2011

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Ecotoxicology and Environmental Safety 74 (2011) 1081–1088

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Relationships between physiological characteristics and trace metal body burdens of banded garden spiders *Argiope trifasciata* (Araneae, Araneidae)

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ARTICLE INFO

Article history:
Received 15 July 2010
Received in revised form
4 February 2011
Accepted 6 February 2011
Available online 5 March 2011

Keywords:
Trace metals
Coastal wetland
Orb-weaving spider
Body size
Fecal material
Oxidative degradation

ABSTRACT

Banded garden spiders (*Argiope trifasciata*) were collected at the Ballona Wetlands, a metal contaminated salt marsh. The relationship between spider body size and individual metal loads was investigated. Biochemical markers were identified in spider fecal material and found to correlate to body metal levels. Body metal dry weight concentrations of Cd, Cr, Cu, Zn and total metals in female *A. trifasciata* exhibited distinct patterns of spatial and annual variation during 2006 and 2007. Spider body size was homogeneous across sites in both years, while increased Cd and Cr concentrations were sometimes associated with a reduction in spider size, though the influence of Cr was quite minor. Spiders with higher body Cu levels showed a reduction in peak area for hypoxanthine and an un-identified component in fecal material chromatograms. Spatial and annual differences in metal bioaccumulation are likely mediated by variation in site-specific environmental parameters and rainfall, while the negative relationships between body size and metal levels are presumably a consequence of a spider's expenditure of energy for metal tolerance mechanisms vs. foraging and growth. Finally, correlating body metal levels with excreta products constitutes a novel method to non-invasively predict metal levels in spiders.

1. Introduction

Salt marshes are highly productive ecosystems located in temperate coastal areas throughout the world (Chapman, 1977). Located at the interface between terrestrial and aquatic habitats, salt marshes harbor considerable biodiversity and are important contributors to food chains beyond their borders since they are net exporters of organic matter (Teal and Howes, 2000). Moreover, salt marshes generate some of the most important ecosystem services of any natural ecosystem, including disturbance regulation (e.g., storm protection), waste treatment, food production (e.g., aquaculture) and recreation (e.g., birdwatching) (Gedan et al., 2009). However, salt marshes worldwide have also experienced centuries of anthropogenic perturbations (e.g., land development, tidal restriction, resource extraction, pollution), particularly when located near growing cities and urban areas (Wilen and Bates, 1995; Gedan et al., 2009), making it vital to better understand and remediate current threats to marsh ecosystems and their biota (Zedler, 2001; Silliman et al., 2009).

The negative impacts of human population growth and urbanization for coastal ecosystems are especially evident at the Ballona Wetlands, a degraded salt marsh located on the western edge of the city of Los Angeles and the last remaining major coastal wetland in Los Angeles County, California (West, 2001). Its main water source is tidal flow via flap gates from the adjacent, concrete-lined Ballona Creek (Fig. 1), which serves $\approx 316\ km^2$ of highly urbanized western Los Angeles County and contributes 2–9% of the total pollutant mass emissions, which enter the Santa Monica Bay (Maguire-Thomas Partners, 1991). Water quality monitoring of the Creek has measured elevated levels of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), coliform and enteric bacteria, pesticides and trace metals (Los Angeles County Watershed Management, 2006). Given the daily cycling of contaminated Creek water through the Ballona

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doi:10.1016/j.ecoenv.2011.02.003

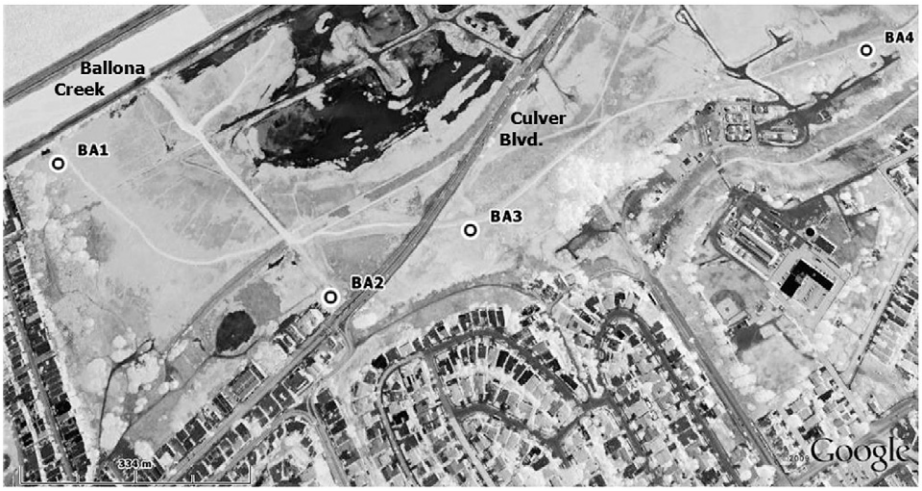

Fig. 1. Location of four sites (BA1, BA2, BA3, BA4) at the Ballona Wetlands Ecological Reserve (open space north and east of housing) sampled for *Argiope trifasciata*. The Ballona Creek flood protection channel and Culver Boulevard (Blvd.) are also indicated.

