FLUCTUATING ASYMMETRY IN CRAYFISH POPULATIONS INHABITING LEAD AND CADMIUM POLLUTED AQUATIC SYSTEMS

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Due to past mining practices, high concentrations of lead and cadmium have been found in the streams and rivers of southeastern Cherokee County, Kansas. These heavy metals are known to cause gross developmental anomalies in a diverse group of organisms. To assess more subtle effects on the crayfish of Cherokee County, specimens were examined for asymmetrical development of bilateral characteristics (fluctuating asymmetry). Sediments and crayfish from seven locations were analyzed for lead and cadmium concentrations. Metal concentrations from the sediments and crayfish tissues of locations in close proximity to heavily mined areas were significantly higher than those from upstream control locations.

Three axial and three appendicular measurements from both sides of the plane of symmetry were taken from 155 *Orconectes neglectus* and 125 *O. virilis* from seven populations. Variances of right side minus left side character differences were compared between the two species and among the different locations. No significant differences were found to exist between fluctuating asymmetry values of the two species. Linear regression analysis revealed that no relationship exists between the level of fluctuating asymmetry and the concentration of heavy metals in body tissues. These results suggest that chronic exposure to lead and cadmium at the levels in the streams of Cherokee County does not affect the homeostatic mechanisms operating during the ontogenetic development of *O. neglectus* and *O. virilis*.

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INTRODUCTION

Between 1876 and 1970, approximately 111 million tons of lead and zinc ores were mined from southeastern Cherokee County, Kansas near the cities of Galena and Baxter Springs (Spruill, 1987). Because of this mining activity, over one trillion cubic feet of rock were extracted and displaced. According to Spruill (1987), approximately 32,000 acre-feet of water has entered these open mine shafts as a result of groundwater seepage and surface runoff. During the years of mining activity, this mine water was pumped directly into nearby streams and reservoirs. Mills were located near the shaft openings for processing of the metal ores, and the chat piles resulting from the discarded waste rock would reach heights up to 300 feet and encompass several acres (Spruill, 1987).

These past mining activities have had a detrimental effect on the water quality of the surrounding aquatic systems (Environmental Protection Agency, 1990). Conditions within the mine groundwater are conducive to the oxidation of sulfide ores due to the influx of oxygen through the mine openings (Spruill, 1987). High levels of sulfate ions and a lowering of the pH are consequences of this oxidative process. Because of the geology of this region, the aquatic systems are poorly buffered against decreases in the pH. The leaching of acidic water through these mines has elevated concentrations of dissolved trace metals (Young and Harvey, 1991). Spruill (1987) found that mine water around

Galena contained concentrations of the heavy metals, lead (median = 240 µg/l) and cadmium (median = 180 µg/l), at such levels that it was unsuitable for any use.

A water quality study conducted by Spruill (1987) of the streams and rivers around the Galena and Baxter Springs mining areas concluded that surface waters are also adversely affected by past mining activities. Water seepage through the mines and chat piles, which contain high concentrations of lead and cadmium (Environmental Protection Agency, 1990), contributes to the high metal concentrations in the nearby surface waters (Spruill, 1987). As dissolved lead and cadmium enter the surface water they begin to precipitate, due to the increase in pH, and settle on the beds of the streams and rivers. Therefore, sediment of surrounding streams typically contains high metal concentrations (Spruill, 1987; Environmental Protection Agency, 1990). Also, direct deposition of chat may elevate lead and cadmium concentrations in the sediment.

Lead and cadmium play non-essential roles in the physiology of plant and animal species, and can be toxic above certain thresholds (Hiatt and Huff, 1975; Anderson, 1978; Anderson and Brower, 1978; Thorp et al., 1979; Thorp and Gloss, 1986). These metals were found to have long half-lives within animal tissues due to sequestering and storage mechanisms (Gillespie et al., 1977; Giesy et al., 1980; Roldan and Shivers, 1987). Therefore, these metals accumulate in animal tissues and may display some toxic effects as the animal ages. Toxicological effects, such as renal tubular dysfunction, bone demineralization, sterility, pulmonary edema, hypertension, cancer, and death may occur due to exposure (Hiatt and Huff, 1975). Concentrations of lead and cadmium in the aquatic systems around the southeastern Cherokee County mining area are at such a level that concern for the public's health has prompted the Environmental Protection Agency to target it for reclamation as part of a Superfund project (Environmental Protection Agency, 1990).

Crayfish have been used as indicators of lead and cadmium pollution due to the bioaccumulation of these metals within their tissue (Anderson and Brower, 1978). Because of their ubiquitous distribution in rivers and streams in southeastern Cherokee County and their benthic life histories, crayfish were selected as the target organism for my study. Past studies have investigated acute toxicities of lead and cadmium on crayfish (Anderson, 1978; Thorp and Gloss, 1986). However, acute toxicity testing would not be appropriate for crayfish of southeastern Cherokee County because the metals are at nonlethal concentrations in most of the streams and rivers. Deviations of bilateral characteristics from perfect symmetry (fluctuating asymmetry) could determine the effects of chronic exposure to these nonlethal concentrations on the developmental homeostasis working in crayfish embryos.

Deviations from bilateral body symmetry can be placed into three categories depending on the mean and variance of the population distribution of right minus left sided character measurements (Van Valen, 1962; Palmer and Strobeck, 1986). Directional asymmetry occurs when greater character development is consistently biased toward one side of the plane of symmetry. The distribution of right minus left character differences for a species or population expressing directional asymmetry will be normal around a mean significantly different from zero. An example of directional asymmetry is the consistent bias of greater development on the left side of the human heart (Van Valen, 1962). Another form of asymmetry, antisymmetry, occurs when the more developed character is expressed randomly relative to the plane of symmetry. The chance of greater development on either side is nearly identical. Antisymmetry results in a platykurtic or bimodal distribution of right minus left differences around a mean of zero. The oversized signalling claw of the male fiddler crab displays antisymmetry due to the nearly equal frequency of occurrence on either side of the body (Palmer and Strobeck, 1986). According to Leary and Allendorf (1989), directional asymmetry and antisymmetry occur naturally within animal species and are genetically controlled.

The final category of bilateral asymmetry identified by Van Valen (1962) and Palmer and Strobeck (1986) is fluctuating asymmetry. This type of asymmetry occurs when greater character development is randomly expressed relative to the plane of symmetry. The distribution of right minus left character differences is normally distributed around a mean of zero. Unlike the other two types of asymmetry, fluctuating asymmetry has little or no heritable basis (Palmer and Strobeck, 1986).

