

Life Stage and Population Variation in Resistance and Tolerance of *Hyaella azteca* (Amphipoda) to Low pH

R. L. France and P. M. Stokes

Institute for Environmental Studies and Department of Botany, University of Toronto, Toronto, Ont. M5S 1A4

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Lethality experiments revealed that resistance of *Hyaella azteca* (Amphipoda) to low pH was directly related to size and developmental stage. Exposure of adults to water below pH 5.0 during pulses of acid snowmelt, or of juveniles to below pH 5.5 through gradual lake acidification, could result in population decline. *Hyaella azteca* from moderately acidic Ontario lakes (pH 5.6–5.7) survived longer at lethal pH than did amphipods from circumneutral lakes (pH 6.4–7.2) where spring pH depressions do not occur. Resistance and tolerance to low pH was neither readily lost by tolerant amphipods exposed to neutral water for 10 d nor readily gained by nontolerant amphipods exposed to sublethal low pH for a similar duration. This absence of physiological plasticity of individual *H. azteca* suggests that population differences in acid tolerance may result from processes of selective mortality with or without a genetic basis.

Des expériences de létalité ont révélé que la résistance de *Hyaella azteca* (Amphipodes) à un faible pH est directement liée à la taille et au stade de développement. L'exposition d'adultes à de l'eau acide à pH inférieur à 5,0 provenant de la fonte des neiges ou de juvéniles à de l'eau à pH inférieur à 5,5 suite à l'acidification lacustre graduelle peut entraîner une baisse des effectifs. *Hyaella azteca* peuplant des lacs ontariens modérément acides (pH 5,6–5,7) ont survécu plus longtemps à un pH létal que des amphipodes provenant de lacs presque neutres (pH 6,4–7,2) où une baisse du pH n'a pas lieu au printemps. La résistance et la tolérance à un faible pH n'étaient ni facilement perdues par des amphipodes tolérants exposés à des eaux neutres pendant 10 j ni aisément acquises par des amphipodes non tolérants exposés à un faible pH subléthal pendant la même période. Cette absence de plasticité physiologique chez les individus de *H. azteca* porte à croire que les différences de tolérance aux acides peuvent être le résultat d'une mortalité sélective à caractère génétique ou non.

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Tolerance refers to an organism's ability to survive indefinitely when exposed to certain environmental conditions. Resistance implies a temporal effect under a set of stressful conditions which will eventually prove lethal outside the range of tolerance (Fry 1947; Duncan and Klaverkamp 1983). Due to the episodic nature of acid snowmelt and severe storm events on the Canadian Shield (Jeffries et al. 1979; Keller 1983; LaZerte 1984), survival of any species here will depend not only on its tolerance to the magnitude of pH depression, but also on its resistance to the duration of perturbation.

Although short-term laboratory experiments and survey data indicate that amphipods are sensitive to pH below 5.5–6.0 (Borgstrom and Hendrey 1976; Roff and Kwaitkowski 1977; Brehm and Meijering 1982; Meinel et al. 1985; Sutcliffe and Carrick 1973a; Okland and Okland 1985; Stephenson and Mackie 1986), tolerance and resistance may vary over the life cycle and among animals from different populations depending upon their history of exposure.

The objectives of this study were to (1) determine how the tolerance and resistance of *Hyaella azteca* (Amphipoda) to low pH varies in relation to organism size and instar stage, and to (2) ascertain interpopulation variability in low pH resistance of *H. azteca* collected from five lakes of different acidity (pH 5.7–7.2). If population variation was demonstrated, we then

planned to investigate whether these differences in resistance were due to the ionic composition of testing media or to processes of short-term acclimation or acclimatization. In this paper, acclimation refers to the physiological alteration of an organism's homeostasis in a compensatory response to experimental manipulation of a few environmental variables; acclimatization occurs through exposure for much longer periods to modifications of the organism's natural habitat (Duncan and Klaverkamp 1983). Decisions regarding the operation of these two processes are based on comparisons of survival rates (e.g. Swarts et al. 1978). Where reference is made to low pH or acid-induced resistance or tolerance, it is understood that the toxic agent that the amphipods are responding to is the hydrogen ion (Brehm and Meijering 1982).

Study Area

Hyaella azteca were collected from five lakes within the Haliburton–Muskoka region of south-central Ontario, an area susceptible to, and currently receiving, acidic precipitation (Dillon et al. 1978; Jeffries et al. 1979; Schieder et al. 1979; LaZerte and Dillon 1984). Glen Lake has hard water (Table 1) and a pH that does not decrease much below the average annual value of 7.23. Blue Chalk and Harp lakes are circumneutral with moderate alkalinities (Table 1); the pH over wide-

TABLE 1. Summary of selected chemistry for lakes of origin of *H. azteca* used for experiments in Paint Lake water. Data are from P. Dillon and K. Holtze, MOE, Dorset Research Centre.

Lake	pH	Alk. ($\mu\text{eq}\cdot\text{L}^{-1}$)	Colour (Hazen units)	Cond. ($\mu\text{S}\cdot\text{cm}^{-1}$)	Ca ($\text{mg}\cdot\text{L}^{-1}$)	Al ($\mu\text{g}\cdot\text{L}^{-1}$)	Mn ($\mu\text{g}\cdot\text{L}^{-1}$)
Glen	7.23	1 200	13	169.0	25.0	26	59.3
Blue Chalk	6.47	103	9	30.3	2.6	8	36.7
Crosson	5.65	58	29	27.8	2.1	80	39.6
Harp	6.22	101	22	36.1	2.9	39	64.1
Heney	5.74	46	13	25.0	2.0	41	30.0
Paint	6.15	83	26	62.3	4.2	33	80.0

spread areas of the littoral zone never drops to below 6.0 (P. Dillon, Ontario Ministry of the Environment (MOE), Dorset Research Centre, Dorset, Ont., unpubl. data). The more acid lakes, Crosson and Heney, have average annual pHs of 5.65 and 5.74, respectively, which, however, can decrease to below 5.0 during spring snowmelt (P. Dillon, MOE, unpubl. data). The higher colour value for Crosson Lake suggests that it may have always been somewhat acidic. Both Crosson and Heney lakes have experienced biotic disturbances related to acidification (Harvey 1982; Allison and Harvey 1981; Rooke and Mackie 1984). Stephenson and Mackie (1986) found a 2-wk delay in *H. azteca* recruitment in Heney Lake compared with other circumneutral lakes such as Blue Chalk and Harp where springtime pH depressions did not occur; they suggested that this is indication of a stressed *H. azteca* population.