Wetlands, along with additional pollutant inputs from developed land and roads surrounding the Wetlands, it is not surprising that elevated heavy metal concentrations have been documented for both Wetlands water and animals (fish, bivalves) by Cohen et al. (2001). While the fitness or survival consequences of metal accumulation for these marine organisms was not assessed, Swift and Frantz (1981) found that 26% of the striped mullet (*Mugil cephalus*) they sampled from tidal channels at Ballona exhibited eroded fins or anatomical deformities, defects which they suspected were pollution related. Aside from this study, we are not aware of other investigations, which have dealt with the biological consequences of pollutant presence for Ballona Wetlands organisms.

With over 41,000 described species (Kuntner and Coddington, 2009), spiders are perhaps the fifth largest animal order (Foelix, 1996) and are a major predatory group in most ecosystems (Wise, 1993), including salt marshes, where they are usually the top arthropod predators (Finke and Denno, 2004; Gratton and Denno, 2006). Spiders assimilate trace metals from their prey (Zhang et al., 2009); from direct contact with contaminated substrates (Larsen et al., 1994); by the ingestion of metals on their body surface via grooming behavior (Clausen, 1986) and by the consumption of metal contaminated webs prior to building new ones for some web-building spiders (Hose et al., 2002; Xiao-li et al., 2006). Yet, relative to their biodiversity and ecological importance, knowledge concerning heavy metal accumulation by spiders and its biological consequences is taxonomically limited (Heikens et al., 2001; Schipper et al., 2008).

Given this context, one topic which merits further investigation is the relationship of metal loads to oxidative stress markers in spiders. Wilczek et al. (2008) found correlations between metal loads and cellular stress reactions in the midgut gland of three spider species. They observed negative correlations of caspase-3-like proteins and number of depolarized mitochondria, and positive correlations of the number of necrotic cells and catalase activity, for copper (Cu) and zinc (Zn). As the Wilczek study involved lethal methods, we hypothesized that metal stress correlations might be observed in spider feces, which led to our interest in developing a method of monitoring metal-induced stress via the analysis of fecal material. The main excretory components of spiders are purine bases (Foelix, 1996). We hypothesized that metal stress could directly effect the levels of nucleobases in fecal matter through direct metal-induced oxidative reactions or from changes in cellular stress reactions as observed by Wilczek et al. (2008). It is well documented that purine bases and nucleosides are the prime targets of metal-mediated oxidative degradation and oxidative nucleobase stress markers are linked to many human diseases (Marx, 1987; McLachlan et al., 1996; Burrows and Muller, 1998; Evans et al., 2010). Guanine, the major organic component of spider fecal matter, is the most easily oxidized base and a great deal of research has been devoted to studying products from the oxidation of guanine and its derivatives (Burrows and Muller, 1998; Cadet et al., 2002; Cooke et al., 2003; McCallum et al., 2004). The authors are not aware of any reports monitoring nucleobase oxidation products in spiders. However, there is literature precedence in the use of noninvasive methods to measure stress in a variety of birds and mammals via the monitoring of steroid hormone metabolites in feces (Eriksson et al., 2004; Touma and Palme, 2005; Wasser and Hunt, 2005) and numerous examples of monitoring nucleobase oxidation compounds in the urine of mammals and humans (Marx, 1987; Cooke et al., 2003; Evans et al., 2010).

Thus, to explore the fitness consequences for a web-building spider which experiences heavy metal uptake in a salt marsh ecosystem, this study investigated the relationship between body size and individual metal loads in the banded garden spider *Argiope trifasciata*, a cosmopolitan orb-weaving species (Levi, 2004) which is common at the Ballona Wetlands (Ramirez et al., 2003). We also report on the development of a non-invasive, non-lethal method of monitoring metal-induced stress in this species by identifying and measuring biochemical markers associated with oxidative stress in spider fecal material.

2. Materials and methods

2.1. Spider and fecal matter sampling

During October 2006 ($n=29$) and 2007 ($n=51$), adult female *A. trifasciata* were collected from four sites at the Ballona Wetlands Ecological Reserve (Fig. 1), with each site being adjacent to potential pollution sources (Ballona Creek, highway, housing). In the laboratory, spider body size [carapace width (mm)] was determined using a dissecting microscope with a calibrated ocular micrometer. Carapace width is fixed at maturation and does not change with the feeding status of the spider, making it superior to more plastic fitness measures (e.g., mass, body condition). After being measured, each spider was maintained in a glass petri dish sheltered by a plastic tumbler (diameter 8.5 cm; height 21.5 cm) for 4–5 days to facilitate web construction and the deposition of fecal matter onto the dish, similar to the protocol of Curtis and Carrel (2000). Excreta samples for each spider were collected, weighed and stored in glass vials pending sample preparation for HPLC. Spiders were subsequently frozen at −85 °C and then oven dried at 70 °C for 48 h pending metal analysis.

