# GROWTH RATE VARIATION IN CAPTIVE SPECIES: THE CASE OF LEOPARD GECKOS, *EUBLEPHARIS MACULARIUS*

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*Abstract.*—Captive breeding is becoming increasingly attractive for conservation programs where re-introduction is anticipated. Such technique requires implementation of rigorous protocols starting with the understanding of life-history traits under captive conditions. To this end, Leopard Geckos, *Eublepharis macularius*, are useful models because they are often maintained under artificial living conditions, using dietary manipulation. As natural prey is not readily available in captivity, substitute prey items are required to maintain these captive animals, thus allowing testing the potential for environmental factors to affect the animal phenotype. Using three different treatments (House Crickets, *Acheta domestica* only, Beetle Mealworm, *Tenebrio molitor* larvae only, or a mixture of both Beetle Mealworm larvae and House Crickets), we investigated the relationship between diet and phenotypic plasticity in young siblings of Leopard Geckos over a 120-day period to identify the diet that leads to the fastest growth rate. Not only did diet affect the growth rates and overall body mass of the Leopard Geckos fed with the mealworm diet have a significantly greater body mass and larger body traits (head width, head length, basal tail width and snout-vent length) as compared to geckos fed with crickets only or a mixed diet, therefore supporting the notion that these traits can be influenced by an environmental resource after birth. Our study adds to the limited data concerning the effect of diet on the growth rates of captive animals and discusses the effects of mixed diet for Leopard Geckos.

Key Words.—captive species; diet manipulation; Eublepharis macularius; growth; Leopard Gecko; phenotypic plasticity; reptile

### INTRODUCTION

It is estimated that within the next decade, 20 to 50% of the earth's species will become extinct (Rahbek 1993). As a result, captive breeding and zoological conservation may prove as useful tools in the preservation and biodiversity of animal populations (Rahbek 1993). Captive breeding has been established throughout the globe in attempts to manage animals including endangered species. However, captive environments can be radically different from natural habitats and may not be suitable or conducive to a species requirement (Lynch and O'Hely 2001). It has been suggested, therefore, that attempting to manage species in captivity results in a double-edge sword relationship (Gippoliti and Carpanito 1997). For instance, the use of propagation for supplementation purposes can regularly cause changes in genetics, reducing the sustainability of wild populations (Araki et al. 2007). Problems may also arise because animal species in captivity often undergo significant evolutionary changes in their morphology, behavior, and physical traits, in turn compromising their fitness as compared to natural populations (Lynch and O'Hely 2001). There are also instances where animal species are kept in captivity for personal enjoyment or as a commodity to sell (i.e. selective breeding for color or

pattern enhancement). When animals are being maintained as part of a breeding colony for monetary gain, this situation leads to trade offs between expenditure on current reproduction and future reproductive success (Shine 1980). Simply because a captive environment is established does not necessarily ensure that these captive animals will breed and provide viable offspring.

The expression of phenotypic traits is the ability of an organism to change its phenotype in response to changes in the environment (Stearns 1992). While numerous studies have demonstrated the effects of the environment on early development (Sorci et al. 1996; DeWitt et al. 1998; Miaud et al. 1999), it remains unclear as to whether or not such environmental cues can modify more prolonged aspects of ontogeny (Pigliucci 2005). For instance, Bonnet et al. (2001) showed captive-born Gaboon Vipers, Bitis gabonica, raised with abundant food differed from their less well-fed siblings across several fitness-related traits including body mass, snoutvent length, and feeding apparatus morphology. In the context of captive breeding, it is therefore crucial to understand the implications of the local environment on development that could impact the success of reintroduction programs or other conservation plans (Gippoliti and Carpanito 1997; Bonnet et al. 2001).

