THE EFFECTS OF LOW pH, SELENIUM AND CALCIUM ON THE BIOACCUMULATION OF ^{203}Hg BY SEVEN TISSUES OF THE CRAYFISH, *ORCONECTES VIRILIS*

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CHANG, P.S.S., D.F. MALLEY, N.E. STRANGE and J.F. KLAVERKAMP. 1983. ~~1981.~~ The effects of low pH, selenium and calcium on the bioaccumulation of ^{203}Hg by seven tissues of the crayfish, *Orconectes virilis*. Can. Tech. Rep. Fish. Aquat. Sci. 1151: 45-67p.

The effects of low pH (5.4 and 5.0), selenium (10 µg L^{-1}) and elevated calcium concentrations (5 and 15 mg L^{-1}) on the bioaccumulation of radioactive isotopes of Hg, Se, Cd and Zn by seven tissues of the crayfish, *Orconectes virilis*, were studied in 1 m diameter enclosures in Lake 302, Experimental Lakes Area, northwestern Ontario. Tubes contained lake communities plus added pearl dace, white suckers and crayfish. Only bioaccumulation of ^{203}Hg by the crayfish is discussed here.

Concentration of ^{203}Hg increased with time in seven tissues of the crayfish until at least 15 days after isotope addition. General rank order of ^{203}Hg accumulation by tissues (cpm g^{-1} wet weight) was green glands>hepatopancreas>gills>gut>carapace>ovary>abdominal muscle. Low pH in a number of cases appeared to retard accumulation of ^{203}Hg in tissues, selenium consistently retarded bioaccumulation, and elevated calcium concentrations were associated with a few cases of enhanced ^{203}Hg bioaccumulation. ^{203}Hg declined exponentially in the water of tubes. Loss rate of ^{203}Hg was significantly faster in the tubes with selenium and was associated with a higher proportion of the ^{203}Hg bound to suspended particulates than in other tubes. Water in tubes contained spuriously high concentrations of zinc, 30 to 200 µg L^{-1} compared with lake water of <10 µg L^{-1}. Effect of high zinc concentration on bioaccumulation of ^{203}Hg by crayfish is unknown.

Key words: low pH; selenium; mercury; calcium; crayfish tissues; bioaccumulation.

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Les effets d'un pH faible (5.4 et 5.0), du sélénium (10 µg ℓ^{-1}) et d'une concentration élevée de calcium (5 et 15 mg ℓ^{-1}) sur la bioaccumulation de quatre isotopes radioactifs de Hg, Se, Cd et Zn par sept tissus d'écrevisse ont été étudié dans des enclos de 1 m de diamètre au lac 302 de la Région de Lacs Expérimentaux du nord ouest ontarien. Chaque tube contenait un échantillon représentatif de la communauté aquatique de lac et on y avait ajouté des mulets perlés, des meuniers noirs et des écrevisses. Seule la

bioaccumulation de ^{203}Hg par l'écrevisse est discutée.

Les concentrations de ^{203}Hg augmentent progressivement dans sept tissus d'écrevisse pendant au moins 15 jours après son introduction. La concentration de ^{203}Hg dans les différents tissus (cpm g^{-1} poids frais) est de l'ordre suivant: glandes vertes hépatopancréas>branchies>intestin>carapace>ovaire> muscle abdominal. Un faible pH semble inhiber dans plusiers cas l'accumulation de ^{203}Hg par les tissus, le sélénium retarde de façon consistante la bioaccumulation et les concentrations de calcium élevées sont liées dans quelques cas à une accumulation accrue de ^{203}Hg. Le ^{203}Hg diminue exponentiellement dans l'eau des tubes. Le taux de disparition est significativement plus rapide dans les tubes contenant du sélénium et y est associé à une proportion plus élevée de ^{203}Hg lié aux particules en suspension que dans les autres tubes. L'eau a l'intérieur des tubes contient des concentrations anormalement élevées de zinc, 30 à 200 µg ℓ^{-1}, comparees a celles du lac (<10 µg ℓ^{-1}). Les effets de la concentration élevée de zinc sur la bioaccumulation de ^{203}Hg par les tissus d'écrevisse ne sont pas connus.

INTRODUCTION

An experiment to study the effects of low pH and additions of selenium or calcium on the behaviour of the radioisotopes, ^{109}Cd, ^{75}Se, ^{203}Hg and ^{65}Zn, was conducted from mid-August to mid-October, 1980 in a series of 1 m diameter enclosures (tubes) in Lake 302 in the Experimental Lakes Area (ELA), northwestern Ontario. It was designed as an initial study to determine whether acidification, the addition of selenium or elevated calcium concentrations have major effects on the rates of accumulation of these isotopes by various biological and physical compartments in small aquatic ecosystems. These compartments included water, suspended particulates, zooplankton, chlorophyll, periphyton, crayfish, white suckers and pearl dace.

Low pH altered the solubility of radioisotopes of several heavy metals in and their rate of loss from the water column in 10 m diameter tubes in another ELA lake (Schindler et al. 1980a). The presence of selenium in 10 m diameter tubes in Clay Lake, northwestern Ontario, affected the movement of mercury among various compartments in the water column and retarded rate of mercury bioaccumulation by fish, crayfish and haptobenthos (Rudd et al. 1980). Calcium has been found to reduce bioaccumulation of zinc by the crayfish *Austropotamobius pallipes pallipes* (Bryan 1967) and cadmium by the amphipod *Gammarus pulex* (Wright 1980). Calcium concentration in lake water appeared to be inversely related to accumulation of several heavy metals in liver of two fish species (McFarlane and Franzin 1980).

This paper presents data on the uptake of ^{203}Hg by seven tissues of the crayfish, *Orconectes virilis*. Results on the bioaccumulation of the other three isotopes by the crayfish will be reported elsewhere. General experimental design and the behaviour of the four isotopes in other compartments will be presented by Klaverkamp et al. (in prep.).

MATERIALS AND METHODS

DESCRIPTION OF THE LAKE

Lake 302 is a double-basin lake located at 49°41'N latitude and 93°46'W longitude in the Experimental Lakes Area, northwestern Ontario (Brunskill and Schindler 1971). North and south basins have surface area and maximum depth of 12.8 ha, 13.8 m and 10.9 ha, 10.6m, respectively. The north basin of the lake has been used previously to study the effects of the injection of nutrients into the hypolimnion. Additional lake morphometry and the results of that experiment are described by Schindler et al. (1980b). Average composition of epilimnetic water of the south basin between June 10 and October 28, 1980 is given in Table 1.

TUBE CONSTRUCTION AND TREATMENTS

The experiment was conducted in fifteen 1 m diameter enclosures (tubes) in 2.5 to 3 m of water in a sheltered bay of the south basin of Lake 302. These tubes were constructed of cross-laminated polyethylene. The top openings of the tubes were supported by a wood and styrofoam frame. PVC tubing was inserted into the tubes to provide additional structural strength. Galvanized metal culverts, 1 m in diameter, were placed externally on the bottom skirts of the tubes to seal tubes to the sediments. The skirts were further stabilized on the sediments by sandbags.

The tubes were arranged in 5 series of 3 tubes each, each series consisting of tubes labelled A, B and C (Table 2). Sufficient NaCl was added to each tube on August 18 to increase Na^+ concentration about 3-fold above the lake background average of 0.69 mg L^{-1} (N = 6 between August 15 and September 16) in order to monitor tubes for leakage. No major leakages were detected during the experiments. Half-times for Na^+ concentration in tubes to return to background level ranged from 17 to more than 200 days, but most commonly were about 70 days (Table 3). Half-times were calculated from 6 water samples between 0 and 58 days, but the last data point was dropped when this was lower than the trend between 0 and 29 days.