Materials and Methods

Life Stage Response to Low pH

Experiment A

During August 1984, *H. azteca* were collected by dip samples and gentle sieving from the littoral zone of Glen Lake (Table 1). Amphipods were held for 3 d in aerated aquaria containing a thin layer of flocculent organic sediment from Glen Lake that covered most of the aquaria bottoms and served both as substrate and food supply (Hargrave 1970). Water from Paint Lake was used for both this laboratory adjustment period and the following test. The concentrations (milligrams per litre) of selected ions were as follows: Ca^{2+} , 4.2; Na^+ , 2.5; Mg^{2+} , 1.5; K^+ , 0.7; Cl^- , 4.1; SO_4^{2-} , 9.6; total Mn, 0.08; and total Cu, 0.002 (K. Holtze, MOE, unpubl. data).

Experiments were conducted in 7-L plastic aquaria, each containing two 100-mL plastic beakers with their sides partially removed and covered with Nitex® screening. To limit the possibility of stress-related antagonism, amphipods for each treatment were separated on the basis of size and contained within the testing beakers. The size range of the larger amphipods was 0.58–0.82 mm head length (age 1+ yr adults) and for the smaller amphipods was 0.32–0.60 mm head length (age 1+ mo juveniles).

Water from Paint Lake was acidified to the test pH with H_2SO_4 and vigorously aerated to ensure removal of excess CO_2 (France 1982). A natural photoperiod and continuous aeration was used for the test. Mortality and pH were checked four to six times daily. At each check the plastic beakers containing the amphipods were raised until most of the water was drained free and were then resubmerged enabling water renewal. Periodic additions of acid were required to maintain each treatment pH. Measured pH values never fluctuated more than

± 0.20 unit from the nominal levels, and ses were within 0.01–0.03 pH unit of the mean for all treatments. Dead animals were removed, preserved in 90% ethanol, and measured. Further details of this experiment and any deviations in protocol for subsequent tests are summarized in Table 2.

Experiment B

To confirm that the mortality observed in Experiment A was size dependent, an additional test was undertaken in July 1985 using animals from acidic (pH 5.65) Crosson Lake (Table 2).

Population Variation in Acid Resistance

Experiment C

In late May 1985, an experiment was undertaken to compare the low pH sensitivities of overwintering adult *H. azteca* from three populations that differed in their history of pH exposure (Table 2). Amphipods were collected from Glen Lake (pH 7.23) and from Blue Chalk and Crosson lakes (pH 6.40 and 5.34 at time of collections, respectively; cf. Table 1 for mean annual values). Amphipod head lengths from two randomly chosen Blue Chalk treatments were not significantly different from either Crosson or Glen Lake treatments (ANOVA test, $p < 0.05$), indicating that differences in rate of mortality cannot be ascribed to differences in organism size. Amphipod sizes were similarly not significantly different among treatments for the other experiments (Table 2).

Experiment D

In early July 1985, we compared the low pH resistance of adult *H. azteca* from acidic Heney Lake (pH 5.74) and circumneutral Harp Lake (Table 2).

Testing Media Comparison (Influence of Ionic Strength on Acid Resistance)

During early July 1985, we investigated (Table 2) whether the large differences in "low pH" resistance found between adult amphipods from Glen and Crosson lakes could have been due to an effect of ionic stress on Glen Lake animals following their transfer from Glen Lake to the much softer test water of Paint Lake. The following experimental media were used: unmodified Paint Lake water as before ($43.4 \mu\text{S}\cdot\text{cm}^{-1}$, $4.0 \text{ mg Ca}\cdot\text{L}^{-1}$); Paint Lake water to which CaCl_2 had been added ($48.1 \mu\text{S}\cdot\text{cm}^{-1}$, $10.0 \text{ mg Ca}\cdot\text{L}^{-1}$); untreated Glen Lake water ($105.23 \mu\text{S}\cdot\text{cm}^{-1}$, $23.0 \text{ mg Ca}\cdot\text{L}^{-1}$); and water from Glen Lake diluted 50% by volume with distilled water ($56.7 \mu\text{S}\cdot\text{cm}^{-1}$, $13.0 \text{ mg Ca}\cdot\text{L}^{-1}$).

Influence of Previous Exposure on Acclimation to Low pH

During September 1984, adult *H. azteca* were collected

TABLE 2. Details of experimental methodology.

	Life stage		Population variation		Testing media	Acclimation induction	Acclimatization loss
	Exp. A	Exp. B	Exp. C	Exp. D			
Source of animals	Glen	Crosson	Blue Chalk Glen Crosson	Heny Harp	Glen Crosson	Glen	Blue Chalk Crosson
Adjustment period (d)	3	2	1	2 ^a	2 ^a	10 ^b	10
Treatment pHs	3.5, 4.0, 4.2, 4.5, 4.8, 5.0, 5.5, 6.4	4.0, 4.3, 4.6	4.1, 4.5, 4.8, 5.2, 6.3	4.0, 4.3, 4.6, 4.9, 6.2	4.0, 4.3, 4.6 ^b	4.0, 4.2, 4.5, 4.8, 5.0	4.0, 4.2, 4.5, 4.8, 5.0, 6.4
Replicates	3	1	3	3	1	1-3 ^b	3
Organisms per rep.	12-16	15	11-13	12-17	11-15	10-12	10
Head length (mm ± SE)	0.32 ± 0.60 0.58 ± 0.82	0.31 ± 0.68	0.67 ± 0.03 0.70 ± 0.04 0.69 ± 0.02	0.68 ± 0.07 0.70 ± 0.03	0.64 ± 0.11 0.70 ± 0.10	0.62 ± 0.11 0.68 ± 0.08	0.48 ± 0.08 0.51 ± 0.08
Food source	Tetra-min, floc	Floc	Floc	Floc	None	Tetra-min, floc	None
Temperature (°C)	19-25	15-22	12-21	17-24	16-24	12-20	15-24
Water renewal	days 3 and 6	None	day 3	None	None	day 3	None
Exptal duration (d)	8	8	8	8	8	8	10

^aIn water of lake of origin.

^bDescribed in text.