Leopard Geckos, *Eublepharis macularius*, can be well

Food Source	Ca (%)	Mg (%)	P (%)	Crude Fat (% dry matter)	Total N (% dry matter)
Tenebrio molitor mealworms	0.12	0.28	1.42	$31.1 \pm 3.9$	$8.3 \pm 0.9$
House Crickets	0.21	0.08	0.78	$9.8 \pm 1.4$	$8.8 \pm 0.5$

**TABLE 1.** Selected macromineral and nutrient composition (dry weight) of *Tenebrio molitor* mealworms and House Crickets (*Acheta domestica*; Barker et al. 1998).

maintained and propagated under artificial living conditions, making them a good model for the investigation of phenotypic plasticity under controlled environment. This species is native to Afghanistan, Pakistan, and the north-western regions of India, with a majority of the captive stock being imported from Pakistan (de Vosjoli 2004). Hatchling Leopard Geckos weigh 2.5–3.0 g on average, with average adult weight between 30-60 g (de Vosjoli 2004). Adulthood is generally achieved around 18 months of age, while individuals are able to successfully breed at 30-35 g (de Vosjoli 2004). As natural prey is not readily available in captivity, substitute prev items are required to maintain captive populations of geckos. The two most common prey items that contribute to the diet of captive bred Leopard Geckos are House Crickets (Acheta domestica) and/or Mealworm Beetle larvae (Tenebrio molitor). Insects and other invertebrates comprise a large portion of diets of many animals maintained in captivity. Invertebrates in general have high levels of protein but are poor sources of calcium (an important factor for bone development; Allen et al. 1986). Of the two commonly fed insects, crickets contain slightly higher levels of protein whereas mealworm larvae have a significantly higher crude fat content (Table 1; Barker et al. 1998). There are conflicting notions regarding which prey species is favorable over the other, or if a mixed diet is most beneficial (Wagner 1980). For instance, some lizard species fed exclusively with mealworm larvae have experienced intestinal and vent compaction resulting in death or other complications (Mattison 1982). Although there are many controlled studies on the effect of differential diet and growth on fish (Diehl and Eklov 1995; Lemke and Bowen 1998), invertebrates (Leighton and Boolootian 1963), and other reptile species (Bjorndal 1991; Donoghue et al. 1998; Lemm et al. 2004), there are no such published results pertaining to Leopard Geckos (but see Rich 1995).

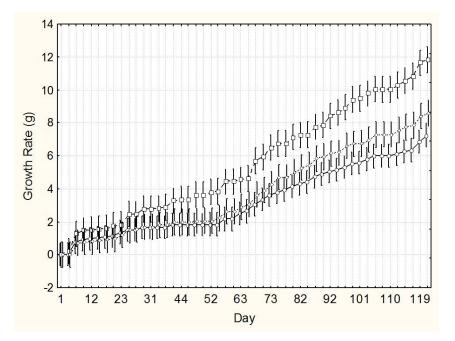
To better understand the influence of food resources on development, we set out to determine the relationship between the type of diet and morphological characters of the Leopard Gecko. Due to the nutrient content of diets commonly used for Leopard Geckos, we predicted that overall mass and morphological characters (head width, head length, basal tail width and snout-vent length) will be positively influenced by mixed feeding treatments as

it is a more nutritionally balanced diet. A mixed diet should produce larger Leopard Geckos with larger morphological traits.

#### MATERIALS AND METHODS

We obtained three-week old unsexed Leopard Geckos (n = 15) from Sciensational Sssnakes and Port Credit Pet Centre, Orillia, Ontario, Canada. The Leopard Geckos were collected from different clutches, all of which were incubated at 29.4°C (85° F). We kept incubation temperature constant to avoid differences that would affect growth and morphology once animals hatch (Crews et al. 1998). We housed the animals in plastic containers (27.9 cm long, 17.1 cm wide and 7.6 cm high); each vivarium had a lid to prevent escaping and the container was perforated to allow for constant air flow between the vivarium and the external environment. The dimensions of the vivarium were the same as those commonly used in breeding facilities so that the mobility of the geckos was not affected (Sakata et al. 2002). We used a paper towel as a substrate and each unit was equipped with a cardboard hide, a water dish, and a food dish. A heat cord was running under each vivarium to establish a heat gradient, which allowed the geckos to thermoregulate by changing location within the cage (Autumn and De Nardo 1995). We placed 15 vivariums on three different shelves, with five vivariums (side by side) on each shelf. We randomly placed individual Leopard Geckos among the vivariums to prevent confounding environmental effects on a particular individual or treatment.