In bilaterally symmetric organisms, coadapted gene complexes work to create homogeneous character development on either side of the plane of symmetry (Jagoe and Haines, 1985). Fluctuating asymmetry results as factors disrupt the expression of these gene complexes and bilateral characteristics fail to develop in a synchronous manner (Van Valen, 1962). This disruption may occur by the interference of a stressor on the genome of an organism, or the level of stress may exceed some threshold in which the organism's homeostatic mechanisms fail to buffer against such perturbations (Owen and McBee, 1990). Environmental stress, such as the introduction of toxins, may have an effect on developmental homeostasis of an organism and increase the level of fluctuating asymmetry displayed (Palmer and Strobeck, 1986). Valentine and Soule (1973) found significant increases in fluctuating asymmetry of the pectoral fin rays of grunion (<u>Leuresthes</u> <u>tenuis</u>) exposed to increasing concentrations of the toxin p,p'-DDT. Valentine et al. (1973) studied three species of marine fish that exhibited significant correlations between increases in fluctuating asymmetry and the distribution of toxins. Therefore, fluctuating asymmetry may be a useful tool for evaluating sublethal conditions of environmental stress.

Crayfish inhabiting the streams and rivers of southeastern Cherokee County are likely to be under considerable stress from chronic exposure to lead and cadmium pollution. One objective of my study was to compare lead and cadmium loads in the sediment and crayfish tissue from populations exposed to minewater drainage to upstream control populations. Another objective was to investigate levels of fluctuating asymmetry among populations of crayfish exposed to different concentrations of heavy metal pollutants. The hypotheses to be tested were that lead and cadmium concentrations would be higher in the sediment and tissue of crayfish which were in close proximaty to the mining area, and fluctuating asymmetry would increase as populations were exposed to increased lead and cadmium concentrations.

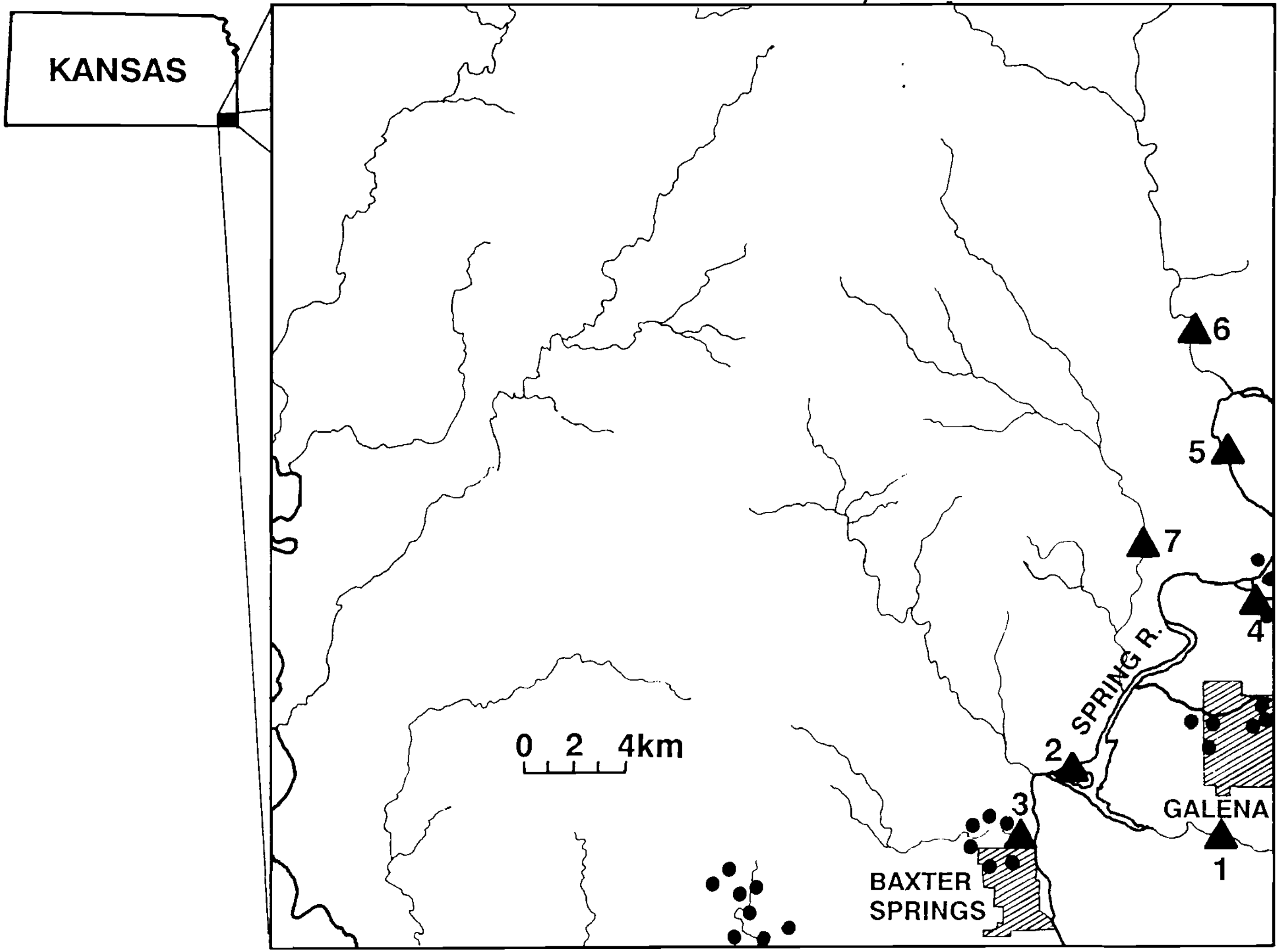
MATERIALS AND METHODS

Crayfish from seven different populations were collected from the Spring River and its tributaries in southeastern Cherokee County, Kansas (Figure 1). Four of these populations (1-4) were located downstream from nearby mining sites and were expected to be polluted with lead and cadmium. Three populations (5-7) upstream from the mining sites were selected as controls not affected by mining pollution.

Specimen collection took place during the summer of 1991. Collection methods included flipping rocks and picking up crayfish by hand or dipnet, or by seining. Seven specimens from each population were collected for heavy metal analysis. These individuals were placed individually in sterile storage bags, transported to Emporia State University in an ice chest, and then preserved for future analysis by freezing at -75° C. A sediment sample was collected at each site for metal analysis. Sediment samples were refrigerated in glass jars and preserved with 5 ml concentrated nitric acid. Additional crayfish were collected from each site for fluctuating asymmetry analysis. These individuals were preserved on site in 4% formalin and later transferred to 70% isopropyl alcohol. Collected crayfish were sexed and identified in the laboratory. Form I and II males and females were identified with the aid of keys by Williams and Leonard (1952), Beasley and Branson (1971), Hobbs (1972), Pennak (1989), and Hobbs (1991). Two

Figure 1. Map of sample sites in Cherokee Co., Kansas. Darkened circles represent abandoned lead or zinc mines. Darkened triangles represent sample sites. Sample site 1 = Shoal Creek, sample site 2 = Spring River, sample site 3 = Willow Creek, sample site 4 = Turkey Creek, sample site 5 = north Spring River, sample site 6 = Cow Creek, sample site 7 = Shawnee Creek.

