ELEMENT CONCENTRATIONS AND FLUCTUATING ASYMMETRY IN A TERRESTRIAL ISOPOD (*ARMADILLIDIUM VULGARE*) IN METROPOLITAN CHICAGO PARKLANDS

Eric L. Peters[1], Bettina Kaufmann-Daszczuk[1], Heather A. Brant[2], Charles H. Jagoe[2], Robert Richter[3]

[1]Department of Biological Sciences, Chicago State University, Chicago, IL, USA. [2]University of Georgia Savannah River Ecology Laboratory, Aiken, SC, USA. [3]Department of Chemistry & Physics, Chicago State University, Chicago, IL, USA

ABSTRACT

Terrestrial isopods have long been known to bioaccumulate high levels of Cu, Fe, Zn, Cd, and Pb, and are established biological indicators of these metals. Southern metropolitan Chicago, Illinois has a long history of contamination from industrial activities (e.g., metal smelting, electroplating, and paint manufacture) and associated waste production (e.g., steel mill slag, coal and coke ash). Many of the sources of these contaminants were located within or near residential areas. We sampled populations of the terrestrial isopod *Armadillidium vulgare* collected neighborhood parks and other natural areas in metropolitan Chicago and its surrounding suburbs, including NW Lake County, Indiana (total area > 600 km^2). We sampled isopods ($n \geq 60$) from 21 sites in late June-early July 2004, and again from 12 of these sites in September. We analyzed these isopods for 21 elements by ICP-MS. Only Ag was consistently below method detection limits: most populations were above the MDLs for Sb and Hg, and all populations had detectable concentrations of the other elements. As expected from previous studies, isopod populations within the city accumulated higher levels of Cu, Fe, Zn, Cd, and Pb than did isopods in suburban areas. We also found elevated concentrations of other less common but potentially hazardous elements, e.g., V, Cr, Ni, As, and Ba. We noted apparent effects of these contaminants on postnatal development: concentrations of toxic metals and metalloids (and combinations of these elements) were positively correlated with the degree of fluctuating asymmetry in compound eye lens (ommatidia) number. As an abundant (and invasive) species in habitats ranging from woodlands, residential neighborhoods, and parks to industrialized areas throughout the Northern Hemisphere, *A. vulgare* may be an ideal sentinel animal for monitoring the presence and effects of metal and metalloid pollutants in urban environments.

INTRODUCTION

Many areas of metropolitan Chicago represent a perfect example of the need for early warning of the effects of pollutants on urban environments. The 19th Century practice of situating heavy industries away from the city center, filling in wetlands with industrial wastes, and interspersing industrial zones with newly-spawned 'company neighborhoods', resulted in an admixture of industrial, residential and recreational areas that exists in few other places in the U.S. Many of these industries centered around the alloying, processing, and fabricating of metals. There are (or were) numerous potential sources of metal contaminants in the region, including smelters, coke ovens, steel mills, paint factories, and electroplating facilities. Highly concentrated organic waste products (e.g., coal ash, sewage sludge) are also known to contain high concentrations of metals, and these sources are also abundant throughout the area [1,2].

Terrestrial isopods (e.g., *Armadillidium vulgare*, Fig. 1) are abundant native and introduced soil arthropods in both urban industrial sites and residential districts. They are readily found in gardens and vacant lots, along drainage ditches, house foundations, and in basements. Terrestrial isopods also show a high degree of site fidelity, radiating outward from refuge sites at night, thus providing excellent sampling of the surrounding microhabitat [3,4]. They are easily collected in numbers from these sites by active or passive sampling.

There have been numerous studies on the uptake, tissue distribution, and toxicity of metals in terrestrial isopods [5-10]. Few, however, have examined the effects of of these pollutants on in these animals at levels that may be found in urban residential areas. Although fluctuating asymmetry (FA) analyses have been employed in determining effects of metal pollutants on aquatic insect larvae [11], little information is available on other species. In fact, of several hundred FA studies to date, few involved metals, and none were conducted on synanthropic animals [12].

Our objective was to examine the potential use of FA in ommatidia number (Fig. 1) in A. vulgare as an indicator of potential effects of urban metal and metalloid pollutants.

