Calcium and Trace Metal Composition of Crayfish (*Orconectes virilis*) in Relation to Experimental Lake Acidification

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Subjective estimates indicate that the carapaces of crayfish from experimentally acidified Lake 223 (pH 5.4–5.6) in the Experimental Lakes Area are becoming less rigid. Decreased carapace rigidity was inversely correlated with carapace dry weight and Ca^{++} content. *Orconectes virilis* from L223 have 25–35% less Ca^{++} in their exoskeletons (mean % dry wt ± SE = 13.90 ± 0.54) than do those from reference lakes (19.82 ± 0.33, 20.34 ± 0.63, and 22.18 ± 0.51). Lake 223 crayfish have accumulated higher tissue concentrations of both Mn (L223 value of 240 $\mu g \cdot g^{-1}$ dry wt compared to a mean ± SE for reference populations of 48 ± 11 $\mu g \cdot g^{-1}$ dry wt) and Hg (L223 value of 0.52 $\mu g \cdot g^{-1}$ dry wt compared to reference mean of 0.26 ± 0.05 $\mu g \cdot g^{-1}$ dry wt). Mn content of carapaces in crayfish from acidified L223 were also elevated threefold over background levels for the ELA region.

Des estimations subjectives révèlent que la carapace des écrevisses peuplant le lac 223, artificiellement acidifié (pH 5, 4–5, 6), de la Région des lacs expérimentaux (RLE) devient moins rigide. La baisse de rigidité présente une corrélation inverse avec le poids sec et la teneur en Ca^{++} de la carapace. L'exosquelette des *Orconectes virilis* du lac 223 contient de 25 à 35 % moins de Ca^{++} (% poids sec moyen ± É.-T. = 13,90 ± 0,54) que celui des écrevisses peuplant des lacs témoins (19,82 ± 0,33; 20,34 ± 0,63; 22,18 ± 0,51). Les écrevisses du lac 223 ont accumulé des concentrations somatiques plus élevées de Mn (240 $\mu g \cdot g^{-1}$ poids sec dans le lac 223 v. 48 ± 11 $\mu g \cdot g^{-1}$ poids sec (moyenne ± É.-T.) chez les populations témoins) et de Hg (0,52 $\mu g \cdot g^{-1}$ poids sec dans le lac 223 par rapport à une moyenne témoin de 0,26 ± 0,05 $\mu g \cdot g^{-1}$ poids sec). La teneur en Mn de la carapace des écrevisses du lac acidifié 223 est aussi trois fois plus élevée que la teneur ambiante dans la RLE.

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Disturbances in Ca^{++} metabolism leading to inhibited calcification or structural deformation of carbonate body parts have been observed in many animals exposed to acid water (Jewell 1922; Beamish et al. 1975; Hultberg 1976; Singer 1981; Nelson 1982; Fraser and Harvey 1982). For crustaceans, Borgstrom and Hendrey (1976) proposed that the effect of low pH may be to interfere with the uptake of Ca^{++} at molting. It has long been known in aquaculture practice that crayfish in acid waters tend to have thinner shells (e.g., LeCaze 1970). Appelberg (1980) found a significant decrease in dry weight and Ca content of *Astacus astacus* carapaces with decreasing pH. Similarly, *Orconectes virilis* held at pH 5.0 in ELA water for 10 d had slower rates of calcification and lower Ca contents than crayfish held at pH ≥6.0 (Malley 1980). It is not known if these results would be expected to be duplicated in non-laboratory environments.

The crayfish *O. virilis* is common in northwestern Ontario lakes that average 1.6 $mg \cdot L^{-1}$ Ca (Armstrong and Schindler 1971), and may be existing close to its lower environmental limit (Greenaway 1974; Capelli 1975). Therefore, relatively minor disruptions in Ca metabolism might be a critical factor for maintenance of crayfish populations. The close interrelationships between Ca and pH necessary for crustacean survival have been demonstrated previously in other systems (Sutcliffe and Carrick 1973; Okland 1980).

The geochemistry of trace metals in freshwaters is now widely known to be altered by acid deposition causing increases in their concentrations in acidified lakes (Dickson 1975; Schindler et al. 1980a). Despite these changes in water concentration, little is known about the effects of acidification on the metal concentrations of aquatic biota, and the consequences of these changes for populations in nature.

