Hydrobiologia **184**: 89–97, 1989.

The effects of short-term laboratory pH depressions on molting, mortality and major ion concentrations in the mayflies *Stenonema femoratum* and *Leptophlebia cupida*

Locke Rowe[1], Michael Berrill, Lois Hollett[1] & Ronald J. Hall[2]
Watershed Ecosystems Program, Trent University Peterborough, Ontario, Canada K9J 7B8; Present addresses: [1]Department of Zoology, University of British Columbia Vancouver, British Columbia, Canada V6T 2A9; [2]Dorset Research Centre, Ontario Ministry of Environment, Dorset, Ontario, Canada POA 1EO

Received 31 March 1988; in revised form 30 June 1988; accepted 10 September 1988

Key words: acid, toxicity, mayflies, ion loss

Abstract

Field surveys of the distribution of mayfly nymphs suggest that *Stenonema femoratum* are more acid-sensitive than *Leptophlebia cupida*. To assess whether this apparent difference in sensitivity of nymphs is reflected in differences in the degree of whole-body loss of [Na], [Cl], [Ca] or [K] under laboratory conditions, we exposed nymphs of both species to low pH for 96–192 h in soft water ([Ca] = 0.1 mM). Although mortality and loss of whole-body [Na] and [Cl] occurred in both species at pH 3.5, unexpectedly they were considerably greater in *L. cupida* than in *S. femoratum*. Ion loss was not size related within the range of nymphal weights used (2–14 mg dry wt) for *S. femoratum*. Exposure to the environmentally more common pH 4.5 had no effect on whole-body [Na] and [Cl] or on mortality in either species. However, in *L. cupida*, molting by nymphs increased at both pH 3.5 and 4.5. A decrease in whole-body [Ca] occurred, and the loss of whole-body [Na] and [Ca] at pH 3.5 appeared to cease following the period of molting. In *S. femoratum* no molting or Ca loss occurred and whole-body [Na] and [Cl] decreased between 96 and 192 h exposures.

Introduction

Stream pH depressions commonly occur during spring snowmelt in areas receiving acidic depositions (Dillon *et al.*, 1984) and synoptic surveys of such streams consistently demonstrate a loss of aquatic insect species (particularly mayflies; Hall & Ide, 1987; Kimmel *et al.*, 1985; Mackay & Kersey, 1985; Sutcliffe & Carrick, 1973; Raddum & Fjellheim, 1984). Proposed explanatory hypotheses include reduction of food supplies (Sutcliffe & Carrick, 1973), changes in trophic relationships (Eriksson, 1980) and increased toxicity of mobilized or aqueous metals such as aluminum (Herrmann, 1987). Little quantitative evidence exists to support these hypotheses (Okland & Okland, 1986; Herrmann, 1987).

More often, the loss of these species is attributed to the direct toxicity of H^+, due to a failure to regulate major body ions (Na, Cl, Ca, and K) (Hall *et al.*, 1980; Havas, 1981; Havas &

Hutchinson, 1983; Lechlietner *et al.*, 1985; Okland & Okland, 1986). However, no laboratory studies of acid-sensitive species or comparative studies of species that appear to vary in acid-sensitivity have been undertaken (Mierle *et al.*, 1986). Therefore, we have little indication to what degree toxicity or ion loss is correlated with the observed loss of aquatic insects from streams currently undergoing acidification.

In contrast, the degree of toxicity and whole-body Na and Cl loss during laboratory exposures to low pH is correlated with interspecific differences in the acid sensitivity of some fish, amphibians and crustaceans observed in the field (Fraser & Harvey, 1984; Freda & Dunson, 1984; 1985; Havas & Likens, 1985; Berrill *et al.*, 1985; Hollett *et al.*, 1986). Given that aquatic insects, including mayflies, share similar mechanisms of ionic regulation as these taxa (Shaw & Stobbart, 1963; Komnick, 1977) we should expect greater mortality and whole-body ion loss in species lost from acidified streams than those that are not.

Mayfly species (Ephemeroptera) are particularly suited for examining this response. Although field surveys indicate that mayflies are relatively sensitive to low pH, interspecific variation is considerable (Sutcliffe & Carrick, 1973; Raddum & Fjellheim, 1984). In Haliburton Co., Ontario, where lakes and streams have undergone gradual acidification, the mayfly community was reduced in moderately acidic streams of yearly pH range of 5.0–6.3 and almost eliminated from strongly acidic streams of pH range 4.3–4.8 (MacKay & Kersey, 1985). We selected two species from this region for testing; an acid-sensitive species, *Stenonema femoratum* (Heptageniidae) and an acid-tolerant species *Leptophebia cupida* (Leptophlebidae). *Stenonema* spp. (including *S. femoratum*) occur in circumneutral streams of Haliburton Co. but are absent in moderately acidic streams (pH < 5.3) (MacKay & Kersey, 1985; Hall unpub. data). The loss of *Stenonema* spp has also been correlated with the long-term acidification of a stream in the same area (Hall & Ide, 1987). In contrast, *L. cupida* is abundant in both circumneutral and acidic streams (pH 4.2–4.8; MacKay & Kersey, 1985).

