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INTRA- AND INTERSPECIFIC VARIATIONS IN CALCIUM CONTENT OF FRESHWATER MOLLUSCA IN RELATION TO CALCIUM CONTENT OF THE WATER

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ABSTRACT

Analyses of calcium content of molluscs collected from 53 freshwater habitats on a naturally occurring calcium gradient between the Canadian Shield and limestone formations in southern Ontario showed both intraspecific and interspecific variations in relation to pH, total alkalinity, total hardness and calcium hardness of the water. The calcium content of individuals is related to the calcium concentration of the water for only about half of the species analysed. Two species (*Sphaerium rhomboideum* and *Sphaerium simile*) showed negative correlations, while six (*Cincinnatia cincinnatiensis, Pisidium casertanum, Pisidium compressum, Sphaerium striatinum, Anodonta grandis grandis,* and *Elliptio complanata*) showed positive correlations beteen calcium content of individuals and environmental calcium content. In general. species associated with the sediments (e.g. bivalves) showed better calcium concentrations than did species associated with macrophytes (e.g. gastropods).

INTRODUCTION

For molluscs, the most important chemical parameter is reported to be calcium (Hunter, 1964). This study examines the interspecific variations in the calcium content of whole bodies (shell and tissues) of twenty-eight species of freshwater mollusc in relation to a naturally occurring gradient of calcium concentrations between the Canadian Shield and limestone formations. Since some species were represented by fewer than two populations only sixteen of these species could also be examined for intra-specific variations.

Similar studies (e.g. Russell-Hunter, Apley, Burky & Meadows, 1967; Lee & Wilson, 1969, 1974; Hunter & Lull, 1977; Burky, Benjamin, Catalano & Hornbach, 1979) have been done in North America for only a few species, but none includes calcium-poor waters, such as those in the Canadian Shield. These studies demonstrated either strong intraspecific relationships between calcium content of shell of some species and the environmental calcium content or no relationship at all for other species in similarly medium to hard waters. Only Burky *et al.* (1979) attempted to find interspecific relationships between calcium content of pisidiid shells and water hardness but no such relationships could be demonstrated with the few populations of some species from waters of low calcium content.

McKillop & Harrison (1972) have determined the relationships which exist between the distribution and relative density of indigenous populations of freshwater gastropods and the calcium content of waters in the gradient across the same interface between the Canadian Shield and limestone formations as in the present study. Their results showed a direct correlation between calcium content of water and densities for most pulmonates; an inverse correlation was found for many prosobranchs. Studies on the relationships between the calcium content of water and density and distribution of freshwater molluscs are well documented for European species (Williams, 1970a & 1970b; Dussart, 1976, 1979).

STUDY AREA

Fifty-three freshwater habitats were sampled within an area bounded by Post Lake of the Vermillion River to the north (81°12′W, 47°02′N, Britannia Bay of the Ottawa River to the east (75°47′W, 45°22′N, With my compliments Gerry M.

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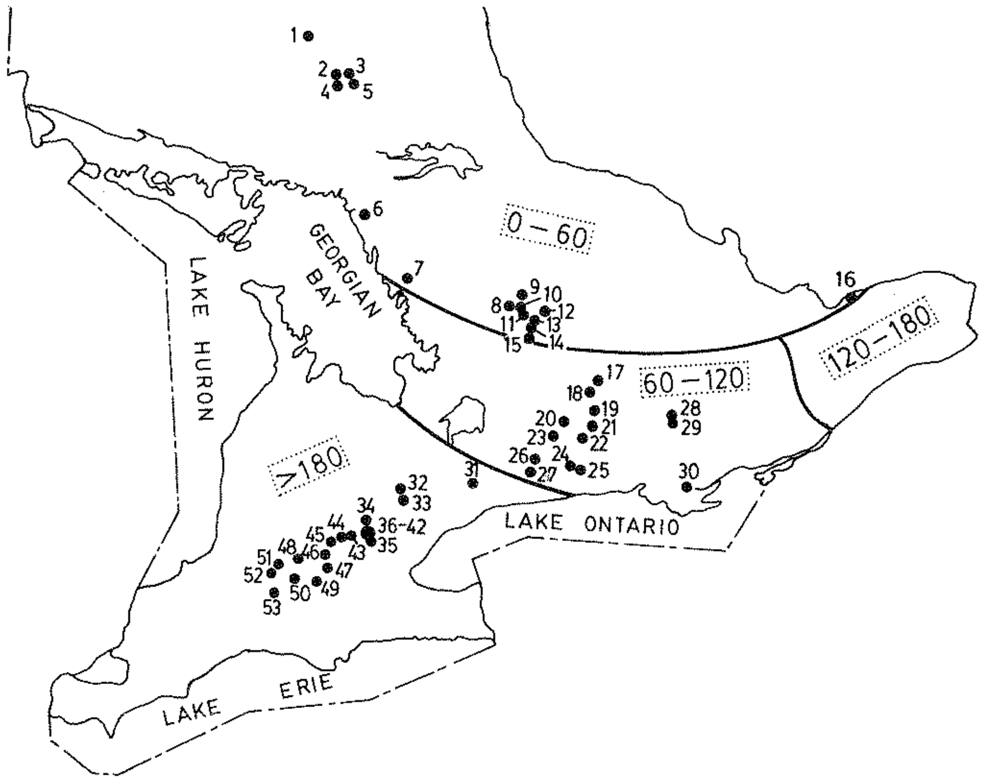

