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Lead Elimination and Size Effects on Accumulation by Two Freshwater Gastropods

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Abstract. Freshwater gastropods, *Physa integra* and *Campeloma decisum,* inhabiting a small reservoir with elevated levels of lead displayed widely differing soft tissue lead concentrations. Analysis of environmental factors did not fully explain these differences. Several physiological characteristics of the two gastropods were examined. The influence of snail size on concentrations of naturally-accrued lead was examined as was the rate of lead elimination from the two species. Lead concentrations in *P. integra* were independent of animal size, while those of *C. decisum* were described by the curvilinear model: μg Pb/g dry wt = 2.29(g dry wt)$^{-0.25}$. Naturally-accrued lead in *P. integra* was eliminated rapidly during the first four days of clearance but slowly thereafter; a portion of the lead is apparently bound strongly. In contrast, no significant change in lead concentration of gravid or nongravid *C. decisum* occurred during a three to four week period in which the animals were maintained in a lead-free environment.

Physiological as well as environmental factors may determine the tissue lead concentrations of a mollusc (Coughtrey and Martin 1977). Examinations have been made of the contribution of selected environmental factors to tissue lead concentrations in the freshwater gastropods, *Physa integra* and *Campeloma decisum* (Newman and McIntosh 1982). Widely differing tissue lead concentrations occurred in specimens of these species collected from two lead-enriched reservoirs. Speculation based on regression analysis and the known ecology of these molluscs did not fully explain the observed species differences in tissue lead concentrations. Investigations into physiological factors controlling body lead concentrations were necessary to more fully explain these differences and, also, to verify the results of the regression analyses. Therefore, elimination rates of naturally-accrued lead, as well as the effect of size on lead accumulation, were examined.

Materials and Methods

Influence of Size: Samples of the gastropods were collected from Weston's Mill Pond, a moderately lead-contaminated New Jersey reservoir described earlier by Newman and McIntosh (1982). Fifty-seven *C. decisum* were collected in September and October 1979. Snails larger than 9 mm in length (approximately 5 mg dry wt) were analyzed individually, while smaller snails were pooled to obtain sufficient tissue for analysis. Snails of the same size (within 1 mm deviation in shell length) were pooled. Pooled samples were dried at 105° C for 12 hr. Average weights were calculated for pooled snails by dividing the total dry weight of the sample by the number of snails. Large numbers of *P. integra* were collected in February 1980 from Weston's Mill Pond and were sorted by size (length) and pooled as described for small *C. decisum*. The tissues were digested at 55° C for six hr in concentrated nitric acid. Samples were analyzed with a Perkin-Elmer 503 atomic absorption spectrophotometer equipped with an HGA flameless unit as described in Newman and McIntosh (1982). National Bureau of Standards Reference Material 1577 (Bovine Liver Standard) was used to determine the accuracy of the lead analyses. The log of lead content (μg Pb/individual) or concentration (μg Pb/g dry wt) was regressed against the log of the mean dry weight of the individuals using the method of least squares.

Lead Elimination: A continuous-flow system (Figure 1) provided a constant flow of relatively lead-free water through 12 tanks containing snails. Part B (Figure 1) was constructed inside a Freas 815 Low Temperature Incubator. A controlled light/dark cycle and temperature regime were afforded by the system. Each of four triplicate sets of tanks received artificial freshwater (EPA 1978) from a 50-L Nalgene® carboy. The artificial freshwater was made by adding four salts ($MgSO_4$, $NaHCO_3$, $CaSO_4 \cdot 2H_2O$ and KCl) to distilled-deionized water. The salt concentrations were adjusted to yield a hardness, total alkalinity, and pH within the range of those measured in Weston's Mill Pond (34 mg/L as