Beginning on August 18, B and C tubes in all series were acidified to average pH 5.44 and 5.01, respectively, using 0.1 N H_2SO_4. The pH of these tubes was held constant by the addition of acid on a daily basis. Tubes A were allowed to remain at average lake pH of about 6.73.

On August 15, prior to acidification, Na_2SeO_3 was added to tubes in series 2. Concentrations declined with time (Table 4) and were intended to be approximately 10 µg L^{-1} on the day of isotope addition. On August 18, $CaCl_2$ was added to series 3 and 4 to bring tubes to nominal concentrations of 5 mg L^{-1} and 15 mg L^{-1}, respectively. Ca^{++} in series 3 tubes remained very constant up to 22 days, after which it declined slowly in A and B but remained constant in C. In series 4 tubes Ca^{++} declined exponentially with time and half-times of disappearance of the added Ca^{++} ranged from 48 to 88 days (Table 5). Tubes in series 1 and 5 received no chemical additions other than acid.

On August 26 (designated day 0) each tube received 0.33 mCi of each of ^{203}Hg, ^{109}Cd, ^{65}Zn and 0.13 mCi of ^{75}Se (1 Ci = 37 GBq). A summary of the

Table 1. Average composition ($\bar{x} \pm$ S.E.) of epilimnetic water sample between June 10 and October 28, 1980 from Lake 302.

No. of samples	Conductivity $\mu S\ cm^{-1}$ at 25°C	Dissolved inorganic carbon, $\mu moles\ L^{-1}$	pH range
6	23.0±1.7	73.8±8.7	6.40-6.74

Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	SO_4^{--}
		mg L^{-1}			
0.85±0.04	0.38±0.02	1.59±0.02	0.58±0.01	2.03±0.08	2.98±0.07

Table 2. Treatments to tubes in Lake 302, 1980.

Tube series	Tube (mean pH in parentheses)			Additions
1	A(6.7)	B(5.4)	C(5.0)	isotopes[a] + H_2SO_4[b]
2	"	"	"	10 $\mu g\ L^{-1}$ selenium + isotopes[a] + H_2SO_4[b]
3	"	"	"	5 mg L^{-1} calcium + isotopes[a] + H_2SO_4[b]
4	"	"	"	15 mg L^{-1} calcium + isotopes[a] + H_2SO_4[b]
5[c]	"	"	"	isotopes[a] + H_2SO_4[b]

[a] $^{109}Cd + ^{75}Se + ^{203}Hg + ^{65}Zn$

[b] Tubes B and C only.

[c] Replicate of series 1.

Table 3. Half-times for disappearance of added Na^+ from water in tubes in Lake 302. Half-times were calculated between 0 and 58 days except for values marked by * which denotes calculations over 0 to 29 days.

Series	Tube	Half-times of disappearance of added Na^+, days A	B	C
1		46.0	78.6	58.9
2		19.9*	69.3	29.6*
3		17.1*	70.9	155.6
4		112.2	70.5	84.3
5		200+	181.3	200+

Table 4. Concentrations of Se in tubes of series 2 initially after its addition, at the time of addition of isotopes 11 days later, and half-time of disappearance.

Tube	Concentration of Se, $\mu g\ L^{-1}$ Initially after addition	On day of isotope addition	Half-times of disappearance, days
2A	21.6	7.6	11.3
2B	23.7	7.4	15.1
2C	33.4	5.8	7.7

Table 5. Concentrations of Ca^{++} in tubes of series 3 and 4 and half-times of disappearance of added Ca^{++}.

Tube	Initial Conc. $mg\ L^{-1}$	Average Conc. 0-22 d $mg\ L^{-1}$	Half-times of disappearance, days
3A	4.71	4.60 ± 0.01	-
3B	5.07	5.11 ± 0.04	-
3C	5.24	5.08 ± 0.06	-
4A	13.9	-	88.0
4B	14.3	-	48.1
4C	14.0	-	48.8

nominal treatments of each tube is given in Table 2. The introduction of fish, periphyton strips and water sampling scheme are described by Klaverkamp et al. (in prep.).

COLLECTION AND SAMPLING OF CRAYFISH AND ANALYSIS OF TISSUES

All crayfish were collected at night from another ELA lake, 239, at a depth of less than 1 m of water. Only mature females were selected for the experiment because they had completed a molt earlier in the summer and would not molt again. Crayfish were weighed and measured and placed singly into individual compartments (12 x 12 x 7 cm) of wire-mesh cages (60 x 12 x 7 cm). These cages were previously covered with several coats of Varathane® to ensure that no bare metal was exposed. Caged crayfish were maintained in Lake 239 in shallow water for several days before the experiment began.

The day before the addition of isotopes, caged crayfish were transported to Lake 302. Two cages containing a total of 10 crayfish were introduced into each of the 15 tubes and allowed to rest on the bottom. No food was added to the tubes during the experiment.

Two to four crayfish, normally three, were removed from each tube at 9 ± 2, 15 ± 1 and 35 ± 1 day, after isotope introduction. The radioactive crayfish were transported to the ELA laboratory where wet weights, carapace lengths and molt stages were recorded. Crayfish were dissected to obtain the following tissues for isotope analyses: carapace, hepatopancreas, gills, green glands (antennal glands), ovary, gut (proventriculus and intestine) and abdominal flexor muscle. Fresh tissue samples were placed in plastic petri dishes (50 mm in dia., 9 mm in height) with locking lids and were kept at 5°C. Length of time required for dissection determined the number of days required for sampling. Crayfish were dissected within a few hours of removal from a tube.

The activities of the four isotopes in the tissues were determined within 4 months of the isotope addition to the tubes by gamma spectroscopy performed on a lithium-drifted germanium (GeLi) crystal detector connected to a 4000 channel analyzer. Spectra were processed by a programmable computer (Hesslein et al. 1980). Samples were counted for an appropriate time period, usually 1 to 2 h. Samples were corrected for radioactive decay by normalizing the counts to the day of isotope addition to the tubes. Data were expressed as counts per minute per gram wet weight of tissue (cpm g^{-1}). Water and particulate samples were analyzed for the activity of the four isotopes according to Hesslein et al. (1980).

Ionic and other analyses on water were performed using the methods of Stainton et al. (1977). Concentrations of zinc in water samples and tissues of crayfish were determined by the Analytical Services Laboratory of the Freshwater Institute (A. Lutz, unpublished methods).

DATA ANALYSIS AND STATISTICAL TESTS

The disappearances of ^{203}Hg from the water in the tubes was modelled by:

$$C_t = C_o e^{-bt}$$

or its linear form:

$$\ln C_t = \ln C_o - bt$$

where C_t is cpm L^{-1} ^{203}Hg at time t, C_o is the cpm L^{-1} ^{203}Hg at time 0, b is the loss rate and t is time in days. The parameters C_o and b were estimated by linear regression. Half-time of disappearance was calculated as ln2/b. 95% confidence interval around each loss rate, b, was calculated from tabulated Student's t and the standard error of b (Steel and Torrie 1960). Confidence intervals around the half-times were calculated from the upper and lower limits of b.