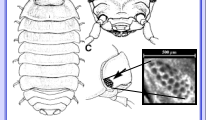

Figure 1. The terrestrial isopod *Armadillidium vulgare*, depicting (A) dorsal view, (B) ventral view of head, and (C) lateral view of head. The arrows indicate one of the animal's two compound eyes, each of which are composed of about 25 ommatidia at maturity.

METHODS

Sampling Procedures

A. vulgare were collected by hand from 21 parklands in June-July 2004 (Fig. 2). Many of these sites experienced impacts from nearby metal-generating industrial sites and some (e.g., Chicago State University) are actually remediated industrial sites. Other parks have likely only have received aerial depositions of pollutants.

Figure 2. Map of terrestrial isopod sampling locations in metropolitan Chicago, Illinois, and adjacent NW Lake County, Indiana. The 21 parks and other recreational areas are shown in green, and all were sampled during the early summer of 2004. The 12 locations sampled a second time in the fall of 2004 are shown in italics. Dashed lines indicate the axes of transects sampled, at 5, 10, and 16 km from the Chicago State University campus. Locations of major highways and waterways, towns, and other landmarks are included as reference points.

Because both asymmetry frequencies and metal concentrations may change as the population age structure of the isopods shifts toward increased numbers of older animals over the summer, repeat collections of isopods were made at 12 of the locations (spanning the range of observed asymmetry values from the summer sample) in early fall of 2004.

Fluctuating Asymmetry Assessments

Based on prior studies, at least 60 *A. vulgare* were examined to obtain sufficient numbers for FA estimates. The isopods were maintained in polyethylene food containers on damp paper towels for 24 hr to allow their guts to clear and then frozen. The number of ommatidia in each eye was determined by examining each isopod with an Olympus dissecting microscope with 20× oculars and a 20× magnifier lens.

An asymmetry index (A) for the isopods sampled from each location was estimated using the equation at the right [13], where: R_i and L_i were the number of ommatidia in the eyes of the ith isopod, and n was the number of isopods in the sample.

$$A = \sqrt{\frac{\sum_{i=1}^{n}(R_i - L_i)^2}{n}}$$

Metal Analyses

After FA measurements were made, the isopods were sonicated twice in DI water for two minutes to remove surface soil. The samples from each site were randomly divided into three 250-mg (dry mass) subsamples, placed in Teflon vessels, and a 1:1 mixture of concentrated HNO_3:H_2O (v/v) added. The samples were heated in a Milestone ETHOS SEL microwave oven to 130 °C over 5 min, heated to 180 °C over an additional 15 min, and held at 180 °C for 10 min. Once the vessels cooled to 50 °C, the contents were diluted with DI water to a 10% acid content, transferred to plastic centrifuge tubes, closed with a screw top and sealed with Parafilm for transport to the Savannah River Ecology Laboratory for analysis.

Concentrations of Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Ag, Cd, Sb, Cs, Ba, Hg, Tl, Pb, and U were determined using a Perkin Elmer 6000 ICP-MS. Acid blanks, and samples of the ultrasonic water bath solution and the plasticene used to mount isopods on microscope slides for FA assessments were also analyzed to assess potential outside sources of contamination. Tissue reference standards of known metal content (TORT-2 and DORM-2, National Research Council, Ottawa, ON, Canada) were analyzed in tandem with all samples.

Figure 3. Frequency distributions of R – L differences (absolute values) In ommatidia numbers of *A. vulgare* collected in metropolitan Chicago.

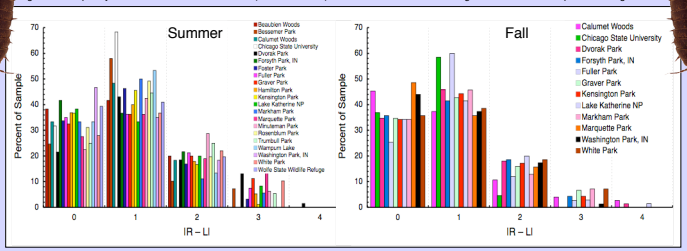

Figure 4. Asymmetry indices (*A*) for *A. vulgare* collected in metropolitan Chicago parklands.

