding in reptiles is influenced by a variety of endogenous and exogenous factors. Shedding frequency is generally associated with thyroid activity (Kar and Chandola-Saklani, 1985; Chandola-Saklani and Kar, 1990).

Chiu and Maderson (1980) proposed that molting frequency is most appropriately viewed as a reflection of general metabolic status. Maderson (1984) suggested that molt is controlled by a complex interaction between exogenous and endogenous factors. Investigations of molt frequency regulation have focused on endogenous (primarily hormonal) regulation (Licht and Jones, 1967; Maderson and Licht, 1967; Maderson et al., 1970; Maderson and Chiu, 1981; Chandola-Saklani and Kar, 1990). Other factors studied include growth (Fitch, 1940; Cliburn, 1976; Semlitsch, 1978; Macartney et al., 1990), food consumption (Cliburn, 1976; Spellerberg, 1982), reproductive status (Kar and Chandola-Saklani, 1985; Bauwens et al., 1989), and temperature (Semlitsch, 1978). Therefore, because molting is influenced by a variety of factors, molting frequency may serve as an indicator of the overall health of reptiles maintained in captive environments.

Shedding regularly and frequently represents a significant energy investment. Smith (1976) determined that energy loss from shedding ranges from 3-11% of ingested energy. Cutaneous water loss is also high during shedding (Zucker and Maderson, 1980). Therefore, relatively few species shed frequently and those that do apparently must shed frequently to maintain certain epidermal specializations (Lillywhite and Maderson, 1982; Maderson, 1984).

The leopard gecko (Eublepharis macularius) is a long lived, xeric adapted species which molts regularly and frequently. The first molt usually occurs within one week of hatching and molting continues regularly at 3 - 4 week intervals thereafter (Thorogood and Whimster, 1978). This species is relatively easily maintained in captivity and may serve as an appropriate model for evaluating the effect of various factors on molt.

The impact of crowding on the leopard gecko is not known. Leopard geckos are thought to live in communal colonies in the wild (Miller, 1980). In captivity, adults are commonly grouped with one adult male and several females per cage (Wagner, 1974; Wagner, 1980; Bull, 1987). Although communal caging is widely practiced, some recommend individual housing (Thorogood and Whimster, 1978). Hatchlings are more aggressive than adults and appear to be stressed by frequent interactions with conspecifics. Injuries and death reportedly occur when hatchlings are group caged and individual caging is recommended (Miller, 1980; Nuygen, 1985). Similar observations have been made for other eublepharine geckos, particularly banded geckos Coleonyx sp. (Benefield et al., 1981; Cooper et al., 1985; Neitman, 1985). Although a variety of opinions are reported, regarding the impact of crowding on this and other species, supportive evidence is not available.

The objective of this study was to determine the effect of crowding on leopard gecko molting frequency and to evaluate the relationship between growth in uncrowded and crowded conditions and molting frequency.

METHODS

Hatchling leopard geckos were obtained from a breeding colony maintained at Oklahoma State University. Individual geckos were weighed to the nearest 0.1 g and SVL was measured to the nearest mm within 24 hours of hatching. All hatchlings used in this study were from eggs incubated at 28 degrees C. Each individual was marked by toe - clipping and randomly assigned to either individual or grouped (4 per cage) housing conditions. A total of 40 geckos were used with 20 assigned to each treatment.

Opaque plastic animal cages with inside dimensions of 28 L X 17.5 W X 12.5 H cm were used for both treatments. Each cage contained a shelter and water dish. All cages were provided with tops made of 3 mm steel mesh. A thermal gradient of 27 - 35 degrees C was provided by a 40 watt incandescent light bulb with an aluminum reflector at one corner of each cage. Room temperature was maintained at 27 ± 1 degrees C and a LD 14:10 photoperiod was provided throughout the study period.

All geckos were provided mealworm (<u>Tenebrio</u> <u>molitor</u>) larvae and water ad libitum throughout the study. Mealworm larvae were from an established mealworm colony at OSU. A vitamin - mineral mix was provided ad libitum for one week per month of the study.

Each individual was marked dorsally with a black permanent magic marker to permit easy detection of a molt. All individuals were observed daily. Each individual was weighed, measured, and remarked within 24 hours of a molt.

Means for age, mass, and SVL at molts 1-7 were compared by independent samples t tests for geckos housed individually and grouped. Growth (mm/day and g/day) and total number of molts, during the 6 month period observed, were also compared between treatments by independent samples t tests. All statistical analyses were performed using SYSTAT (Wilkinson, 1989).