The metal Mn increases greatly in acidified lakes (Dickson 1975; Beamish and Van Loon 1977; Schindler and Turner 1982), and seems to be accumulated to a much higher degree in fish from such habitats compared to fish from circumneutral lakes (Lockhart and Lutz 1977; Fraser and Harvey 1982; Moreau et al. 1983). Field investigations suggest that uptake by fish of other trace metals such as Hg are accelerated at low lake pH (Scheider et al. 1979; Tomlinson et al. 1980). Although individuals of *O. virilis* are known to accumulate certain metals at a rate dependent on their external concentrations (Verner 1972; Anderson and Brower 1978), body metal concentrations in acidified waters have not been studied.

The purpose of this study was to document the calcium and

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TABLE 1. Mean ice-free epilimentic trace metal concentrations ($\mu g \cdot L^{-1}$) for ELA study lakes (values for L239 are for lake outflow). All data from D. W. Schindler (unpublished) except for L240 from Beamish et al. (1976).

Lake	Year	Mn	Al	Zn	Cu	Cd	Pb
L224	1976–77	3.8	11.0	2.4	1.2	<1	<1
L239	1976–77	5.4	—	3.9	1.1	<1	<1
L240	1975	—	—	<2	<2	<1	<1
L223	1980	127.0	27.0	5.6	1.8	<1	<1

trace metal concentrations in a population of *O. virilis* in Lake 223, an experimentally acidified lake in the Experimental Lakes Area (ELA) of northwestern Ontario (Schindler et al. 1985), and to compare these to similar data for crayfish from three reference lakes.

Description of Study Lakes

To simulate the effects of acid precipitation the pH of Lake 223 (L223) was decreased from natural values of 6.5–6.8 in 1976 to 5.4–5.6 by 1979 and 1980 at the time of this study. Details of lake acidification are given by Schindler et al. (1980b), Schindler and Turner (1982) and Schindler and others (1985). The whole-lake average concentration of Ca in L223 ($\simeq 2.2\ mg \cdot L^{-1}$) remained unchanged by experimental acidification. Three other lakes (L239, L224, L240) are circumneutral and considered to be reference lakes for the region (cf. France 1985) with Ca concentrations between 1.7 and 2.4 $mg \cdot L^{-1}$ (Prokopowich 1979).

Trace metal concentrations in ELA waters are low, characteristic of an unpolluted region (Beamish et al. 1976; Table 1). However, as a result of the acidification of L223, several increases have been observed in volume-weighted average concentrations of metals. The most dramatic change has been a steady increase in Mn concentration from 12.5 $\mu g \cdot L^{-1}$ in 1976 to 127.0 $\mu g \cdot L^{-1}$ in 1980. Increases in Al from 15.4 to 27.0 $\mu g \cdot L^{-1}$, and Zn from 1.9 to 5.6 $\mu g \cdot L^{-1}$ are also attributed to lake acidification, while concentrations of Cu, Cd, Cr, and Pb remained near lower detection limits (D. W. Schindler, unpubl. data).

Methods

Carapace Rigidity and Calcium Content

Crayfish were sampled by SCUBA diving with the aid of a suction gun designed to prevent mechanical damage to specimens. Details of sampling methods are presented in France (1985). The rigidity of postmolt and intermolt crayfish was subjectively assessed by gently squeezing the carapace (I. J. Davies, Freshwater Institute, unpublished data). A six-level gradation progressing from very soft (VS), soft (S), intermediate soft-hard (S/H) and hard-soft (H/S), hard (H), to very hard (VH) was adopted. All determinations were performed by the author. Reassessment of individual crayfish indicated that the precision of the technique was over 95%. Comparative determinations on the same crayfish by another experienced researcher (I.J. Davies) using the same technique, generally agreed. Collections of between 193 and 294 crayfish for estimates of carapace ridigity were made from the four study lakes during autumn 1979 and 1980 following the last period of molting (France 1985). The proportion of the total catch that had lost or were regenerating one or both chelae was also calculated from the autumn 1980 collections.