CHEROKEE CO., KANSAS



species of crayfish were identified in the aquatic systems of southeastern Cherokee County. *Orconectes neglectus* was collected at sample sites one through six, but not at site seven. *Orconectes virilis* was collected at site seven, and in sympatry with *O. neglectus* at sites two, four, five, and six. *Orconectes virilis* was not found at sites one and three.

Preparation of sediment and tissue samples for metal analyses followed a modified procedure of Standard Methods for the Examination of Water and Wastewater (1985). Approximately 35 g of sediment was dried in 125 ml Erlenmeyer flasks to a constant weight at 105° C (approximately 18 hours). Two sediment samples from each site were prepared for analysis. These samples were then digested in 20 ml of concentrated nitric acid, and slowly boiled on a hot plate until a volume of approximately 5 ml remained. Glass beads were used to facilitate smooth boiling. After digestion, the sediment samples were filtered through glass microfiber filters and diluted with distilled water to 100 ml.

Preparation of whole body crayfish for metal analysis followed a different procedure from that used for sediment samples. Crayfish were dried to a constant weight at 105° C (approximately 18 hours). Specimens were then placed in 125 ml Erlenmeyer flasks and digested in 20 ml concentrated nitric acid and 20 ml distilled water. Samples were slowly boiled for about two hours until approximately 5 ml remained. They were then filtered and diluted to 100 ml. To avoid contamination, all glassware, glass beads, filters, and utensils that would come into contact with the samples were soaked for at least 12 hours in a 10% nitric acid bath, then rinsed thoroughly in distilled water.

All prepared samples were analyzed for lead and cadmium with a Perkin-Elmer model 603 atomic absorption spectrophotometer at Emporia State University. The indicated wavelength used to analyze lead and cadmium were 284.5 and 229.8 nm, respectively. To detect any lead or cadmium contamination in the acid used, a blank with 5 ml of nitric acid added to it was compared to a blank free of the acid. For quality control, 5 mg/l and 10 mg/l lead standards and 1 mg/l and 2 mg/l cadmium standards were used. Also, each sample was analyzed twice, and the calibration of the machine was checked after every 10 samples were analyzed. The absorbance value for each sample was used to determine the concentration of each metal per microgram (μg) of sample using the formula:

$$C_x = (((A_x \times C_s) / A_s) \times 100) / W_d$$

where, C_x = concentration (μg/g or ppm) of metal in sample material, A_x = absorbance value of sample, A_s = absorbance value of standard, C_s = concentration of metal in standard, and W_d = dry weight (g) of sample material (Standard Methods for the Examination of Water and Wastewater, 1985). Mean concentrations of lead and cadmium in the sediment and crayfish tissue from each sample site were then calculated.

Detection limits were set by comparing the differences between absorbance value units for each of the two sample runs, and applying the range method described by Kenner and Busch (1979) to determine the standard deviation. This value was then divided by the sensitivity of the machine to obtain a detection limit.

Simple linear regression was accomplished by BIOSTAT I (Pimentel and Smith, 1986) in order to determine whether lead and cadmium concentrations in crayfish tissues are dependent on concentrations in the surrounding sediment. The resulting slopes of the regression lines from both species of crayfish were then compared with a Student's _t_-test (Zar, 1984) to determine if uptake of metals from the environment into the tissue was similar between species.

To determine if concentrations of lead and cadmium were significantly greater in the sediment and crayfish tissue at sample sites located downstream from abandoned mining operations (sites 1-4) than upstream (sites 5-7), a _t_-test comparison of two samples was performed (Pimentel and Smith, 1986). Because of the high variance of concentrations within each sample group, the data were log transformed for this test (Sokal and Rohlf, 1981), such that the variance of each sample group was independent of the mean.

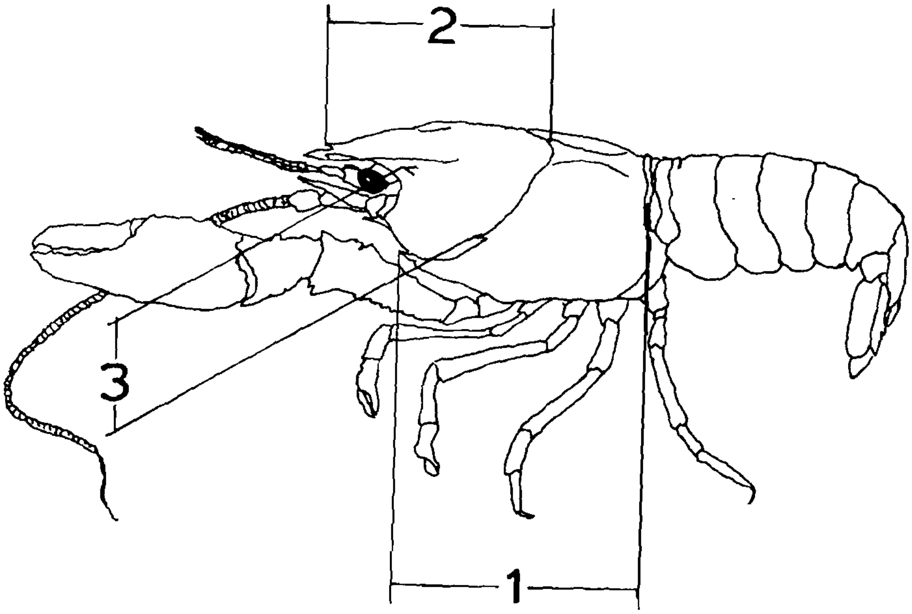
Measurements for asymmetry analyses were made on all collected specimens, including those used for metal analysis, to the nearest 0.1 mm with a vernier caliper. Ten axial and 10 appendicular characteristics were selected on a single individual to test for repeatability of the measurements. Both right and left sides of these selected characters on the individual were measured daily for 15 consecutive days. The three axial and three appendicular characters with the lowest coefficient of variation for the 15 daily measurements were selected for this investigation (Figure 2). Both axial and appendicular characters were selected to test for the effects of limb loss and subsequent regeneration on symmetry.

A difference value for each character was obtained by subtracting the left-sided measurement from the measurement of its right-sided counterpart. Total length was also measured for each individual. A population distribution of character differences was then drawn for each of the six characters by inputting the data into SAS (SAS Institute, Inc., 1988).