The purpose of the present study is to test the hypothesis that the degree of whole-body Na and Cl loss from nymphs of two mayfly species, exposed to low pH in the laboratory, reflects their relative sensitivity reported in field surveys. We also determine the effect of these short-term exposures on mortality, molting and whole-body concentrations of K and Ca, as well as the relationship between body-size and whole-body Na and Cl conentrations.

Methods

Stenonema femoratum was collected from Thompsons Creek, a small relatively hard water (Ca = 0.95 mM) stream in Peterborough Co. and *L. cupida* from Plastic Lake outflow, a soft water (Ca = 0.05 mM) stream in Halibuton Co. Prior to exposures nymphs were maintained at 15 °C for 24 h in aerated water from which they were collected. Nymphs were then transferred to aerated experimental water (pH 6.5), at the same temperature, for 48 h prior to exposures. Experimental water was collected from two soft water lakes, Lake of Bays and Paint Lake, with similar chemical (mM) composition (Paint Lake: Ca = 0.10, Na = 0.10, Cl = 0.09, K = 0.02, Al = 0.001; Lake of Bays: Ca = 0.10, Na = 0.07, Cl = 0.06, K = 0.02, Al.001). Exposures were carried out in 2.5 L static aerated glass containers, each containing fiberglass screening to which nymphs could cling.

In preliminary experiments whole-body Na and Cl concentrations remained constant in controls from day 0 to day 8, but mortality of nymphs often occurred after 10 days, therefore, exposures were limited to 8 days. Mortality was probably due to starvation and not the static nature of the exposures, since both species commonly occur naturally in static waters (pers. obs.). In these experiments Na and Cl concentrations of *S. femoratum* did not vary between exposures in their home water and the more dilute Paint Lake water. These results indicate that *S. femoratum* was not stressed due to the transfer to Paint Lake water for the following experiments (Rowe, 1986; unpub. data).

To compensate for the short duration of our current experiments, we used pH levels (3.5, 4.5 and 6.5) that encompass, but go below the range of spring pH depressions experienced in Haliburton Co. (Jefferies *et al.*, 1979; La-Zerte, 1984). *S. femoratum* and *L. cupida* nymphs were exposed to one replicate each of pH 3.5, 4.5 and 6.5 (*S. femoratum:* Lake of Bays; *L. cupida:* Paint L.) for both 96 and 192 h. pH of the water, molting and mortality of the nymphs were monitored at least every 24 h. Dead nymphs and molts were recorded and discarded. pH was maintained with 0.1 M H_2SO_4 or 0.1 M KOH and varied no more than $\pm$ 0.2 pH units.

Since neither species grows through the winter (unpub. data), body size of nymphs used in these exposures (*S. femoratum,* 2–14 mg dry wt; L. cupida, 1–2 dry wt) were those that would typically be present during spring pH depressions. Mean body weight of nymphs were not significantly different, within species or between treatments ($p > 0.1$). The wide range in body size (approx. 2–14 mg dry wt) of *S. femoratum* allowed us to assess the relationship between ion concentration and body size, following exposures at the various pH levels.

Following exposure, remaining live nymphs were removed and dried to a constant weight at 60 °C. Analysis of whole-body ion concentrations (Na, Cl, Ca and K) was determined by Instrumental Neutron Activation Analysis. Individual nymphs were palced in capped vials and irradiated for 3–5 minutes, depending on size, at a neutron flux of $5^* 10^{12}$ neutrons/cm^2/sec. Following irradiation, individual nymphs were placed in the tip of a non-irradiated eppendorf pipette with the tip heat sealed. Irradiated samples were counted with an Atec hyper-pure germanium detector coupled to a Canberra Series 90 multichannel analyser with a built in pile up rejection unit. Counting efficiency of the detector was 21% and a FWHM (full width at half maximum) resolution of 2.2 kev for the 1332 kev gamma ray of Co^{60}.

Significance of differences between mean ion concentrations in nymphs of each treatment was established using an analysis of variance. Significance of differences in cumulative molting and mortality rates were established using the Kolmogrov-Smirnov test.

Results

96 h exposures

96 h exposure of S. femoratum to pH 3.5 or 4.5 had no significant effect on mortality (5% at pH 3.5), molting rate (0) and whole-body [Na], [Cl], [K], or [Ca], relative to the control of pH 6.5 ($p > 0.05$, Fig. 1).