During early July 1980, late postmolt and intermolt crayfish were collected for analysis of exoskeletal Ca^{++}. Because the concentration of exoskeletal Ca^{++} increases with crayfish size (McWhinnie et al. 1969; Greenaway 1974) only adults within the size range 23–27 mm carapace length were analyzed. Calcium content was determined using procedures similar to those described by Malley (1980). Portions of the carapace branchiostegite were removed with a paper punch, and both carapace and disc were dried for 24 h at 100 ± 5°C to obtain dry weights. Discs were then ashed at 500 ± 30°C for 24 h, dissolved in 0.25 mL of 12 N HCl, and brought to 25 mL with deionized water. Calcium concentrations of solutions were determined by atomic absorption spectrophotometry (Stainton et al. 1977). The ratio of carapace dry weight to length was calculated as an index of organic material (Huner et al. 1978).

Trace Metal Accumulation

Crayfish were collected from the study lakes 1 wk prior to ice formation in autumn 1980. Adult (age 2–3; 20–30 mm carapace length) crayfish of both sexes were frozen within 1 h of returning to the ELA field station. The tail-sections, including abdominal muscle and exoskeletal covering, were dissected from the remaining body with stainless steel instruments. This body section was chosen because it provides some integration of both internally accumulated and surface adsorbed metals that could be available to fish, as well as because previous work has suggested that the highest tissue concentrations in crayfish are found here (e.g. Hamilton 1972b). For each population, a homogeneous sample was prepared by pooling the abdomens of 20 to 30 individuals, then drying at 100°C for 24 h, and finally grinding these into a fine powder in a sample blender. Although only based on single measurements (2 replicates per analysis) for each population, the fact that there was both a close agreement in tissue concentrations with previous determinations on crayfish from L240, and a demonstrated correlation between increasing metal levels in both water and crayfish in L223 through acidification, reinforced confidence in any differences between populations in average tissue concentration. Determinations of tissue concentrations of Mn, Al, Zn, Cu, Cd, Pb, Hg, and Se via atomic absorption spectrometry were carried out by the Analytical Chemistry Unit at the Freshwater Institute, Winnipeg (cf. Nero and Schindler 1983). Manganese was also analysed by atomic absorption from carapace discs previously prepared for Ca measurements.

Results

Carapace Rigidity and Calcium Content

The median carapace rigidity of crayfish from Lake 223 was lower than those from reference lakes (Fig. 1). During autumn 1979, the majority of crayfish in reference populations were in the VH or H category in contrast to the modal H/S grouping prevalent in speciments from L223. Occurrence of small numbers of softer individuals, even in reference lakes, suggests that at the time of these collections some crayfish had not reached their final overwintering state of exoskeletal hardness. To avoid this problem, in 1980 collections were delayed until one week prior to freeze-up and taken over a shorter time span

TABLE 2. Relationship of subjective carapace rigidity index to carapace ratio (carapace dry wt (mg)/unit length) and carapace punch (area = 33.2 mm^2) dry wt (mg) in *O. virilis* from ELA. Standard errors about the mean averaged ±0.26 for the carapace ratios and ±0.32 mg for the punch dry weights.

	L239 n = 43 Carapace	punch	L240 n = 37 Carapace	punch	L224 n = 38 Carapace	punch	L223 n = 55 Carapace	punch
Index	ratio	dry wt	ratio	dry wt	ratio	dry wt	ratio	dry wt
VH	4.04	5.71	4.89	5.84	3.35	4.79	3.32	4.44
H	3.20	5.01	3.44	5.70	3.00	4.32	3.15	3.58
H/S	2.50	4.34	2.15	4.74	2.89	4.29	2.69	3.72
S/H	2.35	4.14	1.65	4.19	2.65	3.99	2.12	3.22
S	1.24	2.67	1.32	3.14	1.17	3.02	1.08	1.97