RESULTS

Average age at molts 1 - 7 did not differ significantly ($p>0.05$) during the study (Table 1). However, differences in mass were significant ($p<0.01$) by molt 3 and persisted through molt 7. Geckos on the crowded treatment were molting at smaller average masses than those caged individually (Table 2).

SVL differences followed the same trend as mass with the geckos on the crowded treatment molting at significantly smaller SVL by molt 3 and continuing to do so through molt 7 (Table 3). Also, cumulative average daily growth (mm/day and g/day) over the 6 month study period differed significantly ($p<0.01$). Geckos housed under crowded conditions grew less in mass and length than individually housed geckos. Mean mass and SVL at 6 months were significantly ($p<0.01$) greater for the individually housed geckos. Geckos on the crowded treatment had a significantly ($p<0.01$) greater number of molts during the 6 month period observed (Table 4). Significant correlations were not found between growth and molt for either treatment.

DISCUSSION

All individuals grew and molted regularly during the 6 month study period. Cumulative average daily gain in mass and length was greater for geckos on the individual housing treatment. Mean mass and SVL at six months was greater, for both treatments, than reported in a study by Allen et al. (1986) in which a maximum of 27.6 g mass was obtained in a 7 month period.

Our results indicate that crowding influences molt. Whether the effect on molt is due simply to growth differences, or to a more complex interaction involving physiological stress from crowding, cannot be ascertained from this study. However, since correlations between growth and total number of molts were not found, we have less support for growth as the predominant influence. This observation is consistent with that of Maderson and Licht (1967) who found no correlation between growth and molting frequency in Anolis carolinensis.

Some studies have reported correlations between growth and molting frequency. Positive correlations were reported by Cliburn (1976) for the Black Pine Snake, Pituophis melanoleucus lodingi. Macartney et al (1990) observed that juvenile Western Rattlesnakes, Crotalus viridis, with the highest growth rates also had increased molt frequencies. However, Semlitsch (1978) found a strong positive correlation between body size and molt frequency in Natrix fasciata, but not for N. taxispilota.

In our study, geckos that grew slower molted more frequently and at smaller sizes. The significance of increased molt frequency in lizards that grow slower is not apparent. Because increased molt frequency incurs energy cost (Smith, 1976), there may be some adaptive advantage for allocating less energy to growth and more to molting when subjected to certain kinds of stress. In our study stress could have been from either increased social contact with conspecifics or competition for resources. Food should not have been a factor because it was provided ad libitum throughout the entire study. However, other resources, such as space or preferred shelter sites, may have been limiting factors.

Mason and Gutzke (1990) reported on the presence of skin-derived semiochemicals and their use by adult leopard geckos to determine sex of conspecifics. Semiochemicals may also play some similar role in other life stages as well and the increased molt frequency may be more adaptive under crowded conditions where maintaining higher frequency of skin shedding enhances the semiochemical role. An alternative explanation may be that increased molt frequency helps maintain color brightness or pattern. Because leopard gecko hatchlings are brightly colored with distinct banding patterns, increased molt frequency may help maintain brighter color or distinctive banding over a longer period of time. This may have increased the survival potential of smaller geckos in crowded environments where increased social interactions are likely.

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Table 1. The effect of crowding on mean leopard gecko age (days) at molts 1 - 7. Standard error of the means are in parentheses. N = 20 for each treatment.

	MOLT						
Treatment	1	2	3	4	5	6	7
Individual	4 (1.110)	20 (1.375)	33 (1.331)	45 (1.610)	59 (1.638)	75 (2.485)	90 (2.718)
Grouped	6 (1.608)	21 (2.245)	33 (2.346)	44 (2.624)	59 (2.766)	71 (2.788)	85 (2.944)

Table 2. The effect of crowding on mean leopard gecko mass (g) at molts 1 - 7. Standard errors of the means are in parentheses. N = 20 for each treatment.

	MOLT						
Treatment	1	2	3	4	5	6	7
Individual	3.92 (0.173)	5.87 (0.257)	8.82* (0.397)	11.57* (0.447)	14.53* (0.572)	17.87* (0.588)	20.69* (0.679)
Grouped	3.82 (0.133)	5.49 (0.228)	7.29 (0.272)	8.97 (0.316)	11.29 (0.431)	13.68 (0.495)	16.07 (0.459)

* Significant difference (p<0.01) between treatment means using paired t test.