In order to combine data from various tubes which differed somewhat in C_o, data from several tubes were expressed as ln C_t/C_o, fraction of the initial cpm L^{-1} of ^{203}Hg remaining at time t, and regressed against time. Loss rates from two sets of pooled data were compared using Student's t-test to determine homogeneity of regression coefficients (Steel and Torrie 1960).

For comparisons of content of ^{203}Hg in tissues from tubes differing somewhat in C_o, cpm g^{-1} of wet weight of tissue were expressed as bioconcentration factors:

$$\frac{\text{cpm } g^{-1} \text{ tissue at time } t}{\text{cpm } mL^{-1} \text{ water at time 0 } (= C_o/1000)}$$

Differences in average bioconcentration factors at different sampling times and from different experimental conditions were compared using Tukey's ω procedure (Steel and Torrie 1960).

RESULTS AND DISCUSSION

BEHAVIOUR OF ^{203}Hg IN THE WATER COLUMN

Total ^{203}Hg (cpm L^{-1}) in the water column declined exponentially over the period of 43 days (Table 6). Half-times of disappearance of this isotope from the water ranged from 6.6 to 14.3 days with an average of 10.8 days (Table 7). When all series were pooled, ^{203}Hg was removed at a faster rate from A tubes, at near neutral pH, than from acidified B and C tubes (Table 8). This is reflected in generally shorter half-times of disappearance for A tubes (Table 7). The rates of removal of ^{203}Hg in the series 2 tubes (with Se) were faster than in all other tubes combined (Table 8). Elevated calcium concentration (series 3 and 4) did not affect rate of disappearance of ^{203}Hg (Table 8)

Table 6. Total ^{203}Hg (cpm L^{-1}) in the water column one day after addition of isotopes to 1 m diameter tubes in Lake 302, 1980. Values under days 3 to 43 are the total ^{203}Hg in the water column expressed as a percentage of the day 1 value.

Tube	Days after additon of isotopes					
	1	3	7	14	22	43
		% of day 1				
1A	8986	85	91	40	26	5
1B	7932	91	92	84	45	13
1C	7718	88	91	44	25	8
2A	8163	80	82	49	6	2
2B	9487	78	87	59	27	7
2C	7166	81	92	39	15	3
3A	7342	83	78	37	6	4
3B	7282	81	74	40	24	8
3C	8378	86	77	50	26	13
4A	8413	76	88	34	32	8
4B	9210	89	90	34	31	7
4C	11325	78	84	39	35	7
5A	10474	74	80	28	21	6
5B	7986	83	93	27	30	9
5C	10531	75	63	36	42	7

Table 7. Half-times in days for disappearance of ^{203}Hg from the water column of tubes in Lake 302, 1980. 95% confidence intervals are given in parentheses.

Tube unit	1	2 (Se)	3 (Low Ca)	4 (High Ca)	5
A (pH 6.7)	9.8 (8.4-11.8)	6.6 (4.8-10.6)	8.2 (5.5-16.3)	11.9 (9.2-16.8)	10.4 (8.2-14.2)
B (pH 5.4)	14.3 (10.8-21.3)	10.7 (8.7-13.7)	11.7 (10.3-13.5)	10.6 (8.6-14.0)	12.0 (8.5-20.8)
C (pH 5.0)	10.9 (9.3-13.3)	8.0 (6.6-9.9)	13.9 (11.3-17.9)	11.5 (9.3-15.2)	12.0 (9.0-18.1)

Table 8. Statistical test (homogeneity of regression coefficients) for differences in rates of disappearance of total ^{203}Hg from the water columns of tubes in Lake 302, 1980.

Data pooled for regression analysis	Probability that the regression coefficients are not different
1A vs 1B, 1C	P > 0.05
2A vs 2B, 2C	P < 0.001
3A vs 3B, 3C	P < 0.02
4A vs 4B, 4C	P > 0.1
5A vs 5B, 5C	P > 0.2
all A tubes vs all B tubes	P < 0.01
all A tubes vs all C tubes	P < 0.02
series 2 vs series 1, 3, 4 and 5	P < 0.001
series 3 and 4 vs series 1 and 5	P > 0.5

Table 9. Average percentages of total ^{203}Hg associated with particulates (± S.E.). Data from the A, B and C tubes in each series were pooled. Lines connect pairs which are different at the 0.01 level using Tukey's ω procedure. All other pairs were not different at the 0.05 level.

1	2	3	4	5
39.7 ± 2.3	54.7 ± 3.4	43.7 ± 2.5	38.4 ± 2.8	40.0 ± 3.6

Table 10. Average percentages of total ^{203}Hg associated with particulates (± S.E.). Data from series 1 to 5 are pooled in each comparison. Lines connect pairs which are different at the 0.05 level using Tukey's ω procedure.

A pH 6.7	B pH 5.4	C pH 5.0	B + C
40.3 ± 2.5	46.5 ± 2.5	43.1 ± 2.5	44.8 ±

or half-time of disappearance (Table 7) compared with series 1 and 5 without added calcium.

Proportions of the total ^{203}Hg in the water column were associated with suspended particulates. Similarly to total ^{203}Hg, particulate ^{203}Hg declined exponentially with time in all tubes ($b = 0$, $P < 0.05$ for all tubes). Percentage of ^{203}Hg associated with particulates ranged from 17 to 80% and in 12 of the 15 tubes showed no tendency to change with time. Three tubes (1A, 4B, 4C) showed small increases in proportion of ^{203}Hg associated with particulates with time ($b = 0$, $P < 0.05$). The percentage of ^{203}Hg in the particulate material was significantly higher in the Se tubes (55%) than in those without the addition of Se (Table 9). There were no differences between other series (38-44%). Thus, elevated levels of calcium had no effect on proportion of ^{203}Hg associated with particulates. The acidified tubes (B and C) had a slightly higher proportion of the ^{203}Hg associated with the particulates than the control pH tubes (A) although A tubes overall were not different from C tubes (Table 10).

The average half-time of disappearance of ^{203}Hg determined in this study of 10.8 days was similar to that reported by other workers. Half-time of disappearance of ^{203}Hg from the epilimnion of an entire lake, 224, also in the Experimental Lakes Area was 14.3 days (Hesslein et al. 1980). ^{203}Hg disappeared with half-time of about 13 days from 10 m diameter tubes in Lake 223, ELA (Schindler et al. 1980a) and about 17 days from 10 m diameter tubes in Clay Lake, northwestern Ontario (Rudd et al. 1980).

According to Hesslein et al. (1980), settling of particulate material is an important pathway of removing radioactive metals from the water column. The Se tubes (series 2) behaved in a manner consistent with this. The higher proportion of ^{203}Hg present on particulate materials in series 2 tubes was associated with the fastest half-removal times. The study of Rudd et al. (1980) also showed that in the presence of selenium (100 $\mu g\ L^{-1}$), ^{203}Hg was associated with particulates to a somewhat greater extent than without selenium. But the presence of selenium in the latter experiment was not associated with faster half-removal time for ^{203}Hg.

The relationship between a high proportion of particulate ^{203}Hg and short half-removal time is not demonstrated when acidic and non-acidic tubes are compared. Under acidic conditions (tubes B and C), the proportion of ^{203}Hg in the particulates was higher than in neutral (A) tubes, but the half-removal time was shortest in A tubes.