Fig. 1. Locations of 53 mollusc samples in southern Ontario. The calcium gradient is indicated by the zones of water hardness (mg $CaCO_3.l^{-1}$, in boxes with dotted lines) according to Fisheries & Environment Canada (1978)

Waubuno Creek to the South (81°06′, 43°02′N), and the North Thames River to the west (81°14′W, 43°14′N) in Ontario (Fig. 1). The area covers a survey of the limestone formations in Southern Ontario and the granite basement rock in the Sudbury District of Ontario. The study area presents a gradient of water ranging from 2.5 to 150.0 mg $Ca^{++}l^{-1}$. Thirty-two of the habitats sampled were lentic systems, the remainder were lotic. The PH, alkalinity, total hardness, and calcium hardness of each habitat are depicted in Fig. 2.

MATERIALS AND METHODS

Water samples were collected in 200 ml glass bottles just before the mollusc samples. These were analysed in the laboratory for pH, total alkalinity, total hardness, and calcium hardness within 24 h of sampling. pH was measured with a Fisher Accumet pH meter, model 144. Titrimetric EDTA methods (A.P.H.A., 1980) were used to measure total and calcium hardness. Total alkalinity was measured as total inflection point alkalinity (O.M.E., 1979) on waters with total hardness less than 20 mg $CaCO_3l^{-1}$ and a total endpoint alkalinity (A.P.H.A., 1980) on waters with more than 20 mg $CaCO_3l^{-1}$.

Molluscs were sampled from less than 1 m water depths using a sieve with a 1 m long handle and mesh openings of 0.32 mm. Sampling continued until at least 10 specimens (usually 25) of a wide range of size classes of each species were taken. This number was based on preliminary observations of size class variance on the smallest (Pisidiidae) and the largest (Unionidae) molluscs and a 95% confidence interval for calcium content to be within 10% of the mean for the size classes sampled (Steel & Torrie, 1980). All molluscs were hand-sorted from the bottom sediments at the site and stored in water from the habitat. Identification to species of most molluscs was made at the site; small species (e.g. some Pisidiidae) were identified later in the laboratory. Identifications were based on descriptions and taxonomic keys in Clarke (1973), Harman & Berg (1971), and Mackie, White & Zdeba (1980).