We provided food every other day and beetle larvae and House Crickets were the prey items used in the various treatments. Every other day, we fed both mealworms and crickets grated carrots *ad libitum* to enhance their overall nutritional value. We also dusted them with a multi-mineral supplement called "Miner-All<sup>©</sup>" (calcium minimum 34% maximum 36%, vitamin D3 4,400.00 I.U, manganese 453.66 mg, zinc 544.39 mg, iron 136.10 mg, copper 113.42 mg, iodine 36.29 mg, cobalt 3.63 mg, magnesium 453.66 mg, and selenium 11.023 mg; Sticky Tongue Farms, Sun City, California, USA). We placed the insects in a plastic bag, which was then shaken to evenly coat the food items



**FIGURE 1.** Growth curves (Mass  $Day_{1}$  – Mass  $Day_{1}$ ) of Leopard Geckos over a four month period in response to a House Cricket diet (°), Beetle Mealworm (¢). Vertical bars denote 95% confidence intervals.

with the mineral supplement prior to feeding. The dusting of the prev sources was completed to ensure that each Leopard Gecko received a nearly equal amount of calcium and other important nutrients. It is also important to note that each invertebrate species fed as a prey item was different in its original nutrient content (Table 1; Barker et al. 1998). During the experiment, we gave each of the 15 Leopard Geckos an equal amount of food from one of the following treatments: crickets only (C; 5 individuals), mealworms only (M; 5 individuals), or a mix of crickets and mealworms (CM; 5 individuals). For the first 50 days, we fed the geckos 0.4 g of food in each of the treatments. After 50 days, we raised the amount of food given to 0.6 g and this amount was used for the duration of the experiment to meet the nutritional requirements of the growing geckos (0.6 g of crickets [C], 0.6 g of mealworms [M], and 0.3 g of crickets plus 0.3 g of mealworms [CM]). The weight of the prev items was calculated by weighing the insects on a digital balance (± 0.05 g; Salter Brecknell Model PB-500<sup>©</sup>, Fairmont, Minnesota, U.S.A.).

Every other day we took morphological measurements of each Leopard Gecko with electronic digital callipers ( $\pm 0.1$  mm). We measured the following

traits: maximum head width, head length, basal tail width, and body size (snout-vent length). We also recorded body mass using a digital balance (Salter Brecknell Model PB-500<sup>°</sup>). To control for temporal variation, we used measurements of each individual in a repeated measures ANOVA with time of measurement being the repeated measure and food treatment the fixed factor (Sokal and Rohlf 1995). As all traits were highly correlated with body size (r > 0.71, P < 0.05 in all cases), we then analysed the morphological character measurements using a MANCOVA with the traits as dependent variables, body size as covariate, and food treatments as factors. As time was constant for all the individuals, we calculated growth using the following equation: Mass  $Day_{x}$  – Mass  $Day_{1}$ . Upon finding significant differences, we used Tukey's HSD test to determine significant differences among individual treatments. All statistical analyses were performed using STATISTICA v.6 (StatSoft Inc. 2001) with an alpha level of 0.05.

# RESULTS

Dietary manipulations affected morphological

**TABLE 2.** Mean weight ( $\pm$  SE) in grams of Leopard Geckos under three feeding treatments over a four month period. All treatments and months were significantly different from each other (P < 0.001).