Schindler et al. (1980a) found no effect (at the 0.05 probability level) of pH on the proportion on ^{203}Hg associated with particulates in 10m diameter tubes in Lake 223. Half-removal times for ^{203}Hg were 12.7 days at pH 6.8, 11.1 days at pH 5.7 and 16.3 days at pH 5.1. These few data are consistent with our result of longer half-removal times in acidified tubes than in near-neutral tubes. Jackson et al. (1980) speculate that mercury may be deposited in sediments as "humic" complexes whose assimilation into sediments is inhibited by acidification.

ELEVATED ZINC CONCENTRATIONS IN TUBES

An unexpected complication of this tube experiment was discovered in 1981 when identical tube design was used to study the effects of low pH on the bioaccumulation of unlabelled aluminum and cadmium on these small ecosystems (S. Lawrence, pers. comm.). Analysis of water in tubes in 1981 showed that the concentrations of zinc ranged from about 30 to 200 $\mu g\ L^{-1}$ (Table 11) and tended to rise with time. In comparison, zinc concentrations in open lake water were <10 $\mu g\ L^{-1}$. The contamination may have arisen from the galvanized metal culverts positioned outside the tubes on the sediment (S. Lawrence, pers. comm.), from the tube fabric itself (R. Hesslein, pers. comm.), or from other possible sources such as the PVC tubing within the tubes, the wire mesh of the crayfish cages or rubber gloves (S. Lawrence, pers. comm.). Samples of water from tubes in 1980, but analyzed in 1981, contained 20 to 250 $\mu g\ L^{-1}$ zinc. In 1981, precautions were taken during sampling of tube water to avoid contamination by zinc and the 1981 values are considered to be more reliable.

As a possible indication of whether the zinc present in tube water in 1980 could have affected the bioaccumulation of the isotopes, tissues of crayfish from the tubes in 1981 were analyzed for zinc content. Tissues from several crayfish were pooled in some cases to provide sufficient tissue for metal analysis. Zinc concentrations in the hepatopancreas of crayfish from the tubes in 1981 were 1.5 to 8 times higher, and in green gland, 2 to 3 times higher, than in tissues from crayfish freshly-collected from Lake 239. There was no difference in zinc concentration in abdominal muscle in the two groups of crayfish (Table 11).

Bryan (1967) reported that the crayfish *Austropotamobius pallipes pallipes* held in 0.1% sea water containing less than 4 µg/L zinc had average zinc concentrations of 109, 11.5 and 7 μg^{-1} wet weight in hepatopancreas, abdominal muscle and green gland, respectively. Bryon (1967) also found that in the presence of elevated external zinc concentrations, zinc concentration increased in the hepatopancreas but not in the abdominal muscle.

Thus, there is evidence to suggest that the crayfish in tubes in 1980 would have absorbed zinc and accumulated it in some tissues to higher than normal levels. The effect of high external zinc or higher than normal zinc concentrations in body tissues on the uptake and accumulation of ^{203}Hg by the crayfish is unknown.

ACCUMULATION OF ^{203}Hg BY CRAYFISH TISSUES

^{203}Hg (cpm g^{-1}) inreased with time in all tissues even though ^{203}Hg (cpm L^{-1}) in water decreased exponentially with time. Mean bioconcentration factors were higher on day 35 than on day 7 in every case and for half the tissues were higher on day 35 than on day 15 (Table 12).

^{203}Hg (cpm, whole tissue) was accumulated to different extents by the seven crayfish tissues. Generally, the order was hepatopancreas > gills > gut > green glands > carapace > ovary > abdominal muscle. Order of concen-

Table 11. Concentration of zinc (μg g^{-1} wet weight tissue) in three tissues from crayfish held in tubes in Lake 302 in 1981 and from 20 crayfish freshly-collected from Lake 239. Hep = hepatopancreas; mus = abdominal muscle; gr gl = green gland.

Experimental conditions	Zn conc. in water μg L^{-1}	Tissue	No. of crayfish	No. of chemical analyses	Zn conc. in tissue ($\bar{x}$ ± S.E.)
Freshly collected					
	<10	hep	20	16	49.9 ± 3.7
		mus	20	16	16.5 ± 0.63
		gr gl	19	1	35
Crayfish held in tubes					
40 μg L^{-1} Al, pH 6.7	180	hep	9	1	223
		mus	9	1	16
		gr gl	14	1	85
40 μg L^{-1} Al, pH 5.3	20	hep	5	1	91
		mus	5	1	18
		gr gl	pooled with 40 μg L^{-1} Al, pH 6.7		
No metal addition, pH 6.7	110	hep	14	2	332
		mus	14	2	17
		gr gl	14	1	72
No metal addition, pH 5.3	135	hep	10	2	94
		mus	10	2	18
		gr gl	10	1	62
1 μg L^{-1} Cd, pH 6.7	50	hep	15	2	214
		mus	15	2	16
		gr gl	15	1	75
1 μg L^{-1} Cd, pH 5.3	85	hep	13	2	156
		mus	13	2	16
		gr gl	13	1	69
3 μg L^{-1} Cd, pH 6.7	115	hep	12	2	301
		mus	12	2	18
		gr gl	12	1	65
3 μg L^{-1} Cd, pH 5.3	150	hep	16	2	302
		mus	16	2	16
		gr gl	16	1	73

Table 12. Average bioconcentration factors ($\bar{x}$ ± S.E.) for Hg-203 in seven tissues at three sampling times. Data from series 1 to 5 are pooled. Lines connect pairs which are different at the 0.01 level using Tukey's ω procedure. All other pairs are not different at the 0.05 level.

	Days after addition of isotopes		
Tissue	7	15	35
Carapace	22.2 ± 3.2	56.5 ± 6.4	52.7 ± 6.2
Hepatopancreas	147.9 ± 36.3	265.3 ± 40.0	403.1 ± 46.9
Gills	207.4 ± 42.4	258.1 ± 39.0	346.0 ± 40.3
Green glands	336.8 ± 56.1	475.1 ± 65.0	873.1 ± 157.7
Ovary	27.1 ± 6.0	44.3 ± 8.5	44.1 ± 5.3
Gut	48.0 ± 11.1	146.0 ± 20.8	139.0 ± 16.6
Abdominal muscle	4.0 ± 0.8	8.4 ± 1.2	11.3 ± 1.3

tration of ^{203}Hg (cpm g^{-1} wet weight tissue) was similar: green glands > hepatopancreas > gills > gut > carapace > ovary > abdominal muscle (Figs. 1 to 6). Although green glands showed the highest rank order in concentration they were intermediate in total accumulation because they weighed 10 to 30 times less than the other tissues.

These results and other studies on the accumulation of metals (Cd, Cu, Pb and Zn) by *O. virilis* (Anderson and Brower 1978; Leonhard 1979), indicate that hepatopancreas and gills are the main sites of metal accumulation. Thus, it seems reasonable to select hepatopancreas or gill as target tissues for assessing metal accumulation in crayfish. On the other hand, Hamilton (1972) found the highest concentrations of mercury in crayfish to be in abdominal muscle when populations were exposed chronically to elevated mercury levels. Possibly mercury is concentrated early during exposure in the hepatopancreas and longer times of exposure are required for slower accumulation by the muscle.

EFFECT OF LOW pH

In some cases, average bioconcentration factors for ^{203}Hg in the several tissues were lower in crayfish from the tubes at pH 5.4 or 5.0 than at pH 6 (Table 13). Thus, low pH appears to retard accumulation of ^{203}Hg at least under some conditions.