In contrast, 96 h exposure of *L. cupida* to low pH resulted in increased molting and mortality and a decrease in whole-body [Na], [Cl] and [Ca]. Molting occurred more often in nymphs at low pH (3.5 and 4.5) relative to those in pH 6.5 ($p < 0.001$), while there was no significant difference between these rates at pH 3.5 and 4.5 ($p > 0.5$, Fig. 2). Exposure to pH 3.5 also resulted in 30% mortality while no mortality occurred in either pH 4.5 or pH 6.5 (Fig. 2). Nymphs surviving 96 h exposure to pH 3.5 had significantly lower whole-body [Na] (23%, $p < 0.005$), [Cl] (31%, $p < 0.005$) and [Ca] (29%, $p < 0.001$) relative to those exposed to pH 6.5 (Fig. 1). The mean loss of whole-body Na (116 μM/g dry wt) was similar to the loss of Cl (109 μM/g dry wt). Nymphs exposed to pH 4.5 had significantly lower whole-body [Ca] (33%, $p < 0.001$), while whole-body [Na] and [Cl] were unchanged relative to those exposed to pH 6.5 (Fig. 1). Whole-body [K] was unaffected by pH ($p > 0.05$, Fig. 1).

192 h exposures:

In contrast to 96 h exposure, S. femoratum nymphs surviving 192 h at pH 3.5 suffered a significant increase in mortality (25%; $p < 0.05$) and decrease in whole-body [Na] (36%; $p < 0.001$) and [Cl] (59%; $p < 0.001$) (Fig. 1). Mortality began only after 104 h exposure. The mean loss of [Na] (90 μM/mg dru wt) was less

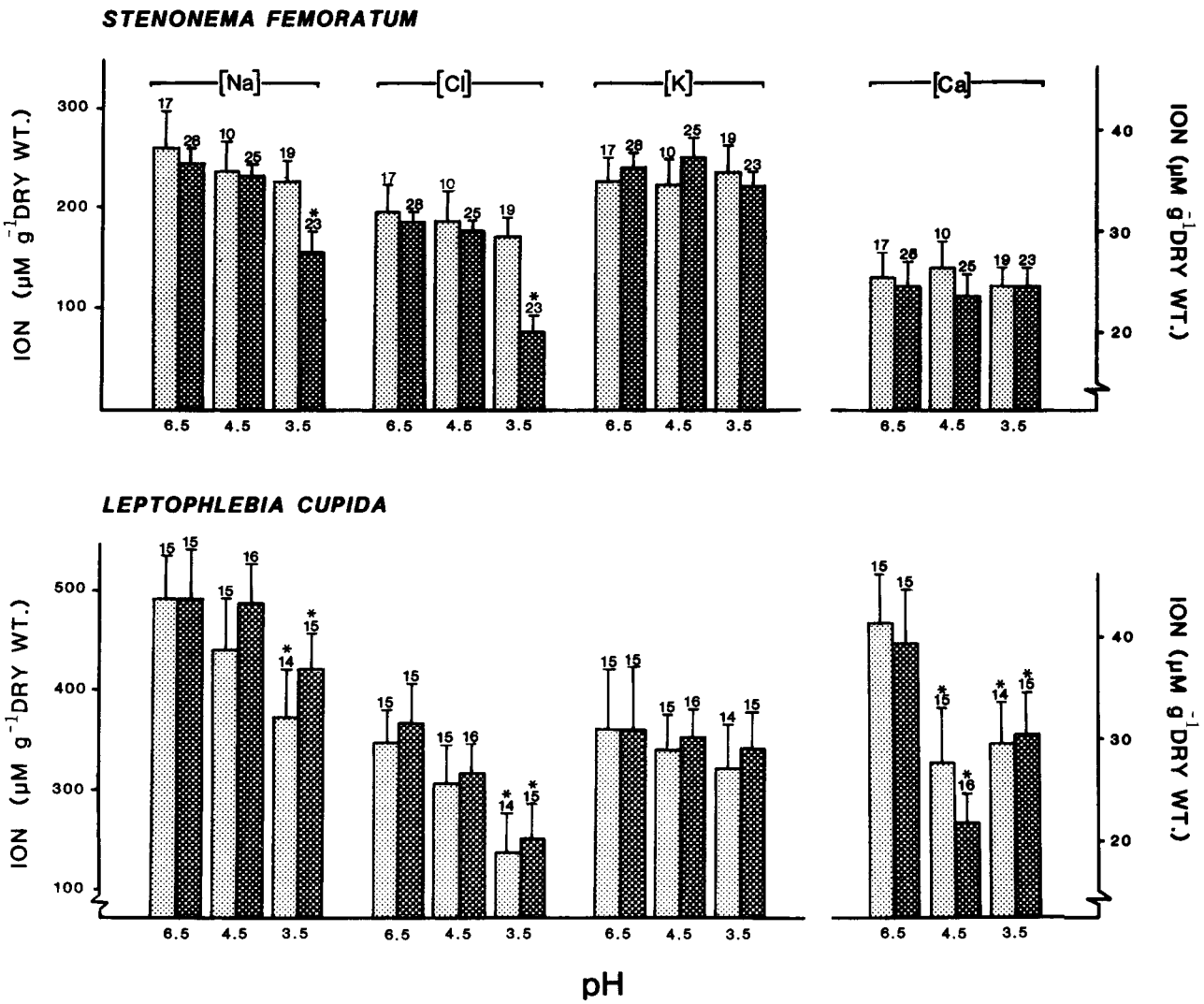

Fig. 1. Whole-body Na, Cl, K and Ca concentrations for nymphs of *S. femoratum* and *L. cupida* following 96 h (light bars) and 192 h (dark bars) exposure to three pH levels. Bars represent mean concentrations ($\pm$95% C.I.) for the number of individuals shown above bar. * indicates a significant difference relative to pH 6.5.

