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THE MEDITERRANEAN GECKO AS A SENTINEL TO EVALUATE HEAVY METAL EXPOSURE

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***Abstract.*—The Mediterranean Gecko (*Hemidactylus turcicus*) is a small, nocturnal lizard that lives on buildings throughout the southeastern United States. Because the buildings it lives on are also occupied by humans and other organisms (e.g., pets), the Mediterranean Gecko may be a good sentinel or bioindicator species to monitor the potential exposure of humans and pets to heavy metals. We measured the concentrations of eight metals (Pb, Cu, Zn, Cd, Cr, Fe, Mn, and Ni) in tail clips from Mediterranean Geckos captured from buildings in an industrialized, urban area and from buildings in an isolated rural area in southwestern Louisiana, USA and compared metal concentrations between sites as well as between sexes within sites. There were no significant differences in metal concentrations in gecko tails from the rural site versus the two urban sites, but there were significant differences in Cu, Zn, and Fe between the two urban sites. Females tended to exhibit higher metal concentrations than males for all eight metals at two sites and seven of eight metals at a third site, and there were significant differences in Cu, Zn, and Fe concentrations in tails of female (but not male) geckos collected from two urban sites. These results suggest that the Mediterranean Gecko can be a viable sentinel species to monitor potential exposure to heavy metals and the metal concentrations in gecko tails should be analyzed separately for each sex.**

Key Words.— bioindicator; heavy metals; *Hemidactylus turcicus*; Louisiana; Mediterranean Gecko; sentinel species; sex; tails

INTRODUCTION

Organisms living in industrialized urban areas are potentially at risk of exposure to heavy metals from industrial emissions, auto exhaust, and other sources (Loumbourdis 1997; Campbell and Campbell 2000). Some heavy metals, such as mercury (Hg), lead (Pb), and cadmium (Cd), are known to cause serious health problems in humans and other animals if they accumulate in high enough concentrations in tissues (Gardner and Oberdorster 2006). One way to evaluate the potential of heavy metal exposure to organisms in industrialized areas is to measure the concentration of metals in the tissues of other animal species that live in close proximity to the organisms of interest. Such species are termed sentinel species (Selcer 2006) or bioindicator species (Lambert 1999). To be useful, a sentinel species should (1) have a wide geographic distribution so it can be found in areas with a range of contaminant profiles to facilitate comparisons (Loumbourdis 1997; Burger et al. 2005); (2) exist in large populations that can be easily sampled (Lambert 1999); (3) exhibit high site fidelity to maximize exposure (Campbell and Campbell 2000; Fletcher et al. 2006); and (4) should be a species about which information on basic life history is available (Selcer 2006).

The Mediterranean Gecko (*Hemidactylus turcicus*) is a small, nocturnal lizard that exhibits the qualities necessary to be a candidate sentinel species for monitoring potential exposure of humans and other resident organisms to heavy metals (Selcer 2006). In industrialized areas, the Mediterranean Gecko lives on and in buildings occupied by humans (Punzo 2001; Meshaka et al. 2006). Moreover, female geckos lay their eggs in soil around buildings or in secluded corners within buildings (Selcer 1986; Paulissen and Buchanan 1991). The Mediterranean Gecko can be found in both urban and rural areas (Meshaka et al. 2006), allowing for quantification of the increased potential for exposure in urban areas by comparison to rural "reference" sites (Fletcher et al. 2006). This species commonly occurs in large populations on the buildings it occupies (Selcer 1986; Punzo 2001; Locey and Stone 2006; Hibbs et al. In press), making it possible to obtain adequate sample sizes for analysis. In addition, Mediterranean Geckos rarely move from one building to another (Trout and Schwaner 1994; Locey and Stone 2006) and can live for up to four years (Paulissen et al. 2004). Small home ranges and long site tenure insure the metal concentrations found in an individual will be a reliable indicator of exposure at the specific site at which it is captured. Mediterranean Geckos can accumulate heavy metals by inhalation, or by consuming invertebrates that have accumulated metals (Lambert 1999; Fletcher et al.

