Bull. Environ. Contam. Toxicol. (1999) 62:122-129

Bulletin of Environmental Contamination and Toxicology

Small-Scale Spatial Variation of Selenium Concentrations in Chironomid Larvae

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Received: 23 September 1998/Accepted: 14 December 1998

Selenium is an important contaminant of many freshwater wetlands receiving agricultural drainage water (Ohlendorf et al. 1986) or natural runoff from seleniferous soils (Maier and Knight 1994). One such wetland is the Benton Lake basin at Benton Lake National Wildlife Refuge in central Montana. Selenium-contaminated water originating from agricultural drainage and selenium-containing natural runoff enters the refuge primarily by way of its main tributary. After entry into the lake, water moves through the diked wetland units as water flow is manipulated for management purposes within this closed basin (Nimick 1997). We have been monitoring selenium concentrations in aquatic invertebrates at Benton Lake since 1988. At least some samples of all invertebrate taxa collected exceeded the 5 µg/g selenium dry wt concentration which, in the diets of migratory birds, has been associated with embryo teratogenesis under some field and laboratory conditions (Skorupa and Ohlendorf 1991). Selenium concentrations were consistently higher in the sediment-dwelling larvae of the family Chironomidae (Order: Diptera) than in any other taxon sampled. Chironomids are an important food for many species of migratory birds breeding at Benton Lake (Bartonek and Hickey 1969, Sugden 1973).

Investigators have traditionally collected a few, usually one to three, samples to characterize the selenium concentration in aquatic macroinvertebrates inhabiting a wetland unit. A sample is a composite of hundreds of individuals collected from an area of usually unstated size. Invertebrates can be collected as random samples (e.g., Schuler et al. 1990, Saiki et al. 1993), as uniformly distributed samples, or as what Manly (1992) called non-random convenience samples (e.g., Nimick et al. 1996). From 1988 to 1995, we collected convenience samples of chironomid larvae from Benton Lake. Our samples were composites drawn from areas of from 0.001 to 0.1 ha along shorelines. Within a wetland unit, the year-to-year variation in selenium concentrations in chironomid larvae collected in that way was large and inexplicable by variation in water inflows.

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We modified our monitoring program in 1996 to minimize the effects of spatial and temporal scale on variation in selenium concentrations in samples of chironomid larvae. Our objective was to collect samples at the same time of year from uniformly distributed subplots within small areas established as permanent sampling stations, and thus determine accurately selenium concentrations in chironomid larvae from those sites. To our dismay, we found that variation within sampling stations was very great. We here report the extent of that variation and warn other investigators of the problem it presents. We also suggest a sampling scheme to estimate accurately selenium concentrations in the invertebrate foods of migratory birds.

MATERIALS AND METHODS

We established five permanent sampling stations along the shore of Benton Lake. Stations were located 0, 0.2, 2.0, 6.0, and 6.1 km down gradient from the point where selenium-contaminated water enters Benton Lake via its main tributary. Sampling stations were marked with numbered steel fenceposts driven into the ground at the shoreline. Each sampling station was composed of several sampling lanes marked with surveyor's flagging. Each sampling lane was 10 m wide and extended 5 m beyond the shoreline into the water (Fig. 1). In 1996, we established and sampled from four sampling lanes at each station. Preliminary results of the 1996 sampling indicated that sampling effort should be increased in order to detect trends at each station and differences between stations with acceptable statistical power, so in 1997 we added an additional six lanes to each station, bringing the total to ten. The sampling lanes were separated by boundary strips 2 m wide, so the centers of adjacent lanes were 12 m apart and the external boundary of each station sampled in 1997 enclosed an area 118 m x 5 m in size.