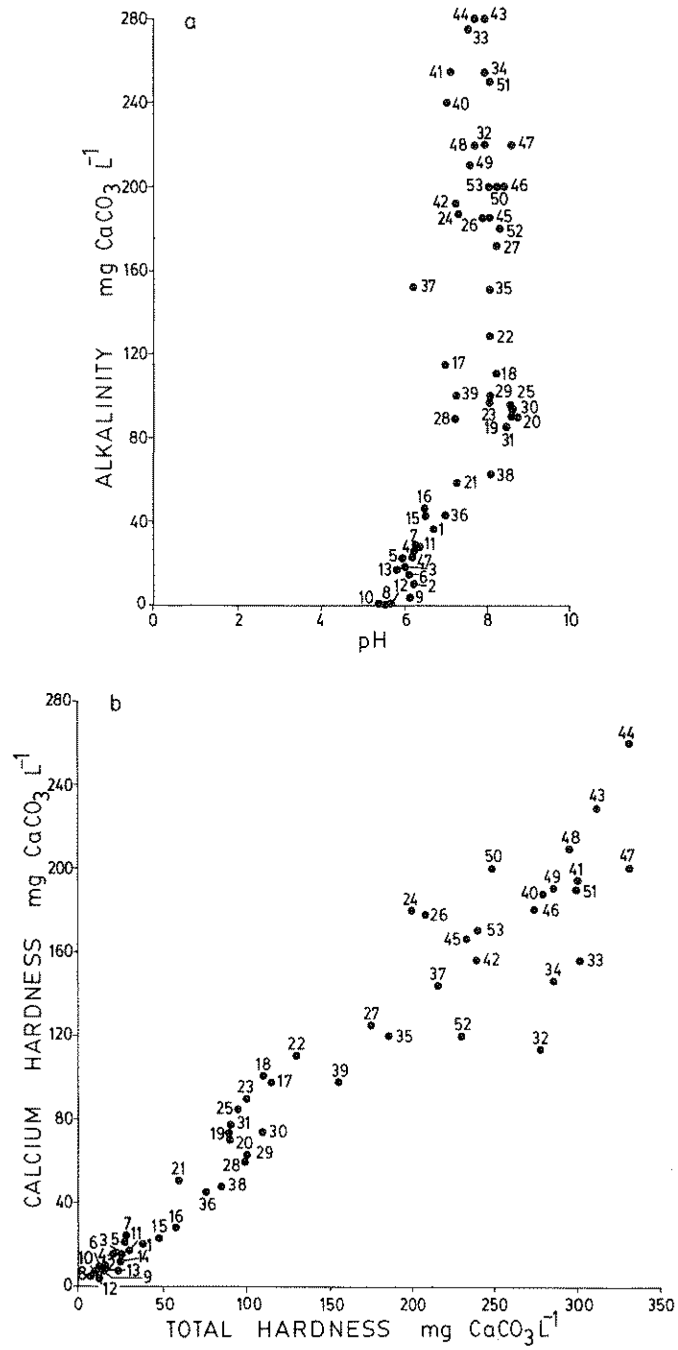

Fig. 2. Some water chemistry of the 53 habitats showing (a) pH and alkalinity and (b) total and calcium hardness. All plots are based on measurements from single samples.

Lengths of shells of bivalves (maximum anterior to posterior distance), height of shell of spired gastropods (maximum distance between top of nuclear whorl and lower lip of aperture), and the greatest diameter of planorbid snails were measured with a modified slide rule (Mackie, 1980) on shells greater than about 2 mm (for dimension measured), and with a stereomicroscope equipped with an ocular grid on shells less than about 2 mm. The specimens were oven-dried at 100°C to constant weight and weighed to five decimal places on a Sartorius microbalance, model 2444. Hence, all weights are based on fresh, not preserved, oven-dried specimens.

Each specimen was then digested in 2 N (4 N for Unionidae) HCl until the dissolved calcium content, measured by atomic absorption, was constant. The specimens were not ashed because preliminary studies showed that there was no significant difference ($P > 0.20$) between calcium content of specimens digested in HCl after ashing at 500°C and other specimens of the same species and similar size classes digested in HCl without previous ashing. All calcium measurements are of whole specimens (i.e. shell and tissue).

Calcium was measured on either 1000, 100 or 10 μl aliquots of HCl digestant (depending on size of mollusc) diluted to 100 ml with 1 N HCl containing 1 mg $LaCl_3 l^{-1}$. The lanthanum reduces interference by phosphorus (Willis, 1961). For large unionids further dilutions often had to be made. A Unicam Atomic Absorption Spectrophotometer, Model SP 1950, at a wavelength of 422 nm and a slit width of 0.20 mm, and an air-acetylene mixture, was used to measure calcium in the 100 ml samples.

Relationships between length and weight, length and calcium content, and weight and calcium content were determined using the power function,

$$y = Ax^b$$

for each species from each population, where A is the y intercept and b is the slope.

Correlations (r^2) between mean calcium content (g $Ca.g^{-1}$ animal) of molluscs and calcium content of the water were determined for each species using a CMS computer program for calculating r^2 according to the methods of Steel & Torrie (1980).

RESULTS

Twenty-eight species of mollusc were found in the 53 habitats sampled (Table 1). Most of the common species occur over a wide range of

TABLE 1. Ranges of pH, total alkalinity, total hardness and calcium hardness of waters in which each species of mollusc was found.