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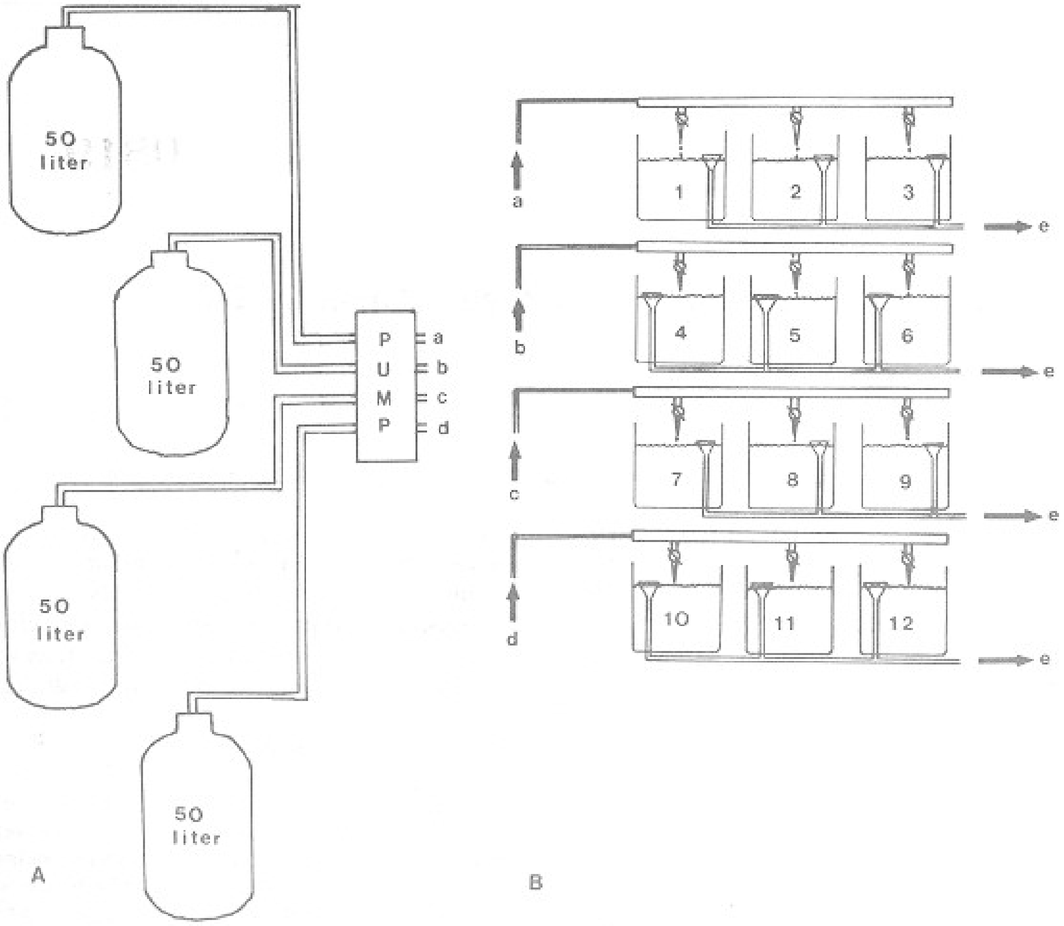

Fig. 1. A continuous-flow system with no metallic components in contact with the water stream. Artificial freshwater was placed into four 50-L Nalgene® carboys. From each carboy, water was pumped separately through four lengths of Tygon® tubing via a peristaltic pump. The four separate water streams were pumped into Part B. Each of the four tubes containing the flow of water (a, b, c and d) entered a plexiglass hydraulic manifold. The water was then allowed to drip at an equal rate through three glass stopcocks fitted with teflon® valves. One of the three tanks received water from each stopcock. The water flowed out of each tank, down an overflow (e) and out of the incubator. In this manner, a slow flow of artificial freshwater was maintained in the four sets of triplicate tanks (designated 1 through 12)