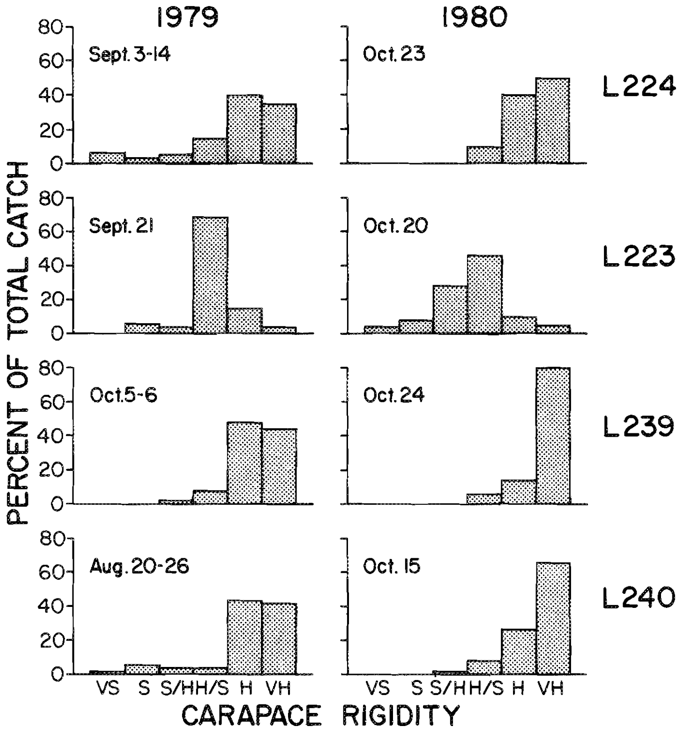

FIG. 1. Frequency distribution of subjective carapace rigidity for *O. virilis* in acidified L223 and three reference lakes. Scale ranges from very soft (VS), soft (S), intermediate soft-hard (S/H), and hard-soft (H/S), hard (H), to very hard (VH). Sample sizes were between 193 and 294 for all collections.

to facilitate inter-lake comparisons. Most crayfish in the reference populations had attained the VH level by this time, while individuals in L223 formed a mode about the S/H–H/S divisions. The difference in carapace rigidity between the L223 and reference populations was not simply due to slower exoskeletal hardening as carapaces in L223 were still soft in the next year's spring collections. The proportion of animals with lost chelae or undergoing limb regeneration was higher in L223 (6.7%) than in reference lakes (L224 — 1.5%; L240 — 2.1%; L239 — 2.7%).

For speciments from each lake there was a correlation between both the mean carapace dry wt to length ratio or the dry wt of carapace disc, and the index of exoskeleton rigidity (Table 2). This indicated that subjective division of populations into hardness classes had some structural basis. An among-lake comparison showed that the dry wts of L239 and

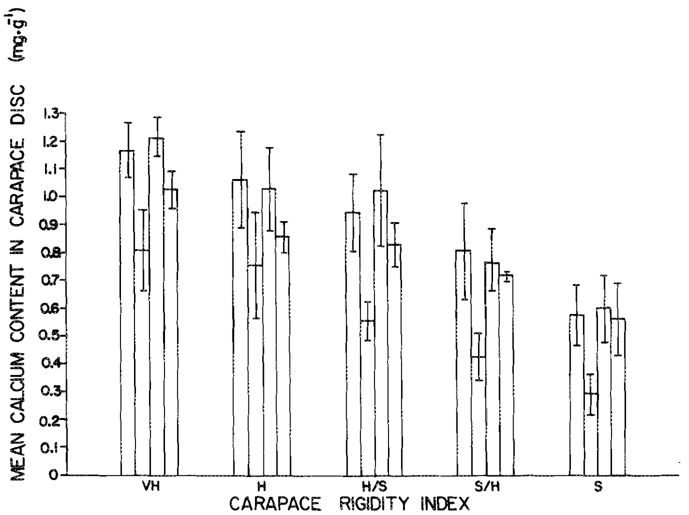

FIG. 2. Comparison of mean calcium content (± 95% C.I.) related to carapace rigidity index among ELA study lakes. Groupings for each index set arranged in order of decreasing lake calcium concentration: L239–L223–L240–L224. Sample sizes are the same as in Table 2.

L240 carapaces were similar, while the L224 carapaces in the upper rigidity classes tended to weigh less, and those from L223 were almost always lowest in mass.

There were strong correlations between carapace disc dry wt and Ca content for each lake (France 1983). From these data the Ca content ($\bar{x}$ ± 95% C.I.) for each index level of carapace rigidity was calculated (Fig. 2). In all lakes there was a decrease in Ca content with progression from VH to S rigidity levels. Calcium content was significantly lower (ANOVA, LSD test, $P < 0.05$) in VH L224 individuals than in VH L239 and L240 animals. Values of Ca content were also significantly lower (ANOVA, $P < 0.001$) in L223 crayfish compared to those from reference lakes at every level of carapace rigidity.