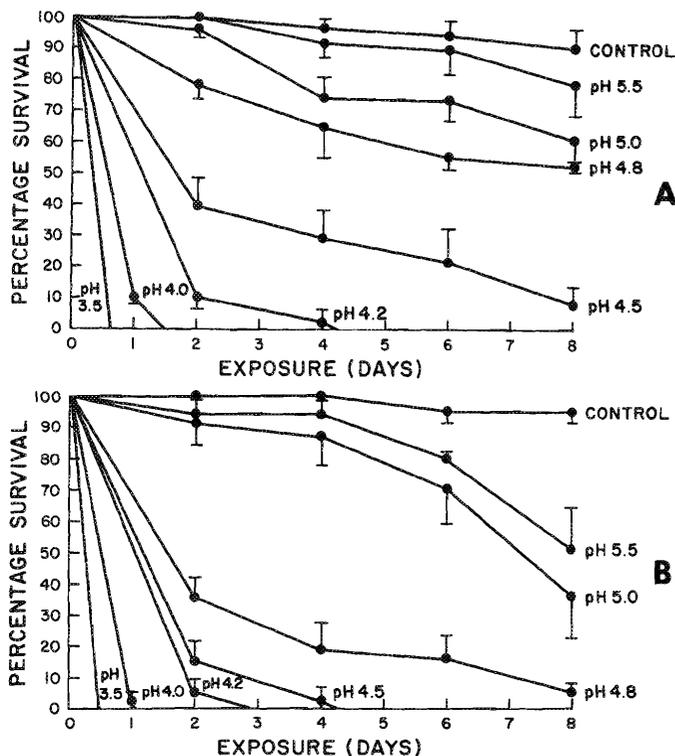


FIG. 1. Survival ± SE ($n = 3$) of (A) age 1+ yr adult (0.58-0.82 mm head length) and (B) age 1+ mo juvenile (0.32- to 0.60-mm head length) *H. azteca* in depressed pH. Control pH was 6.4. Source of amphipods was Glen Lake (pH 7.23).

from Glen Lake and held for 10 d in Paint Lake water adjusted to mean pH 6.56 ± 0.03 (SE) ($n = 24$), 5.86 ± 0.03 (39), 5.56 ± 0.02 (41), and 5.19 ± 0.03 (41). These are nominally represented as pH 6.6 (control), 5.9, 5.6, and 5.2. The following experimental design was used (Table 2): amphipods from the control and pH 5.9 preexposures were tested at lethal pHs 5.0 (two replicates), 4.8, 4.5, and 4.2 (three replicates at each pH), and pH 4.0 (one replicate); amphipods from pH 5.6

preexposure were tested at pH 4.8, 4.5, and 4.2 (three replicates at each pH); and amphipods from pH 5.2 preexposure were tested at pH 4.8, 4.5, and 4.2 (one replicate).

Influence of Exposure to Neutral pH on Loss of Acclimatization

In late August 1985, we examined whether amphipods from an acidic lake would lose their resistance to low pH through short-term exposure to neutral conditions. Late juvenile - early adult amphipods were collected from Blue Chalk (pH 6.47) and Crosson (pH 5.65) lakes and held in Paint Lake water (pH 6.45) for 10 d prior to testing (Table 2).

Statistical Analysis

Median lethal concentrations (LC50) and survival times (MST) were calculated and compared using the procedures of Litchfield (1949) and Litchfield and Wilcoxon (1949). Because of the potential for misapplication of fiducial limits when testing differences (Hodson et al. 1977), the average MSTs were calculated independently from replicate MSTs in the acclimation experiment. Significant differences between survival curves were determined with the nonparametric Kolmogorov-Smirnov (K-S) test for cumulative distributions (Siegel 1956). Differences in tolerance and resistance to low pH are indicated by differences between LC50 values and between survival times, respectively (Duncan and Klaverkamp 1983).

Results

Life Stage Differences in Acid Resistance and Tolerance (Experiments A and B)

Acidification to pH < 5.0 for adults and to pH < 5.5 for juvenile *H. azteca* caused increased mortality (Fig. 1). Acute toxicity occurred below pH 4.2 for adults and below pH 4.5 for juveniles. By 8 d, mortality had stabilized for the adults but not for the juveniles.

With the exception of pH 5.0, the MSTs between adults and juveniles were different ($p < 0.05$) for all treatments (Table 3).

TABLE 3. Median survival times (days) \pm 95% fiducial limits and slope functions (S) of amphipods after exposure for 8 d to low pH. RR = reaction time ratio; SR = slope function ratio; *difference at $p < 0.05$. Control water was pH 6.4.

pH	Adults		Juveniles		RR	SR
	MST	S	MST	S		
Control	Indefinite	—	Indefinite	—	—	—
5.5	23.75 \pm 8.54	3.17	8.54 \pm 1.71	1.64	2.78*	1.93*
5.0	9.79 \pm 2.94	2.73	7.16 \pm 1.39	1.56	1.37	1.75*
4.8	8.13 \pm 4.74	5.90	1.79 \pm 0.66	2.32	4.54*	2.54*
4.5	1.88 \pm 0.90	2.99	0.79 \pm 0.13	1.56	3.62*	1.92*
4.2	1.18 \pm 0.28	1.71	0.52 \pm 0.10	1.52	2.27*	1.13
4.0	0.56 \pm 0.12	1.71	0.25 \pm 0.03	1.32	2.40*	1.30*
3.5	0.32 \pm 0.09	2.02	0.17 \pm 0.04	1.66	1.88*	1.21

TABLE 4. Median survival times (days) \pm 95% fiducial limits and slope functions (S) of *H. azteca* collected from five lakes after exposure to low pH for 8 d. Control water was pH 6.2.

pH	Crosson		Blue Chalk		Glen		pH	Heney		Harp	
	MST	S	MST	S	MST	S		MST	S	MST	S
Control	Indefinite	—	Indefinite	—	Indefinite	—	Control	Indefinite	—	Indefinite	—
5.2	Indefinite (?)	—	83.33 \pm 10.32	4.90	9.17 \pm 1.95	1.83	4.9	Indefinite (?)	—	66.80 \pm 21.57	3.81
4.8	Indefinite (?)	—	26.67 \pm 4.98	3.27	6.54 \pm 1.04	1.57	4.6	Indefinite (?)	—	28.30 \pm 10.63	3.78
4.5	166.70 \pm 36.30	8.35	13.33 \pm 2.67	1.77	2.71 \pm 0.59	1.91	4.3	15.00 \pm 3.97	2.76	7.08 \pm 2.74	4.25
4.1	6.92 \pm 0.85	1.38	3.25 \pm 0.69	1.86	1.29 \pm 0.19	1.47	4.0	9.17 \pm 2.37	2.75	2.50 \pm 0.70	2.17