The inhibition of the uptake of cations by crayfish in low pH has been reported. Below pH 6.0, Na^+ absorption by the crayfish *Astacus pallipes* is inhibited (Shaw 1960). pH below 5.75 progressively inhibits uptake of Ca^{++} by crayfish during postmolt (Malley 1980). Two possible hypotheses are suggested here to explain the retardation of ^{203}Hg uptake by low pH. Either ^{203}Hg is in cationic form and its uptake is inhibited by low external pH, or alternatively, the tendency for ^{203}Hg to bind to particulates to a greater extent at low pH may make the ^{203}Hg less available for uptake by the crayfish.

EFFECT OF SELENIUM

Selenium had the most pronounced effect of all the treatments on the bioaccumulation of ^{203}Hg. The addition of selenium to tubes in series 2 in many cases significantly decreased the bioaccumulation of ^{203}Hg in all tissues at each sampling time when these crayfish were compared with those from series 1, 3, 4 or 5 (Table 14). In addition, concentrations of ^{203}Hg in most tissues of crayfish from tube 2A vs 1A and 5A, tube 2B vs 1B and 5B and tube 2C vs 1C and 5C are shown in Figs. 2 to 4. These results have clearly demonstrated that selenium reduced the accumulation of ^{203}Hg by crayfish tissues under normal and acidic conditions.

Rudd et al. (1980) found that selenium appeared to retard the rate of ^{203}Hg accumulation by fish, crayfish and haptobenthos in the 10 m diameter tubes in Clay Lake, Ontario which has been severely contaminated by mercury for more than a decade. These results led Rudd et al. (1980) to suggest that a concentration of 10 µg L^{-1} of selenium could be used to ameliorate the mercury pollution problem in freshwater ecosystems. Our data support the finding of Rudd et al. (1980), and also indicate that selenium retards

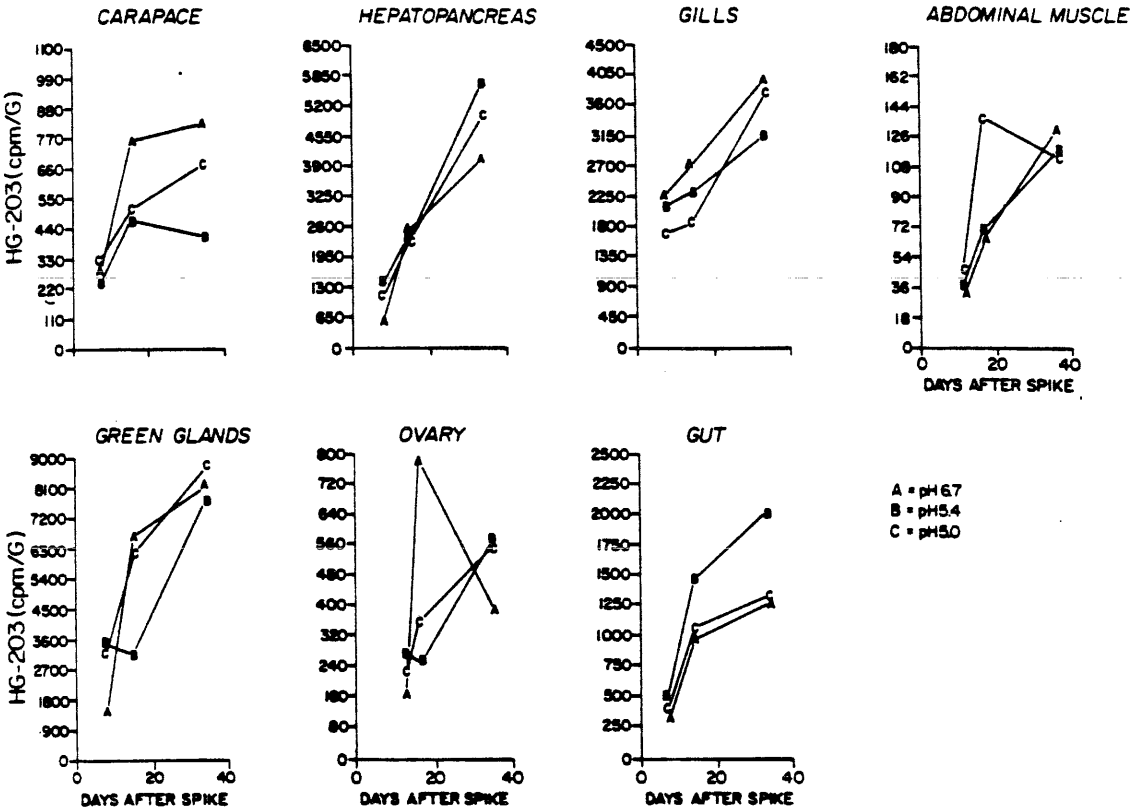

Fig. 1. Bioaccumulation of Hg-203 (cpm g^{-1} wet weight) by seven tissues of crayfish from tubes at pH 6.7, 5.4 and 5.0. A = tubes 1A + 5A, B = 1B + 5B, C = 1C + 5C. Each point represents the mean of 5 to 8 individuals.

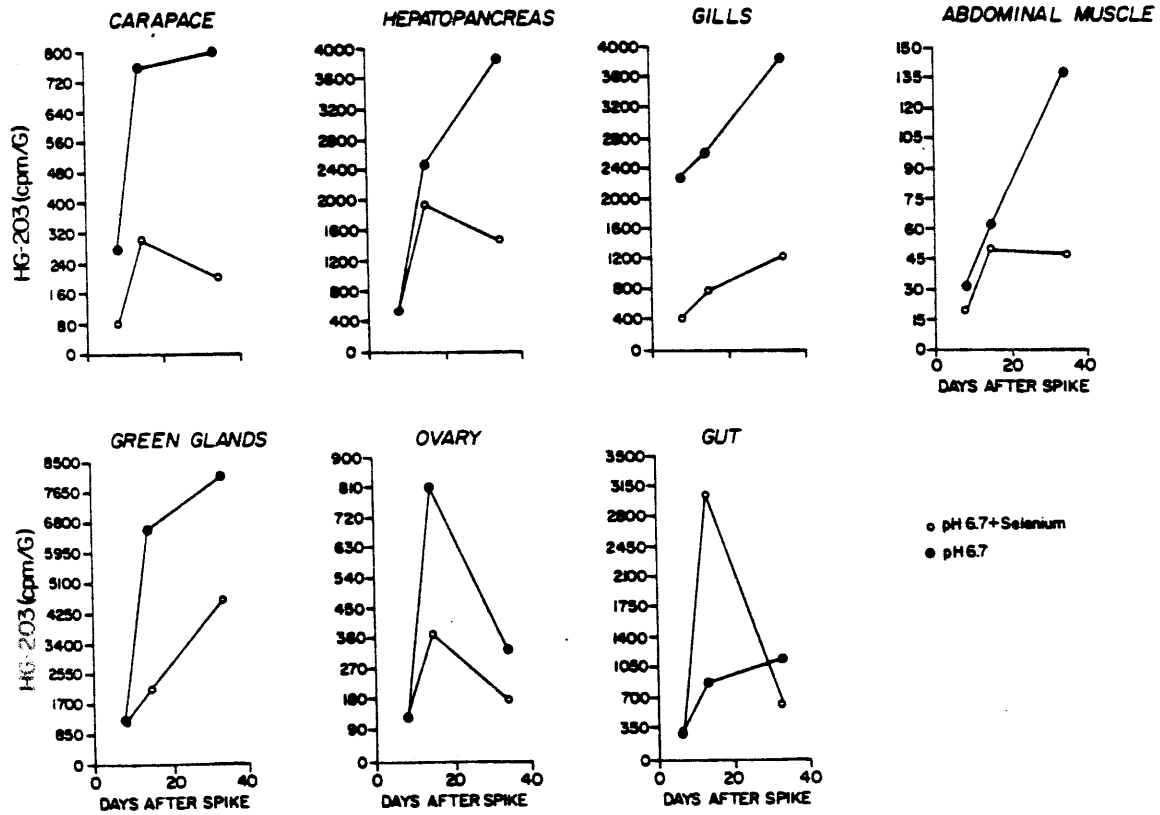

Fig. 2. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes at pH 6.7 with and without the addition of 10 ug L^{-1} selenium. pH 6.7 + selenium = tube 2A; pH 6.7 = 1A + 5A. Each point represents the mean of 3 to 8 individuals.