Species	Number of Populations	pH	Alkalinity mg $CaCO_3$ l^{-1}	Total Hardness mg $CaCO_3$ l^{-1}	Ca Hardness mg $CaCo_3$ l^{-1}
Lymnaea palustris (Müller)	2	6.23 - 7.37	152 - 187	200 - 216	144 - 180
Lymnaea stagnalis (L.)	2	7.31 - 7.55	100 - 187	155 - 220	98 - 180
Physella gyrina (Say)	14	6.32 - 8.37	25 - 280	28 - 310	20 - 230
Helisoma anceps (Menke)	6	5.50 - 8.28	1 - 280	10 - 310	2 - 230
Helisoma trivolvis (Say)	2	7.31 - 8.64	100 - 220	155 - 330	98 - 200
Gyraulus parvus (Say)	4	7.03 - 8.28	42 - 187	76 - 200	45 - 180
Gyraulus hirsutus (Gould)	2	8.21 - 8.28	90	90	70 - 73
Amnicola limosa (Say)	20	5.50 - 8.52	0 - 255	10 - 286	2 - 180
Cincinnatia cincinnatiensis (Anthony)	6	6.00 - 7.93	5 - 220	15 - 265	10 - 114
Valvata tricarinata (Say)	6	6.00 - 8.37	22 - 255	29 - 286	21 - 146
Campeloma decisum (Say)	9	5.87 - 8.28	14 - 130	8 - 110	
Goniobasis livescens (Menke)	1	7.08	240	280	188
Musculium lacustre (Müller)	2	7.34 - 8.64	187 - 220	200 - 330	150 - 200
Musculium securis (Prime)	7	6.05 - 8.21	18 - 152	21 - 216	15 - 155
Musculium transversum	1	6.51	43	60	28
Pisidium adamsi Stimpson	2	7.56 - 7.93	220 - 275	265 - 302	114 - 156
Pisidium casertanum (Poli)	19	5.50 - 8.34	0 - 280	10 - 332	2 - 260
Pisidium compressum Prime	20	7.08 - 8.64	58 - 280	60 - 332	50 - 260
Pisidium ferrugineum Prime	1	5.50	0	10	4
Pisidium nitidum Jenyns	2	6.51 - 7.31	43 - 100	60 - 155	28 - 98
Pisidium variabile Prime	5	7.72 - 8.64	90 - 280	90 - 332	70 - 260
Sphaerium fabale Prime	1	7.08	240	280	188
Sphaerium rhomboideum	4	7.03 - 7.37	42 - 187	76 - 200	45 - 180
Sphaerium simile (Say)	8	7.05 - 8.64	115 - 280	115 - 330	98 - 230
Sphaerium striatinum (Lamarck)	13	6.51 - 8.64	43 - 280	60 - 332	28 - 260
Anodonta grandis grandis Say	12	7.05 - 8.64	36 - 280	38 - 332	20 - 260
Elliptio complanata (Lightfoot)	13	5.50 - 8.63	2 - 172	5 - 175	4 - 125
Lampsilis radiata radiata (Gmelin)	4	6.15-8.63	14 - 280	14 - 332	9 - 260

Table 2. Relationships ($y = Ax^b$) between shell size (i.e. length for bivalves, height for spired gastropods, diameter for planorbid snails, mm) and weight (g), shell size (mm) and calcium content (g), and weight (g) and calcium content (g) for twenty-eight species of freshwater molluscs, where A = y intercept and b = slope. Weights and calcium contents are based on whole animals (see text for explanations). Standard error (S.E.) of each estimate is given in parenthesis. All correlation coefficients (r^2) are significant to at least 99.99% level. The number of populations to obtain the degrees of freedom (D.F.) for each species may be found in Table 1.