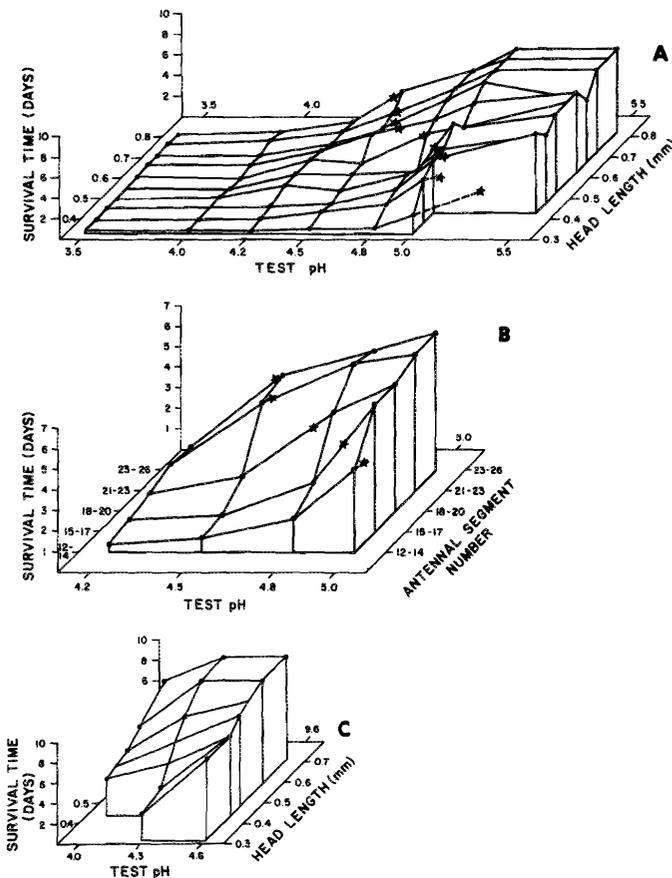


FIG. 2. Relationship of mean survival time of Glen Lake amphipods to (A) head length and (B) antennal segment number and of Crosson Lake amphipods to (C) head length. *Median tolerance limit (LC50) of particular size or age class.

Slope functions also differed ($p < 0.05$) for five of the seven treatments indicating that the time course of mortality was dissimilar. Chi-square tests (Litchfield and Wilcoxon 1949) showed that eye-fitted toxicity lines provided a satisfactory fit to the experimental results. The asymptotic LC50 calculated at 8 d for the adults was pH 4.95 \pm 0.16 ($S = 1.05$), which differed ($p < 0.05$) from the 8-d LC50 of pH 5.30 \pm 0.17 ($S = 1.06$) for juveniles. Compared with 1+ mo old juveniles, 1+ yr old adults were twice as tolerant to low pH.

Differences in low pH resistance of amphipods designated as either "adults" or "juveniles" within the pH range 4.2–5.0 were due to size-dependent mortality (Fig. 2A). Below pH 4.2, death was so rapid as to negate benefits of larger size; above pH 5.0, mortalities were rare and independent of amphipod size. The LC50 ranged from pH 4.80 for 0.80–0.85 mm head length amphipods to pH 5.35 for 0.30–0.35 mm head length amphipods. Resistance to low pH can also be explained by instar developmental stage (Fig. 2B). Here, the LC50 ranged from pH 4.48 for *H. azteca* with 22–26 antennal segments to pH 5.08 for amphipods with 12–14 segments.

A size-dependent relationship to pH mortality was also evident for *H. azteca* from Crosson Lake (Fig. 2C). These amphipods were much more resistant to low pH than similarly sized amphipods from Glen Lake.

Population Differences in Acid Resistance (Experiments C and D)

The nonparametric K-S two-sample test showed that mortality of Glen Lake amphipods occurred more rapidly ($p < 0.05$) than mortality of Blue Chalk or Crosson Lake amphipods at every pH treatment (Fig. 3A, 3B). The survivorship curve of Blue Chalk Lake amphipods was statistically different from that for Crosson Lake amphipods at pH 4.1 but not at other

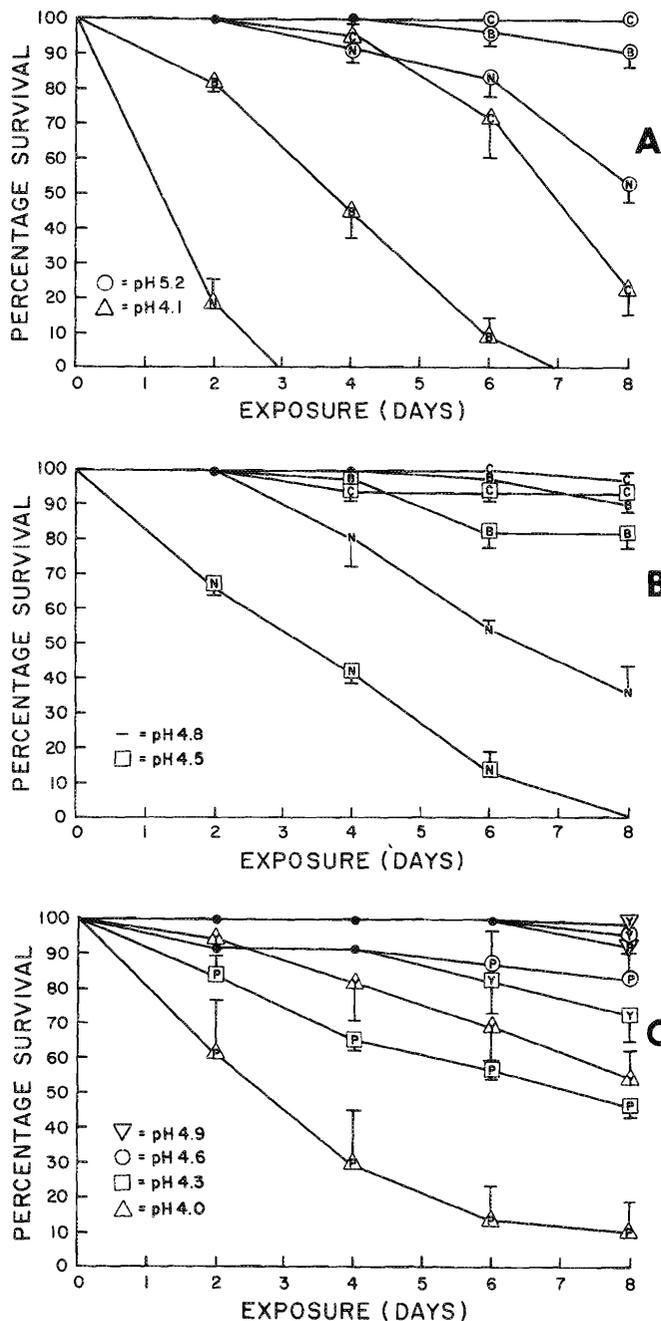


FIG. 3. Comparative survival \pm SE ($n = 3$) of (A and B) *H. azteca* from Crosson (C) (pH 5.65), Blue Chalk (B) (pH 6.47), and Glen (N) (pH 7.23) lakes and of (C) *H. azteca* from Heney (Y) (pH 5.74) and Harp (P) (pH 6.22) lakes, to H^+ toxicity. Control water was pH 6.2–6.6.

pHs. Survival of amphipods from Heney Lake was significantly greater than that of Harp Lake amphipods at pH 4.0 and 4.3, but not at pH 4.6 and 4.9 (Fig. 3C).