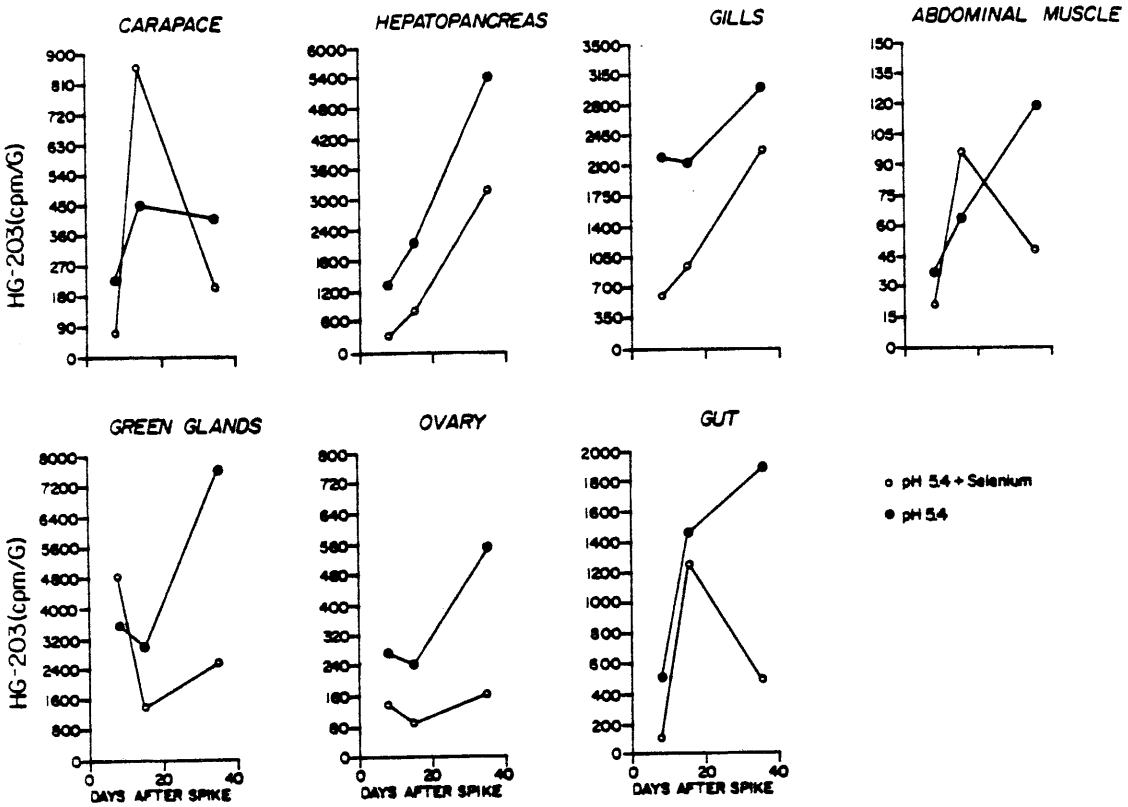

Fig. 3. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes at pH 5.4 with and without the addition of 10 µg L^{-1} selenium. pH 5.4 + selenium = tube 2B; pH 5.4 = 1B + 5B. Each point represents the mean of 3 to 8 individuals.

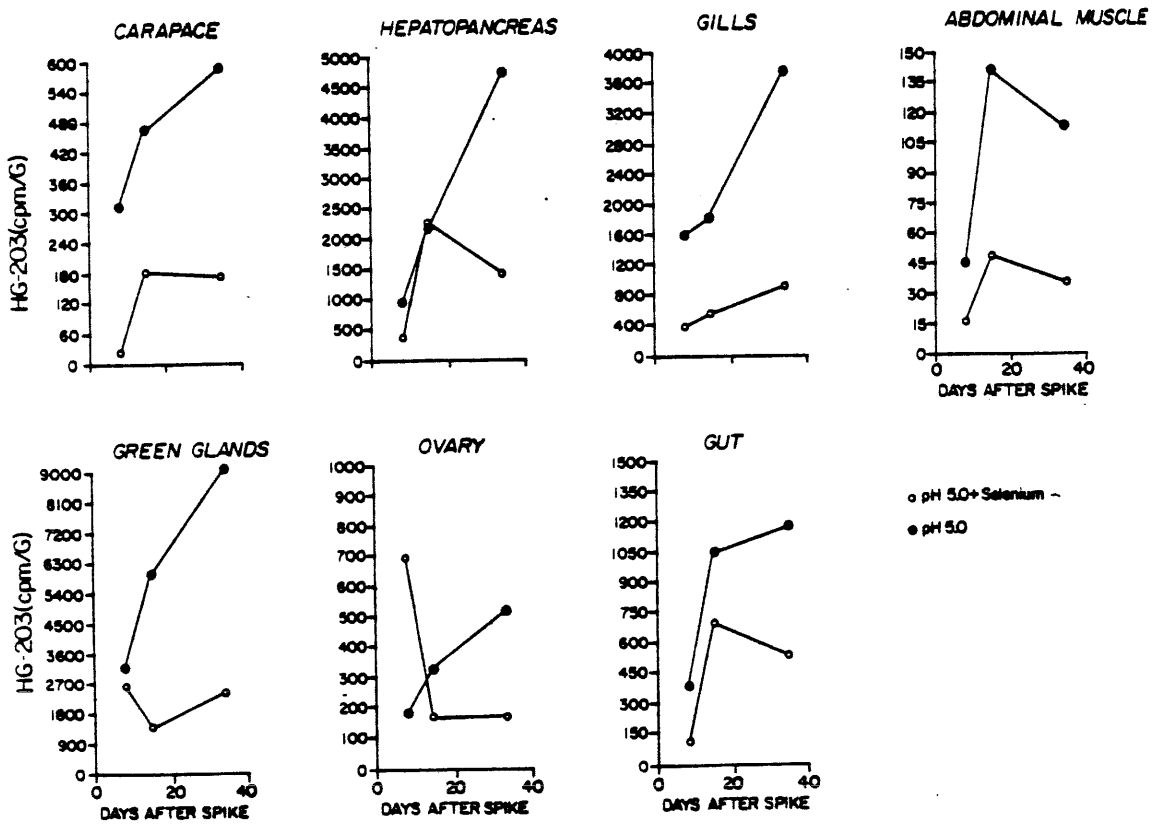

Fig. 4. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes at pH of 5.0 with and without the addition of 10 µg L^{-1} selenium. pH 5.0 + selenium = tube 2C; pH 5.0 = 1C + 5C. Each point represents 3 to 8 individuals.