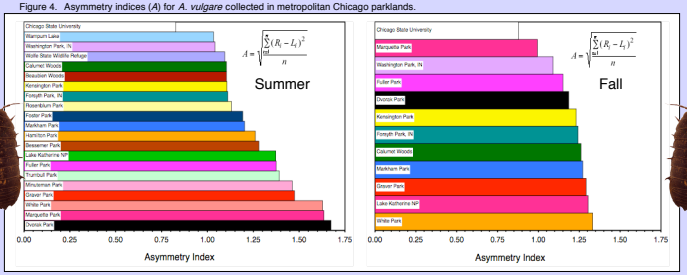

Table 1. Comparisons of FA in ommatidia number of *A. vulgare* for metropolitan Chicago parklands. The Chicago State University campus (CSU). CSU was selected as the comparator because of its central location in the metropolitan area, its known history as an industrial site (the manufacturing center for Pullman railroad cars), and the extensive site remediation (including importing new topsoil) that preceded the establishment of the current campus.

Location	*n*	Mean	Mean Diff. *t**	*P*	Variance	*F**	*P*
Beaubien Woods	60	-0.383	-1.933	0.056	1.088	1.572	0.041
Bessemer Park	69	-0.043	0.036	0.971	1.66	2.398	<0.001
Calumet Woods	60	-0.383	-1.933	0.056	1.088	1.572	0.041
Chicago State University	60	-0.050			0.692		
Dvorak Park	65	-0.062	-0.050	0.960	2.84	4.104	<0.001
Forsyth Park, IN	60	-0.300	-1.422	0.158	1.163	1.681	0.023
Foster Park	95	-0.179	-0.736	0.463	1.404	2.029	0.002
Fuller Park	80	-0.087	-0.184	0.854	1.904	2.751	<0.001
Graver Park	80	-0.575	-2.631	0.009	1.868	2.699	<0.001
Hamilton Park	95	-0.200	-0.821	0.413	1.566	2.263	<0.001
Kensington Park	90	-0.133	-0.466	0.641	1.218	1.760	0.010
Lake Katherine Nature Preserve	60	-0.583	-2.746	0.007	1.569	2.267	0.001
Markham Park	90	-0.244	-1.101	0.273	1.4	2.023	0.002
Marquette Park	58	-0.845	-3.742	0.000	1.993	2.880	<0.001
Minuteman Park	80	-0.562	-2.579	0.011	1.844	2.665	<0.001
Rosenblum Park	61	0.000	0.275	0.784	1.3	1.879	0.008
Trumbull Park	91	-0.275	-1.137	0.257	1.89	2.731	<0.001
Wampum Lake	60	-0.433	-2.356	0.020	0.894	1.292	0.182
Washington Park, IN	60	-0.150	-0.582	0.562	1.079	1.559	0.044
White Park	66	-0.588	-2.427	0.017	2.335	3.374	<0.001
Wolfe State Wildlife Refuge	61	-0.148	-0.554	0.580	1.195	1.727	0.018

*All comparisons of location means and variances were made with the CSU campus

Table 2. Element concentrations in the summer and fall samples of *A. vulgare*, compared with typical concentrations of these elements in terrestrial animals (data from [14]). Highlighted entries are elements in which the lower range of the isopod samples exceeded the literature concentrations.