Analysis of MSTs (Table 4) confirmed trends evident from survival curve testing. Calculated MSTs among populations were different ($p < 0.05$) at every pH treatment. In general, the range of population resistance was Crosson = Heney > Blue Chalk = Harp > Glen. Therefore, *H. azteca* from moderately acidic lakes which experience spring pulses of acidic snowmelt have greater resistance to lethal low pH than do those from circumneutral lakes.

TABLE 5. Median survival times (days) \pm 95% fiducial limits and slope functions (S) of amphipods collected from two lakes, held in pH 6.45 water for 10 d, and then tested at depressed pH for 10 d.

pH	Crosson		Blue Chalk	
	MST	S	MST	S
4.8	116.67 \pm 68.00	6.75	9.58 \pm 1.46	1.64
4.5	41.67 \pm 20.80	5.26	7.50 \pm 1.35	1.75
4.2	6.25 \pm 1.29	1.92	2.92 \pm 0.82	1.93
4.0	5.63 \pm 1.01	1.73	2.04 \pm 0.34	1.68

Effect of Testing Media on Acid Resistance

Calculated MSTs for amphipods from Crosson Lake were longer ($p < 0.05$) than those derived for Glen Lake amphipods at every pH treatment in all four testing media (Fig. 4). Clearly, differences in low pH resistance between *H. azteca* from these two lakes were not due to an effect of Ca stress on Glen Lake amphipods following their transfer to softer test water.

Effect of Previous Exposure on Acid Resistance and Tolerance

No mortality occurred during the pH 6.6 and 5.9 pretreatments, but approximately 10 and 40% mortality occurred in the pH 5.6 and 5.2 pretreatments, respectively. Survival of *H. azteca* preexposed to pH 5.6 and 5.9 and tested at pH 4.5 was greater than control animals (K-S test, $p < 0.05$), suggesting acclimation (Fig. 5). Mortality of amphipods preexposed to pH 5.6 and 5.2 was more rapid than control animals when tested at pH 4.8, indicating a reverse of acclimation, or sensitization. There were no statistical differences in survival curves for preexposed treatments compared with controls for other combinations.

Analysis of MSTs (Fig. 6) confirmed results from survival curve testing. Of 11 comparisons, seven preexposure MSTs (combined data) were not different ($p < 0.05$) from the controls, while four were lower ($p < 0.05$). Two of these were from preexposure treatments to pH 5.2, indicating sensitization. Of the seven possible parametric comparisons, six preexposure MSTs were not different (t -test, $p > 0.05$) from controls, while preexposure to pH 5.9 significantly reduced the MST at pH 5.0 ($p < 0.10$), again indicative of sensitization and not acclimation of resistance. Preexposure to pH 5.2, 5.6, and 5.9 did not increase tolerance of *H. azteca* tested at lethal pH (Fig. 7). In fact, reduced tolerance was shown for amphipods preexposed to pH 5.2 and 5.6 on days 2 and 4, respectively.

Effect of Exposure to Neutral pH on Acid Resistance and Tolerance

Mortality at all pHs was more rapid for amphipods from Blue Chalk Lake than for those from Crosson Lake ($p < 0.05$) (Fig. 8). Calculated MSTs for *H. azteca* from Crosson Lake remained larger ($p < 0.05$) than those for Blue Chalk amphipods at every test pH despite pretreatment exposure (Table 5). The asymptotic LC50 calculated at 10 d for Crosson Lake amphipods was pH 4.32 \pm 0.09 ($S = 1.06$), which differed ($p < 0.05$) from the 10-d LC50 of pH 4.78 \pm 0.14 ($S = 1.05$) for Blue Chalk amphipods. Further, survival times of adults from Crosson lake were similar to those calculated for this life stage from Experiments B and C. Short-term exposure to

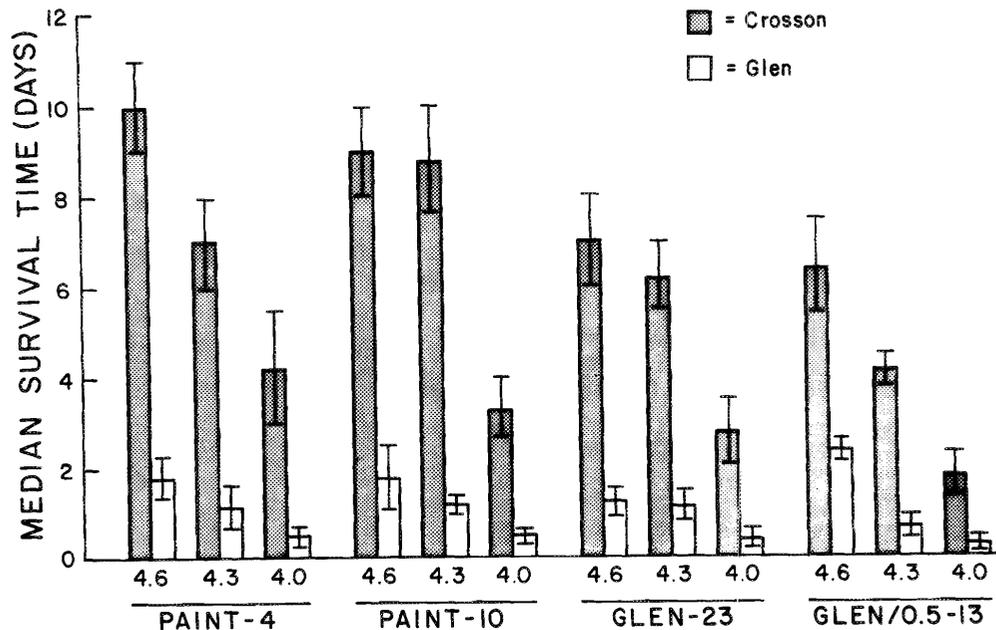


FIG. 4. Median survival times \pm 95% fiducial limits of *H. azteca* from Crosson and Glen lakes exposed to pH 4.6, 4.3, and 4.0 in four different testing media. Paint-4 and Paint-10 = Paint Lake water of 4 and 10 mg $\text{Ca}\cdot\text{L}^{-1}$, respectively; Glen-23 and Glen/0.5-13 = untreated (23 mg $\text{Ca}\cdot\text{L}^{-1}$) and diluted 13 mg $\text{Ca}\cdot\text{L}^{-1}$ Glen Lake water, respectively.