		Shell Size (mm) vs Weight (g)			Shell Size (mm) vs Calcium Content (g)			Weight (g) vs Calcium Content (g)		
	D.F.	Intercept (S.E.)	Slope (S.E.)	r^2	Intercept (S.E.)	Slope (S.E.)	r^2	Intercept (S.E.)	Slope (S.E.)	r^2
Lymnaea palustris	49	0.800 (0.2600)	1.43 (0.2520)	0.402	0.216 (0.2710)	1.55 (0.2637)	0.419	0.445 (0.0170)	1.06 (0.0097)	0.996
Lymnaea stagnalis	31	2.010 (0.2585)	1.13 (0.2254)	0.457	3.010 (0.1861)	0.58 (0.1620)	0.296	0.071 (0.0988)	0.51 (0.0686)	0.646
Physella gyrina	298	0.064 (0.0373)	2.69 (0.0489)	0.911	0.018 (0.0491)	2.77 (0.0640)	0.862	0.414 (0.0201)	1.05 (0.0084)	0.979
Helisoma anceps	121	0.112 (0.0318)	2.82 (0.0370)	0.980	0.030 (0.0358)	2.97 (0.4160)	0.977	0.438 (0.0169)	1.05 (0.0096)	0.990
Helisoma trivolvis	18	0.120 (0.0830)	2.73 (0.0730)	0.988	0.016 (0.1076)	3.07 (0.0946)	0.984	0.416 (0.0188)	1.12 (0.0172)	0.996
Gyraulus parvus	58	0.013 (0.0376)	2.71 (0.0725)	0.961	0.045 (0.0382)	2.73 (0.0731)	0.960	0.359 (0.0403)	1.00 (0.0157)	0.986
Gyraulus hirsutus	35	0.126 (0.0820)	2.80 (0.1567)	0.904	0.045 (0.0787)	2.80 (0.1514)	0.909	0.349 (0.0329)	0.99 (0.0130)	0.994
Amnicola limosa	424	0.229 (0.0235)	2.59 (0.0475)	0.875	0.353 (0.0201)	1.54 (0.0406)	0.774	0.052 (0.0267)	0.59 (0.0111)	0.869
Cincinnatia cincinnatiensis	107	0.248 (0.0359)	2.41 (0.0672)	0.923	0.039 (0.0563)	2.99 (0.1054)	0.884	1.117 (0.0591)	1.24 (0.0250)	0.959
Valvata tricarinata	114	0.626 (0.0428)	2.71 (0.1248)	0.807	0.236 (0.0494)	2.72 (0.1442)	0.759	0.409 (0.0437)	1.01 (0.0187)	0.963
Campeloma decisum	179	0.194 (0.0280)	2.72 (0.0275)	0.982	0.053 (0.0317)	2.83 (0.0312)	0.979	0.430 (0.0049)	1.04 (0.0040)	0.997
Goniobasis livescens	24	0.341 (0.1740)	2.42 (0.1647)	0.904	0.182 (0.1654)	2.26 (0.1565)	0.901	0.313 (0.0069)	0.93 (0.0071)	0.999
Musculium lacustre	28	0.138 (0.0844)	2.29 (0.1429)	0.904	0.016 (0.1110)	2.85 (0.1880)	0.895	1.019 (0.0759)	1.24 (0.0289)	0.986
Musculium securis		0.087 (0.0552)	2.61 (0.0910)	0.845	0.017 (0.0663)	3.00 (0.1090)	0.833	0.346 (0.1163)	1.01 (0.0457)	0.764
Musculium transversum	23	0.078 (0.1214)	2.35 (0.1832)	0.882	0.007 (0.1746)	3.05 (0.2636)	0.859	1.535 (0.1133)	1.30 (0.0438)	0.976
Pisidium adamsi	39	0.052 (0.0597)	3.61 (0.1146)	0.963	0.009 (0.0627)	4.09 (0.1205)	0.968	0.615 (0.0531)	1.12 (0.0217)	0.986
Pisidium casertanum	312	0.082 (0.0358)	3.24 (0.0840)	0.827	0.007 (0.0710)	4.35 (0.1665)	0.687	2.084 (0.0981)	1.34 (0.0352)	0.823
Pisidium compressum	425	0.117 (0.0203)	3.64 (0.0422)	0.946	0.027 (0.0318)	4.08 (0.0663)	0.899	0.688 (0.0285)	1.12 (0.0127)	0.948
Pisidium ferrugineum	24	0.090 (0.0970)	2.68 (0.3174)	0.757	0.001 (0.4387)	5.59 (1.4360)	0.397	0.008 (1.4153)	1.98 (0.4355)	0.472
Pisidium nitidum	48	0.180 (0.1497)	2.23 (0.4592)	0.335	0.010 (0.2132)	4.25 (0.6544)	0.472	1.396 (0.4144)	1.30 (0.1365)	0.658
Pisidium variabile	61	0.157 (0.0723)	3.29 (0.1583)	0.896	0.035 (0.1338)	3.74 (0.2930)	0.764	0.990 (0.1008)	1.19 (0.0423)	0.941
Sphaerium fabale	24	0.036 (0.0509)	3.47 (0.0544)	0.994	0.010 (0.0519)	3.55 (0.0555)	0.994	0.375 (0.0068)	1.02 (0.0050)	0.999
Sphaerium rhomboideum	72	0.047 (0.0242)	3.20 (0.0276)	0.995	0.001 (0.1242)	4.24 (0.1411)	0.927	0.807 (0.0705)	1.33 (0.0429)	.0.931
Sphaerium simile	152	0.121 (0.0353)	2.81 (0.0346)	0.978	0.033 (0.0421)	2.95 (0.0411)	0.971	0.437 (0.0076)	1.05 (0.0066)	0.994
Sphaerium striatinum	283	0.064 (0.0390)	3.27 (0.0470)	0.945	0.019 (0.0422)	3.40 (0.0509)	0.941	0.431 (0.0099)	1.04 (0.0061)	0.990
Anodonta g. grandis	166	0.024 (0.1059)	3.00 (0.0581)	0.942	0.014 (0.1205)	2.88 (0.0662)	0.920	0.391 (0.0068)	0.97 (0.0069)	0.992
Elliptio complanata	222	0.015 (0.1063)	3.22 (0.0586)	0.932	0.007 (0.1127)	3.16 (0.0622)	0.921	0.391 (0.0061)	0.99 (0.0054)	0.993
Lampsilis r. radiata	44	0.058 (0.1840)	2.93 (0.1008)	0.951	0.023 (0.1770)	2.88 (0.0970)	0.953	0.409 (0.0081)	0.98 (0.0065)	0.998