than the loss of [Cl] (110 μM/mg dry wt). Exposure to pH 3.5 had no significant effect on whole-body [K] or [Ca] relative to pH 6.5 ($p > 0.05$) and exposure to pH 4.5 had no significant effect on molting, mortality rate or whole-body ion concentrations relative to pH 6.5 ($p > 0.05$; Fig. 1). Losses of Na and Cl at low pH do not appear to be related to body size; whole-body [Na] and [Cl] of individual nymphs were not significantly correlated to individual body size in control exposures or following Na and Cl losses at pH 3.5. Using the equation [ion] = a + b (dry wt), correlation coefficients (r) were all below 0.20.

Similar to 96 h exposure, 192 h exposure of *L. cupida* to low pH (3.5 and 4.5) again resulted in increased molting ($p < 0.001$) relative to those in pH 6.5, while there was no significant difference between pH 3.5 and 4.5 ($p > 0.05$; Fig. 2). Exposure to pH 3.5 also resulted in a significant increase in mortality (40%; $p < 0.001$) and lower whole-body [Na] (14%; $p < 0.05$), [Cl] (31%; $p < 0.02$) and [Ca] 23%, $p < 0.02$) relative to

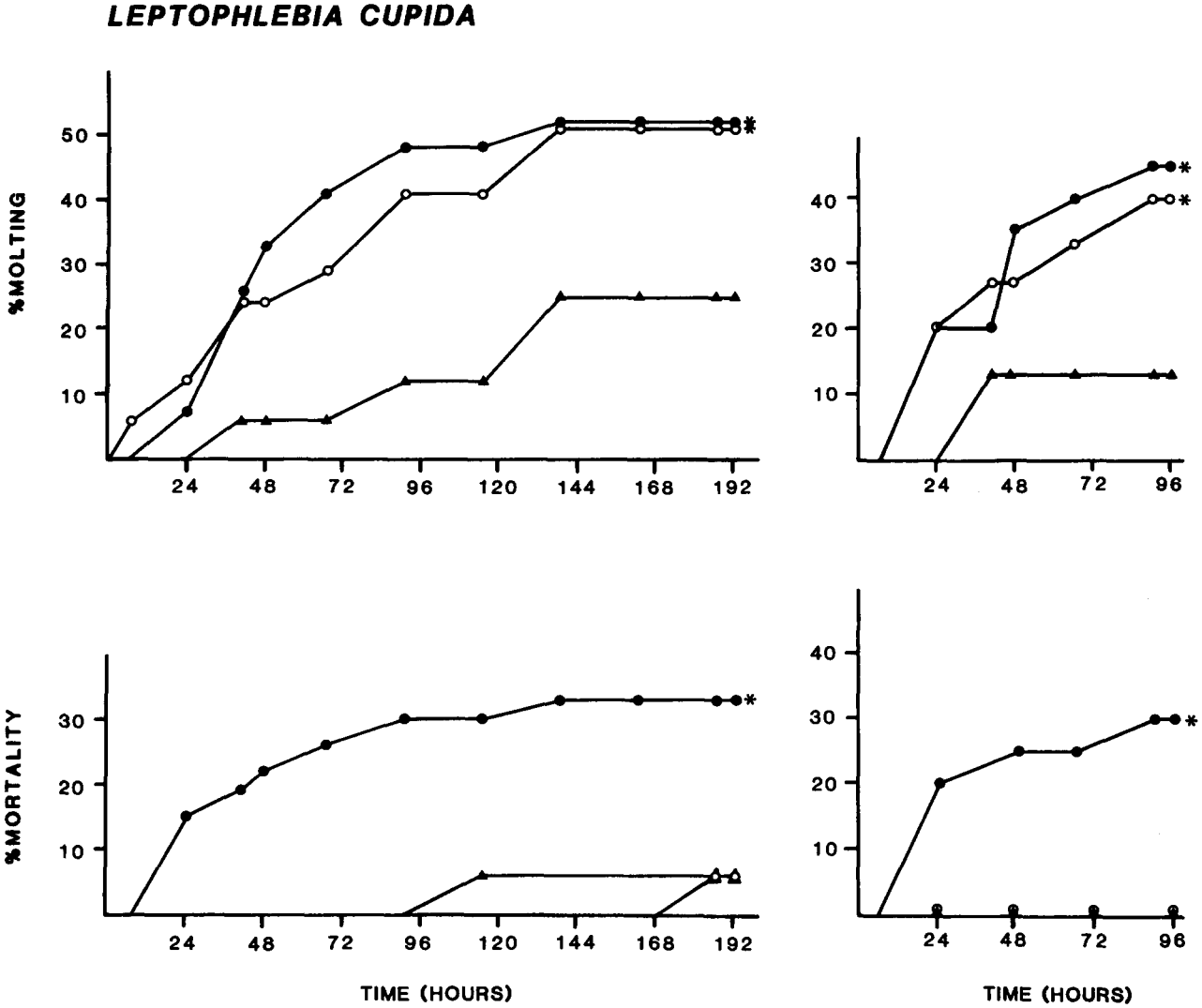

Fig. 2. Cumulative% molting and mortality of *L. cupida* during 96 h and 192 h exposure to pH 6.5 (▲), 4,5 ○) and 3.5 (●). The number exposed in each treatment ranged from 15–27. * indicates a significant difference relative to pH 6.5.

those exposed to pH 6.5 (Fig. 1 & 2). The mean loss of whole-body Na (116 μM/g dry wt) was similar to the loss of Cl (109 μM/g dry wt). Exposure to pH 4.5 resulted in significantly lower whole-body [Ca] (45%, $p < 0.001$), while whole-body [Na] and [Cl] were unchanged relative to those exposed to pH 6.5 (Fig. 1). Whole-body [K] was unaffected by pH ($p > 0.05$; Fig. 2).

Comparison of 96 and 192 hour exposures

Over the first 96 h of the 192 h exposure cumulative molting and mortality was not significantly different then the 96 h exposure at any pH in either species ($p > 0.05$). Similarly, whole-body [Na], [Cl], [Ca] or [K], in either species, were not significantly different ($p > 0.05$) following 96 or 192 h exposure to pH 6.5. Although, mortality and decreases in whole-body [Na] and [Cl] following exposure of *S. femoratum* to pH 3.5

occurred only after 96 h exposure, decreases in whole-body [Na], [Cl] and [Ca] of *L. cupida* nymphs following 192 h exposure were not significantly different ($p > 0.05$) from those exposed to 96 h.