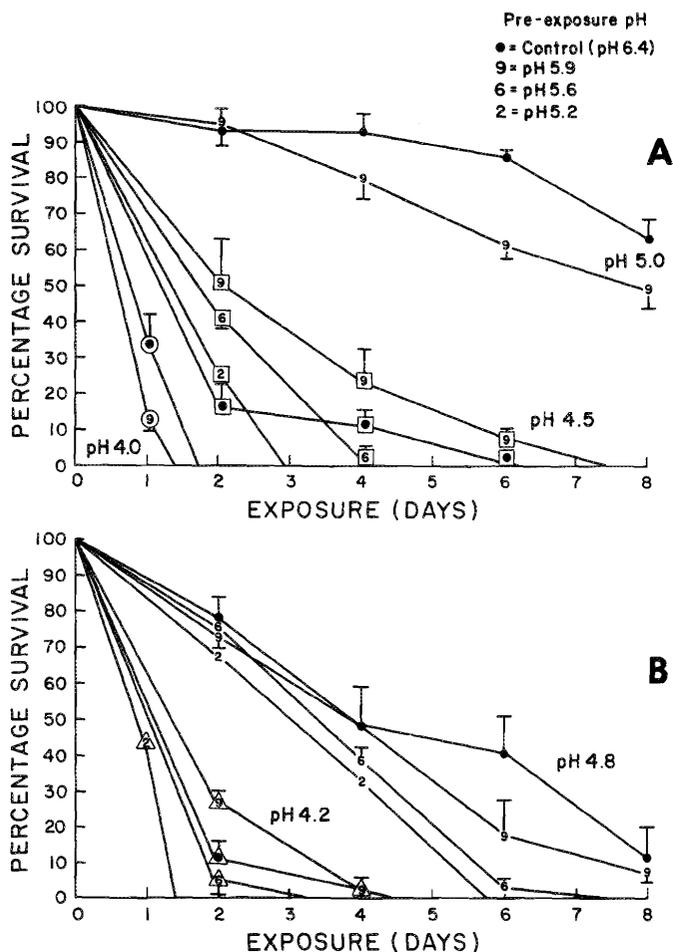


FIG. 5. Survival \pm SE of *H. azteca* from Glen Lake in lethal acidity (pH 5.0, 4.8, 4.5, 4.2, and 4.0) following pretest exposure for 10 d to pH 6.4 (control), 5.9, 5.6, or 5.2. Data are graphed separately only for clarity.

circumneutral pH did not alter resistance or tolerance of *H. azteca* from a low pH lake.

Discussion

Low pH Sensitivity

Our tolerance experiments corroborate synoptic data on amphipod sensitivity to low pH. The asymptotic LC50 ranged from pH 4.32 to 4.95 depending on lake of origin. These values are similar to the 4- and 10-d LC50s of pH 4.4 and 4.5, respectively, determined for *H. azteca* from Harp Lake by Stephenson and Mackie (1986). The LC50 for Glen Lake amphipods increased from pH 4.3 on day 1 to pH 4.7 on day 4 and then further to pH 4.9 on day 8. This was the result of protracted mortality. The ultimate pH tolerance level for *H. azteca* is expected to be slightly higher. For example, Zischke et al. (1983) found that *H. azteca* were almost completely eliminated from an experimental channel acidified to pH 5 over a period of several months. The early spring population of *H. azteca* in Canadian lakes consists entirely of adults overwintering from the previous summer (e.g. Hargrave 1970). At pH 4.2-4.6, which can occur during the spring melt of acid snow in Ontario, the MSTs were between 3 and 23 d. This implies that these animals could be vulnerable to spring pH depressions (France and LaZerte 1987).

The susceptibility of *H. azteca* to toxic levels of acidity is strongly dependent on body size and instar stage. *Hyaella azteca* of 0.30-0.35 mm head length are only one third as tolerant as those 0.80-0.85 mm in size. Similarly, *H. azteca* with 12-14 antennal segments, representing amphipods of about instar stage 3 (L. Richman, University of Toronto, Toronto, Ont., unpubl. data), were one fifth as tolerant as 9th instar adults (22-26 antennal segments). It is not known if this age-specific tolerance of *H. azteca* to low pH is due to processes of physiological maturation or simply to changes in