$CaCO_3$, 15 mg/L as $CaCO_3$, and 6.85, respectively). Water was delivered through Tygon® tubing via a 12-channel Durrum Model 1100 peristaltic pump to the 3.5 L capacity plastic tanks (flow rate = 7 L/day/tank). Clean sand (lead concentration less than 3.6 μg Pb/g dry wt) was provided as a substrate for burrowing, and the snails were fed pulverized Purina Mouse Chow® (lead concentration of 0.1 μg Pb/g dry wt). A light/dark cycle of 14 hr light/10 hr dark and a temperature range of 18°−20° C were maintained for the duration of the experiments. Fifteen *C. decisum* taken from Weston's Mill Pond two days before the experiment began were placed into each of six tanks in the continuous-flow system. One snail was removed from each tank after 0(4 hr), 1, 2, 4, 8, 12, 16, 20, 25 and 30 days. The snails were allowed to clear material from their guts in containers of lead-free water for four hr before being frozen. After thawing, snails were measured (length), removed from their shells, and analyzed for lead. This experiment was repeated with gravid *C. decisum* collected in December 1980. In this manner, the rates of lead elimination were determined from this species during different stages of reproduction. The duration of the second experiment was 22 days, due to failure of the peristaltic pump. Snails were fed every four days. At each feeding, any food remaining from the previous feeding was removed. During the second *C. decisum* lead clearance experiment, the six tanks remaining unused in the continuous-flow system were used to study the clearance of lead from *P. integra*. Specimens were collected from Weston's Mill Pond in December 1980. As in the *C. decisum* experiments, *P. integra* were exposed to water, sediment, and food containing low lead concentrations. An identical feeding schedule was used for both species. After 0 (4 hr), 1, 2, 4, 8, 12, 16 and 22 days, five snails were removed from each of the six tanks. The snails were allowed to clear their guts for four hr before being frozen. Snails from randomly paired tanks were pooled for each sampling time to yield three samples of 10 snails each for lead analysis. After thawing, the tissues from each sample of 10 snails were pooled, dried, and analyzed for lead concentration.

The statistical model for lead elimination by *P. integra* was derived by regressing the log of lead concentration against log days of clearance. *C. decisum* data were not transformed before regression analysis.

Table 1. Water chemistry and snail size for the three clearance experiments

	Campeloma decisum (non-gravid)		*Campeloma decisum* (gravid)		*Physa integra*	
	Mean ± 1 S.D.	N	Mean ± 1 S.D.	N	Mean ± 1 S.D.	N
Dissolved lead (μg Pb/L)	0.34 ± 0.35	20	0.20 ± 0.20	14	0.23 ± 0.20	14
Hardness (EDTA) (mg/L as $CaCo_3$)	12.0 ± 2.7	20	24.3 ± 3.5	14	23.8 ± 4.1	14
Total alkalinity (mg/L as $CaCo_3$)	9.2 ± 0.9	20	11.6 ± 1.7	14	12.2 ± 1.8	14
pH	6.14 ± 0.16	20	6.80 ± 0.25	14	6.64 ± 0.50	14
Dissolved oxygen (mg O_2/L)	7.1 ± 0.5	20	8.3 ± 0.6	14	8.3 ± 0.8	14
Temperature (°C)	19.5 ± 1.6	20	18.6 ± 0.5	14	18.7 ± 0.6	14
Shell length (mm)	22.2 ± 1.9	60	21.7 ± 3.7	48	5.9 ± 0.9	236

Results

Size Effects: C. decisum examined during the survey ranged in size from 0.0046 to 0.2775 g dry weight (5 to 33 mm shell length). Within this range, the relationship between lead content (μg Pb/individual) and size was calculated by the methods of least squares to be the following: Log lead content (μg Pb/individual) = 0.75 (Log dry wt) + 0.34. The associated r-value for this statistically derived linear model was significant at $\alpha = 0.05$ ($r = 0.90$, $t = 15.68$, $n = 57$). This relationship transforms to the following: Lead content = 2.21(Weight)$^{0.75}$. The above model describes a curvilinear relationship between lead content and snail size. When the data were used to develop a model for lead concentration and size, the following relationship was obtained: Lead concentration (μg Pb/g dry wt) = 2.29 (Weight)$^{-0.25}$. Smaller individuals had higher lead concentrations than larger individuals. The size range noted for *P. integra* in this survey was 0.0007 to 0.0070 g dry weight (3.6 to 8.1 mm shell length). Within this range, a significant r-value ($\alpha = 0.05$, $r = 0.92$, $t = 8.82$, $n = 16$) was calculated when the log of lead content was regressed against the log of the dry weight. The statistical model transformed to the nearly linear relationship: Lead content (μg Pb/individual) = 46.7(Weight)$^{0.97}$. In the model developed for lead concentration and size of this species, the slope was not significantly different from zero ($P > 0.05$), indicating that lead concentration was independent of size for the *P. integra*.