Discussion

Surprisingly, *L. cupida* appears more sensitive to high [H^+] than *S. femoratum* in short-term (96–192 h) exposures, suffering higher mortality and greater decreases in whole-body [Na] and [Cl]: field surveys (MacKay & Kersey 1985; Hall & Ide 1987; unpub. data) had led us to predict the opposite. Nonetheless, where mortality occurred, surviving nymphs of both species suffered significant decreases in whole-body [Na] and [Cl]. Similar losses of whole-body Na and Cl in acid-stressed aquatic insects have been reported elsewhere, although at somewhat lower pH's (pH 2.8–3.0) and were also associated with mortality (Vangenechten & Vanderborght, 1980; Havas & Hutchinson, 1983; Lechleitner *et al.*, 1985). The combination of these toxicity and whole-body ion results, suggest a high degree of tolerance to low pH by aquatic insects relative to some fish (Wood & McDonald, 1982), amphibians (Freda & Dunson, 1984; 1985) and crustaceans (Havas & Hutchinson, 1972; 1983). In these taxa, mortality and acute ion loss typically occur at higher (and more relevant) pH levels (4.5–5.5) in short-term exposures.

Decreases in whole-body [Ca] of *L. cupida* at low pH (3.5 and 4.5) was associated with molting and not with mortality. Similarly, no correlation was found between whole-body [Ca] and mortality at low pH in a midge *(Orthocladius consobrinus)* and a caddisfly *(Limnephilus pallens)* (Havas & Hutchinson, 1983). Whole-body burdens of multivalent cations, such as Ca, in starved aquatic insects appear to be adsorbed on the integument surface and lost to the exuviae following molt (Kormondy, 1965; Smock, 1983; Harvey, 1971).

During acid-stress, the loss of whole-body K in fish and crayfish are generally associated with blood/haemolymph acidosis (Lade & Brown, 1963; Wood & Rogano, 1986). Another indicator of blood/haemolymph acidosis in these taxa is that Na losses exceed those of Cl (Wood & Rogano, 1986). These did not occur in the present study. Instead, no change in [K] was observed and losses of Na and Cl were equimolar or Cl losses were much in excess of Na. Although there was an association between mortality and Na and Cl loss, the actual cause of death is not known, our results suggest that mortality is probably due to ion loss and not haemolymph acidosis.

