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INTERSPECIFIC DIFFERENCES IN MANGANESE LEVELS IN FRESHWATER BIVALVES

(Research Note)

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Abstract. Vital effects may be important in determining metal levels in bivalves and must be taken into account before the environmental 'meaning' of the data may be interpreted. Manganese concentrations in shells and soft tissues of several species of freshwater bivalves from three recent environments in northern Ohio and one archeological site from southern Ohio were determined by neutron activation analysis and show a species effect where *Fusconaia flava* Mn levels > *Anodonta grandis grandis* levels ≥ all *Lampsilis* species levels. Manganese is concentrated by a factor of 2 to 25 in soft tissues compared to shells. In addition, within-taxon variability of Mn concentration is high. The high intraspecies variability points to the necessity of processing many individuals before reliable numbers may be obtained.

1. Introduction

Bivalve molluscs are used as biological monitors of environmental pollution because it is believed that the presence of environmental contaminants in bivalve soft tissue and shell is related to the presence of contaminants in the environment (International Mussel Watch, 1980; Forester, 1980; Imlay, 1982; Koide *et al.*, 1982; Dermott and Lum, 1986; Duncan *et al.*, 1987). Nevertheless, the factors affecting the encoding of environmental signals by bivalves are poorly understood. For example, Dermott and Lum (1986) concluded that trace metal levels in the shell of the freshwater mussel *Elliptio complanata* may not directly correspond to environmental levels because their uptake is affected by factors such as physiology and availability during growth periods. Thus, additional study of the factors that affect the relationship between environmental contaminant levels and shell/soft tissue levels is necessary (see also Hinch and Stephenson, 1987).

We examine the influence of species type on Mn levels in shells and soft tissues of bivalves collected from three sites within the Lake Erie drainage and one archeological site in southern Ohio. Mn concentrations were compared among species

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within each site. In addition, the same species from different environments were compared to see if Mn values were more correlative with species affinity than environmental character. Manganese was selected because it is a reasonably abundant transition metal that is easily detected in $CaCO_3$ samples. The purpose of this study is to evaluate these preliminary results and determine if a more extensive investigation of possible species effects on the monitoring potential of freshwater bivalves is warranted.

2. Methods

The following live and freshly dead articulated bivalves were collected by hand, by wading or with SCUBA from the locations listed below:

(1) Western Basin, Lake Erie (41°40′ N; 82°57′ W), 8.6 m water, Summer 1981: *Lampsilis radiata luteola; Leptodea fragilis.*

(2) Vermilion River, Ohio, near Banks Roads (41°21′ N; 82°19′ W), 0.5 m water, Summer 1979; *Lampsilis radiata luteola; Lampsilis ventricosa.*

(3) Black River, Ohio, near Rivers Corners (41°6′ N; 82°4′W), 0.5 m water, Fall, 1986: *Lampsilis radiata luteola; Andonta grandis grandis; Fusconaia flava.*

(4) Archeological (Graham) Site, near Athens, Ohio (39°32′ N; 82°23′ W), C^{14} dated AD 950–1000: Lampsilinae fragments.

After collection, the bivalves were measured with vernier calipers, depurated, and then the soft tissues were immediately removed from the shell with nonmetal implements, frozen in liquid nitrogen and then freeze-dried. The age and sex of individuals were determined where possible by the examination of the shell exterior. The air dried shells were stored until needed for analysis. One valve from each individual was then cleaned of extraneous surface material, baked in a muffle furnace for 10 min at 500 °C to remove the periostracum and organic matrix, and then ground in a porcelain mortar and pestle for 1 min. All prepared samples were placed in polyvials and shipped to Buffalo, N.Y. for neutron activation analysis (NAA).

For NAA, 10 to 15 mg aliquots of the shells were weighed on a Mettler analytical balance and heat sealed (to prevent contamination by radioactive 'dust') in either one or 2/5 dram polyvials (1 dram = 3.887 g). Sets of 4 samples and NBS Standard Reference Material Orchard Leaves were placed in pneumatic conveyor #2 of the 2 MW Pulstar Reactor operated by Buffalo Materials Research Center at the State University of New York at Buffalo. Shells and standard were subjected to a neutron flux of 3×10^{12} neutrons cm^{-2} s^{-1} for 5 min. Samples were allowed to decay for approximately 60 min and then placed in a lead cave with an intrinsic Germanium detector connected to either a Canberra 8100 or a Nuclear Data 65 multi-channel analyzer. The 1811.2 keV gamma decay emissions of Mn 56 were counted over a period of 15 min and corrected for the low degree of Compton scatter at that energy. Activity at the time of removal from the reactor was calculated using a half life of 2.58 hr. Concentrations of Mn were calculated by multiplying the ratio

of total counts in a sample to total counts in the standard by the concentration of Mn in the standard (91 $\mu g\ g^{-1}$). The weights of sample and standard were the same. The procedure had a minimum detection limit of 10 ppm and an accuracy within about 10%.