Individual measurements of carapace disc Ca^{++} were expressed for each population on a mean percentage dry wt basis. Carapace Ca content was significantly lower (t-test, $P <$ 0.05) in L223 crayfish ($\bar{x}$ % dry wt ± SE = 13.90 ± 0.54) than in animals from any of the three reference lakes (19.82 ± 0.33; 20.34 ± 0.63; 22.18 ± 0.51).

Individuals of *O. virilis* at ELA do not achieve the same degree of hardness as do individuals from harder waters (Malley 1980). If the assumption is made that there is a relationship between Ca content of crayfish exoskeletons and the Ca concentration of the lake water they inhabit, then the

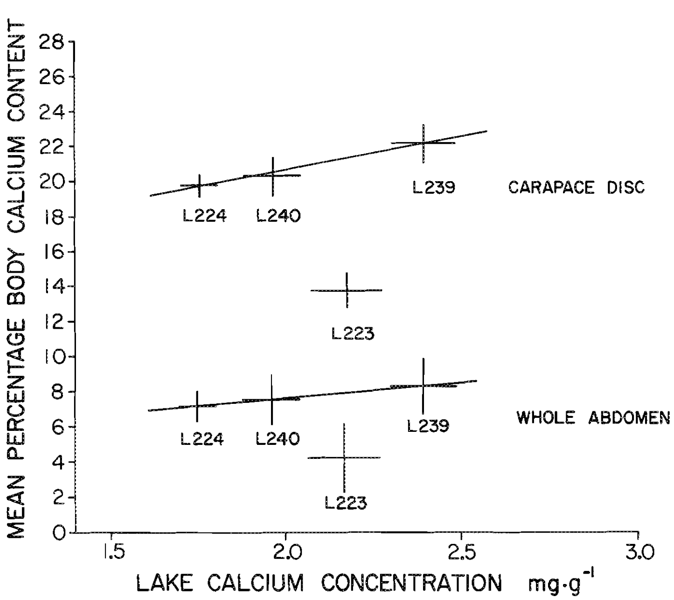

FIG. 3. Plot of mean percentage calcium content of carapace (n = 37–55) and whole abdomen (n = 4) preparations against average lake calcium concentration. Vertical and horizontal lines denote 95% confidence limits. Lake chemistry data from Prokopowich (1979).

TABLE 3. Abdominal muscle trace metal concentrations in $\mu g \cdot g^{-1}$ dry wt (average of 2 replicate determinations) for a composite sample of 20 to 30 adult *O. virilis* from each ELA study lake.

Lake	Mn	Al	Zn	Cu	Hg	Cd	Pb	Se
L223	240	46	74	79	0.52	0.10	3.8	1.6
L224	33	37	66	77	0.20	0.22	1.1	2.1
L240	70	58	69	66	0.36	0.12	1.7	1.3
L239	41	52	48	64	0.24	0.15	3.1	0.86

expected Ca^{++} concentrations of carapaces of L223 crayfish in the absence of acidification can be estimated, and the additional effect of acidification can be derived. From this it was calculated that crayfish in L223 have 35% less carapace Ca^{++} than background levels for the ELA region (Fig. 3). To compensate for small samples sizes of 37 to 55 for the Ca content determinations, the weighted mean population exoskeletal Ca content was estimated by multiplying the percentage values from the rigidity distribution (Fig. 1), based on sample sizes of between 193 and 294, with the results in Fig. 2: L224 — 20.57%; L239 — 20.59%; L240 — 20.07%; and this was compared with the L223 value — 14.77%, about 25% lower than the reference populations. Measurements of Ca concentration performed on whole abdomens used for trace metal analysis provide further evidence that L223 crayfish have significantly less body Ca than animals from reference lakes (Fig. 3).

Trace Metal Accumulation

Five of the seven metals had their highest concentration in L223 crayfish while four of the seven were lowest in the L224 sample (Table 3). A Friedman test (Hollander and Wolfe 1973) was performed on ranked data and indicated that there was no overall significant difference (t = 5.07, d.f. = 6, $P > 0.05$) in the ordering of tissue metal concentrations among lakes. Individually, however, L223 crayfish have accumulated higher levels of both Mn (L223 value of 240 $\mu g \cdot g^{-1}$ dry wt compared to a mean ± SE for the reference populations of 48 ± 11 $\mu g \cdot g^{-1}$ dry wt) and Hg (L223 value of 0.52 $\mu g \cdot g^{-1}$ dry wt compared to reference mean of 0.27 ± 0.05 $\mu g \cdot g^{-1}$ dry wt).