alkalinities and total and calcium hardnesses. The only exceptions are *Campeloma decisum*, *Musculium securis* and *Elliptio complanata* which occur in waters with alkalinities less than about 170 mg $CaCO_3.l^{-1}$ and *Sphaerium simile* which occurs in waters with alkalinities greater than about 100 mg $CaCO_3.l^{-1}$.

Some habitats had very low buffering capacities and showed a high degree of acidification. The lowest pH of water sampled was 5.50. Only five common species of molluscs were found at this site (Table 1).

The mean length-weight, length-calcium content and weight-calcium content relationships of each species are given in Table 2. Although nearly all relationships have highly significant coefficients of correlation ($P < 0.0007$), many show considerable variations in slope and intercept values, as indicated by the standard errors. However, much of the variation in slope and intercept values is due to variance in these parameters within populations and not entirely to variance among populations.

For some species the variation in calcium content of whole individuals correlates well with the calcium content of their environment (Table 3). Six species (*Cincinnatia cincinnatiensis*, *Pisidium casertanum*, *P. compressum*, *Sphaerium striatinum*, *Anodonta grandis*, and *Elliptio complanata*) show a direct correlation and two species (*S. simile* and *S. rhomboideum*) show an inverse correlation between calcium content of whole individuals and calcium hardness of the environment (Table 3). Most of the species that show a significant correlation are bivalves. *C. cincinnatiensis* was the only gastropod to show a significant correlation between calcium content of the individual and calcium hardness of the environment. However, most gastropods showed a correlation between calcium content of the individual and either pH or alkalinity (Table 3).

DISCUSSION

The ranges of pH, alkalinity, and total and calcium hardness within which each species was found are generally wider than those reported in other studies (Clarke & Berg, 1959; Harman & Berg, 1971; Starrett, 1971; Harman, 1974; Buckley, 1977). Usually the discrepancies are with the minimum values where the present study seems to be the first to examine molluscs in habitats with poorly buffered waters.

Acid precipitation is affecting or already has affected many habitats in the Canadian Shield (ACSCEQ, 1981). Although we did not examine lakes with pH < 4.5 (i.e. acidified lakes), we did sample molluscs in lakes with pH 5.5 and no apparent buffering capacity (i.e. alkalinity = 0). These lakes still had molluscs (e.g. *A. limosa*, *P. casertanum*, *P. ferrugineum*, Table 1). These results compare favourably with those in other studies. In a survey of 1350 Norwegian lakes for

Table 3. Correlation coefficients (r^2) and the significance level of r^2 (in parenthesis) for the relationships between calcium content of the whole animal and pH, total alkalinity, total hardness and calcium hardness of the water. The asterisks accentuate the significant correlations.