2.2. Microwave acid digestion

Following drying, each spider body was pulverized by hand using a mortar and pestle and approximately 0.09 g was placed in a 23 mL PTFE sample cup along with 3 mL of concentrated HNO_3. Each sample cup was placed in a Parr outer vessel. The resulting bomb assembly was tightly secured and microwaved on high power for 24 s in a 1.4 kW microwave. Bombs were removed from the microwave and placed on ice to chill for 30 min. Samples were then filtered and transferred into a 10 mL volumetric flask. The 10 mL volumetric flask was then diluted with deionized water. Samples were transferred to plastic sample storage containers pending atomic absorption analysis.

2.3. Atomic absorption spectroscopy

Concentrations of cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn) in the resulting digests for each spider were determined using graphite furnace (Cd, Cu, Cr) and flame (Zn) atomic absorption spectroscopy (AAS). A Shimadzu 6300 AA spectrometer was used for all analyses. Flame atomization employed a continuous flame with deuterium background correction. Metal concentrations were determined by the average of 5 measurements. Graphite furnace atomization used Ar gas with replicate runs carried out on all samples to ensure reproducibility. Standards were prepared fresh and run with method blanks and samples. For all metals tested, method blanks gave digest solution concentrations less than samples tested. In addition, select samples were analyzed in two separate runs (digestion followed by AA analysis) and yielded comparable results.

2.4. High performance liquid chromatography

Fecal samples were dried by heating overnight at 80 °C. The samples were prepared by adding 800 μL of deionized water and 200 μL of 2% H_3PO_4, followed by vortexing to dissolve the samples. The solution was then filtered through a Teflon© 0.45u PTFE filter, diluted ten-fold and assayed with a Beckman Gold HPLC with PDA detector fitted with a Phenomenex Synergi 4m Hydro reverse-phase column (80 A, 250 × 4.6 mm). The samples were run in a mobile phase consisting of a flow rate of 100% phosphate buffer solution (20 mM) at pH = 2.4 at a flow rate of 1 ml/min at 254 nm and peaks were detected using a photo-diode array detector. The identification of peaks was performed by comparing the retention times and UV/vis spectra of known standards via HPLC. The following standards were used to identify the main organic components: adenine, adenosine, allantoin,