Animals from L223 displayed a large variation in carapace Mn content (Fig. 4). No correlations were found between exoskeleton rigidity and Mn concentration in crayfish from any lake. Concentrations of carapace Mn were threefold higher in *O. virilis* from L223 than in crayfish from reference lakes. The ratio of carapace Ca to Mn was decreased from approximately 1000:1 in reference animals to 230:1 in L223 crayfish.

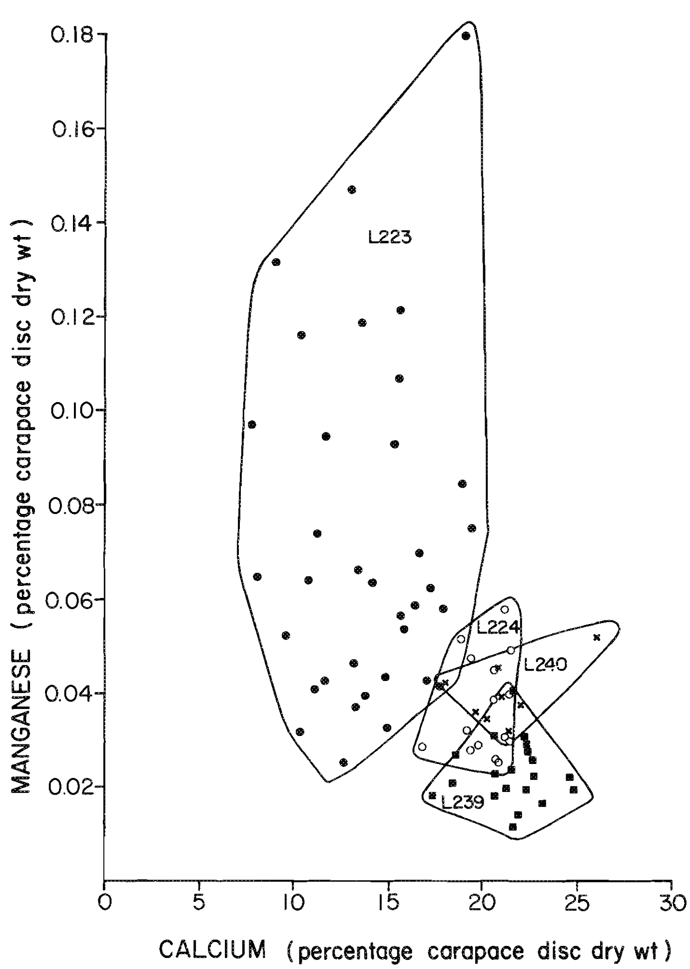

FIG. 4. Manganese and calcium content of *O. virilis* carapaces collected from ELA lakes during 1980.

Discussion

Calcium Content

Postmolt crayfish depend largely upon uptake of Ca^{++} from the aquatic environment to recalcify their exoskeletons because the amount stored in the gastroliths or taken up through feeding is insufficient (Adegboye et al. 1977). Postmolt Ca^{++} uptake was reduced 60% in *Austropotamobius pallipes* in the absence of external HCO_3^- Greenaway 1974), and was inhibited below pH 5.75 and completely blocked at pH 4.0 in *O. virilis* (Malley 1980). Based on the experimental data in Fig. 2 in Malley (1980), one can empirically predict that during 1979 the L223 population experienced a 13% inhibition in Ca^{++} uptake at a mean lake epilimnion pH of 5.6. Further acidification in 1980 to pH 5.4 caused a 30–35% reduction in calcification. The present findings from the field verify these laboratory predictions. The carapace Ca^{++} content as mean % dry wt ± SE for L223 intermolt crayfish was 16.8 ± 0.4 in 1977 (Malley 1980), significantly ($P < 0.05$) higher than the value 13.90 ± 0.54 recorded during 1980 in this study. The % dry wt of Ca in intermolt *O. virilis* from reference lakes was 20–23%, comparable to values of 20–25% previously recorded for this species (Travis 1963; McWhinnie et al. 1969). Other processes of decalcification (Morgan and McMahon 1982) or reduced sclerotization (Yonge 1932) may have also contributed to the reduced exoskeleton rigidity observed in L223 crayfish.