Species	pH	Alkalinity	Total hardness	Ca hardness
GASTROPODA				
Physella gyrina	0.105 (0.071)	0.114 (0.048)*	0.103 (0.076)	0.036 (0.533)
Helisoma anceps	0.179 (0.061)	0.022 (0.814)	−0.017 (0.851)	−0.049 (0.592)
Gyraulus parvus	−0.405 (0.002)*	−0.075 (0.570)	*.108 (0.414)	0.030 (0.819)
Amnicola limosa	−0.003 (0.948)	0.088 (0.067)	0.074 (0.127)	0.053 (0.274)
Cincinnatia cincinnatiensis	0.306 (0.001)*	0.354 (0.001)*	0.341 (0.001)*	0.340 (0.001)*
Valvata tricarinata	−0.292 (0.002)*	−0.083 (0.373)	−0.021 (0.822)	−0.155 (0.098)
Campeloma decisum	−0.056 (0.485)	0.041 (0.614)	0.062 (0.439)	−0.016 (0.841)
BIVALVIA				
Musculium securis	−0.002 (0.983)	0.074 (0.366)	0.087 (0.284)	0.099 (0.226)
Pisidium casertanum	0.113 (0.046)*	0.251 (0.001)*	0.310 (0.001)*	0.271 (0.001)*
Pisidium compressum	−0.160 (0.001)*	0.222 (0.001)*	0.218 (0.001)*	0.240 (0.001)*
Pisidium variabile	0.073 (0.608	0.192 (0.173)	0.196 (0.164)	0.231 (0.099)
Sphaerium rhomboideum	−0.328 (0.005)*	−0.345 (0.003)*	−0.336 (0.004)*	−0.345 (0.003)*
Sphaerium simile	−0.432 (0.001)*	−0.123 (0.129)	−0.209 (0.009)*	−0.226 (0.005)*
Sphaerium striatinum	0.150 (0.012)*	0.166 (0.005)*	0.125 (0.035)*	0.132 (0.026)*
Anodonta g. grandis	0.222 (0.004)*	0.173 (0.026)*	0.222 (0.004)*	0.220 (0.004)*
Elliptio complanata	0.233 (0.001)*	0.146 (0.021)*	0.147 (0.021)*	0.178 (0.005)*
Lampsilis r. radiata	0.014 (0.927)	−0.204 (0.179)	−0.211 (0.165)	−0.210 (0.167)

gastropods in relation to nine environmental variables, snails showed a low frequency of occurrence in the pH range 4.4-4.6 (Økland, 1969). Most molluscs disappeared from Norwegian waters when the pH fell below 5.0 (Økland, 1969; Økland & Økland, 1980 Økland & Kuiper, 1980). In the English Lake District few molluscs were found below pH 6.0 (Macan, 1950). Roff & Kwiatowski (1977) found that in the relationship between diversity index for zoobenthos and pH in six lakes southwest of Sudbury, Ontario, the inflection point (i.e. the point at which diversity changed) occurred at pH 4.8; *Pisidium* was the only mollusc to be found below pH 5.0 but no molluscs were found below pH 4.8.

The ranges of pH, alkalinity and hardness in this study represent the values measured on single samples during June and July. Total alkalinity and hardness fluctuate greatly from one season to the next (Wetzel, 1975) so that the ranges observed above may in fact be even greater. However, the extremes of these fluctuations often occur in the winter and early spring when most molluscs are dormant. The values reported here were measured when molluscs were active and growing. Hence, any relationships between molluscan calcium content and environmental calcium content are considered more valid under active growing conditions than during dormant periods. Moreover, the values of pH, alkalinity, total hardness and calcium hardness observed in June and July are close to the annual means documented in other studies (Mackie, 1971; Conroy, Hawley & Keller, 1978; Mackie, 1979; Seidl, 1980; Bailey, 1982). This has been verified for about 60% of the habitats studied (the remaining 40% having no documented data for annual variations in water chemistry).

If, indeed, spring pH values are considered, many molluscs can be found in acidifying lakes with pH as low as 4.7 (i.e. spring depression value), such as in Chub Lake (No. 10) and Heney Lake (No. 8). Molluscs found in these lakes include *A. limosa, E. complanata, P. casertanum,* and *P. ferrugineum.* Servos (pers. comm.) found *P. casertanum* in lakes with a spring pH depression value as low as 4.4

It would appear from Table 3 that the calcium content in bivalves is influenced by environmental calcium more than in gastropods, although correlations do not necessarily imply cause-effect relationships. Even if there is a cause-effect relationship, some species (e.g. *A. limosa* and *P. casertanum*) are found over such a broad range of calcium concentrations that factors such as lake acidification appear to have little effect on their distribution. Clearly other factors dictate the distribution of such molluscs.

The inverse relationships between calcium content of some molluscs (e.g. *S. rhomboideum* and *S. simile*) and calcium content of the water is interesting but difficult to explain because it would seem that less energy would be needed to build the most calcareous shells in the hardest water. Burky *et al.* (1979) also found this inverse relationship but for *S. striatinum*, a species for which a positive correlation was found in this study (Table 3). However, their study examined waters with a narrower range of calcium content (25-82 $mg.l^{-1}$) than our study (11-104 $mg.l^{-1}$); this represents the broadest range of calcium reported for this species to date. At present no explanations can be offered for the interspecific variations in the correlations between calcium content of bivalve molluscs and calcium concentration of the environment.