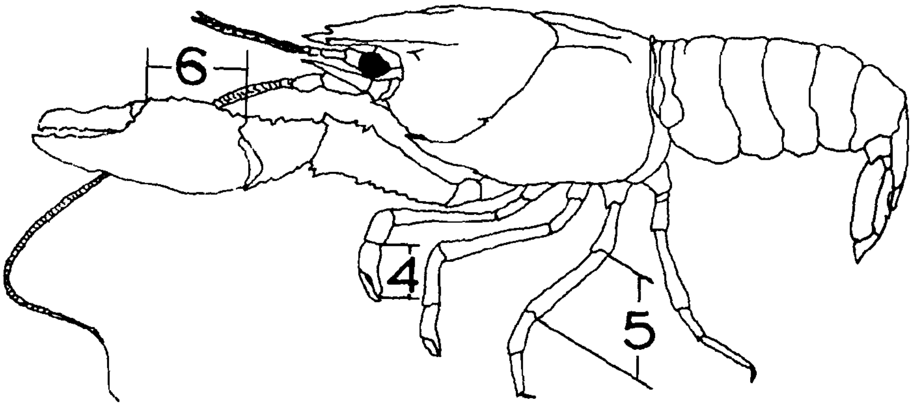
If the mean of a population distribution is significantly greater than or less than zero, the character is expressing directional asymmetry. A *t*-test (SAS Institute, Inc., 1988) was used to determine if means were significantly different from zero. A mean significantly greater than zero indicates bias of that character toward

Figure 2. Measurements used for fluctuating asymmetry analyses. The axial measurements are: measurement 1 = tip of branchiostegal spine to end of carapace, measurement 2 = tip of marginal spine to intersection of cephalic groove and areola, measurement 3 = tip of postorbital spine to tip of cervical spine. The appendicular measurements are: measurement 4 = length of propodite of the second pereiopod, measurement 5 = dorsal length of meropodite of the fourth pereiopod, measurement 6 = palm length of chela.

AXIAL MEASUREMENTS



APPENDICULAR MEASUREMENTS



the right side of the body, and a mean significantly less than zero indicates bias toward the left side. Directional asymmetry can be corrected for by subtracting the mean, if it is greater than zero, or adding the mean, if it is less than zero, to every individual's character difference value. Significant deviations of the distributions from normality indicate that the character is displaying antisymmetry. Antisymmetry was evaluated for each character by applying the Shapiro-Wilk statistic (SAS Institute, Inc., 1988).

The level of fluctuating asymmetry that a character displays within a population is equal to the variance of the distribution. Because this index is equal to the variance of the distribution, the level of fluctuating asymmetry will not be affected by directional asymmetry. Therefore, it was not necessary to correct for directional asymmetry in this investigation. However, antisymmetry may have a strong influence on fluctuating asymmetry values. The population distribution should therefore be normal in order to obtain the best estimate of fluctuating asymmetry.

Levels of fluctuating asymmetry for each of the six characters were compared among all populations by Bartlett's test for homogeneity of variances (Pimentel and Smith, 1986). If the asymmetry levels were significantly different among populations, the population with the highest value was removed and the test run again. This was repeated until there were no significant differences among population variances. This procedure was done to determine if a particular population showed a significantly higher fluctuating asymmetry level from the others.

I compared the levels of fluctuating asymmetry for each character from populations downstream of the mining activity (sites 1-4) to levels from populations upstream (sites 5-7) by a _t_-test. This analysis was performed to determine if fluctuating asymmetry levels from each of the six characters were significantly different between upstream and downstream populations.

Simple linear regression analysis was performed to determine whether lead and cadmium levels in the tissues of crayfish had a direct influence on fluctuating asymmetry values. That is, I wanted to determine if an increase in heavy metal concentrations linearly increased fluctuating asymmetry levels.

To evaluate the effects of limb loss and subsequent regeneration on asymmetry, I used a _t_-test to compare fluctuating asymmetry values between the three axial and three appendicular characters for each population. Molting is another factor which may affect body symmetry in crayfish. To test for differences in asymmetry due to age, and subsequent molting, all crayfish were placed into six size classes (<40 mm, 40-49 mm, 50-59 mm, 60-69 mm, 70-79 mm, >79 mm) based on total body length. Bartlett's test for homogeneity of variances was then used to determine if significant differences existed in fluctuating asymmetry values among size classes.

RESULTS

Significant differences in lead and cadmium concentrations in sediment were found between sample sites 1-4 and sites 5-7 (Table 1). Sediments at sites downstream from lead and zinc mines were higher in both lead ($\underline{t}$ = 4.637; d.f. = 5; $\underline{P}$ = 0.006) and cadmium ($\underline{t}$ = 4.740; d.f. = 5; $\underline{P}$ = 0.005) than upstream sediments.

Both *O. neglectus* and *O. virilis* were analyzed for heavy metals (Table 2). A significant linear relationship was found between the concentration of lead in the sediment and the tissues of *O. virilis* ($y = 0.018x + 21.6$; $\underline{F}$ = 37.4; d.f. = 1, 3; $\underline{P} < 0.01$), but a non-significant relationship was found between lead in the sediment and *O. neglectus* tissue ($\underline{F}$ = 1.42; d.f. = 1, 4; $\underline{P} > 0.25$). It appears that an upper maximum exists in the bioaccumulation of lead in the tissues of crayfish. As lead increases in the sediment, it also increases proportionally in the tissues until a certain level has been reached. After the maximum body load has been met, the accumulation of lead in the tissue begins to taper off (Brisbin and Newman, 1991). Therefore, the outlier, sample site 4, which had lead concentrations 4.5 times greater than the next highest, was excluded and regression analysis run again. Excluding sample site 4, a significant linear relationship was found between sediment lead concentrations and concentrations in both *O. neglectus* ($y = 0.073x + 19.7$; $\underline{F}$ = 18.6; d.f. = 1, 3; $\underline{P} < 0.025$) and *O. virilis* ($y = 0.052x + 19.7$; $\underline{F}$ = 31.9; d.f. = 1, 2; $\underline{P} < 0.05$)

Table 1. Lead and cadmium concentrations in sediment samples from streams in Cherokee Co., Kansas. See Figure 1 for specific collecting sites.

Site	Lead (µg/g)	Cadmium (µg/g)
1	242.0	23.80
2	169.0	29.60
3	190.0	10.40
4	1080.0	103.00
5	16.4*	0.56*
6	27.4	1.66
7	32.6	1.47

* Below detection limit (lead = 24.8 µg/g; cadmium = 1.25 µg/g)

Table 2. Lead and cadmium concentrations in tissues of crayfish from streams in Cherokee Co., Kansas. See Figure 1 for specific collecting sites.