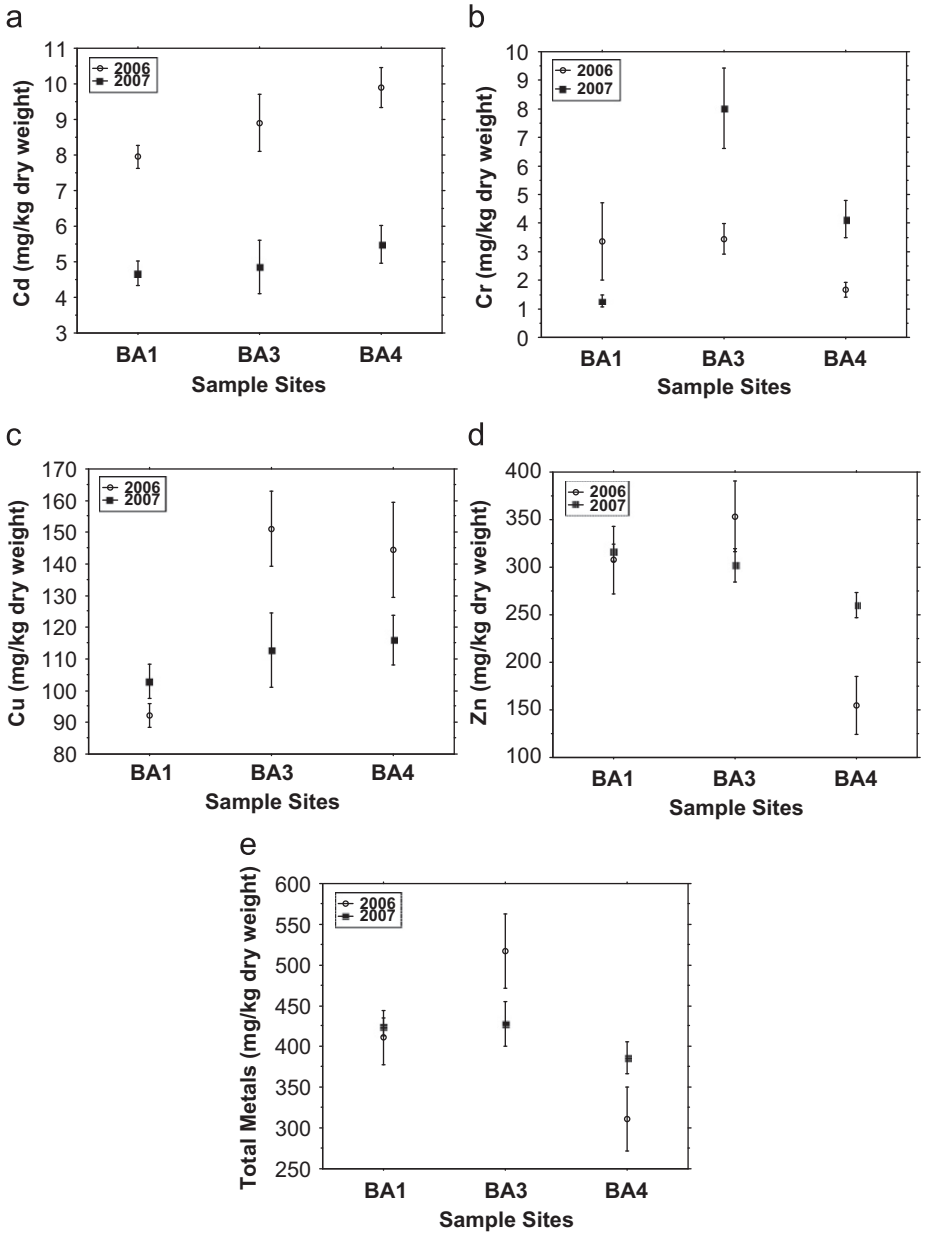

Fig. 2. Means (± SE) of metal mg/kg dry weight for *Argiope trifasciata* by sample site and year for sites BA1, BA3 and BA4. (a) Cadmium (Cd); (b) chromium (Cr); (c) copper (Cu); (d) zinc (Zn); (e) total metals.

cytidine, cytosine, guanine, guanosine, hypoxanthine, ionosine, thymidine, thymine, uric acid, uracil, uridine, xanthine and xanthosine. All chemicals were purchased from Sigma-Aldrich.

2.5. Statistical analyses

Since three sites (BA1, BA3, BA4) were sampled in both 2006 and 2007, we used two-way ANOVA to examine the effects of sample site and year on means of the carapace width and spider body metal concentration (mg/kg dry weight) for each metal and for total metals using the two years of data for these sites. In addition, since a fourth sample site (BA2) was added in 2007, we also conducted one-way ANOVA to test for differences in means of the carapace width and spider body metal concentration for each metal and for total metals among the four 2007 sample sites. In cases where a significant difference among means for the three or four sample sites was indicated by ANOVA, the Tukey–Kramer test (Dunnett, 1980) was used to conduct post-hoc pairwise comparisons.

To determine the relationship between spider size and metal load, regressions of carapace width (y) vs. body metal dry weight concentrations (x) were conducted for each metal (Cd, Cr, Cu, Zn) and for total metals for the largest site sample each year (BA3 in 2006, BA1 in 2007); for all samples combined in 2006 and 2007; and for the total samples collected over 2006 and 2007, which resulted in the generation of 25 regression relationships. For the HPLC data, we examined the relationship between chromatogram peak area per mg of fecal sample (y) and body metal dry weight concentrations (x) (Cd, Cr, Cu, Zn) for every peak in each chromatogram using regression analysis and pooled site samples for 2006 and 2007. In order to better meet the assumptions of regression, all regression analyses used natural logarithm transformed data (Sokal and Rohlf, 1995), following recent examples (e.g., Bhavsar et al., 2010; Shoults-Wilson et al., 2010). All analyses were carried out using the StatView 5.0.1 (SAS Institute Inc., 1999) statistical analysis program.

3. Results

3.1. Spatial variation in metal concentrations and spider size

For the three sites (BA1, BA3, BA4) which were sampled in both 2006 and 2007, two-way ANOVA revealed significant effects of site, year and/or their interaction on spider body metal dry weight concentrations for all four metals and for total metals, as outlined below. However, carapace width showed no significant effects for site ($F_{2,69}=1.09$, $p=0.343$), year ($F_{1,69}=0.63$, $p=0.430$) or their interaction ($F_{2,69}=0.20$, $p=0.820$), so spider size was homogeneous across these sites during 2006 and 2007.