Table 3. The effect of crowding on mean leopard gecko SVL (mm) at molts 1 - 7. Standard error of the means are in parenatheses. N = 20 for each treatment.

	MOLT						
Treatment	1	2	3	4	5	6	7
Individual	57 (0.805)	64 (0.783)	71* (0.887)	76* (0.964)	83* (0.972)	87* (1.077)	92* (1.163)
Grouped	56 (0.555)	62 (0.624)	67 (0.716)	72 (0.820)	77 (1.042)	82 (0.973)	87 (0.958)

* Significant difference (p<0.01) between treatment means using paired t test.

Table 4. The effect of crowding on mean leopard gecko growth (mm/day, g/day), final mass (g), final SVL (mm), and total number of molts during a six month study. Standard error of the means are in parentheses. N = 20 for each treatment.

	Gecko Growth		Final Mass (g)	Final SVL (mm)	Total Number Molts
Treatment	(g/day)	(mm/day)			
Individual	0.195* (0.006)	0.339* (0.006)	36.5* (1.076)	111* (1.105)	11.1* (0.228)
Grouped	0.157 (0.004)	0.313 (0.005)	29.9 (0.580)	106 (0.884)	12.2 (0.264)

* Significant difference ($p<0.01$) between treatment means using t test.

CHAPTER X

CONCLUSION

The western fence lizard (Sceloporus occidentalis) may offer some potential as a laboratory reptile but appears to be less easily adapted to artificial environments than the leopard gecko (Eublepharis macularius). Our experience with the western fence lizard suggests the need for more extensive analysis of vitamin and nutrient requirements. However, the diet study with this species demonstrated that mealworm diets were not detrimental to hatchling growth. This finding was significant because mealworms are typically considered less desirable prey items and are often viewed as potentially harmful (Wagner, 1980; Wynne, 1981; Mattison, 1982). Mealworms are attractive for laboratory use due to the relative ease of maintaining, propagating, and handling mealworm larvae as prey items.

The leopard gecko also grew well on either crickets or mealworm larvae. The preliminary study comparing single prey item diets with combined prey diets demonstrated that leopard gecko growth did not vary significantly with either single or combined prey item diets. Using single prey item diets simplifies the experimental design and prevents dietary selection preferences from becoming a confounding factor. The use of mealworm larvae permitted evaluation of prey consumption separate from selenium consumption by providing mealworm larvae and the selenium - spiked sand mixture in separate dishes. This distinction would not have been possible with crickets as prey.

Leopard gecko housing requirements are not as flexible as prey choices. Average daily growth rates were significantly higher for geckos caged individually. Although group caging would be desirable to facilitate maintenance and save space, hatchling growth was reduced by crowded housing conditions and therefore should be maintained in individual cages for most growth studies. Also, individual consumption measurements are not generally possible in group caging.

The observation that leopard geckos regularly ingest large amounts of substrate (averaging approximately 50% of their body weight per week) suggests an exposure route that can be accomplished easily without stressing the animal by excessive handling. This route is also ecologically relevant to several reptiles.

Data from this study appear to support the hypothesis that reptiles are more sensitive to environmental contaminants than other terrestrial vertebrates typically used for establishing safety criteria. These results differ from those of Hall and Clark (1982) who reported that *Anolis caroliniensis* sensitivity to organophosphates did not differ significantly from other vertebrates. Whether our conclusions differ because of species differences, differences in test substances, or end points is not known. Our conclusion regarding sensitivity differences is based on effects on growth rate. Growth rate effects occur at lower levels of chronic exposure and should be assessed because of their importance in nature. Effects at other life stages (Peakall, 1994) and sensitivity to other contaminants should also be investigated. Our results also suggest the need for expanding this assessment to include long-term studies to evaluate potential reproductive effects.

Other reptile species need to be evaluated to determine the range of sensitivities within this class. Lizards from other families, snakes, turtles, and crocadilians that differ anatomically, ecologically, and physiologically should be assessed since the diversity of the class is so great. The leopard gecko is a member of the family Gekkonidae which consists of about 750 species and is represented on all continents except Antarctia and is widespread on oceanic islands (Stebbins, 1985). The leopard gecko is strictly terrestrial and is representative of some reptile species but no single test species is representative of the entire Class Reptilia.

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