Element	Expected Concentration (ppm) in Animals (from [14])	Mean (Range) Concentration in Isopods (ppm) Summer	Fall
Al	4-100	100.67 (14.16–284)	90.66(15.59–274)
V	0.1	0.32 (0.14–0.67)	0.34(0.16–0.68)
Cr	0.075	0.82 (0.33–4.74)	0.53 (0.35–1.01)
Mn	2-4	15.72 (6.95–41.05)	14.91 (7.93–34.12)
Fe	40-160	3390 (2623–4238)	3107 (2568–3769)
Co	1	1.87 (1.24–2.37)	1.78 (1.22–2.23)
Ni	0.8	2.05 (1.13–6.86)	1.59 (1.11–3.19)
Cu	2-4	346.7 (137.2–907.4)	410 (197.7–744.6)
Zn	160	341.3 (187.8–648.2)	332.8 (182.2–595.4)
As	0.04-0.09	0.647 (0.299–3.047)	0.786 (0.403–2.594)
Se	<1	1.693 (0.865–3.179)	1.778 (0.987–2.692)
Rb	1-25	3.833 (1.864–18.482)	3.638 (2.039–7.306)
Cd	0.1-0.5	1.885 (0.391–5.291)	1.738 (0.51–4.347)
Sb	40-400 (liver)	0.0536 (0.0081–0.5001)	0.0521 (0.0129–0.3004)
Cs	0.00064	0.021 (0.007–0.099)	0.017 (0.007–0.04)
Ba	0.8	262.1 (139.9–510.8)	251.2 (153–388.9)
Hg	0.040-0.050	0.086 (0.022–0.319)	0.087 (0.035–0.204)
Tl	0.1	0.0199 (0.0037–0.0619)	0.0214 (0.0008–0.0711)
Pb	2	4.087 (0.874–13.64)	5.376 (1.036–23.148)
U	0.013	0.0175 (0.0051–0.0398)	0.0177 (0.0063–0.0522)

Table 3. Pearson correlation coefficients (*r*) between element concentrations and asymmetry index (*A*) for the summer sample of *A. vulgare*.

Element	*r*	*n*	*z*	*P*
Al	0.341	62	2.729	0.006
V	0.337	62	2.693	0.007
Cr	0.342	62	2.741	0.006
Mn	-0.189	62	-1.467	0.142
Fe	0.224	62	1.749	0.080
Co	0.369	62	2.974	0.003
Ni	0.269	62	2.119	0.034
Cu	0.305	62	2.420	0.016
Zn	0.266	62	2.093	0.036
As	0.346	62	2.769	0.006
Se	0.385	62	3.116	0.002
Rb	-0.325	62	-2.592	0.010
Cd	-0.128	62	-0.990	0.322
Sb	0.192	44	1.242	0.214
Cs	-0.189	44	-1.222	0.222
Ba	0.315	62	2.505	0.012
Hg	0.432	52	3.236	0.001
Tl	0.206	52	1.459	0.145
Pb	0.470	61	3.880	< 0.001
U	0.420	62	3.434	0.001

RESULTS & DISCUSSION

- The FA of >2300 isopods was examined (>1500 isopods in the 21 summer samples, and >800 in the 12 fall samples). There was no evidence of directional asymmetry and no significant skewness or kurtosis in ommatidia number in either time period.
- Frequency distributions of R – L differed among sites (Fig. 3). The range of *A*-values was narrower in the fall than in the summer (Fig. 4), but the paired differences of *A*-values of the sites sampled in both the summer and fall were not significantly different ($t = 0.171$; $P = 0.867$).
- Sample mean ommatidia numbers remained centered on a difference of zero in nearly every site, and most sites did not show a significant difference from Chicago State University (Table 1). The sample variances, however, differed from the CSU site in nearly every case (Table 1).
- In addition to elements known to hyperaccumulate in terrestrial isopods (i.e., Cu, Fe, Zn, Cd, and Pb). **Isopods showed evidence of accumulating other metals and metalloids** to high concentrations (Table 2).
- **Of the 21 elements examined, only Ag was below the MDL in all samples**. Hg and Tl were below the MDL in 16%, and Sb and Cs were below the MDL in 30% of the summer samples (Table 3).
- FA is valid only if observed asymmetry can be positively correlated with exposure to an environmental stressor. **This suggests that the levels of elements present in *A vulgare* in recreational parklands located within residential neighborhoods do in fact correlate with FA in ommatidia number**. Concentrations of two-thirds of the elements analyzed were significantly correlated with the asymmetry index, *A*. In most cases, correlations were greatest with the elements considered to be the most hazardous. Concentrations of these elements (e.g., Hg, Pb) were generally positively correlated with *A* (Cd was the exception in that the correlation was not significant, but the range of concentrations of this metal was also narrow). Correlations between *A* and concentrations of less toxic elements (e.g., Mn, Rb) were negatively or not significantly correlated (Table 3).
- It is possible that other pollutants (e.g., hydrocarbons, organochlorines) may act alone or in tandem with metal and metalloid contaminants to produce the FA we observed. For example, the asymmetry observed for isopods in Minuteman Park (which borders Midway Chicago Airport, Fig. 2) was one of the highest observed, and organic chemicals from jet exhaust and fuel vapors may be a contributing factor.
- Future studies will examine the induction of FA in the laboratory at contaminant levels similar to those experienced by isopods in the field and will refine sampling protocols to increase replicability of FA estimates.