With Cd, only the effect of year was significant ($F_{1,69}=49.80$, $p<0.0001$) and body metal dry weight concentration levels in 2006 were nearly twice the 2007 levels (Fig. 2a). With Cr, there were significant effects for site ($F_{2,69}=13.36$, $p<0.0001$), year ($F_{1,69}=7.54$, $p<0.008$) and their interaction ($F_{2,69}=10.97$, $p<0.0001$). Among sites, spiders at BA3 had significantly greater Cr burdens those at sites BA1 and BA4 (Tukey–Kramer tests, p's <0.05). Cr levels in 2007 were over twice the levels in 2006, except at site BA1, where this relationship was reversed (Fig. 2b), reflecting the significant interaction between site and year. With Cu, there were significant effects for site ($F_{2,69}=6.52$, $p=0.003$) and year ($F_{1,69}=4.54$, $p=0.037$), though the interaction between these factors was nearly significant ($F_{2,69}=3.01$, $p=0.056$). Among sites, spiders at BA1 had significantly lower Cr burdens those at sites BA3 and BA4 (Tukey–Kramer tests, p's <0.05). Cu levels in 2006 were considerably higher than those in 2007, except at site BA1, where this relationship was reversed (Fig. 2c), presumably reflecting the nearly significant interaction between site and year. In this regard, the pattern for Cu by site and year was the reverse of that for Cr (compare Fig. 2c and b). With Zn, there were significant effects for site ($F_{2,69}=11.08$, $p<0.0001$) and the interaction between site and year ($F_{2,69}=4.32$, $p=0.017$). Among sites, spiders at BA4 had significantly lower Zn burdens than spiders at the other two sites (Tukey–Kramer tests, p's <0.05). The site-year interaction was reflected in the distinct Zn concentration profiles from site to site: at BA1, the 2006 and 2007 values were virtually the same; at BA3, the 2006 value was greater and at BA4, the 2007 value was considerably greater (Fig. 2d). Finally, with total metals, there were significant effects for site ($F_{2,69}=7.00$, $p=0.002$) and the interaction between site and year ($F_{2,69}=3.26$, $p=0.044$). Among sites, only spiders at BA3 and BA4 significantly differed in total metal levels (Tukey–Kramer test, $p<0.05$), with BA4 having lower values. The site-year interaction resulted in a total metals dry weight concentration profile much like that for Zn: at BA1, the 2006 and 2007 values were virtually the same; at BA3, the 2006 value was greater and at BA4, the 2007 value was greater (Fig. 2e). Thus, the two-way ANOVA results for each trace metal were unique, while the results for total metals largely followed those for Zn.

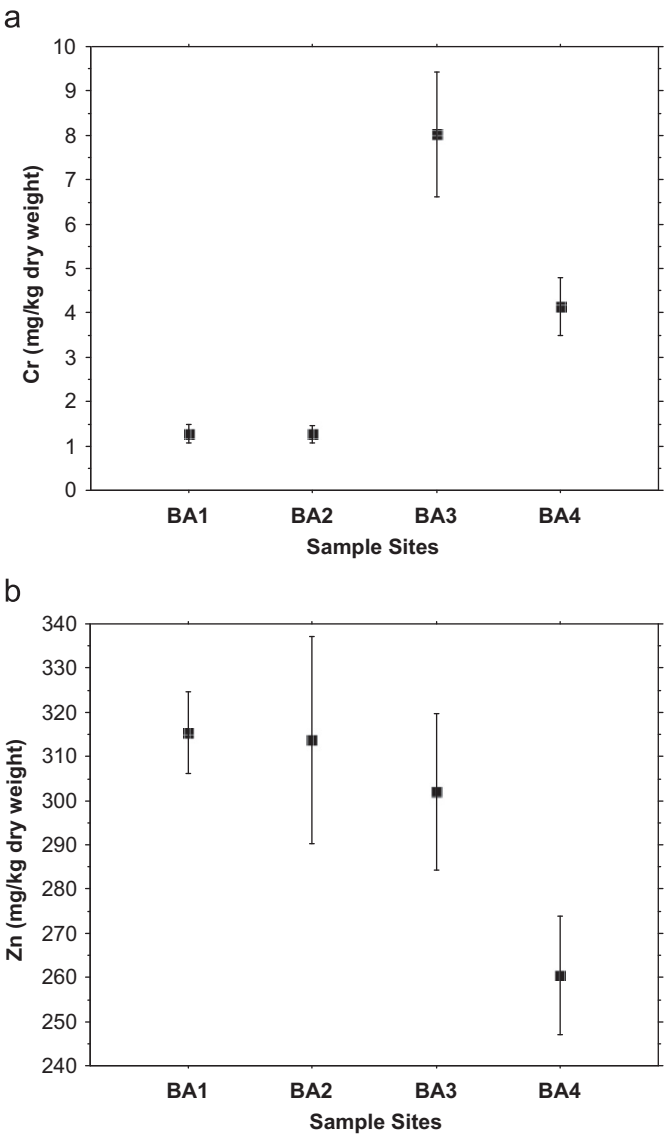

Fig. 3. Means ($\pm$SE) of the metal mg/kg dry weight for *Argiope trifasciata* by sample site in 2007. Sample sizes are indicated in parentheses. (a) Chromium (Cr); (b) zinc (Zn).