Lead Elimination: The water chemistry data, as well as snail sizes, are summarized in Table 1. Mean dissolved lead concentrations in the soft waters was less than 0.5 μg Pb/L for the three experiments. The average water hardness and pH during the first *C. decisum* clearance experiment were somewhat lower than those measured during the other two experiments. These differences were assumed to have minimal influence on the rate of lead elimination.

Figure 2 shows the changes in lead concentrations in the two species in a relatively lead-free environment. *P. integra* soft tissue lead concentrations were 32.2 ± 7.8 μg Pb/g dry wt initially. The body lead concentration decreased rapidly to 12.4 ± 2.5 μg Pb/g dry wt during the first four days of clearance. Thereafter, lead concentrations in this species remained relatively constant. When the log of the lead concentration of this species was regressed against the log of the days of clearance, a significant r-value ($\alpha = 0.05$, $r = -0.87$, $t = -8.33$, $n = 24$) was calculated. The resulting log-log linear model transformed to the following: Body lead concentration (μg Pb/g dry wt) = 19.95 (Days)$^{-0.25}$.

In sharp contrast to the rapid clearance of lead noted for *P. integra*, no significant decrease in body lead concentrations was detected in *C. decisum* (Figure 2). Data from the experiment utilizing non-gravid *C. decisum* produced a non-significant r-value ($\alpha = 0.05$, $r = 0.06$, $t = 0.46$, $n = 60$) when body lead concentration was regressed against days of clearance. Similar results were obtained from the experiment employing gravid *C. decisum* ($\alpha = 0.05$, $r = 0.22$, $t = 1.53$, $n = 48$).

Discussion

Influence of Size: Within the size range examined, smaller *C. decisum* displayed higher lead concen-