Site	Species	Sample Size	Lead (µg/g)	Cadmium (µg/g)
1	_O. neglectus_	7	38.3	2.86
2	_O. neglectus_	1	35.2	2.74
	O. virilis	6	28.3	2.86
3	_O. neglectus_	7	29.3	2.63
4	_O. neglectus_	6	35.0	4.60
	O. virilis	1	40.0	3.69
5	_O. neglectus_	3	19.4*	1.97
	O. virilis	4	19.4*	2.34
6	_O. virilis_	7	21.2*	1.79
7	_O. neglectus_	1	23.7*	2.10
	O. virilis	6	22.5*	1.96

* Below detection limit (lead = 24.8 µg/g; cadmium = 1.25 µg/g)

tissues (Figure 3). Also, a significant linear relationship was found between cadmium concentrations in the sediment and cadmium concentrations in the tissues of *O. neglectus* ($y = 0.024x + 2.14$; $F = 115.8$; *d.f.* = 1, 4; $P < 0.01$) and *O. virilis* ($y = 0.016x + 2.08$; $F = 23.1$; *d.f.* = 1, 3; $P < 0.025$) (Figure 4). It does not appear that a maximum load for cadmium accumulation in the body tissues was met. Therefore, all populations were included in this analysis.

When slopes of the regression lines (Figures 3 and 4) for *O. neglectus* and *O. virilis* were compared, no significant differences were found between the species in the uptake of lead ($t = 0.814$; *d.f.* = 5; $P > 0.20$) and cadmium ($t = 1.935$; *d.f.* = 7; $P > 0.05$). Therefore, because both species accumulate lead and cadmium in their tissues from the sediment in the same manner, both species were pooled to increase sample size (Table 3). Although Hurlbert (1984) suggests that pooling may create problems, the levels of significance found in these *t*-tests are great enough to avoid error. A significant linear relationship was found between lead concentrations in the tissues of the pooled species and the concentrations in the sediment after site 4 was excluded ($y = 0.068x + 19.0$; $F = 47.67$; *d.f.* = 1, 4; $P < 0.01$). Also, a significant linear relationship was found between cadmium concentrations in the sediment and pooled crayfish tissues from all populations ($y = 0.024x + 2.09$; $F = 84.22$; *d.f.* = 1, 5; $P < 0.001$). In general, crayfish

Figure 3. Linear regression analysis of lead concentrations in the sediment and the whole body tissues of *Orconectes neglectus* (squares and solid line) and *O. virilis* (triangles and dotted line) from streams in Cherokee Co., Kansas.

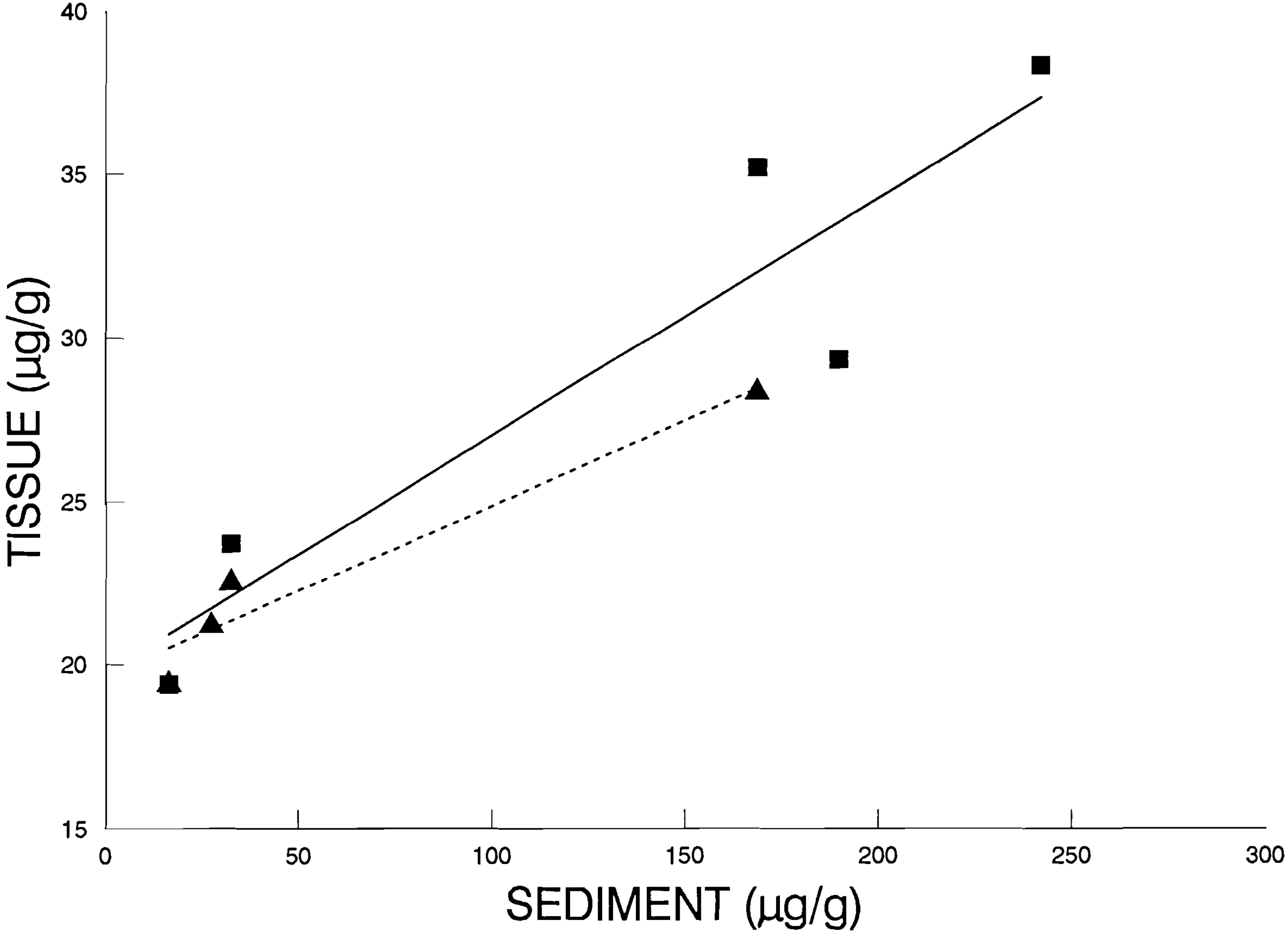

40
35
30
25
20
15
0
50
100
150
200
250
300
TISSUE (µg/g)
SEDIMENT (µg/g)

Figure 4. Linear regression analysis of cadmium concentrations in the sediment and the whole body tissues of *Orconectes neglectus* (squares and solid line) and *O. virilis* (triangles and dotted line) from streams in Cherokee Co., Kansas.