The one-way ANOVA results for the four 2007 sample sites (BA1, BA2, BA3, BA4) showed that the spider body metal dry weight concentrations of Cd, Cu and total metals were homogeneous among sites (F's $=0.71$–1.24, p's $=0.306$–0.553). Spider size (carapace width) was also homogeneous across these sites in 2007 ($F=0.96$, $p=0.419$). However, Cr dry weight concentration did vary significantly by site ($F=21.35$, $p<0.0001$) and spiders at sites BA1 and BA2 had mean Cr concentrations of less than 1.5 mg/kg dry weight, while spiders at sites BA3 and BA4 had Cr

loads, which were approximately six and three times higher, respectively (Fig. 3a). Spiders at BA3 exhibited a significantly higher Cr dry weight concentration than at any other site (as with the two-way ANOVA results for Cr), while the concentration level at BA4 was significantly higher than that at BA1 (Tukey–Kramer tests, p's < 0.05). The Zn dry weight concentrations also varied significantly by site ($F=3.61$, $p=0.020$), with the mean values decreasing across the four sample sites (Fig. 3b), though only the sites with the highest (BA1) and lowest (BA4) means differed significantly (Tukey–Kramer test, $p < 0.05$). Thus, spider body dry weight concentrations of Cr and Zn in 2007 varied significantly across sites and displayed distinct concentration profiles, as with the two-way ANOVA results for these metals, while spider size was once again homogeneous among sites.

3.2. The influence of metal load on spider size

Of the 25 regressions between spider body size and metal dry weight concentrations, two regressions involving Cr proved to be significant, that for the 2007 samples ($n=51$, $y=1.72–0.04x$, $p=0.048$) and that for the total 2006 and 2007 samples ($n=80$, $y=1.73–0.04x$, $p=0.028$). However, the amount of variation explained by these regression models was low [$r^2=0.08$ (2007) and 0.06 (2006 and 2007)], indicating that these negative linear relationships between spider size and increasing Cr dry weight concentration were weak. On the other hand, at site BA3 in 2006, there was a nearly significant negative relationship between spider size and Cd dry weight concentration ($n=16$, $y=2.33–0.30x$, $p=0.059$), with Cd burden being a much better predictor of spider size ($r^2=0.23$) than with the Cr regressions (Fig. 4). Overall, these data suggest that while the size of female *A. trifasciata* is generally unaffected by their trace metal burdens, at some sites and in some years, increased Cd concentration can be associated with a reduction in spider size. An increased Cr dry weight concentration can also contribute to such a size reduction, though its role is clearly much more minor in this regard.

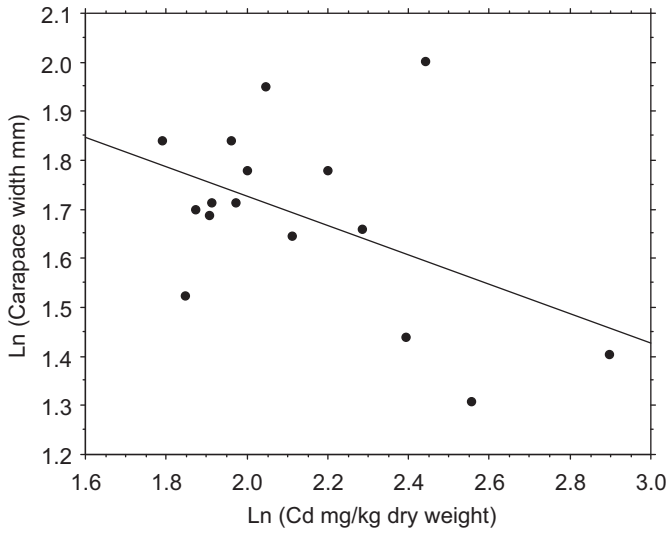

Fig. 4. Relationship between Ln carapace width (mm) and Ln cadmium (Cd) mg/kg dry weight for *Argiope trifasciata* at site BA3 ($n=16$) in 2006.

3.3. High performance liquid chromatography of fecal samples

Excreta chromatograms revealed a minimum of five major peaks for fecal samples (Fig. 5), of which two have been designated alphabetically (F, I). Regression analyses of peak areas vs. spider metal dry weight concentrations revealed significant negative relationships between peak F and Cu dry weight concentrations in 2006 ($n=22$, $y=16.42–1.49x$, $p=0.022$, $r^2=0.24$) and 2007 ($n=32$, $y=25.20–2.44x$, $p=0.007$, $r^2=0.22$). Peak I also exhibited a negative relationship with Cu dry weight concentrations in 2006 ($n=23$, $y=14.24–0.84x$, $p=0.040$, $r^2=0.19$) and 2007 ($n=41$, $y=18.42–0.92x$, $p=0.038$, $r^2=0.11$). All these relationships resulted in regression plots such as that for peak F in 2007 (Fig. 6). Thus, higher body levels of Cu generally coincided with an area reduction for peaks F and I.

In terms of identification, peak I matched the retention time and UV/vis spectrum for hypoxanthine, and has been tentatively identified as hypoxanthine. Peak F has a retention time of 6.8 min and a maximum absorbance at 258 nm. The values for retention time and maximum absorbance for peak F did not match any of the 16 standards we tested. Further work is being conducted to definitively identify peaks F and I.