2006). Metals may also be absorbed through the skin or may be ingested when the gecko cleans its face with its tongue or when the gecko ingests shed skin. Moreover, the Mediterranean Gecko offers an additional advantage of non-destructive sampling. Like many lizards, the Mediterranean Gecko readily autotomizes its tail to escape predation. Because gecko tails are a combination of fat, blood, muscle, bone, and skin tissue, they are more likely to reveal accumulation of metals than single tissues (Hopkins et al. 2001; Fletcher et al. 2006) and so can be used to assess metals accumulation without sacrificing animals.

The basic ecology and life history of the Mediterranean Gecko is well known (Selcer 2006). It is a small species with an adult snout-to-vent length (SVL) of 50-55 mm. It is native to the eastern Mediterranean region but was introduced into the United States in the early twentieth century (Stejneger 1922). Today it is found throughout the southeastern United States and has expanded its range northward to Maryland (Norden and Norden 1991), Missouri (Bufalino 2004), Illinois (McDowell et al. 2006), and South Dakota (Platt et al. 2008). It lives almost exclusively on buildings and other artificial structures, climbing up and down vertical walls at night in search of insects and other arthropod prey (Saenz 1996). In southwestern Louisiana, where this study was conducted, the Mediterranean Gecko is active on outside walls every warm night from March through November, and can occasionally be seen outside during warm evenings from December to February. Geckos, especially juveniles and subadults (SVL < 42 mm), are active inside buildings all year. Therefore, Mediterranean Geckos are likely exposed to whatever heavy metals there are on or in buildings throughout the year.

In 2005, we began a study of the concentration of heavy metals in the tails of Mediterranean Geckos collected from sites in heavily industrialized, urban areas and rural areas in southwestern Louisiana, USA. We hypothesized that geckos collected from more industrialized, urban areas would contain higher metal concentrations in their tails than geckos from a rural site, owing to inter-site differences in metal contamination profiles. Sample sizes from three of the sites were large enough to permit evaluation of the influence of the gecko sex on heavy metal concentrations. Herein, we report the results of this evaluation and offer suggestions for conducting future work.

MATERIAL AND METHODS

Study sites.**—**We conducted this study in Calcasieu and Beauregard Parishes in southwestern Louisiana, USA. Calcasieu Parish includes a metropolitan population of approximately 200,000 people comprised primarily of the city of Lake Charles and surrounding small towns. The central portion of the parish supports a large petrochemical industry with many smokestacks that release particles into the atmosphere; prevailing southerly winds carry the particles northward towards small towns north of Lake Charles, such as Westlake and Moss Bluff. Beauregard Parish lies directly north of Calcasieu Parish and is primarily rural with no heavy industry. To test the hypothesis that Mediterranean Geckos from urban areas contained higher concentrations of heavy metals than in rural areas, we collected Mediterranean Geckos from multiple sites in Calcasieu and Beauregard Parishes. The three sites for which we have the largest sample sizes were: (1) S. P. Arnett Middle School (SPA) in Westlake, LA (Calcasieu Parish, 30° 14’ 34’’N lat.; 93° 15’ 06’’W long.); (2) Moss Bluff Elementary School (MBE) in Moss Bluff, LA (Calcasieu Parish, 30° 18’ 00’’N lat.; 93° 12’ 13’’W long.); and (3) South Beauregard Elementary School (SBE) in rural Beauregard Parish (30° 34’ 11’’N lat.; 93° 14’ 03’’W long.). All three sites were large brick buildings with numerous entrances that were illuminated at night with bright lights that attracted the insects that the geckos consumed. Each building was composed of several connected units most of which were one story, enabling us to reach most of the geckos we saw active on outside walls. S. P. Arnett Middle School is located < 5 km NE of the heavy industry in Calcasieu Parish. Moss Bluff Elementary School is located about 11 km NE of the industries. Both SPA and MBE are in the path of smoke plumes pushed northward from the industries by prevailing winds and are exposed to the exhaust of numerous automobiles traveling in the surrounding cities. By contrast, South Beauregard Elementary School is located in an isolated rural area 16 km N of the Calcasieu/Beauregard Parish line, about 32 km N of the industries of Calcasieu Parish; thus, it receives less contamination from stack emissions from industries from central Calcasieu Parish. In addition, due to smaller human population and less vehicular traffic, SBE presumably is exposed to much less auto exhaust.