Diet Treatment	30 days	60 days	90 days	120 days
House Cricket	4.46 (0.175)	5.30 (0.154)	6.99 (0.208)	9.34 (0.199)
Mealworms	4.21 (0.117)	6.00 (0.084)	8.85 (0.183)	12.43 (0.184)
Mixed	3.72 (0.147)	4.83 (0.126)	7.05 (0.198)	9.88 (0.239)

characters in the Leopard Gecko. With regard to head width, individuals from treatment M had significantly greater head widths than individuals in other treatments  $(F_{8.992} = 135.8, P < 0.001)$ . After 120 days of study, the mean head width increased by 1.53 mm, 2.64 mm, and 2.27 mm in treatments C, M and CM, respectively. Further analysis determined that individuals in treatments M had greater head widths than individuals in C and CM treatments (Tukey HSD = 0.002 and 0.009respectively, P < 0.05) while C and CM treatments were not significantly different (Tukey HSD = 0.945, P >0.05). We observed the same trend for head length  $(F_{8.992} = 135.8, P < 0.001)$  and tail width  $(F_{8.992} = 135.8, P < 0.001)$ P < 0.001), such that individuals in the M diet had a greater head length and tail width. The mean head length increased by 2.45 mm, 4.31 mm, and 2.97 mm in treatments C, M, and CM, respectively, while the mean tail width increased by 1.35 mm, 2.02 mm, and 1.92 mm in treatments C, M and CM, respectively.

Growth also varied as a result of dietary manipulation (Table 2). After controlling for body size and correcting for snout-vent length, individuals in treatment M had significantly greater mass ( $F_{8,992} = 16.097$ , P < 0.001) as compared to the other two feeding treatments (Fig. 1). At the completion of the experiment, the mean body mass of Leopard Geckos increased by 5.78 g, 9.65 g, and 6.98 g (mass increase of 262%, 447%, and 340% of the original mass; Table 2) in treatments C, M, and CM, respectively. Treatment M was significantly different from C and CM treatments with individuals fed mealworms having a greater growth (Tukey HSD = 0.00002 and 0.099, respectively, P < 0.05).

# DISCUSSION

Our results confirm that juvenile Leopard Geckos grew on all three diets and there were no short-term negative growth effects of any of the feeding treatments. Our hypothesis that Leopard Geckos fed a mixed diet would have greater body mass increase was not supported. However, our study showed that Leopard Geckos fed a strictly mealworm diet consistently weighed more and experienced significantly greater growth than geckos from the two other groups. When correcting for snout-vent length, mealworm diet individuals also had larger morphological characteristics such as head length, head width, and tail width at the conclusion of the experiment.

The mealworm diet produced Leopard Geckos with significantly larger traits than either of the other two feeding treatments and the gecko body mass was significantly different per feeding treatment over the four month study period. By contrast, Rich (1995) did not observed any difference between mealworm and cricket diets with regards to gecko mass in an experiment where individuals were fed both food sources *ad libitum*. The

author also noted that Leopard Geckos fed more actively on crickets, which is expected because Leopard Geckos are visual predators and crickets are more active than mealworms (Rich 1995). This increase in mass (in the mealworm diet) that we found could be a result of the nutritional components of beetle larvae (mealworms) in comparison to House Crickets. For instance, calcium levels were different among the food sources (Table 1) and it has been proposed that calcium levels in invertebrates are a better measure of their suitability as alternate food sources compared to fat content (Anderson 2000). However, although House Crickets contained a higher percentage of calcium as compared to mealworms, the latter individuals grew quicker in our study, suggesting that calcium is not the only contributing factor to growth rates in Leopard Geckos. More importantly, the differences in fat content may be one contributing factor to the significantly larger individuals observed in the mealworm only treatment. This conclusion has also been reached by similar experiments conducted on Leopard Geckos (Rich 1995) and Western Fence Lizards (Sceloporus occidentalis; Boykin 1992). However, it is generally believed that a higher percentage of fat in food sources increases the likelihood of hepatic lipidosis or obesity. Obesity in animals results from an improper balance between energy intake and the expenditure of energy. Even though such improper balance may be the case in Leopard Geckos, it does not necessarily result in fat or obese individuals, as large variation in this trade-off has been observed (Seidell 1998). In fact, Case (1979) suggested that these negative notions of increased caloric value in captive diets are actually what would be naturally occurring whereby the abundance of food rather than the caloric content of food might affect diet constitution. If this is true, then the perception that increased caloric value of mealworm diet will produce fatter geckos might not be accurate and more knowledge on the availability of insect larvae (which are high in fat but tend to be fossorial or effectively camouflaged) versus adult insects (which are of lower energy concentration but actively forage on the surface) in the wild is needed. Moreover, Leopard Geckos, as other gecko species, store fat in their tails making it difficult to accurately detail obesity and further studies would be required to explore this hypothesis.