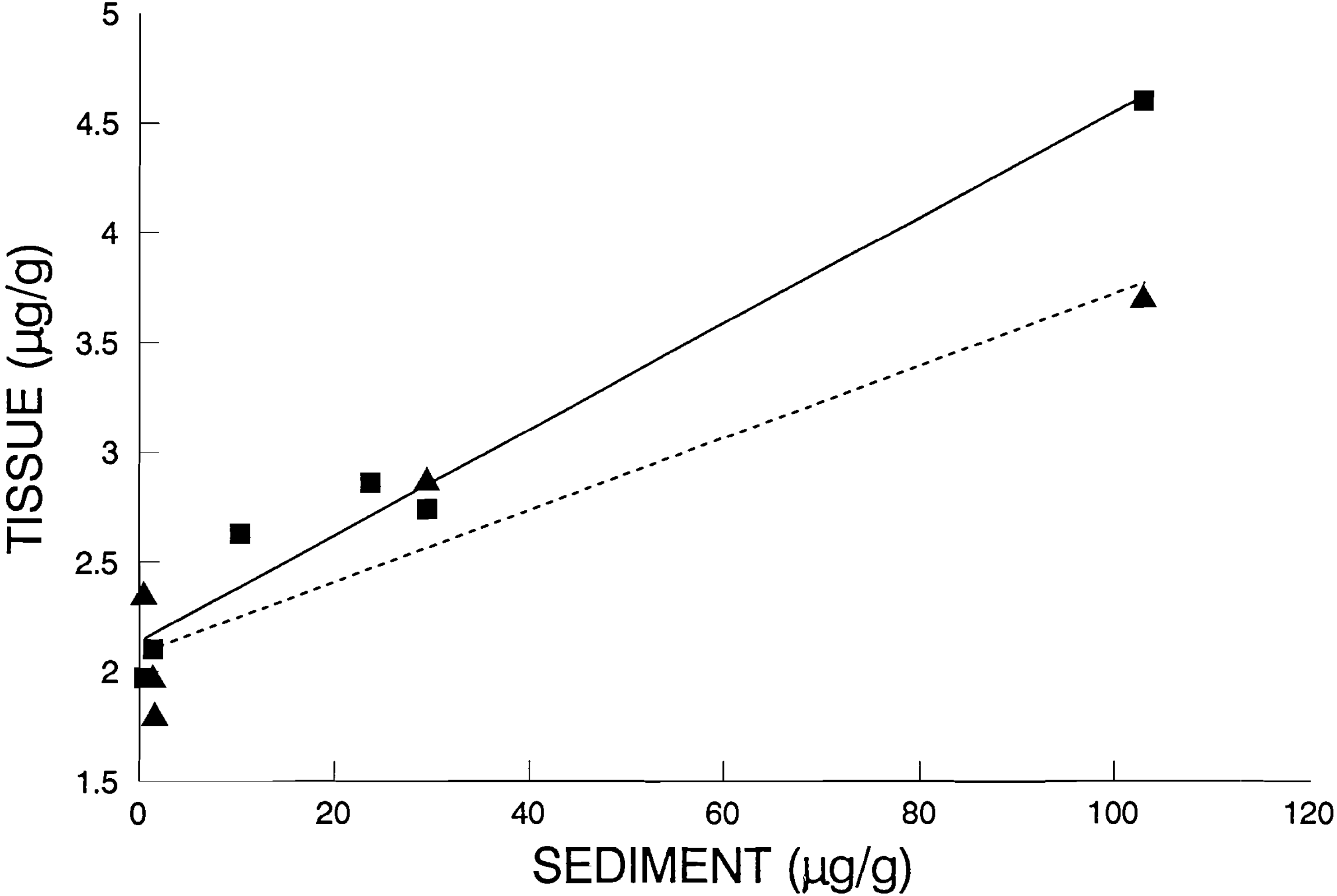

TISSUE (µg/g)
5
4.5
4
3.5
3
2.5
2
1.5
0
20
40
60
80
100
120
SEDIMENT (µg/g)

Table 3. Lead and cadmium concentrations in crayfish tissues, pooled by species, for crayfish collected from streams in Cherokee Co., Kansas. See Figure 1 for specific collecting sites.

Site	Lead ($\mu g/g$)	Cadmium ($\mu g/g$)
1	38.3	2.86
2	29.3	2.84
3	29.3	2.63
4	35.8	4.47
5	19.4*	2.13
6	21.2*	1.79
7	22.7*	1.98

* Below detection limit (lead = 24.8 $\mu g/g$; cadmium = 1.25 $\mu g/g$)

living downstream from nearby lead and zinc mines have significantly higher concentrations of lead (t = 4.947; d.f. = 5; P = 0.005) and cadmium (t = 2.928; d.f. = 5; P = 0.032) in their whole body tissues than crayfish living upstream.

The variance ratio test indicated that population variances for all measured characters, and consequently fluctuating asymmetry values, between male and female crayfish were not significantly different (P > 0.05 for all ratios). Therefore, sexes were pooled for asymmetry analyses. Also, the variance ratio test concluded that fluctuating asymmetry values were not significantly different between *O. neglectus* and *O. virilis* when living in sympatry (P > 0.05 for all ratios). Species were then pooled for each population in order to increase sample size. Table 4 lists the value of fluctuating asymmetry for each measured character after sexes and species were pooled.

The mean value of right minus left character differences for each population are displayed in Table 5. Characters which have means significantly different from zero (P < 0.05) display directional asymmetry. Character 2 was biased towards greater development on the left side of the body in four of the seven populations, and character 5 was also left side biased in three populations. Character 4 was biased towards the right side in four populations.

The Shapiro-Wilk test concluded that 45% of the right minus left character difference distributions were

Table 4. Fluctuating asymmetry values for six characteristics with species and sexes pooled at different sample locations. Numbers in parentheses are sample size at each location. Refer to Figure 1 for specific collecting sites and to Figure 2 for specific measurements.

	Character					
Site	1	2	3	4	5	6
1 (38)	0.0486	0.0788	0.0705	0.0866	0.0273**	1.8596*
2 (41)	0.0542	0.0789	0.0230	0.0554*	0.0390	0.5568*
3 (36)	0.0364	0.0608*	0.0363**	0.1419*	0.0281	1.4872*
4 (38)	0.0403	0.0546	0.0405	0.0495	0.0291	0.9021*
5 (38)	0.0248	0.0646	0.0392**	0.0550*	0.0363	0.5149
6 (46)	0.0346	0.0351*	0.0327*	0.0917	0.0257	0.8185*
7 (43)	0.0205**	0.0501*	0.0364	0.0952*	0.0286*	0.7612*
x^2	13.65	9.44	13.45	16.36	2.97	26.38
P	0.034	0.150	0.037	0.012	0.814	0.000

* Displaying significant leptokurtosis.
** Displaying significant platykurtosis.

Table 5. Directional asymmetry values for six characteristics with species and sexes pooled at different sample locations. Numbers in parentheses are sample size at each location. Refer to Figure 1 for specific collecting sites and to Figure 2 for specific measurements.