CONCLUSIONS

- **Our results support the use of terrestrial isopods as a an indicator of the effects of inorganic contaminants in urban residential environments.**
- **The use of FA in ommatidia number provides an apparently sensitive, yet easily quantifiable estimate of exposure to both hazardous metals and metalloids**.

LITERATURE CITED

1. Colten, C.E. 1985. *Industrial wastes in the Calumet area, 1869-1970*. Research Report 001, Illinois Hazardous Waste Research and Information Center, Champaign, IL.
2. Kay, R. T., T. K. Greeman, R. F. Duwelius, R. B. King, J. E Nazimek, and D. M. Petrovski. 1997. *Characterization of fill deposits in the Calumet Region of Northwestern Indiana and Northeastern Illinois*. Water-Resources Investigations Report 96-4126, U. S. Geological Survey, 34 p. DeKalb, Illinois & Indianapolis, Indiana.
3. Warburg, M.R., K.E. Linsenmair, and K. Bercovitz. 1984. The effect of climate on the distribution and abundance of isopods. Pages 339-368 in S.L. Sutton and D.A. Holdich (eds.) *The biology of terrestrial isopods*. Symp. Zool. Soc. Lond. 53. London, Academic Press.
4. Wieser, W. 1961. Copper in isopods. *Nature* 191:1020.
5. Alikhan, M.A. 1972. Haemolymph and hepatopancreas copper in *Porcellio laevis* Latreille (Porcellionidae, Peracarida). *Comp. Biochem. Physiol.* 42A:823-832.
6. Coughtrey, P.J., M.H. Martin, and E.W. Young. 1977. The woodlouse *Oniscus asellus* as a monitor of environmental cadmium levels. *Chemosphere* 6:827-832.
7. Beeby, A. 1978. Interaction of lead and calcium uptake by the woodlouse, *Porcellio scaber* (Isopoda, Porcellionidae). *Oecol.* (Berlin) 32:255-262.
8. Hopkin, S.P., and M.H. Martin. 1982. The distribution of zinc, cadmium, lead and copper within the woodlouse *Oniscus asellus* (Crustacea, Isopoda). *Oecol.* (Berlin) 54:227-232.
9. Hopkin, S.P., and M.H. Martin. 1984. Heavy metals in woodlice. Pages 143-166 in S.L. Sutton and D.A. Holdich (eds.) *The biology of terrestrial isopods*. Symp. Zool. Soc. Lond. 53. London, Academic Press.
10. Hopkin, S.P., M.H. Martin, and S.J. Moss. 1985. The woodlouse *Porcellio scaber* as a 'biological indicator' of zinc, cadmium, lead and copper pollution. *Environ. Poll.* (Ser B) 10:239-254.
11. Clarke, G.M. 1993. Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. *Environ. Pollut.* 82:207-211.
12. Moller, A., and Swaddle, J. 1998. Asymmetry, developmental stability, and evolution. Oxford University Press, Oxford.
13. Palmer, A.R. 1994. Fluctuating asymmetry analyses: a primer. Pages 335-364 in T.A. Markow (ed.), *Developmental instability: its origins and evolutionary implications*. Kluwer Academic Publishers, Dordrecht, Netherlands.
14. Pais, I., and J.B. Jones. 1997. The handbook of trace elements. St. Lucie Press, Boca Raton, Florida.

ACKNOWLEDGMENTS

This study was supported by the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research of the U.S. Department of Energy, through Financial Assistance Award No. DE-FRC09-96SR18546 to the University of Georgia Research Foundation. Support from Chicago State University allowed for a sabbatical leave for ELP.