Data collection.**—**We collected Mediterranean Geckos by shining flashlights onto the outside walls of buildings at night, then using soft, long-handled cleaning brushes to gently chase geckos to a position where they could be captured by hand. We measured geckos (SVL to the nearest mm), weighed them to the nearest 0.1 g with a Pesola Spring balance, and determined their sex by checking for the presence of pre-anal pores (present in males, absent in females; only for geckos > 42 mm SVL could sex be reliably determined). We assayed adult geckos (45-58 mm SVL) in good condition (i.e., with no obvious injuries or deformities) by removing a 1.0-1.5 cm section of tail by pinching the tail at a fracture plane. Each tail was placed in its own Ziploc™ bag and stored on ice until it was returned to the lab. We

TABLE 1. Mean ± SE concentration of metals in tails of Mediterranean Geckos collected from a rural site (SBE) and two urban sites (MBE and SPA) in southwestern Louisiana, USA. All data are in mg/kg dry tail mass; males and females were pooled. ANOVA plus post-hoc *t*-tests on log-transformed data were used to compare sites: values superscripted by the same letter are not significantly different ($P \geq 0.05$). Sample sizes (number of geckos collected) are given in parentheses.

Heavy Metal	SBE Rural Site (N = 12)	MBE Urban Site (N = 34)	SPA Urban Site (N = 19)
Lead (Pb)	15.04 ± 2.34^{a}	11.98 ± 1.35^{a}	17.31 ± 2.77^{a}
Copper (Cu)	$8.87 \pm 1.12^{a,b}$	8.23 ± 0.85^{a}	12.35 ± 1.80^{b}
Zinc (Zn)	$85.61 \pm 17.27^{a,b}$	62.64 ± 4.72^{a}	90.27 ± 7.15^{b}
Cadmium (Cd)	7.39 ± 1.00^{a}	6.60 ± 0.67^{a}	7.43 ± 0.74^{a}
Chromium (Cr)	11.46 ± 1.55^{a}	10.42 ± 1.04^{a}	12.38 ± 1.25^{a}
Iron (Fe)	$53.28 \pm 6.75^{a,b}$	41.91 ± 3.01^{a}	60.05 ± 4.98^{b}
Manganese (Mn)	7.95 ± 1.02^{a}	7.25 ± 0.83^{a}	8.12 ± 0.76^{a}
Nickel (Ni)	14.59 ± 0.97^{a}	12.23 ± 1.22^{a}	14.86 ± 1.60^{a}

then uniquely toe-clipped each gecko and released it at its site of capture. All geckos were collected between 2030 and 2330 h on the following dates: SPA: 20 July 2006; MBE: 25 April and 5 July 2006; SBE: 12 April 2006.

We stored tails collected in the field in a -80°C freezer until processing. Frozen tails were weighed individually and then dried in an 80°C oven overnight. Dried tails were reweighed then placed individually in clean Teflon chemical digestion bombs with 2 mL HNO_3. The tails were then digested for 20 seconds in a microwave set on high power, then allowed to cool in a refrigerator for five minutes (Friel et al. 1990). The contents of each bomb were then transferred to 10 mL volumetric flasks, diluted to 10 mL with de-ionized water, then transferred to clean plastic vials, which were stored at -20°C until analyzed. These samples were analyzed for the following metals: lead (Pb), copper (Cu), zinc (Zn), cadmium (Cd), chromium (Cr), iron (Fe), manganese (Mn), and nickel (Ni) by inductively coupled plasma analysis (ICP), using a Leeman PS 1000UV instrument (Teledyne Leeman Labs, Hudson, New Hampshire, USA). The ICP was optimized and the optics aligned using a 100 ppm Mn standard at 257.6 nm. Three independent determinations of each metal concentration were obtained for each sample. The mean of the three samples was compared to a standard curve generated from 0, 1, 3, and 5 ppm standards, and the metal concentrations were expressed in mg/kg (ppm) of dry tail mass.