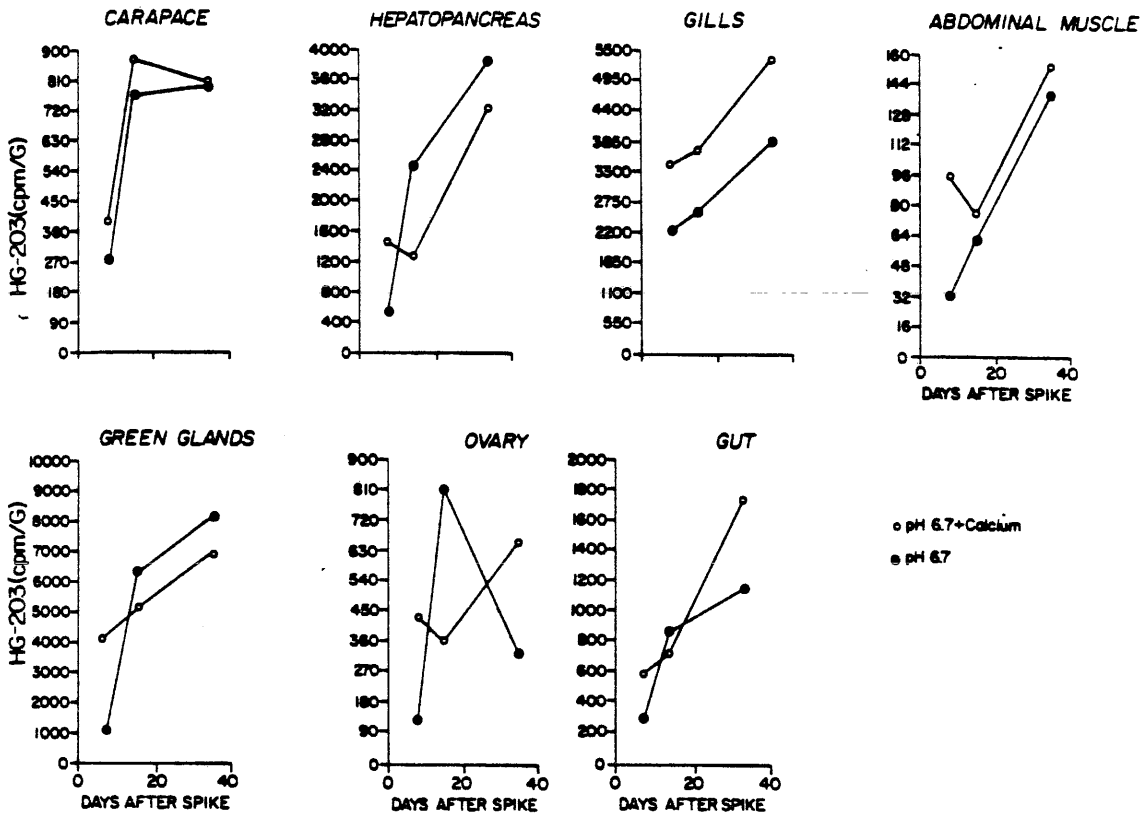

Fig. 5. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes held at average lake pH of 6.7 with and without the addition of 15 mg L^{-1} calcium. pH 6.7 + calcium = 4A; pH 6.7 = 1A + 5A. Each point represents 3 to 8 individuals.

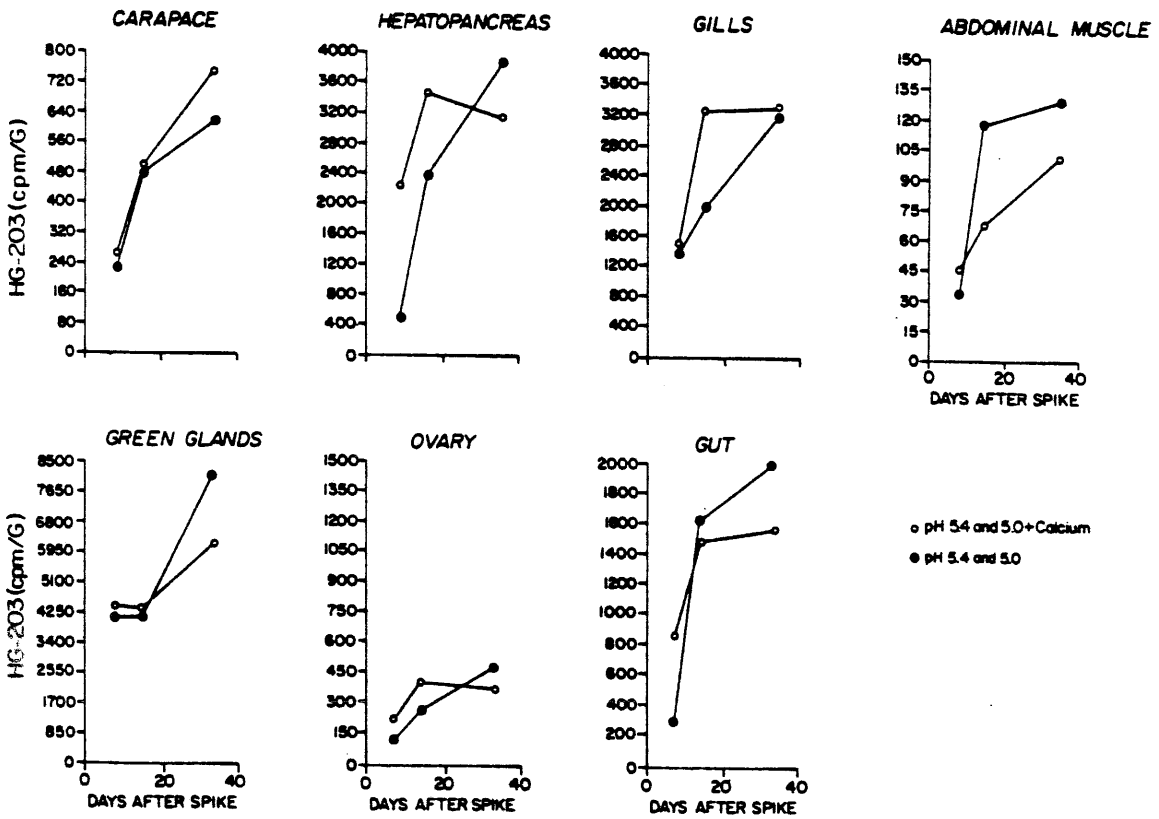

Fig. 6. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes at pH 5.4 and 5.0 with and without the addition of 5 and 15 mg L^{-1} of calcium. pH 5.4 and 5.0 + calcium = tubes 3B + 3C + 4B + 4C; pH 5.4 and 5.0 = 1B + 1C + 5B + 5C. Each point represents 6 to 15 individuals.

Table 13. Average bioconcentration factors ($\bar{x}$ ± S.E.) for Hg-203 in seven tissues in three pH's at three sampling times. Data from series 1 to 5 are pooled. Lines connect pairs which are different at the 0.05 or 0.01 level using Tukay's ω procedure. All other pairs are not different at the 0.05 level.