The increase in molting observed only in *L. cupida* at low pH, has not previously been reported in any taxa. An apparent stabilization of whole-body [Na] and [Cl] in *L. cupida* following molting at pH 3.5, strongly suggest a compensatory mechanism for ion loss. A similar response occured in nymps of the mayfly Callibaetis coloradensis exposed to diluted (1 : 100) media; nymphs molted frequently, had significantly higher densities of chloride cells than animals in control treatments after 15 days. This increase in chloride cell density apparently prevented acute ionoregulatory disruption (Wichard *et al.*, 1973). Increases in chloride cell densities have recently been demonstrated in fish following chronic exposures at low pH (Leino & McCormick, 1984). In contrast to fish, chloride cells of mayflies and other aquatic insect are imbedded in the integument; such insects probably must molt to increase the density of chloride cells and this increase may result in increased Na and Cl uptake rates (Wichard *et al.*, 1973; Komnick, 1977).

Molting may also be a time of increased vulnerability to acid-stress, as well as a mechanism to cope with it. Mortality of the caddisfly *Clistoronia magnifica* was greatest, at low pH, during the molt to be forth instar (van Frankenhuyzen *et al.*, 1985). Significant mortality of another caddisfly, *Lepidostoma liba,* exposed to low pH occurred only during the period of rapid growth and presumably of frequent molting (Burton *et al.*, 1985). In our experiments, molting at pH 3.5 never occurred independently of mortality, suggesting that the causative mechanisms of mortality and molting may be the same, or that mortality may be the result of molting at low pH.

However, mortality of *S. femoratum* exposed to pH 3.5 occurred in the absence of molting, whereas many more nymphs of *L. cupida* molted than died in both pH and 3.5. The relationship between mortality and molting at low pH remains obscure (Rowe *et al.*, 1988a). Future experiments, concentrating on Na and Cl flux rates before and after molting at low pH, should elucidate this phenomenon.

Acid-sensitivity of aquatic insects may vary with body size. A reduction of recruitment success occurreed in an acid-stressed population of the mayfly *Ephemerella funeralis*, perhaps due to the sensitivity of early instars (Fiance, 1978). Similarly, decreased colonization by early relative to late instars occurred in experimentally acidified (pH 4.0) stream channels (Allard & Moreau, 1987). While the apparent sensitivity of these early instars may reflect size-dependent ion loss, as is the case in some fish (Powell & McKeown, 1986), decreases in whole-body [Na] and [Cl] of *S. femoratum* following exposure to pH 3,5 were not size dependent. Therefore, our results do not support the hypothesis that the apparent sensitivity of very early instars in some aquatic insects is due to their high surface to volume ratio (Wiederholm & Eriksson, 1977; van Frankenhuyzen *et al.*, 1985; Correa *et al.*, 1986; 1987). However, we did not stress organisms in the >2.0 mg and these earliest instars may be particularly sensitive, for other reasons (Rowe *et al.*, 1988b).

It is not clear whether the observed loss of whole-body Na and Cl from acid-stressed aquatic insects is due to increased passive loss and/or decreased active uptake of these ions. Surface: volume increases logrithmically with decreasing size, therefore smaller nymphs should suffer much greater ion losses at low pH if these losses were strongly associated with passive loss across the integument; this did not occur in *S. femoratum*. In aquatic Diptera and Hemiptera uptake rates of Na are decreased at low pH (Stobbart, 1971; Wright, 1975; Vangenechten & Vanderborght, 1980) while efflux rates and haemolymph levels may remain constant (Vangenechten & Vanderborght, 1980). On the other hand, equimolar increases in Cl uptake and efflux rates have been reported in aquatic Hemiptera (Vangenechten & Vanderborght, 1980). However, the response of these acid-tolerant Hemipterans is probably not universal to aquatic insects, since they imply whole-body losses of only Na while we report significant whole-body losses of both Na and Cl in *S. femoratum* as have Havas & Hutchinson (1983) in the midge *Orthocladius consobrinus*.

In summary, the degree of mortality and whole-body ion loss following short-term exposures to low pH are not correlated with the sensitivities of *L. cupida* and *S. femoratum* noted in field surveys. Bell (1971) has demonstrated that short-term (96 h) tests of H^+ toxicity to aquatic insects may underestimate long-term (192) tests by as much as 1.0 pH unit. The apparent ability of *L. cupida* to curtail losses of Na and Cl suggests that increased duration of exposures should result in increased mortality only of *S. femoratum*, however, the evidence for this is not strong at present. Both species appear relatively tolerant of low pH in the nymphal stage. Perhaps further tests should concentrate on other life cycle stages such as emergence (Bell, 1971) and hatching (Rowe *et al.*, 1988b) that may be much less tolerant of low pH.

Acknowledgements

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Ontarrio Ministry of Environment. We thank David Lasenby for help throughout the project, Sheldon Lansburger of the McMaster Nuclear Research Centre for his advice on methodology and G. G. E. Scudder for support of the senior author during the preparation of this manuscript. The manuscript was improved by the comments of T. Burton, J. Martin.

References

Allard, M. & G. Moreau, 1987. Effects of experimental acidification on a lotic macroinvertebrate community. Hydrobiologia 144: 37–49.

Bell, H. L., 1971. Effect of low pH on the survival and emergence of aquatic insects. Wat. Res. 5: 313–319.