We statistically compared log-transformed metal concentrations in geckos from the three sites on each of the eight metals using ANOVA and post-hoc *t*-tests. Additional comparisons of metal concentrations in males versus females were performed for each of the three sites using *t*-tests. The *P*-value to reject was 0.05 in all cases.

RESULTS

There were no statistically significant differences in the mean concentrations of any of the eight metals in gecko tails collected from the rural Beauregard Parish site versus gecko tails collected from the two urban Calcasieu Parish sites (Table 1). However, there were a few statistically significant differences between the two urban Calcasieu Parish sites: gecko tails collected from SPA exhibited significantly higher concentrations of Cu, Zn, and Fe than did gecko tails from MBE (Table 1). Comparison of the three sites shows that for all eight metals tested, gecko tails collected from MBE exhibited the lowest mean concentrations; gecko tails from SPA exhibited the highest mean concentrations, whereas gecko tails from the rural SBE site exhibited mean concentration in between the two urban sites (Table 1). Thus, contrary to our hypothesis, geckos from rural Beauregard Parish did not exhibit lower mean concentrations of metals than geckos from Calcasieu Parish.

When metal concentrations were analyzed by sex, an interesting trend emerged. Samples collected from two of the sites (SPA and SBE) showed females had a higher mean concentration of all eight metals than males. For samples from the third site (MBE), this trend was observed for seven of the eight metals tested (the lone exception is Zn; Table 2). The sex difference reaches statistical significance for Cr (and nearly did so for Pb, Cu, Cd, Fe, and Ni) at SPA (Table 2). Furthermore, females at SPA had significantly higher concentrations of Cu, Zn, and Fe than did females at MBE (Cu: $t = 2.47$, $P = 0.020$; Zn: $t = 3.05$, $P = 0.005$; Fe: $t = 2.73$, $P = 0.011$). However this was not observed for males from the two sites (Cu: $t = 0.73$, $P = 0.474$; Zn: $t = 1.86$, $P = 0.076$; Fe: $t = 1.58$, $P = 0.129$).

DISCUSSION

Contrary to our hypothesis, there were no significant differences in the concentrations of any metal in the tails of geckos from the rural site versus the two urban sites. This result is surprising because previous work has shown metal concentrations in lizards were strongly influenced by proximity of lizard populations to urban areas (Campbell and Campbell 2000). In a study with the Moorish Wall Gecko, *Tarentola mauritanica*, Fletcher et al. (2006) found the concentration of heavy metals (especially arsenic, lead, and cadmium) in whole carcasses generally increased the closer the geckos were

TABLE 2. Mean ± SE concentration of metals in tails of male and female Mediterranean Geckos collected from three different sites in southwestern Louisiana, USA. All data are in mg/kg dry tail mass. Probability (*P*) of differences based on a *t*-test on log-transformed data comparing the two sexes is shown in the last column. Sample sizes are given in parentheses.