3. Results and Discussion

No correlations were observed between shell morphology, age, or sex of bivalves and Mn concentrations in the shell or soft tissue. Analysis of variance of Mn concentrations in bivalve shells collected from the Black River shows that there are significant differences among the mean concentrations of the three species analyzed (Table I). Scheffe-type confidence intervals calculated on species contrasts (Table I) shows that the *Fusconaia flava* values for Mn (730.2 $\mu g\ g^{-1}$, s.d. = 253.5) are higher than the *Anodonta grandis grandis* values (455.2 $\mu g\ g^{-1}$, s.d. = 181.4), which in turn are equal to or higher than *Lampsilis radiata luteola* values (372.4 $\mu g\ g^{-1}$, s.d. = 74.0). This gives a general relationship for Mn levels for one collection of *Fusconaia* > *Anodonta* > *Lampsilis* (Figure 1). While it is apparent that the variance of Black River shell Mn is proportional to the mean, a square root transformation of the data does not alter the statistical conclusions. Therefore, analysis of raw data are reported here. In addition, all *Lampsilis radiata luteola* Mn values are less than the single *Leptodea* value from Lake Erie. In the Vermilion River collection, the single *L.r. luteola* Mn value is less than the *Lampsilis ventricosa* values (321 $\mu g\ g^{-1}$, s.d. = 22.0; Figure 1).

Four collections contained *Lampsilis* species (Table II). There is no difference in mean Mn concentrations among the three modern collections (Table III). There is a statistically significant difference between modern and archeological collections of *Lampsilis* ($p = 0.048$, Table III), the older collection having Mn concentrations 34% higher than the modern collection. This suggests that it cannot be assumed that older samples from pre-industrial times necessarily possess lower trace metal

TABLE I

Analysis of variance of Mn content of 17 Black River bivalve shells belonging to three species

Source of Variation	Sum of squares	Degrees of freedom	Mean square	F ratio	p
Species differences	398112.90	2	199056.45	5.49	0.017
Error	507720.87	14	36265.78		

Contrast of Means
(*Fusconaia* – *Anodonta*): 275.0 ± 256.1[a]
(*Anodonta* – *Lampsilis*): 82.8 ± 268.8
(*Fusconaia* – *Lampsilis*): 357.8 ± 268.8

[a] Confidence intervals of contrasts of means were calculated by the Scheffe procedure using a 0.10 overall error rate for multiple comparison suggested by Scheffe (1959) and Seal (1964).