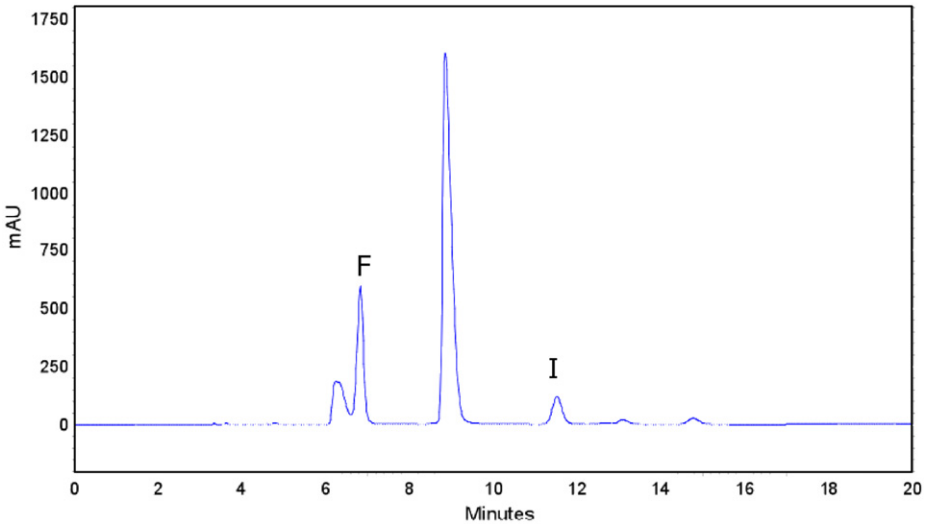

Fig. 5. Chromatogram of typical fecal sample at 254 nm.

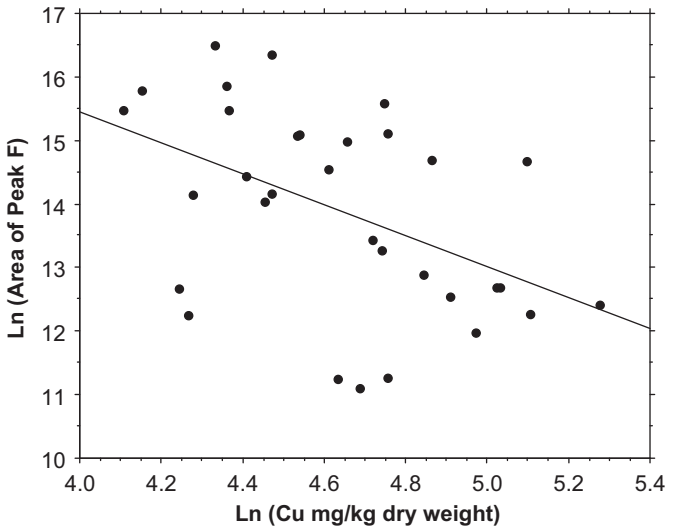

Fig. 6. Relationship between Ln chromatogram peak F area and Ln copper (Cu) mg/kg dry weight for *Argiope trifasciata* in 2007 ($n=32$).

4. Discussion

4.1. Spatial variation in metal concentrations and spider size

The fact that body metal dry weight concentrations of Cd, Cr, Cu, Zn and total metals in female *A. trifasciata* exhibited distinct patterns of spatial and annual variation during this study probably reflects the unique conditions at each sample site in 2006 and 2007, as well as differences in metabolism for each metal (Hunter et al., 1987). Since the trace metal contents of individual spiders result at least in part from the metal body burdens of their insect prey, Wilczek et al. (1997) observed that the prey types and numbers collected by spiders at a given location will depend on the environmental, climatic and prey population dynamics of that site, which could foster site-to-site (Hendrickx et al., 2004) and year-to-year differences in spider metal levels. Moreover, since the trace metal content of prey species at Ballona may partly reflect variability in ongoing metal inputs to the system, it should be noted that the 2006 sample was collected following a wet rainfall year for Los Angeles (2005–2006, 335.03 mm), while the 2007 sample followed the driest year on record (2006–2007, 81.53 mm) (http://www.nwsla.noaa.gov/lox). This means that storm-related runoff of accumulated pollutants from roadways, urban surfaces and storm drains (Svejkovsky and Jones, 2001; Stein and Tiefenthaler, 2005) was more frequent in 2005–2006, which may have increased metal availability and uptake by insects and spiders at our Ballona sample sites. The fact that Cd dry weight concentrations in 2006 were nearly twice those in 2007 at sites BA1, BA3 and BA4 (Fig. 2a) would be consistent with such a rainfall difference. However, this was not the case with the dry weight concentrations of Cr, Cu, Zn and total metals in spiders from these sites, which may indicate that Cd availability to *A. trifasciata* at Ballona is more closely tied to rainfall than is the case with other trace metals. In addition, since flying insects make up a significant portion of the diet of *A. trifasciata* (Brown, 1981), these spiders at Ballona may gather a prey spectrum, which includes both resident insects and those which fly into Ballona from surrounding, less contaminated areas (e.g., Larsen et al., 1994), enhancing the likelihood of spatial and annual variation in spider metal loads. Finally, despite probable spatial and annual differences in prey composition and abundance at Ballona, these did not prevent female *A. trifasciata* from reaching a similar mean size at all study sites, which are of course fairly close together (Fig. 1). Clearly, a comprehensive study of trace metal availability at Ballona, coupled with detailed studies of insect and spider phenologies and dietary intake at Ballona and adjacent areas, will be needed to explicitly evaluate the influence of site-specific environmental parameters on trace metal bioaccumulation and the body size of resident *A. trifasciata*.