Day after addition of isotopes	Tissue	Tube A	Tube B	Tube C
7	carapace	26.6 ± 7.5	19.7 ± 3.4	20.4 ± 5.6
15		65.8 ± 16.0	57.9 ± 16.5	45.8 ± 8.5
35		66.3 ± 14.0	44.5 ± 7.5	47.4 ± 9.4
7	hepatopancreas	136.4 ± 56.3	213.4 ± 93.0	93.8 ± 14.7
15		233.8 ± 45.2	344.9 ± 101.1	217.2 ± 44.8
35		332.3 ± 81.9	469.3 ± 66.6	407.7 ± 97.9
7	gills	294.5 ± 96.3	221.1 ± 67.0	108.2 ± 26.1
15		295.4 ± 69.5	309.8 ± 84.4	169.2 ± 32.0
35		393.8 ± 87.0	336.6 ± 63.1	307.6 ± 67.6
7	green glands	211.9 ± 53.9	355.0 ± 66.3	443.4 ± 137.3
15		593.9 ± 141.0	356.1 ± 88.5	475.2 ± 97.3
35		1291.9 ± 401.7	623.3 ± 131.0	704.3 ± 126.8
7	ovary	26.4 ± 9.3	26.2 ± 10.7	28.7 ± 13.1
15		60.4 ± 20.8	35.3 ± 11.1	37.3 ± 10.0
35		44.3 ± 11.1	47.4 ± 10.3	40.6 ± 7.4
7	gut	45.7 ± 18.2	69.8 ± 26.4	28.3 ± 6.6
15		154.1 ± 45.2	189.6 ± 27.8	94.5 ± 23.5
35		129.5 ± 23.8	168.2 ± 36.4	119.2 ± 26.0
7	abdominal muscle	5.2 ± 2.3	3.2 ± 0.5	3.5 ± 1.0
15		7.2 ± 1.0	8.5 ± 1.2	9.6 ± 3.3
35		13.1 ± 2.6	10.7 ± 2.2	10.1 ± 2.0

Table 14. Average bioconcentration factors ($\bar{x} \pm$ S.E.) for Hg-203 in seven tissues in 5 tube series. Data from tubes A, B and C are pooled. Lines connect pairs which are different at the 0.05 or 0.01 level using Tukey's ω procedure. All other pairs are not different at the 0.05 level.

Day after addition of isotopes	Tissue	Series 1	2	3	4	5
7	carapace	19.2 ± 5.4	5.8 ± 1.5	30.3 ± 6.6	25.1 ± 6.7	31.0 ± 4.7
15		50.8 ± 5.0	36.7 ± 18.9	66.8 ± 3.8	60.1 ± 20.7	66.1 ± 18.6
35		73.0 ± 14.3	19.1 ± 0.5	61.4 ± 2.6	60.0 ± 17.2	50.1 ± 4.1
7	hepatopancreas	37.5 ± 15.2	73.1 ± 34.7	324.8 ± 120.8	158.8 ± 39.9	145.4 ± 63.2
15		294.5 ± 49.7	174.0 ± 53.8	395.9 ± 94.1	274.1 ± 148.1	187.9 ± 57.5
35		511.9 ± 142.8	197.5 ± 45.1	464.7 ± 80.0	357.1 ± 45.6	484.1 ± 113.0
7	gills	134.0 ± 23.4	46.6 ± 3.6	286.5 ± 119.8	208.9 ± 94.1	363.5 ± 90.2
15		206.2 ± 18.1	76.6 ± 5.4	370.5 ± 55	391.1 ± 126.0	246.2 ± 30.0
35		325.7 ± 73.2	144.9 ± 30.2	469.0 ± 13.6	386.1 ± 121.0	404.4 ± 72.8
7	green glands	320.7 ± 135.7	303.6 ± 76.5	495.3 ± 240.6	373.5 ± 28.4	190.8 ± 49.5
15		424.8 ± 155.2	166.0 ± 24.8	666.0 ± 58.1	463.8 ± 109.8	665.0 ± 154.4

Table 14. Cont'd.

35		712.0 ± 158.5	322.3 ± 68.0	1129.3 ± 359.1	1248.4 ± 662.6	953.7 ± 41.0
7	ovary	10.7 ± 4.4	35.7 ± 22.4	21.1 ± 5.1	30.4 ± 14.0	37.6 ± 16.0
15		68.3 ± 35.5	21.9 ± 8.5	69.6 ± 3.4	31.3 ± 9.1	30.6 ± 6.4
35		44.1 ± 8.5	17.5 ± 1.2	54.4 ± 4.0	47.8 ± 15.4	56.5 ± 12.1
7	gut	29.0 ± 8.9	14.0 ± 2.4	106.0 ± 34.1	52.8 ± 20.2	37.6 ± 11.3
15		158.5 ± 22.3	165.3 ± 69.0	192.1 ± 52.4	128.3 ± 51.5	86.0 ± 34.0
35		183.3 ± 30.3	57.4 ± 6.1	152.4 ± 13	182.0 ± 41.0	120.1 ± 36.9
7	abdominal muscle	3.3 ± .9	1.8 ± .1	5.8 ± 1.7	6.1 ± 3.5	2.8 ± .7
15		10.7 ± 5.9	6.4 ± 1.3	9.5 ± 1.0	6.9 ± 1.3	8.5 ± 1.1
35		12.6 ± 1.5	4.3 ± .2	15.4 ± .2	10.8 ± 3.5	13.2 ± 2.1

^{203}Hg accumulation by crayfish under acidic as well as neutral conditions.

The higher proportion of ^{203}Hg associated with particulates in the selenium tubes and the faster half-time of disappearance suggests that selenium decreased bioaccumulation of ^{203}Hg by the crayfish by physically removing the isotope faster from the water column. Therefore, less ^{203}Hg was available for uptake by crayfish.

EFFECT OF ELEVATED CALCIUM CONCENTRATIONS

The addition of calcium to tubes to bring concentration to 5 or 15 mg L^{-1} generally did not affect bioaccumulation of ^{203}Hg by crayfish at normal lake pH of 6.7 (Fig. 5) nor at lower pH's (Fig. 6). Significant differences in mean bioconcentration factors between tubes 3 or 4 and 1 or 5 were rare and in these cases the tissues from the tubes with elevated calcium displayed higher concentration of ^{203}Hg, not lower as expected (Table 14).

High calcium concentration in lake waters was associated with lower accumulation (Cd, Cu and Hg) by fish (McFarlane and Franzin 1980). The highest concentration of Hg in fish muscle and liver was found to correspond with the lowest calcium concentration in a number of lakes and marshes in the Parry Sound area, Ontario, having calcium concentrations ranging from 2 to 25 mg L^{-1} (C. Wren, pers. comm.). However, although we increased the calcium concentrations 3x and 10x over the background level, there was no indication that elevated calcium concentrations reduced ^{203}Hg accumulation by crayfish. Preliminary results indicate that the presence of 5 and 15 mg L^{-1} of calcium also had no consistent effect on ^{203}Hg accumulation by white suckers (J. Klaverkamp, unpubl. data). Therefore, the trend of high calcium concentration and low mercury accumulation may not be applicable to all circumstances, at least not in the case of the crayfish from our study.

ACKNOWLEDGEMENTS

We thank D.A. Hodgins, L.J. Wesson and D.P. Mathew for field assistance, and R.H. Hesslein, W.R. Lillie and P. Wilkinson for gamma isotope counting. A. Lutz analyzed water and tissues for heavy metal concentrations. G. Mueller assisted in data analysis. Constructive reviews of the manuscript were given by P. Campbell, S. Elliott, P. Healey, R. Hesslein, D. Schindler and M. Turner.

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