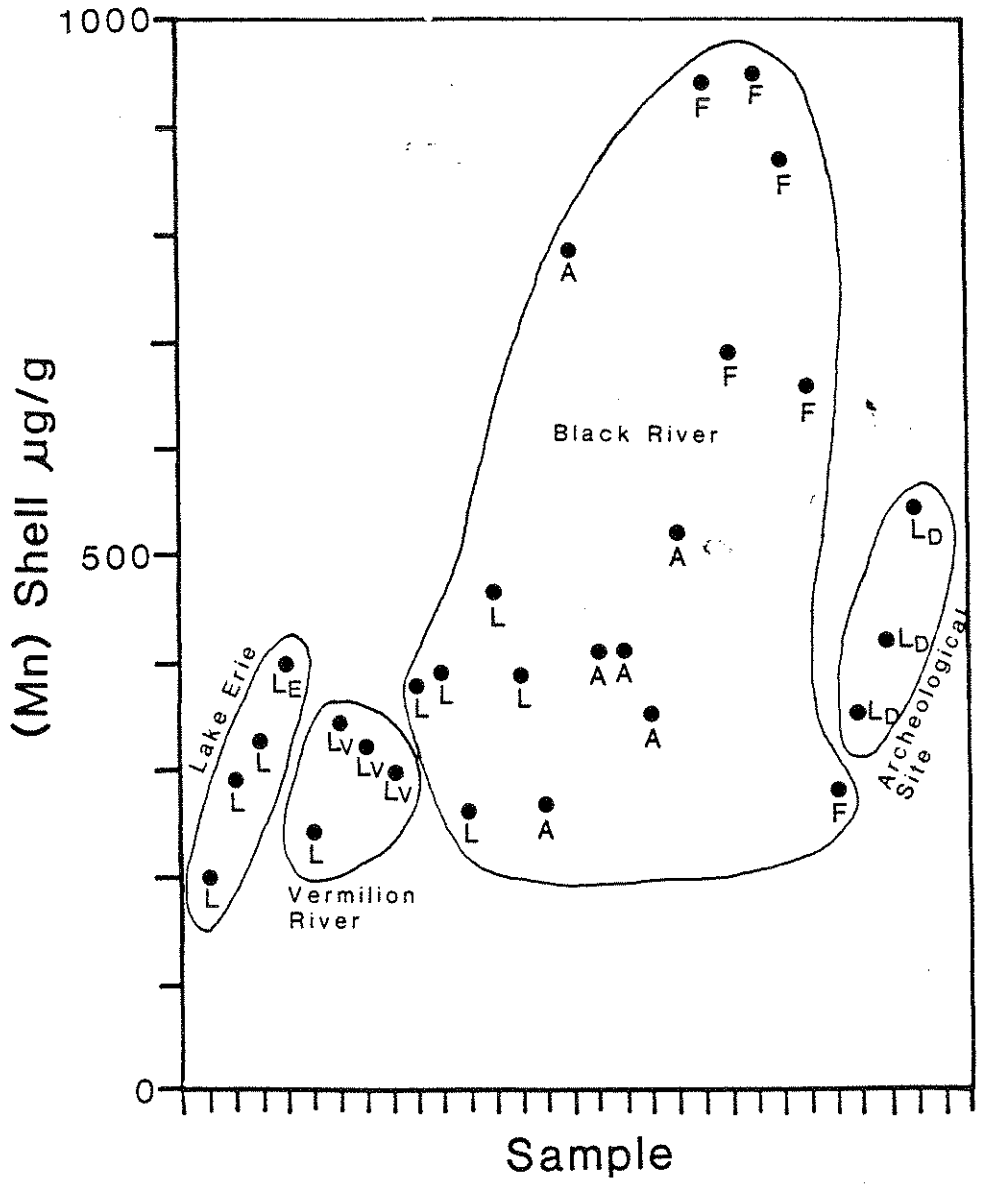

Fig. 1. Mn Values (μg^{-1} g) of each analyzed bivalve shell plotted in environmental groupings. L = *Lampsilis radiata luteola*; LE = *Leptodea fragilis*; LV = *Lampsilis ventricosa*; A = *Anodonta grandis grandis*; F = *Fusconaia flava*; LD = *Lampsilinae*.

concentrations, and that baseline data are needed from older samples before environmental loading may be proven.

Table IV shows that Mn is concentrated by a factor of 2 to 25 in soft tissues compared to shells. Thus, if a short-term view of inferred environmental loading is required, as opposed to a longer-term record equal to the bivalve's life span,

TABLE II

Mean Mn concentrations in Lampsilinae shells from four locations in Ohio

Age	Location	Species	Mean	Standard deviation	Number of specimens
Modern					
	Lake Erie	*L. radiata*	268.0	65.9	4
	Vermilion River	*L. ventricosa*	318.3	22.0	4
	Black River	*L. radiata*	372.4	74.0	5
	(All)		327.2	73.9	13
Ancient (AD 950-1000)					
	Athens, Ohio	Lampsilinae fragments	439.7	96.0	3

TABLE III

Analyses of variance of Mn content of Lampsilinae shells in modern (three species) and ancient collections

Source of variation	Sum of squares	Degrees of freedom	Mean square	F ratio	p
Three modern species differences	20921.77	2	10460.88	2.65	0.131
Error	31527.87	8	3940.98		
Ancient-modern species differences	28773.41	1	30856.64	5.14	0.040
Error	70890.30	14	5998.88		

TABLE IV

Individual shell and soft tissue concentrations of Mn (μ g g^{-1}) (All samples from Black River)

Taxon	Shell	Depurated soft tissue
Lampsilis radiata luteola	375	2292
	386	763
Anodonta grandis grandis	410	4292
	350	7534
	516	5133
Fusconaia flava	866	21928
	659	2472

the soft tissue may be the appropriate part of the animal to analyze. Once again, the extremely high variability in the values for each species suggests that a large number of samples are needed for reliable results. Nevertheless, while there are too few data for statistically significant results, the same rank order of species is produced using soft tissue Mn concentrations as was seen using shell Mn concentrations.

In sum, the preliminary data gathered in this study suggest the possibility of differences in Mn concentrations at least at high taxonomic levels and warrant further investigation. These results also suggest that assessing the correlation of metal concentrations in freshwater bivalves with environmental concentrations may be more complicated (based on unravelling vital effects) and more expensive (based on the need to process many individuals) than has been suggested by previous studies.

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