From each sampling lane, we collected a minimum of 2 g of chironomid larvae by sweeping the bottom sediment with a sweep net. At some stations, collections were supplemented with chironomids collected in light traps (Espinosa and Clark 1972) placed near the center of each lane. The larvae were separated from the sediment in glass pans filled with site water, stored in chemically clean glass jars, and frozen as soon as practicable after collection, usually within 4 hr. Samples remained frozen for 8 mon before being analyzed. From two of the chironomid sampling lanes at each station, we also collected a minimum of 50 g of bottom sediment. In 1996, all 20 chironomid samples were collected between 17 June and 26 June. In 1997, all 50 chironomid samples and all 10 bottom sediment samples were collected

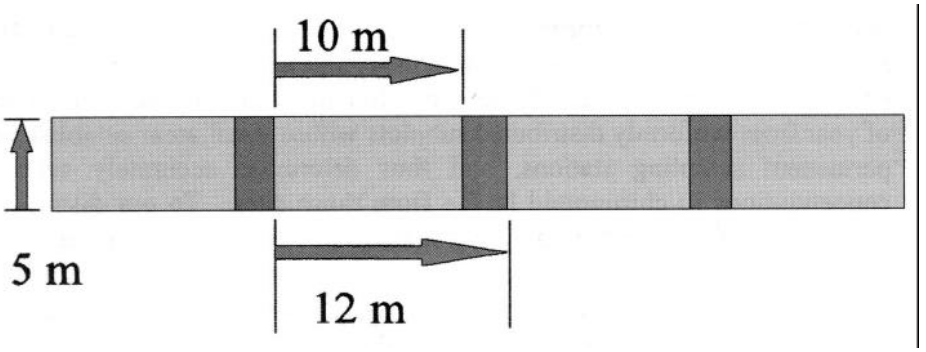

Figure 1. Dimensions of a station, as sampled in 1996. Lightly stippled rectangles represent the sampling lanes, and dark rectangles represent the unsampled buffer strips between sampling lanes. Stations sampled in 1997 were enlarged to include ten sampling lanes, rather than the four shown here.

between 17 June and 27 June. Chironomid samples from the sampling lanes of a single station were collected within no more than 7 d and usually within 3 d.

Samples were analyzed for selenium by graphite furnace atomic absorption spectrometry (Krynitsky 1987) by the U.S. Fish and Wildlife Service's Patuxent Analytical Control Facility in Laurel, MD. Analytical results were reported on a dry wt basis. The precision and accuracy of laboratory analyses were confirmed with procedural blanks, duplicate analyses, test recoveries of spiked material, and reference material analyses. Six chironomid samples were analyzed as duplicates. The relative percent differences of the five duplicate pairs with selenium concentrations above the lower limit of detection ranged from 2.5% to 10.4%. The relative percent difference of the duplicate pair with one determination of selenium concentration below the lower limit of detection and one above it was 103%.

Because sampling lanes were linearly arranged along shorelines, we calculated a simple index of spatial autocorrelation by comparing the selenium concentration in each chironomid sample with the concentrations of its nearest neighbors from the same station. We used Pearson's product-moment correlation coefficient (Norušis 1993) to conduct those analyses on the samples collected in 1997, when sample sizes were large. Because we did not

originally design this study to examine spatial autocorrelation, we could not conduct an a priori power analysis (Hayes and Steidl 1997). Instead, we calculated 95% confidence intervals around the computed correlation coefficients. We also compared selenium concentrations in sediment samples with concentrations in chironomid larvae collected from the same lanes. We assigned values of one-half the lower limit of detection to samples with undetectable concentrations. Data were transformed to natural logarithms for all analyses. After transformation, data were normally distributed (Lilliefors test, $p > 0.05$ in all cases).

RESULTS AND DISCUSSION

Variation of selenium concentrations in chironomid larvae collected from a single station was very great. Samples collected as little as 12 m apart differed up to five-fold in selenium concentration. We found no significant spatial autocorrelations among selenium concentrations in chironomids at any of the five stations (Table 1). One of the correlation coefficients was even negative. However, the 95% confidence intervals for the three stations most distant from the entry point of selenium-contaminated water included values of $r > 0.80$. The evident absence of spatial autocorrelation observed at the two sample stations closest to the main tributary may have been due in some way to their proximity to the source of selenium entering the lake. Even if spatial autocorrelation cannot be conclusively rejected for samples collected from the three stations most distant from the selenium source, they were quite spatially variable. Chironomid larvae from at least one pair of adjacent lanes at each of those three stations differed in selenium concentrations by more than a factor of two. In 1996, chironomid samples collected 12 m apart from the station most distant from the entry point of selenium-contaminated water were on opposite sides of the 5 µg/g selenium dry wt (Skorupa and Ohlendorf 1991) dietary threshold.