Trace Metal Content

The range of Ca concentrations in the ELA study lakes (1.7–2.4 $mg \cdot L^{-1}$) is too narrow to explain differences in metal uptake (France 1981), suggesting that the elevated tissue concentrations of Mn and Hg observed in L223 crayfish are probably related to acidification.

Accumulation of Hg by *O. virilis* occurs directly from the water (Hamilton 1972a). Tissue concentrations of Hg in this study are similar to values previously recorded for *O. virilis* at ELA (0.17 $\mu g \cdot g^{-1}$; Hamilton 1972b), and intermediate between concentrations for *O. virilis* from northern Manitoba (0.04 $\mu g \cdot g^{-1}$) and from Clay Lake (0.95–10.12 $\mu g \cdot g^{-1}$), a mercury polluted system near Dryden, Ontario (Verner 1972; Hamilton 1972a). It is possible that sample preparation at 100°C may have been severe enough to volatize some Hg so that the presented results are underestimates (P.M. Stokes, Univ. of Toronto, pers. comm.).

The uptake of Mn^{++} is due not only to surface adsorption but also to substitution for $CaCO_3$ in the structural layers of invertebrates (Bryan and Ward 1965) as well as the ossified body parts of mammals (Kato 1963; Koshida et al. 1963). This is consistent with the geochemistry of Mn/Ca interactions (McBride 1979) and the chemical competition between these two divalent ions in plants (Ouellette and Dessureaux 1958; Sutton and Hallsworth 1958; Foy 1973). Lockhart and Lutz (1977) suggested that the large increases in Mn^{++} observed in skin and scales of George Lake white suckers may have been due to an impairment in Ca^{++} uptake as a result of competition for active cellular binding sites between the two elements. Although Mn was elevated in L223 exoskeletons coincident with lower Ca^{++} contents, this did not appear to be a process of simple substitution. Fraser and Harvey (1982) reached the same conclusion regarding the bone composition of white suckers. Further work is needed to discriminate possible subtle effects of Mn interference on Ca^{++} metabolism.

Potential Ecological Consequences

Limitation of calcification or other hardening mechanisms producing prolonged periods of soft exoskeletons could make populations more vulnerable to mechanical damage and cannibalism, both of which can limit crayfish distribution and production in softwater environments (Abrahamsson and Goldman 1970; Mills et al. 1976; Bretonne et al. 1969). Because adult crayfish are preyed upon by fish following ecdysis when exoskeletons are still soft and palatable (Scott and Duncan 1967; Stein 1977), they remain in seclusion (Mobberly 1967; Stein 1977), a behavioural trait absent in intermolt crayfish with reduced carapace rigidity caused by acidification. As a result of this, predation could have contributed to the increased population mortality rates observed by France and Graham (1985) for crayfish in L223. Imbalance in Ca^{++}uptake may also eventually disturb the molting cycle (Borgstrom and Hendrey 1976; Malley 1980; Appelberg 1980) and affect growth and onset of sexual maturity. Contrary, however, to implications from laboratory studies, the timing of molting events and growth rate of L223 crayfish had not been altered by acidification to pH 5.1 in 1981 (France 1985). The higher proportion of crayfish in L223 with missing chelae compared to reference populations may reflect increased antagonism among softer animals, or may indicate an inhibition of limb regeneration at low pH (Needham 1947). Chelae serve an important intraspecific behavioral and ecological role (Bovberg 1953), as well as deterring predation (Stein 1977).

Information on Mn toxicity to aquatic organisms, particularly in acidified systems (Anderson and Nyberg 1984), is rare. Despite some data implying that crayfish may be able to tolerate Mn concentrations at least as high as 1000–2000 $\mu g \cdot L^{-1}$ (Water Quality Criteria 1972), its toxicity is known to depend on a number of other factors including interaction with other elements such as Ca (Chandra et al. 1974; Gale et al. 1973) or Fe and Al (Anderson and Nyberg 1984). The content of Mn in acidified Swedish lakes ranges from 200 to 400 $\mu g \cdot L^{-1}$ (Dickson 1975), much higher than the concentration of 100 $\mu g \cdot L^{-1}$ suggested to exert hazardous sublethal effects (Water Quality Criteria 1972). For example, Boutet and Chaisemartin (1972) found that Mn diminished crayfish reproductive capacity by half at concentrations only 40–50% of those producing 30 d LC50's. Such chronic effects of Mn in acid water remain unknown.

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