Diets of other captive reptiles have been studied to understand the link between diet and phenotypic expression of growth. Komodo Dragons (*Varanus komodoensis*) are an important zoological species and are typically fed rodent or poultry based diets in captivity. Comparing dragons fed either a rodent diet containing 24.9% fat or a poultry diet containing 44.4% fat, Lemm et al. (2004) did not observe any difference in body weight or morphological traits. In contrast, the mealworm diet in our study with the higher fat content significantly increased the size of morphological features and overall body mass. In fact, these two studies suggest that calories, not the form of the diet (protein vs. fatrich), drive growth and perhaps morphometrics. When calories were controlled (Lemm et al. 2004), diet did not influence size, but, when mass was controlled leading to greater calories intake (our study), there were morphometric differences among diets. Experimental studies have also been conducted on frogs to determine the relationship between diet and growth. Claussen and Layne (1983) compared growth rates and survival of juvenile Bufo woodhousei fowleri toads maintained on four food types, including crickets and mealworms. The authors noted that all diets were capable of supporting growth. However, individuals that were fed exclusively crickets exhibited poor growth rates in comparison to the other feeding treatments (a mixture of crickets and mealworms or solely mealworms) whereby individuals solely fed mealworms grew rapidly from the onset (Claussen and Layne 1983). Growth in American Toads (Bufo americanus), Couch's Spadefoot (Scaphiopus couchi), and American Bullfrogs (Rana catesbeiana) also showed the same results, with cricket diet producing smaller individuals significantly and reduced morphological characteristics (Modzelewski and Cullev 1974; Dimmit and Ruibal 1980; Jorgensen 1989). These results are consistent with our study on Leopard Geckos suggesting the importance of fat in diets of amphibians and reptiles in the context of captive breeding.

Not only did diet affect the growth rates and overall body mass of the Leopard Geckos, it also influenced the phenotypic expression of several important fitness related traits. Head width, head length, and tail width all differed significantly among feeding treatments, with individuals in the mealworm diet presenting larger morphological characters as compared to individuals in the other two feeding treatments. In fact, as the Leopard Gecko's growth rate increased, so did measurements of specific phenotypic characters. However, a diet that optimises growth is not necessarily one that optimises long term fitness as it may lack essential nutrients whose lack does not become apparent until later. For instance, Furrer et al. (2004) observed that a very fast growth rate in growing Galapagos Giant Tortoises (Geochelone nigra) may lead to an early sexual maturity, in turn shortening the overall longevity. Although it is known that growth rates slow dramatically in Leopard Geckos once sexual maturity is reached (Kratochvil and Frynta 2002), our results confirm that individuals raised with different food resources will present different developmental curves before sexual maturity, which may lead to potential differences in survival and fitness when individuals are released in the wild (DeWitt et al. 1998).

Each of the three feeding treatments (crickets only, mealworms only, and a mixture of crickets and mealworms) we used throughout this study are clearly

capable of supporting adequate growth of juvenile Leopard Geckos. Although House Crickets and mealworms are not the same prey items Leopard Geckos consume in the wild, it appears they are both suitable replacements. However, they are not all equal in their effects on Leopard Gecko's size-related traits. It appears that mealworms are a superior food source for Leopard Geckos in terms of promoting growth during early development. While further studies are required to establish the potential negative consequences of an exaggerated fast growth rate and evaluate the long-term dietary effects from juvenile stages to sexual maturity that different prey items have on growth and ultimately fitness, our study adds to the limited data concerning the effect of diet on the growth rates of captive animals.

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