4.2. The influence of metal load on spider size

Our results suggest that reduced body size in female *A. trifasciata* can sometimes be associated with an elevated body burden of Cd, and secondarily, Cr. Reduced body size in such cases presumably reflects the more limited resources available for foraging and growth due to the expenditure of energy for metal tolerance mechanisms (Maryanski et al., 2002; Velickovic, 2007). While Cr is a well known toxicant (Eisler, 1986) whose negative effects have been documented for both terrestrial and aquatic arthropods (e.g., Trumble and Jensen, 2004; Sorensen et al., 2006), we are not aware of any reports of Cr toxicity in arachnids. On the other hand, there are two studies which document the harmful effects of Cd for spiders. First, Maelfait and Hendrickx (1998) found a positive relationship between fluctuating asymmetry (FA, a measure of developmental stability, with 0=bilateral symmetry) and internal Cd concentration for a sample of wolf spiders (*Pirata piraticus*) taken from a polluted Belgian salt marsh, indicating that spiders with greater Cd loads had more abnormal phenotypes. Second, Jung et al. (2005) found that with another wolf spider (*Pardosa astrigera*), spiders fed a Cd-enriched diet for 8 weeks experienced a reduced growth rate and attained a smaller body weight as compared with spiders fed a Cd-free diet. While these studies might have provided a context for the Cd dry weight concentration observed in the *A. trifasciata* sample (BA3 in 2006) where a nearly significant negative relationship between body size and Cd level was observed (mean $=8.90 \pm 0.80$ mg/kg dry weight), this was unfortunately not the case. Specifically, Maelfait and Hendrickx (1998) did not include a mean Cd concentration for their sample of *P. piraticus*, while Jung et al. (2005) reported their Cd concentrations in terms of μg/spider but did not indicate the body weights of the relevant spiders.

Since larger female spiders are generally more fecund (Marshall and Gittleman, 1994) and are commonly favored by males (Refs. in Huber, 2005), our findings for *A. trifasciata* suggest that when Cd (and possibly Cr) bioaccumulation results in a reduction in adult female size, there may also be reduction in reproductive success. What would be useful in this regard is a multiyear field study of *A. trifasciata* populations of varying metal bioaccumulation levels to determine relative levels of reproductive output and fitness (e.g., Hendrickx et al., 2003). In addition, analyzing the body composition of individual *A. trifasciata* would delineate the distribution of trace metals among organs within the body. Knowing an organ-level metal distribution for *A. trifasciata* would be especially relevant in the present context, since Wilczek and Babczynska (2000) found higher levels of Cd, Pb and Zn in the hepatopancreas than in the gonads of six spider species and suggested that this system allows these spiders to protect their reproductive functions and to thus continue to occur in metal contaminated sites. Whether this is also true for *A. trifasciata* remains to be investigated.

4.3. High performance liquid chromatography of fecal samples

Our analyses revealed significant negative relationships between two excreta products, peak I, tentatively identified as hypoxanthine, and peak F with Cu dry weight concentrations in both 2006 and 2007. Higher body levels of Cu generally coincided

with a decrease of these components. We are currently investigating the definitive identification of peaks F and I via mass spectrometry.

While oxidative degradation of purine bases is well studied, future work is needed to identify the possible biological mechanisms to account for these correlations. It is unclear whether decreased levels of hypoxanthine (or un-identified peak F) result from specific metal-induced reactions to hypoxanthine or as a result from changes to cellular stress reactions, namely apoptosis induced processes or increase in antioxidants as a result from increased stress. Hypoxanthine is a substrate for xanthine oxidase in the production of xanthine and serves as a guanine precursor (Hamdry and Sidrak, 1982; Dusbabek et al., 1991). It is possible that metal induced changes (specific damage, or induction or inhibiton of enzyme expression) to enzymes that involve hypoxanthine as a substrate or product could result in the observed changes. Further work is underway to investigate the possible cause of reduced hypoxanthine levels induced by metal loads. This process does constitute a novel method to predict Cu metal levels in spiders via a non-destructive method.

Acknowledgments

Financial support was provided by a Merck/AAAS Undergraduate Science Research Program Award (2006–2008) and by Loyola Marymount University (William F. McLaughlin Chair in Biology).

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