The paucity of correlations in the gastropods (Table 3) perhaps can be explained on the basis of their habits. Most gastropods are associated with aquatic macrophytes. Perhaps the macrophytes have a greater influence on snail calcium content than does the water chemistry. In this regard, it may be worth speculating further that since most bivalves are associated with the sediments, the strong correlations that occur with water chemistry (Table 3) would be even stronger if sediment chemistry was measured instead of water chemistry. Some support for this argument comes from the strong correlations seen in *C. cincinnatiensis* (Table 3) which was found more closely associate with the sediments than was *A. limosa*, a closely related species which is associated with macrophytes. Weaknesses in this argument are apparent when *C. decisum* is analysed since this species is very strongly associated with sediments yet no correlations were found.

It is worth noting that when correlations between calcium contents of molluscs and environments were present, strong correlations usually occurred with pH, alkalinity and total hardness as well (Table 3). Total alkalinity is a measure of all the dissolved carbonates and bicarbonates (and hydroxides in strongly basic waters) in water. Total hardness is a measure of not only the cations of carbonates and bicarbonates, but of other anions (e.g. chloride and sulphate) as well. These noncarbonate anions are usually in lesser amounts than the monocarbonates and bicarbonates (as subtraction of total alkalinity from total hardness in mg $CaCO_3.l^{-1}$ will verify). Since alkalinity and total hardness are measures of the size of the bicarbonate pool (when expressed as $CaCO_3$, any correlations of calcium content in molluscs with these variables are probably reflecting the importance of the bicarbonate pool (concentration) to shell formation (which accounts for 80-95% of the weight of molluscs). Hence, for *P. gyrina* the bicarbonate pool appears to be important, but for *C. cincinnatiensis* the calcium in the bicarbonate pool seems to be more important (Table 3).

According to Table 3, the calcium content of individuals in eleven species of mollusc are also related to pH and/or alkalinity, implying that acid deposition may affect shell formation in these species. However, of the eleven species, five (*Gyraulus parvus, V. tricarinata, P. compressum, S. rhomboideum,* and *S. simile*) have negative correlations indicating that as pH and/or alkalinity is lowered, calcium content of these molluscs increases. Therefore, acid deposition should not be a factor in shell formation of these species, at least down to pH 6.00 and an alkalinity of 20 mg $CaCo_3.l^{-1}$. Below these values acid deposition may very well have an effect since the species are not found at lower pH and alkalinity values. Even though the species are found in hard water, they cannot be considered calcicolous in habit because the negative correlations indicate that the species do not grow best in hard water.

The remaining six of the eleven species show positive correlations with pH and/or alkalinity, implying that acid deposition could directly affect shell formation in these species. However, three of the species (*P. gyrina, S. striatinum,* and *A. g. grandis*) are found only in well-buffered (pH > 6.00, alkalinity > 20 mg $CaCo_3.l^{-1}$) water and hence are not likely to be affected by acid deposition. The remaining species (*C. cincinnatiensis, P. casertanum,* and *E. complanata*) are found in acidifying water (pH < 6.00) and appear able to concentrate calcium from waters that have little or no buffering capacity. This ability appears to be unrelated to the correlations between calcium content of individuals and calcium content of water since *A. limosa,* which showed no correlations (Table 3), is also found in acidifying waters.

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SUMMARY

(1) Intraspecific and interspecific variations in calcium content were determined for 28 species of mollusc collected from 53 freshwater habitats on a naturally occurring calcium gradient between the Canadian Shield and limestone formations in southern Ontario.

(2) Reported are the ranges of pH, total alkalinity, total hardness and calcium hardness in which the 28 species of mollusc were found.

(3) Sufficient numbers of populations were collected for 16 species to permit analyses of intraspecific and interspecific variations in calcium content of whole individuals relative to pH, total alkalinity, total hardness and calcium hardness of the water.

(4) The calcium content of whole individuals is related to calcium concentration of the water for only 8 species, 7 of these being bivalves. Two species (*Sphaerium rhomboideum* and *Sphaerium simile*) showed negative correlations, while the other six (*Cincinnatia cincinnatiensis, Pisidium casertanum, Pisidium compressum, Sphaerium striatinum, Anodonta g. grandis, Elliptio complanata*) showed positive correlations with environmental calcium. In general, species associated with the sediments showed better calcium correlations than did species associated with macrophytes.

(5) The effect of acid precipitation on calcium uptake by molluscs is discussed.

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