	South Beauregard Elementary School (SBE) (♂ = 7, ♀ = 5)		
Heavy Metal	Males	Females	*P*
Lead (Pb)	12.97 ± 2.51	17.94 ± 4.39	0.55
Copper (Cu)	8.15 ± 1.10	9.88 ± 2.28	0.80
Zinc (Zn)	74.94 ± 7.22	100.54 ± 42.02	0.91
Cadmium (Cd)	6.73 ± 1.11	8.30 ± 1.90	0.75
Chromium (Cr)	10.32 ± 1.57	13.05 ± 3.09	0.72
Iron (Fe)	47.94 ± 3.84	60.75 ± 15.68	0.70
Manganese (Mn)	7.24 ± 1.04	8.93 ± 2.04	0.79
Nickel (Ni)	12.53 ± 2.18	17.47 ± 4.52	0.63
	Moss Bluff Elementary School (MBE) (♂ = 17, ♀ = 17)		
Lead (Pb)	10.94 ± 1.80	13.01 ± 2.04	0.33
Copper (Cu)	7.62 ± 1.06	8.83 ± 1.35	0.48
Zinc (Zn)	64.33 ± 6.96	60.96 ± 6.57	0.59
Cadmium (Cd)	6.12 ± 0.92	7.08 ± 1.00	0.45
Chromium (Cr)	9.86 ± 1.53	10.99 ± 1.42	0.48
Iron (Fe)	37.88 ± 3.62	45.94 ± 4.72	0.26
Manganese (Mn)	6.57 ± 0.95	7.92 ± 1.37	0.46
Nickel (Ni)	11.62 ± 1.81	12.83 ± 1.67	0.53
	S. P. Arnett Middle School (SPA) (♂ = 7, ♀ = 12)		
Lead (Pb)	11.26 ± 1.74	20.55 ± 4.00	0.072
Copper (Cu)	8.61 ± 1.60	14.40 ± 2.54	0.056
Zinc (Zn)	82.33 ± 8.43	94.90 ± 10.22	0.45
Cadmium (Cd)	5.75 ± 0.80	8.41 ± 0.98	0.083
Chromium (Cr)	9.14 ± 1.21	14.27 ± 1.64	0.044
Iron (Fe)	48.88 ± 7.10	66.57 ± 6.14	0.083
Manganese (Mn)	6.53 ± 0.80	9.05 ± 1.04	0.12
Nickel (Ni)	11.22 ± 1.64	16.99 ± 2.16	0.065

to a source of mine tailings contamination. This study also reported that lead concentrations tended to be higher in urban areas than rural ones independent of the location of the geckos along the mine tailings contamination gradient. However, other studies report different patterns. For example, Burger et al. (2004) found site differences in metal concentrations in Brown Anoles (*Anolis sagrei*) but the expected pattern of higher concentrations from lizards in industrialized sites did not hold; indeed the authors could not identify any consistent pattern. Furthermore, Loumbourdis (1997) found that Starred Agamas (*Laudakia stellio*) from a rural site generally had higher concentrations of metals than those from an urban site in a large metropolitan area. Therefore, the lack of significant differences between urban and rural sites in our study is not unprecedented. Our results highlight the need to collect more data to determine if the generally accepted pattern of greater metal accumulation in tissues of lizards living near urban areas is really as "general" as believed.

Though we found no significant differences in metals concentration in gecko tails from urban versus rural sites, we did find significant differences in copper, zinc, and iron concentrations in tails of geckos collected from the two urban sites (both metals were higher at SPA than MBE). These results suggest that analysis of heavy metal concentrations in tails of Mediterranean Geckos is sensitive enough to identify site differences. Recent studies have advocated non-destructive sampling of skin, blood, or tail clips as an alternative to sacrificing whole animals for quantification of metals concentration in snakes (Hopkins et al. 2001; Burger et al. 2005). To our knowledge, the only study that has used this approach on a lizard is that of Fletcher et al. (2006). These authors measured concentrations of metals from whole bodies as well as from tails and concluded tail clips are a viable non-destructive index of metal accumulation for most metals. Comparison of the data we obtained from gecko tails to the data from Fletcher et al. (2006) and other studies is problematic because the comparisons involve different combinations of tissues, obtained from different, unrelated species of lizards, living in different parts of the world with potentially different levels of metal contamination (Campbell and Campbell 2000;