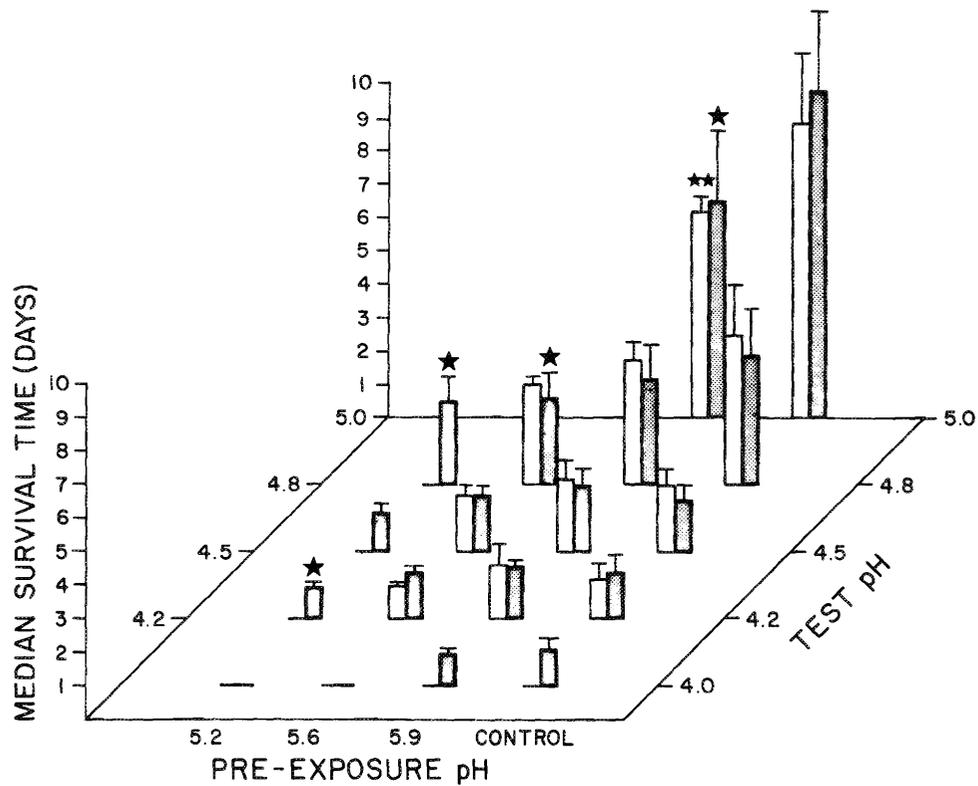


FIG. 6. Median survival times (MST) of amphipods in lethal acidity (test pH 4.0–5.0) following preexposure to pH 5.2, 5.6, 5.9, and 6.4 (control) for 10 d. Open histogram = average MST \pm SE from independently calculated replicate MSTs per treatment; hatched histogram = average MST \pm 95% fiducial limits from combined data per treatment as in Fig. 5. **Significant difference ($p < 0.10$) from control treatment with parametric *t*-test; *significant difference ($p < 0.05$) from control treatment with reaction time ratios.

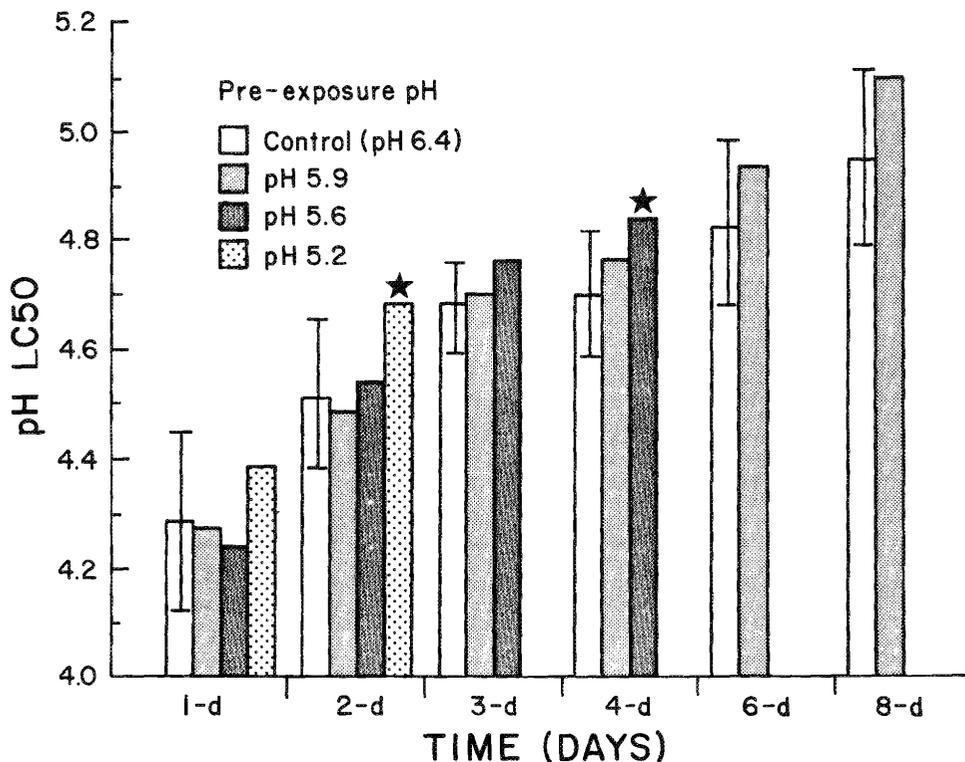


FIG. 7. Low pH LC50 values at six specific times for amphipods exposed to pH 6.4, 5.9, 5.6, or 5.2 for 10 d. Vertical bars represent 95% fiducial limits; *significant difference ($p < 0.05$).

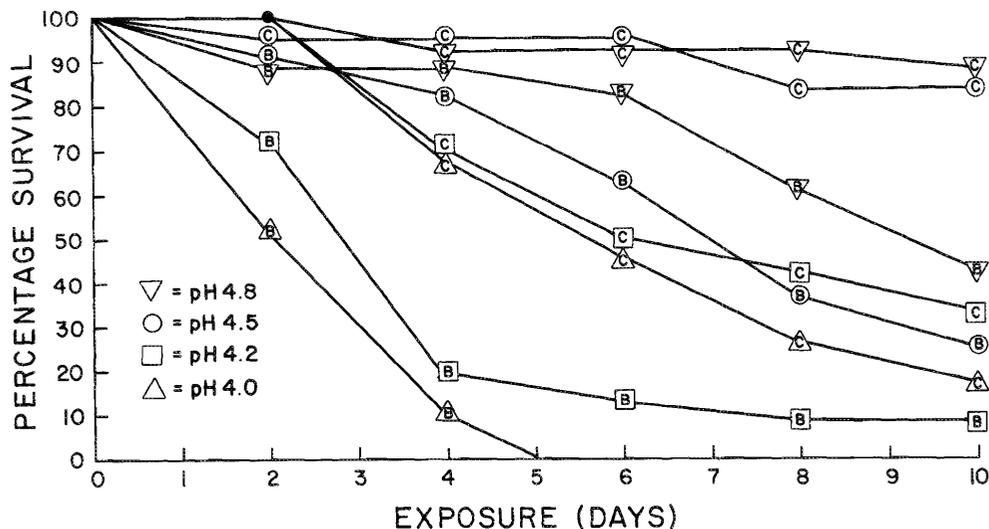


FIG. 8. Comparative survival of *H. azteca* from Crossson (C) and Blue Chalk (B) lakes in lethal acidity after preexposure to control (pH 6.5) water for 10 d. Calculated SES (not shown) were all within $\pm 25\%$ of the mean.

general body size. In common with other crustaceans, H^+ toxicity in amphipods is due to failures in ionic regulation (Meinel et al. 1985). Small organisms have a greater body and gill surface area per unit weight than do larger animals, allowing for a more rapid loss of ions.