Berrill, M., L. J. Hollett, A. Morgosian & J. Hudson, 1985. Variation in tolerance to low environmental pH by the crayfish *Orconectes rusticus, O. propinquus* and *Cambaras robustus*. Can. J. Zool. 62: 2360–2363.

Correa, M., R. Coler & C. Yin, 1985. Changes in oxygen consumption and nitrogen metabolism in the dragonfly *Somatochlora cingulata* exposed to aluminium and acid water. Hydrobiologia 121: 151–164.

— & E. Kaufman, 1986. Oxygen consumption and ammonia excretion in the detritivor caddisfly *Limnophilus* sp. exposed to low pH and aluminum. Hydrobiologia 140: 237–241.

Burton, T. M., R. M. Stanford & J. W. Allan, 1985. Acidification effects on stream biota and organic matter processing. Can. J. Fish. aquat. Sci. 42: 669–675.

Dillon, P. J., N. D. Yan & H. H. Harvey, 1984. Acidic deposition: effects on aquatic ecosystems. C.R.C. Crit. Revue Envir. Control 13: 167–194.

Eriksson, M. O. G., L. Henrickson, B.-O. Nilsson, G. Nyman, H. G. Oscarson, & A. E. Stenson, 1980. Predator-prey relations important for the biotic changes in acidified lakes. Ambio 9: 248–249.

Fiance, S. B., 1978. Effects of pH on the biology and distribution of *Ephemerella funeralis* (Ephemeroptera). Oikos 31: 332–339.

Fraser, G. A. & H. H. Harvey, 1984. Effects of environmental pH on the ionic composition of the white sucker *(Catastomus commersoni)* and pumpkinseed *(Lepomis gibbosus)*. Can. J. Zool. 62: 249–259.

Freda, J. & W. A. Dunson, 1985. Field and laboratory studies of ion balance and growth rate of ranid tadpoles chronically exposed to low pH. Copeia 3: 415–423.

—, 1984. Sodium balance of amphibian larvae during acute and chronic exposure to low environmental pH. Physiol. Zool. 57: 435–443.

Hall, R. J. & F. P. Ide, 1987. Evidence of acidification effects on stream insect communities in central Ontario between 1937 and 1985. Can. J. Fish. aquat. Sci. 44: 1652–1657.

—, G. E. Likens, S. B. Fiance & G. R. Hendrey, 1980. Experimental acidification of a stream in the Hubbaard Brook Experimental Forest, New Hampshire. Ecology 61(4): 976–989.

Harvey, R. S., 1971. Temperature effects on the maturation of midges (Tendipida) and their sorption of radionuclides. Health Physics. 20: 613–620.

Havas, M., 1981. Physiological effects of acid stress. In R. Singer (ed.), Effects of Acid Precipitation on Benthos. North American Benthological Society Hamilton N.Y.

— & T. C. Hutchinson, 1982. Aquatic invertebrates from Smiking Hills N.W.T.: effect of pH and metals on mortality. Can. J. Fish. aquat. Sci. 39: 890–903.

—, 1983. Effect of low pH on the chemical composition of aquatic invertebrates from tundra ponds at the Smoking Hills, N.W.T., Canada. Can. J. Zool. 61: 241–249.

Havas, M. & G. E. Likens, 1985. Toxicity of aluminum and hydrogen ions to *Daphnia catawaba, Holopedium gibberum, Chaoborus punctipennis,* and *Chironomus anthrocinus* from Mirror Lake, New Hampshire. Ca. J. Zool. 63: 114–119.

Herrmann, J., 1987. Aluminum impact on freshwater invertebrates at low pH: a review. In Speciation of metals in water, sediment and Soil Systems. Lecture Notes/Springer-Verlag 1–12.

Hollett, L., M. Berrill & L. Rowe, 1986. Variation in major ion concentration of *Cambarus robustus* and *Orconectes rusticus* following exposure to low pH. Can. J. Fish. aquat. Sci. 43: 2040–2044.

Jeffries, K. S., C. M. Cox & P. J. Dillon, 1979. Depression of pH in lakes and streams in Central Ontario during snowmelt. J. Fish. Res. Bd. Can. 36: 640–646.

Kimmel, W. G., D. J. Murphey, W. E. Sharpe & D. R. DeWalle, 1985. Macroinvertebrate community structure and detritus processing rates in two southwestern Pennsylvania streams acidified by atmospheric deposition. Hydrobiologia 124: 97–102.

Komnick, H., 1977. Chloride cells and chloride epithelia of aquatic insects. Int. Revue Cytol. 49: 385–423.

Kormonday, E. J., 1965. Uptake and loss of zinc-65 in the dragonfly *Plathemis lydia*. Limnol. Oceanogr. 10: 427–433.

Lade, R. J. & E. B. Brown, 1963. Movement of potassium between muscle and blood in response to respiratory acidosis. Am. J. Physiol. 204: 761–764.