Burger et al. 2005; Fletcher et al. 2006). In general, however, our results from Mediterranean Gecko tails fall within the ranges measured in other lizard species for seven of the eight metals we studied. The one exception is cadmium: Mediterranean Gecko tails we sampled had Cd concentrations about an order of magnitude greater than those measured from whole bodies of Starred Agamas (Loumbourdis 1997), Brown Anoles (Burger et al. 2004), or Moorish Wall Geckos (Campbell and Campbell 2000; Fletcher et al. 2006). This suggests that either southwestern Louisiana is subject to higher Cd exposure or that Mediterranean Geckos accumulate Cd in their tails in a way fundamentally different from how other lizards accumulate Cd in their entire bodies. It is interesting to note that Fletcher et al. (2006) found that, of the 16 metals they compared, Cd showed the poorest correlation between whole body and tail concentrations in Moorish Wall Geckos. These results suggest Cd accumulation in lizard tails warrants additional study.

Results of this study suggest it is important to analyze metal concentrations in lizard tissues separately for each sex. Females exhibited higher concentrations of all eight metals than males from the same site (except for Zn at MBE), and the only significant differences in metal concentrations between sites that we found (Cu, Zn, and Fe all higher at SPA than MBE) were statistically significant for females but not for males. Only a few studies have addressed the role of sex in metals accumulation in lizards, and we are aware of only one that showed the trend of females having higher concentrations than males. Burger et al. (2004) studied the concentrations of seven metals in whole bodies (minus stomach contents) of Brown Anoles collected from six sites in southern Florida, USA. They found that females had higher concentrations of cadmium, chromium, manganese, mercury, and selenium than males, though the magnitude of the differences varied among sites. Other studies either show no differences in metal concentrations between sexes (Loumbourdis 1997) or show males having higher metal concentrations than females (reviewed in Burger et al. 2004). The causes of sex differences in metals accumulation in lizard tissues are unknown. Burger et al. (2004) hypothesized the sex differences they found were a consequence of microhabitat separation that led to males and females consuming different types of arthropods. These authors also allowed that sex-specific differences in physiology may also be involved. In our study, physiological differences may be more important that microhabitat differences. This is because male and female geckos were caught from the same "microhabitats" on the sides of buildings (mostly brightly illuminated window and door frames) and so likely consumed the same types of arthropod prey. Furthermore, sex-specific physiological differences that affect the processing of metals have been demonstrated in a lizard species. Hopkins et al. (2005) showed that when juvenile Western Fence Lizards (*Sceloporus occidentalis*) are fed a diet of selenium-laden crickets, males and females accumulated the same amount of Se on a whole body basis, but partitioned it among their organs differently (females partition more to their gonads than do males). Similar sex-specific differences may exist in the Mediterranean Gecko.

The use of tail samples from lizards to measure metal concentrations has several advantages. Tail clips are easy to obtain, especially from lizards that autotomize their tails as part of their suite of antipredator defenses. Furthermore, because lizards regrow lost tails, recapture of a lizard that has regenerated its tail since its initial capture permits measurement of metals accumulation in a single lizard over a known span of time. Finally, use of tails eliminates the need to sacrifice whole animals resulting in less impact on the lizard populations being studied at a specific site (Selcer 2006). We encourage additional studies using lizard tails to measure heavy metal concentrations and recommend that data be analyzed separately for males and females to ensure the maximum amount of information can be obtained with minimal impact to lizard populations.

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ANDREA DESTORIES was a student in Biology at McNeese State University taking classes in preparation for applying to Medical School. She worked on this project during her junior and senior years and presented preliminary results at the Louisiana Academy of Sciences Meeting in 2006. She is currently a medical student at the Louisiana State University Health Science Center in New Orleans, Louisiana.

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MARK PAULISSEN is an Associate Professor of Biology at Northeastern State University in Tahlequah, Oklahoma; prior to 2006 he was a member of the faculty in the Department of Biological and Environmental Sciences at McNeese State University in Lake Charles, Louisiana. Since earning his Ph.D. from the University of Oklahoma, he has conducted research on the biology of parthenogenetic whiptail lizards in Texas and population biology of the Mediterranean gecko in Louisiana. Currently, his main research interest is in spatial learning and antipredator behavior of skinks.