The calculated LC50 of pH 5.35 for 0.30–0.35 mm juvenile *H. azteca* indicates that they are almost as sensitive to low pH as *Mysis relicta* (Nero and Schindler 1983). Nilssen et al. (1984) believed that the marked sensitivity of amphipods to low pH was a result of greater body permeability compared with other benthic macroinvertebrates. Individuals in earlier instar stages are probably even more sensitive, as are postmolt animals due to increases in water uptake and body permeability at this time (Lockwood and Andrews 1969). Our laboratory results support Stephenson and Mackie's (1986) contention that absence of *H. azteca* from lakes below about pH 5.6 could be due to direct H^+ toxicity. Pending detailed life history data on *H. azteca* in Crossson and Heney lakes (e.g. calculation of mortality rates), we suggest that these amphipod populations may be experiencing acidification stress. Support for this hypothesis is based on the greater resistance to lethal low pH for *H. azteca* from the more acid lakes (cf. Luoma 1977; France 1987).

Influence of Testing Media on Survival

Rahel (1983) believed that the greater acid tolerance of yellow perch (*Perca flavescens*) from bog lakes could, in part, be a consequence of physiological adaptation to low electrolyte concentrations in those waters ($9\text{--}22 \mu\text{S}\cdot\text{cm}^{-1}$) compared with the more saline non-bog lakes ($49\text{--}73 \mu\text{S}\cdot\text{cm}^{-1}$). The conductivity range of $25\text{--}36 \mu\text{S}\cdot\text{cm}^{-1}$ in the softwater lakes from which *H. azteca* were collected is too narrow to account for the differences in population tolerance observed in this study. Comparison of resistance times for *H. azteca* from Glen Lake ($169 \mu\text{S}\cdot\text{cm}^{-1}$) held in Paint Lake water ($62 \mu\text{S}\cdot\text{cm}^{-1}$) for either 3 or 10 d before testing (Table 3; Fig. 6) indicated no differences ($p > 0.05$). This agrees with Sutcliffe's (1967) observations that amphipods taken from hardwater localities can quickly and easily readjust their Na balance in response to low environmental concentrations. Population differences in

acid tolerance in the present experiments are not due to shifts in chemistry from lake of origin to testing media.

Because failure in ability to restrict rate of Na loss is the primary cause of mortality of amphipods at low pH (Meinel et al. 1985; Sutcliffe and Shaw 1968), it is not surprising that their occurrence in waters near their lower pH tolerance limits is to some extent controlled by the availability of cations (Okland and Okland 1985; Sutcliffe and Carrick 1973b; Schrimpf and Föeckler 1985). Therefore, at low pH, a direct relationship might be expected between the ionic strength of the water and *H. azteca* survival. However, as amphipods have a high Na affinity that is easily saturated at low external concentrations (Sutcliffe 1967), the range in concentrations from $2.5 \text{ mg}\cdot\text{L}^{-1}$ in untreated Paint Lake water to $17 \text{ mg}\cdot\text{L}^{-1}$ in Glen lake water is not expected to influence amphipod survival (cf. Meinel et al. 1985). Further, amphipod survival at low pH appears independent of variation in external Ca concentration (Meinel et al. 1985; R. France and P. Stokes, unpubl. data).

Population Variation in Acid Tolerance

The difficulty in comparing relative sensitivities among animals that differ in their history of acid exposure is in identification of underlying mechanisms of induced tolerance. Such differences in population tolerance could result from (1) acclimatization of individuals over their life span to high toxicant concentrations by preliminary exposure to sublethal concentrations (Guthrie, cited in Flick et al. 1982), (2) selective mortality of less tolerant animals leaving a more resistant subpopulation in which tolerance is not necessarily a strongly heritable trait (Swarts et al. 1978; Tome and Pough 1982; Scholfield, cited in Flick et al. 1982), and (3) actual evolutionary genetic adaptation over many generations (Rahel 1983; Edwards and Gjedrem 1979).

For acclimation to occur (Flick et al. 1982), it is essential that both the magnitude and duration of change in water quality be sufficient to stimulate physiological adjustment without exceeding the survival ability of the organisms. Resistance and tolerance of *H. azteca* to lethal acidity were not increased after 10 d of preexposure to sublethal pHs. Frequently, survival

remained unaltered; occasionally an acclimation-induced reduction in survival, or sensitization, was observed. These results confirm other investigations which have failed to rigorously demonstrate acclimation of fish to low pH (reviewed by Spry et al. 1981). However, Swarts et al. (1978) cautioned that early attempts may have failed because acclimation pH levels were either too high or too low relative to the test pH levels, which in turn may have been unrealistically low. Our choice of three pretest acidities, exposing *H. azteca* to sublethal pH 5.9, intermediate pH 5.6, and toxic pH 5.2 (with testing of survivors), followed by testing at five concentrations within the pH range 4.0–5.0, was deemed adequate to dispel several of the criticisms raised against earlier studies. It is possible, however, that the exposure period of 10 d may have been too brief to allow development of physiological compensation. There have been no comparable attempts to induce low pH acclimation in other crustaceans.

Loss of tolerance on return of organisms to control water is consistent with a physiological mechanism induced by a specific stress, the removal of which makes the original adaptation redundant, and whose retention would be an energetic detriment (Dixon and Sprague 1981). In the present case, exposure for 10 d to control conditions, in contrast, did not eliminate the differences in acid resistance and tolerance of *H. azteca* from acidic Crosson Lake compared with circumneutral Blue Chalk Lake.

The failure to demonstrate physiological plasticity of individual *H. azteca* to low pH infers that differences in sensitivity may result from selective mortality with the implied possibility of genetic adaptation. For example, Rahel (1983) found that neither acclimation of yellow perch from alkaline lakes (pH 7.6–7.8) to sublethal low pH nor transfer of yellow perch from acid lakes (pH 4.5–4.6) to control water removed differences in acid tolerance, suggesting to him a genetic basis for the greater tolerance of fish from the acid lakes. The possible mechanism of genetic divergence in adjacent amphipod populations, the adaptive significance of genetic variability to other environmental stresses, and the implications of these theoretical considerations for the success of *H. azteca* populations in lakes undergoing chemical recovery are discussed in France (1987).

Whatever the precise mechanism for tolerance induction, the importance of these results for design of future experiments is crucial (cf. France 1987). As with behavioral studies (France 1985), caution must always be applied in assessing biological responses to low pH, as these can be expected to be markedly different in relation to the exposure history of the organisms under investigation (e.g. Price and Swift 1985). Researchers should therefore attempt to avoid the use of test organisms raised in high pH environments.

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