We do not know why selenium concentrations were so variable within such small areas. Trace element concentrations in aquatic invertebrates collected from the same site can be highly variable over the course of a year (Hare and Campbell 1992), and some species have been shown to bioaccumulate selenium very rapidly (Nassos et al. 1980). Nevertheless, we do not think that the differences in selenium concentrations that we observed were the result of temporal differences in collection of samples. During our ten sampling episodes (five stations in each of 2 yr), the chironomid samples with the maximum and minimum selenium concentrations within a station were collected on the same day (i.e., within 8 h) on six occasions and on successive

Table 1. Variation in selenium concentrations (µg/g dry weight) in chironomid larvae and sediment collected from the Benton Lake basin.

	1996	1997		1997 spatial autocorrelation among chironomids (n = 9)		
Station[a]	Chironomids (n = 4)[b]	Sediment (n = 2)	Chironomids (n = 10)	r[c]	95% CL[d]	p[e]
0	13.2 (9.3-18.4)	(1.2-1.3)	17.9 (9.7-28.1)	-0.30	-0.75, 0.39	0.45
0.2	17.5 (12.6-23.1)	(0.4-1.0)	11.4 (8.3-18.3)	0.02	-0.59, 0.61	0.95
2.0	24.5 (8.8-50.0)	(0.8-3.1)	13.6 (7.8-21.9)	0.44	-0.27, 0.80	0.23
6.0	1.2 (<0.8-2.6)	(<0.2-<0.2)	2.8 (1.2-4.4)	0.48	-0.24, 0.82	0.19
6.1	3.3 (2.1-6.2)	(<0.1-<0.2)	1.6 (<1.0-2.4)	0.57	-0.13, 0.85	0.11

[a] Sampling stations are named according to their distance in km from the entry point of selenium-contaminated water.

[b] Concentrations are presented as geometric means with ranges in parentheses.

[c] r = Pearson's product-moment correlation coefficient.

[d] 95% CL = 95% confidence limits for r.

[e] p = two-tailed probability of an equally or more extreme outcome than that observed.

days (i.e., within 32 h) on three occasions. However, if selenium body burdens increase with age in chironomid larvae, and if larvae are spatially segregated by age, the spatial variation we observed could itself vary temporally.

Selenium concentrations in chironomid and sediment samples collected from the same sampling lanes were positively and significantly correlated ($r = 0.87$, $p = 0.001$, $n = 10$), so the variation in selenium concentrations in chironomids probably reflects a spatial pattern in selenium distribution in sediment. The association may have been due to sediment retained in the guts of the larvae. We did not allow larvae to depurate because we were interested in selenium concentrations in the diets of migratory birds consuming chironomids as they would encounter them in the wild. We did not measure physical characteristics of the sediment in the sampling lanes, but selenium concentrations in chironomids may have varied with sediment grain size.

The small scale and great magnitude of spatial variation in selenium concentrations in chironomid larvae collected within the same week surprised us. Knowledge of the selenium concentration in a sample of chironomid larvae from one sampling lane was of little or no value in predicting the selenium concentration in a sample collected 12 m away in this wetland. Spatial scale has traditionally been given little consideration in experimental designs for aquatic research (Frost et al. 1988). Our results suggest that investigators should attempt to control for spatial as well as temporal variation when designing studies of trace element concentrations in benthic invertebrates.

If other wetlands influenced by agricultural drainage are similar to Benton Lake in the spatial distribution of selenium in benthic invertebrates, it will be difficult to estimate accurately selenium concentrations in the diets of migratory birds without extensive sampling. We make the obvious suggestion that investigators randomly collect composite samples over entire wetlands. The expense of chemical analysis might be minimized by creating one composite collected from many (e.g., > 50) randomly distributed points within a wetland, with each point contributing an equal mass of material to the composite sample. If it is necessary to conduct statistical comparisons between wetlands or between years, we see no alternative to analyzing many separate samples collected randomly or uniformly from the wetlands of interest.

Acknowledgments. We thank John Barlow, Liz Bradley, Kate Colenso, Nikki Krisfalusi, Jill Marsik, Christy Raffa, Rachel Ries, and Fern Thompson for help with the collection of chironomid samples. John F. Moore arranged for and supervised the chemical analyses. Our work was funded by the U.S. Fish and Wildlife Service.

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