LaZerte, B. D., 1984. Forms of aqueous aluminum in acidified catchments of central Ontario: a methodological analysis. Can. J. Fish. aquat. Sci. 41: 766–776.

Lechleitner, R. A., D., S. Cherry, J. Cairnes Jr & D. A. Stetler, 1985. Ionoregulatory and toxicological responses of stonefly nymphs (Plecoptera) to acidic and alkaline pH. Arch. envir. Contam. Toxicol. 14: 149–185.

Leino, R. M. & J. H. McCormick, 1984. Morphological and morphometric changes in chloride cells of the gills of *Pimephales promelos* after chronic exposure acid water. Cell. Tissue. Res. 236: 121–128.

Mackay, R. J. & K. E. Kersey, 1985. A preliminary study of aquatic insect communities and leaf decomposition in acids streams near Dorset, Ontario. Hydrobiologia 122: 3–11.

Mierle, G., K. Clark & R. France, 1986. The impact of acidification on aquatic biota in North America: a comparison of field and laboratory results. Wat. Air Soil Pollut. 31: 593–604.

Okland, J. & K. A. Okland, 1986. The effects of acid deppositon on benthic animals in lakes and streams. Experimentia 42: 471–486.

Powell, J. F. F. & B. A. McKeown, 1986. The influence of size on ion regulation and sea-water survival of acid-exposed coho salmon smolts *(Oncorhuncus kisuth)*. Comp. Biochem. Physiol. 85C: 369–373.

Raddum, G. G. & A. Fjellheim, 1984. Acidification and early warning organisms in freshwater in western Norway. Verh. int. Ver. Limnol. 22: 1973–1980.

Rowe, L., 1986. Inter- and intra-specific variation in the physiological response of the mayflies *Stenomema femora-*

tum and *Leptophlebia cupida* to short-term low pH exposures. Thesis. Trent University.

—, M. Berrill & L. Hollett, 1988a. The influence of season and pH on mortality, molting and whole-body ion concentrations in nymphs of the mayfly *Stenonema femoratum*. Comp. Biochem. Physiol.: 90A: 405–408.

—, J. Hudson & M. Berrill, 1988b. Hatching success of mayfly eggs at low pH. Can. J. Fish. aquat. Sci. 45: 1649–1652.

Shaw, J. & R. H. Stobbart, 1963. Osmotic and ionic regulation in insects, p. 315–399. In: J. W. L. Beament, J. E. Treherne & V. B. Wigglesworth (eds.), Advances in Insect Physiology, Vol. 1, Academic Press, New York.

Smoch, L. A., 1983. Relationships between metal concentration and organism size in aquatic insects. Freshwat. Biol. 13: 313–321.

Stobbart, R. H., 1971. Evidence for Na^+/H^+ and Cl-HCO exchanges during undependent sodium and chloride uptake by the larva of the mosquito *Aedes aegyptes* (L.). J. exp. Biol. 54: 19–27.

Sutcliffe, D. W. & T. R. Carrick, 1973. Studies on mountain streams in the English Lake District I. pH, calcium and the distribution of invertebrates in the River Dudden. Freshwat. Biol. 3: 437–462.

van Frankenhuyzen, K., G. H. Geen & C. Koivisto, 1985. Direct and indirect effects of low pH on the transformation of detrital energy by the shredding caddisfly, *Clistoronia magnifica* (Banks) Limnephilidae. Can. J. Zool. 63: 2298–2304.

Vangenechten, J. H. D. & O. L. J. Vanderborght, 1980. Effect of acid pH on sodium and chloride balance in an inhabitant of acid freshwaters: the waterbug *Corixa punctata* (Illig.) (Insecta, Hemiptera), p. 342–343. In: D. Drablos & A. Tollan (eds.), Ecological Impact of Acid Precipitation. SNSF Project, Sandfjord, Norway.

Wiederholm, T. & L. Eriksson, 1977. Benthos of an acid lake. Oikos 29: 261–267.

Wichard, W., P. T. P. Tsui & H. Komnick, 1973. Effect of different salinities on the coniform chloride cells of mayfly larvae. J. Insect Physiol. 19: 1825–1835.

Wood, C. M. & M. S. Rogano 1986. Physiological responses to acid stress in crayfish *(Orconectes):* haemolymph ions, acid-base status, and exchanges with the environment. Can. J. Fish. aquat. Sci. 43: 1017–1026.

-- & D. G. McDonald, 1982. Physiological mechanisms of acid toxicity to fish, p. 197–226. In: R. E. Johnson (ed.), Acid rain/fisheries. Proceedings of an International Symposium on Acidic Precipitation and Fishery Impacts in North-Eastern North America. American Fisheries Society, Bethesda, MD.

Wright, D. A., 1975. The effect of external sodium concentration upon sodium fluxes in *Chironomus dorsalis* and *Camptochironomus tentans*, and the effect of other ions on sodium influx in *C. tentans*, J. exp. Biol. 62: 141–155.