	Character					
Site	1	2	3	4	5	6
1 (38)	-0.02	-0.14*	-0.00	0.08	-0.02	-0.11
2 (41)	-0.01	-0.18*	0.00	0.11*	0.01	-0.08
3 (36)	-0.01	-0.25*	-0.04	0.07	-0.14*	0.04
4 (38)	-0.00	-0.10*	-0.02	0.14*	-0.05	0.30
5 (38)	-0.02	0.02	0.02	0.09*	-0.08*	0.06
6 (46)	-0.03	-0.03	0.04	0.13*	-0.05*	0.04
7 (43	-0.00	0.06	0.05	0.09	-0.05	0.22

* Mean is significantly different from zero ($\underline{P} < 0.05$).

significantly deviant from normal ($P < 0.05$) (Table 4). Leptokurtosis was the primary factor contributing to the deviations from normality. Platykurtosis was also a factor, but not to the extent as leptokurtosis.

Significant heterogeneity in fluctuating asymmetry values among populations was found to exist for character 1, character 3, character 4, and character 6. The Spring River population displayed a level of fluctuating asymmetry for character 1 that was significantly greater than that of other populations. For character 3, the Shoal Creek population displayed the highest level, and the Willow Creek population had a significantly higher level of fluctuating asymmetry for character 4 than the others. Fluctuating asymmetry levels for character 6 in both the Shoal Creek and Willow Creek populations were significantly greater than the levels displayed by other populations. Only those populations (1-4) which are downstream from nearby mining activity and thus exposed to high concentrations of lead and cadmium displayed a significantly greater level of fluctuating asymmetry for characters heterogeneous among populations. However, when fluctuating asymmetry values for the populations exposed to lead and cadmium pollution as a group (populations 1-4) were compared to the values from the control populations (5-7), only character 1 displayed significantly higher values ($\underline{t} = 3.103$; $\underline{d.f.} = 5$; $\underline{P} = 0.027$) in the experimental group. There was no significant difference between groups for the other five characters. Results of the simple linear regression analysis between lead and cadmium concentrations in the crayfish tissue and the level of fluctuating asymmetry for each character indicated that no significant relationship exists. Therefore, an increase in lead or cadmium in crayfish tissue did not significantly increase fluctuating asymmetry values.

Results of the *t*-test comparing fluctuating asymmetry values between axial and appendicular characteristics indicated that no significant differences occurred. Therefore, the levels of fluctuating asymmetry for the three appendicular characteristics were not significantly different from those of the three axial characteristics in all seven populations.

Significant heterogeneity in fluctuating asymmetry values was found between the different size classes for all six characters. However, there was no detectable trend in which specific size classes tended to have highest values.

DISCUSSION

Except for the north Spring River sample site, all sampled locations contained concentrations of lead and cadmium in the sediment that were above the detection limit (Table 1). Lead and cadmium are known to enter aquatic environments by such anthropogenic activities as fossil fuel combustion, atmospheric precipitation from industry, and mining (Hiatt and Huff, 1975; Enk and Mathis, 1977). Application of phosphate fertilizers and pesticides may also be sources of cadmium pollution (Hiatt and Huff, 1975). Because the Cow and Shawnee creek sample sites are upstream from the mining areas, low concentrations of lead and cadmium may be entering the aquatic systems of southeastern Cherokee County by some mechanism other than mining. A coal burning power plant is located approximately 2 km northeast of sample site 2 in the city of Riverton, Kansas. Fuel combustion from automobiles may also be a factor contributing trace metals into the study area. Numerous roads and highways transect the sample sites. The majority of the study area is cropland, and cadmium-containing chemicals used for agriculture may leach into the waterways. Although these factors may be contributing lead and cadmium to the environment, the major contributor to sample sites 1 - 4 is lead and zinc mining. As a group, sediments at sites downstream from the mining activity were found to contain significantly higher metal concentrations than at upstream sites.

Sediments have a high capacity for absorbing lead and cadmium from the water column (Hiatt and Huff, 1975; Timmermans et al., 1989) and this acts as a "sink" (Enk and Mathis, 1977). High metal concentrations at sites downstream from the mining activity are probably due to this sorptive nature of the sediments. Waste rock from mining operations also contains high concentrations of heavy metals (Spruill, 1987; Environmental Protection Agency, 1990). Spruill (1987) concluded that high lead and cadmium concentrations found in sediments of Turkey Creek were due to direct input of waste rock into the stream. These results are consistent with the high metal concentrations found in Turkey Creek sediments in my study (Table 1).

Soil and sediment concentrations of lead and cadmium in excess of 1000 ppm (μg/g) and 25 ppm, respectively, may pose a risk to human health (Environmental Protection Agency, 1990). Although Turkey Creek sediments exceed this limit for both metals, and Spring River sediments exceed the cadmium limits (Table 1), crayfish still inhabit these systems. According to Young and Harvey (1991) and Anderson (1978), crayfish may accumulate relatively high concentrations of lead and cadmium without experiencing adverse effects. However, crayfish appear to have developed strategies for coping with toxic metal exposure. For example, crayfish produce a mucous sheath over the gill surfaces in response to suspended matter in the water column

(Anderson and Brower, 1978). Matter, such as trace metals, tend to adsorb to this protective sheath rather than the sensitive gill membranes. Anderson (1978) found that O. virilis produced a mucous film over the gills in response to increased lead concentrations. Although this film could decrease the efficiency of the gills for oxygen uptake, Anderson (1978) found that crayfish respond by increasing the amount of oxygenated water passing the gill surface. Cadmium exposure also increases mucous production over crayfish gill surfaces (Enk and Mathis, 1977).

Another response of aquatic organisms to heavy metal exposure is to sequester and detoxify the metals by binding them to specific metallothionein proteins (Bouquegneau and Joiris, 1988). During periods of acute or chronic cadmium stress, the crayfish Austropotamobius pallipes increases production of the metallothionein protein in the hepatopancreas (Lyon et al., 1983; Lyon, 1984). Roldan and Shivers (1987) found that the crayfish Orconectes propinquus is capable of detoxifying lead by binding it to metallothioneins. These detoxified metals are then stored in the vacuoles of the hepatopancreatic cells until they are eliminated. Metals, such as copper and zinc, which are essential for biological functions of crayfish tend to be regulated by certain homeostatic mechanisms (Anderson and Brower, 1978). Therefore, concentrations of essential metals within the body tissue remain relatively constant even though environmental concentrations may fluctuate. Lead and cadmium, which are not essential to the organism, are detoxified and stored within the body. Therefore, as environmental concentrations of lead and cadmium increase, concentrations in the tissue also increase (Anderson and Brower, 1978). The positive linear relationship found in this study between lead and cadmium concentrations in sediments and body tissues may be due to the sequestering nature of metallothioneins. This same linear relationship was found between concentrations of cadmium in the crayfish _O_. _propinquus_ and their surrounding environment by Gillespie et al. (1977).