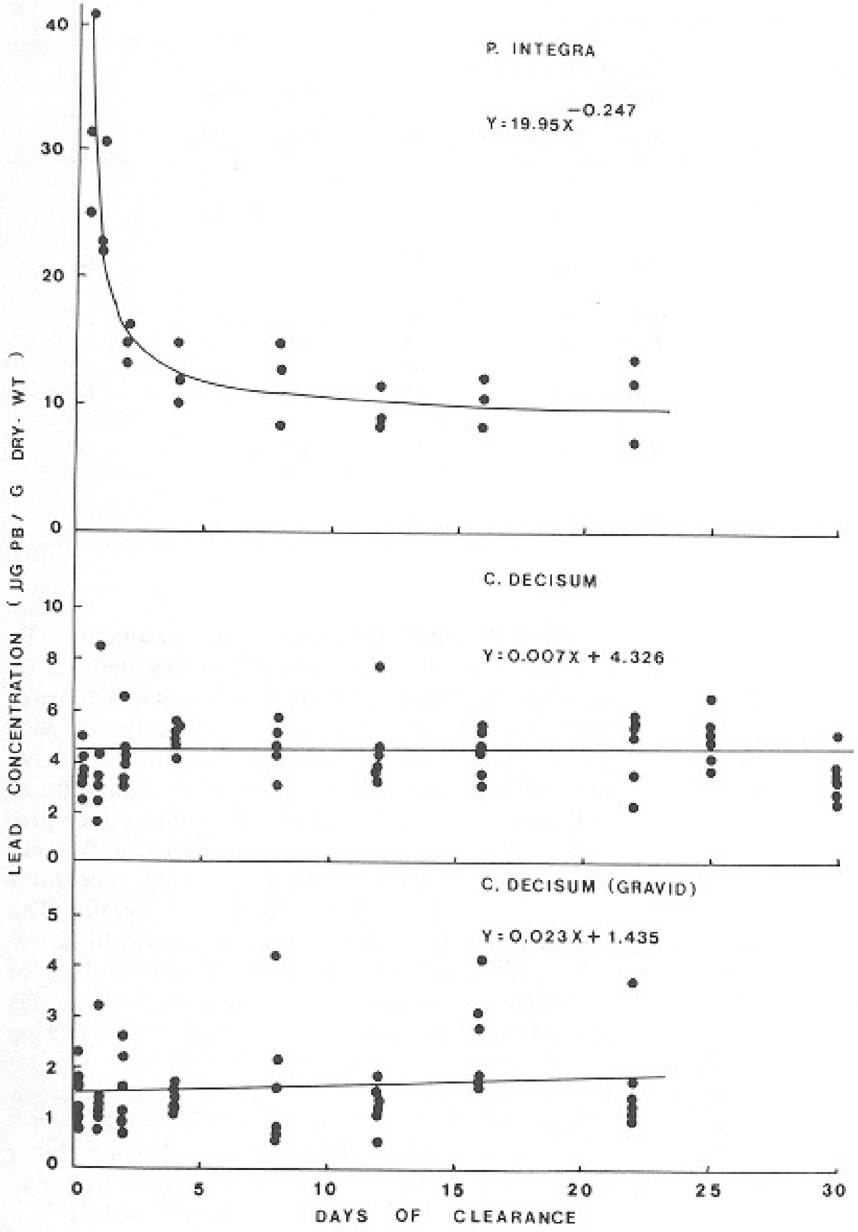

Fig. 2. The clearance of naturally-accrued lead from *P. integra*, non-gravid *C. decisum* and gravid *C. decisum* placed into a relatively lead-free environment

trations than larger individuals. The lead content of this species was proportional to the dry weight of the snail raised to the 0.75 power. This relationship has been interpreted by Boyden (1974, 1977) to indicate that the metal content of the mollusc is controlled by either of the following: 1) an active mechanism linked directly to the organism's metabolism or 2) the surface-to-volume ratio of the animal. In contrast, the *P. integra* had lead concentrations which were independent of size and, generally, ten times higher than those of adult *C. decisum* and five times greater than those of equivalent-sized *C. decisum* (3 mg *P. integra*–59 µg Pb/g dry wt, 4 mg *C. decisum*–12.1 µg Pb/g dry wt). The lower concentrations in equivalent-sized *C. decisum* indicate that other factors are also influencing the realized lead concentrations of this species. The lead content of individual *P. integra* was directly related to animal size *i.e.*, the amount of tissue available to bind the lead.

Lead Elimination: No clearance of lead by gravid or non-gravid *C. decisum* was noted in the two experiments. However, individual *P. integra* rapidly cleared a portion of the body lead from their tissues when exposed to a relatively lead-free environment.

After the initial clearance, lead concentrations remained at a fairly constant level. Similar results have been reported for molluscs exposed to arsenic, mercury, and lead (Cummingham and Tripp 1973; Simpson 1979; Unlu and Fowler 1979). Simpson (1979) found that *Mytilus edulis* transferred from a lead-contaminated site to a clean site rapidly lost lead in the first 30 days but, thereafter, concentrations remained at a level higher than those of mussels native to the site. Simpson interpreted these results to indicate that there existed within mussels a pool of lead which was only slowly eliminated over a long period of time. The findings of the present research suggest a similar phenomenon in both gastropod species studied. *C. decisum* maintained its body lead concentration over the duration of the experiments, while, after a rapid initial clearance, *P. integra* body lead concentrations behaved similarly. The results suggest that the large *C. decisum* maintain a low body lead concentration, perhaps partially due to the relatively small surface-to-volume ratio of the snail. This lead is not eliminated readily when the snail is placed in lead-free environment. The smaller *P. integra* have a relatively higher body lead concentration. Although they rapidly eliminated lead to a certain level, thereafter, only slow elimination occurred. Assuming the two phases of elimination seen in *P. integra* can be approximated with two linear models, the associated constants for the initial clearance and final, slower clearance are 8.84 and 0.07 μg Pb/g dry wt of tissue/day, respectively. The authors are aware that other factors contribute to the large differences in body lead concentrations of these two species when exposed to a contaminated environment.

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