Lead and iron storage mechanisms were examined in _O_. _propinquus_ by Roldan and Shivers (1987). They found that both lead and iron were stored and metabolized similarly in the hepatopancreas. However, they found that lead was also stored in the cells of the antennal gland before being excreted in the urine. Iron did not appear to enter the antennal gland. Roldan and Shivers (1987) concluded that crayfish appear to be more efficient in eliminating lead from the system without experiencing relatively toxic effects than they are with iron. Anderson and Brower (1978) found that the exoskeleton of _O_. _virilis_ acts as a potential "sink" for lead accumulation, and therefore serves as a mechanism for lead elimination during molting. Cadmium accumulation was significantly less in the exoskeleton than lead (Anderson and Brower, 1978). These efficient systems for eliminating lead may be why body tissue concentrations in my study taper off when environmental concentrations reach relatively high concentrations. Because cadmium is not eliminated by molting as efficiently as lead, body cadmium loads tend to increase as environmental concentrations increase (Figure 4).

The use of the entire crayfish body for metal analysis in my study may have introduced some background interference. Young and Harvey (1991) stated that inclusion of gut contents could subject metal analyses to analytical problems. Also, they stated that the specificity of certain tissues to metal binding may create a dilution effect within the organism. For example, shedding of the exoskeleton during molting may eliminate metals, such as lead, that bind effectively to exoskeleton tissues (Anderson and Brower, 1978). However, background interference from these mechanisms was minimized in my study by averaging whole body metal concentrations from seven individuals for each population.

Although lead and cadmium in my study are at concentrations which are non-lethal to the exposed crayfish, they may be introducing significant stress to the populations. Fluctuating asymmetry has provided researchers a method for evaluating the effects of stressful environments on organisms (Leary and Allendorf, 1989). For example, Beacham (1990) found that chum salmon (Oncorhynchus keta) reared in sub-optimal water temperatures displayed greater asymmetry in morphological and meristic characteristics than salmon reared in the most favorable environment. As water temperatures became more extreme, fluctuating asymmetry levels increased significantly. Exposure to non-lethal concentrations of toxic chemicals were demonstrated as a mechanism for increased fluctuating asymmetry values in fish (Valentine et al., 1973; Valentine and Soule, 1973). However, in my study no significant correlation was found between the level of heavy metal exposure and fluctuating asymmetry. Several other studies have also revealed inconclusive trends between environmental stress and fluctuating asymmetry levels (Palmer and Strobeck, 1986). For example, Owen and McBee (1990) compared fluctuating asymmetry in rodents inhabiting a petrochemical waste disposal site and control sites. Although chromosomal aberrations were higher in rodents on the waste site, there were no significant differences in levels of fluctuating asymmetry.

One possibility for the inconsistent results in past asymmetry studies could be the high diversity of indicies used to evaluate fluctuating asymmetry. Twenty-two different indicies have been identified (Palmer and Strobeck, 1986). Palmer and Strobeck (1986) evaluated the effectiveness of the different indicies in detecting real differences in fluctuating asymmetry between samples. The index used for my study, the variance of right minus left differences, is the most common and appropriate for the samples I evaluated (Palmer and Strobeck, 1986). For example, the index used in my study remains unaffected by directional asymmetry. Because significant directional asymmetry was found in my study, other indicies may not have been able to evaluate true differences in fluctuating asymmetry. Palmer and Strobeck (1986) found that the index used in my study was more useful than the others in evaluating fluctuating asymmetry when population sizes are large (N > 25). Population sizes in this study were large enough to justify use of this index.

Use of the variance of signed right minus left differences as an index for evaluating fluctuating asymmetry may be associated with certain problems. Variation in character size could seriously influence this index (Palmer and Strobeck, 1986; Owen and McBee, 1990). However, I chose not to scale for character size due to the small amount of variation within populations. Also, the variation in character size remained relatively constant between populations. With little character size variation, this index was more powerful than other indicies (Palmer and Strobeck, 1986). Antisymmetry may also be a factor introducing error to the analysis of fluctuating asymmetry (Owen and McBee, 1990). Table 4 indicates that 45% of the measured character distributions display significant kurtosis. According to Van Valen (1962), antisymmetry results in a platykurtic or bimodal distribution. Only four of the character distributions displayed significant platykurtosis. Therefore, antisymmetry is probably contributing a minor portion of the observed asymmetry. One possible explanation for the conspicuous leptokurtosis displayed in the appendicular characteristics is limb loss and regeneration. A fraction of each population may have lost the measured appendage, and therefore would have difference values in the tail of the distribution. The majority of the population with the appendage intact would have values centered around the mean. Limb loss and subsequent regeneration was dismissed as a possible factor that may be contributing "background noise" to the analysis of fluctuating asymmetry because axial and appendicular asymmetry values were not significantly different.

The results of Bartlett's test of homogeneity of variances indicate significant heterogeneity of fluctuating asymmetry values in four of the six examined characters. According to Palmer and Strobeck (1986), Bartlett's test is an effective test for comparing asymmetry values. However, they warn that this test is sensitive to deviations from normality, especially long-tailed distributions.

Although significant heterogeneity in asymmetry values was found among the seven populations, no significant correlation between metal loads in the body tissues and fluctuating asymmetry could be determined. However, when significant heterogeneity was found, populations exposed to lead and cadmium consistently had the greatest asymmetry values. It is possible that factors other than, or in addition to lead and cadmium exposure, may be affecting developmental homeostasis. Other factors caused by mining are decreases in pH and increases in zinc, iron, manganese, and silica concentrations (Spruill, 1987). A study conducted by Jagoe and Haines (1985) found that some characteristics of fish were more asymmetrical in populations exposed to conditions of low pH. Although pH was not examined in my study, it could be affecting fluctuating asymmetry values. A more complete survey of environmental parameters should be taken in order to determine if environmental stress is affecting fluctuating asymmetry levels in crayfish exposed to mine drainage.

Although the data in my investigation do not suggest a significant correlation between levels of fluctuating asymmetry and concentrations of lead and cadmium in crayfish populations, the use of fluctuating asymmetry in other systems as a tool for monitoring environmental conditions should be further pursued. As public concern for environmental degradation increases, more effective tools for evaluating environmental conditions will be needed.

Fluctuating asymmetry is a quick and inexpensive method for evaluating environmental pollution.

SUMMARY

Significant concentrations of the heavy metals, lead and cadmium, were found in the sediments of aquatic systems in close proximity to abandoned lead and zinc mines in southeastern Cherokee County, Kansas. A significant linear relationship was found between the concentration of these metals in the sediments and in the body tissue of two resident crayfish species, _Orconectes_ _neglectus_ and _O_. _virilis_. Metal accumulation was similar in both species. To assess effects of these lead and cadmium burdens in the tissues of crayfish, asymmetrical development of six bilateral characteristics was evaluated. No significant relationships were found between the level of fluctuating asymmetry displayed by the characters and the concentrations of lead and cadmium in the body tissue. Also, there was no significant difference between the level of fluctuating asymmetry displayed by crayfish exposed to heavy metals and crayfish inhabiting locations relatively free of metal contamination.

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