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THE INFLUENCE OF SEASON AND pH ON MORTALITY, MOLTING AND WHOLE-BODY ION CONCENTRATIONS IN NYMPHS OF THE MAYFLY *STENONEMA FEMORATUM*

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Abstract—1. Using laboratory exposures (pH 3.5 and 6.5) and instrument neutron activation analysis, the seasonal variation in whole-body ion concentration, mortality and molting of *Stenonema femoratum* were investigated.

2. Winter nymphs (dormant) had significantly lower Na and Cl than other times in the season (growth phase) at both pH levels.

3. Na and Cl levels were consistently lower in nymphs exposed to pH 3.5 relative to pH 6.5.

4. Mortality and molting were associated with ion loss during acid stress and were highest in growth phase nymphs.

5. K levels did not vary with season or pH.

INTRODUCTION

A loss of aquatic insects species from streams currently undergoing acidification has been well documented in eastern N. America, Scandanavia and Britain (Dillon *et al.*, 1984; Okland and Okland, 1986). In such streams, pH depressions of more than one unit typically occur during the few weeks of spring snowmelt and more mild depression may occur in late autumn (Haapla *et al.*, 1976; Jefferies *et al.*, 1979). Given this seasonal variation in stream pH, it is surprising that little is known about the influence of season on the sensitivity of aquatic insects to low pH.

A failure to regulate haemolymph Na and Cl levels appears to be the principal cause of death in acid-stressed fish and amphibians (Wood and McDonald, 1982; Freda and Dunson, 1984). Several studies of fish have demonstrated seasonal variation in acid sensitivity (Falk and Dunson, 1977), blood and muscle ion concentrations and the degree of ion loss during acid stress (Stuart and Morris, 1985). Disruption of ionic regulation in acid-stressed insects has also been indicated by a loss of whole-body Na and Cl and a correlated mortality (Vangenechten and Vanderborght, 1980; Havas and Hutchinson, 1983; Lechlietner *et al.*, 1985; Rowe, 1987). Seasonal variation in ion loss and mortality have not been reported for acid-stressed aquatic insects.

In the present study, we report on seasonal variation in the whole-body ion concentration (Na, Cl and K), (b) mortality and (c) molting of an aquatic insect under two conditions; following short-term exposure to either a circumneutral (pH 6.5) or low pH (pH 3.5) soft water.

METHODS AND MATERIALS

Test organism

The mayfly *Stenonema femoratum* (Ephemeroptera: Heptageniidae) was chosen as a test organism. This species and its congeners are lost from low pH (< 5.3) streams, but are common in circumneutral steams within south-central Ontario, the region of this study (Mackay and Kersey, 1985; Hall and Ide, 1987; unpublished data). As part of a concurrent study (Rowe and Berrill, 1988) we documented the life cycle of *S. femoratum*. *S. femoratum* had a largely univoltine life cycle, overwintering in the nymphal stage and emerging to adults from May through to August. Eggs hatched from June to August. Growth occurred after spring warming, near early May and continued through to October. No growth or development occurred during winter, therefore, winter nymphs are referred to as dormant. Due to the long emergence and therefore oviposition period, most nymphal size classes were present throughout the year. In the region of this study, spring pH depressions commonly occur during the near zero temperatures around March (Jefferies *et al.*, 1979; Lazerte, 1984), when the dormant phase of *S. femoratum* is present. Emergence and egg hatching, two stages that may be particularly acid-sensitive (Bell, 1971; Rowe *et al.*, submitted), occur after the time of spring pH depressions.

Laboratory procedures

We conducted replicate experiments on five dates (12/10/84, 3/12/84, 11/3/85, 27/3/85, 17/5/85 and 16/6/85). Nymphs were in the dormant phase during the December and March exposures, and in the growth phase during the remaining dates. Nymphs used for experiments were collected from Thompsons Ck (pH 7.2–8.0) and transported directly to the laboratory. Immediately after collection, nymphs were brought to 15°C over 24 hr in aerated Thompsons Ck water and then transferred to aerated experimental water (pH 6.5; 15°C) for 48 hr prior to experimental exposures. Following this period, nymphs (10–30) were exposed to either an acid (pH 3.5) or circumneutral (pH 6.5) treatment for 96 hr. Only a pH 6.5 exposure was conducted on the October date.

Acclimation and exposures were carried out in 2.5-L static aerated glass containers, each containing fibreglass screening to which nymphs could cling. Decarbonated water from one of two softwater lakes of similar chemical composition (mM/l) was used in exposures: Lake of Bays (Ca = 0.10, Na = 0.07, Cl = 0.06, K = 0.02) and Paint Lake (Ca = 0.10, Na = 0.10, Cl = 0.09, K = 0.02). The former

was used in the October and December dates and the latter for all other dates. During exposures mortality and molting were monitored at least twice a day and dead nymphs and exuviae were removed. Following exposure, remaining live nymphs were removed and dried to a constant weight at 60°C.

The transfer of nymphs from their home water to the experimental water (pH 6.5) does not significantly effect whole-body [Na] and [Cl] following 96-hr exposure (Rowe, 1987, unpublished data), indicating that the pH 6.5 treatment can be referred to as a control. Individual dry weights of nymphs used in these experiments ranged from 2–15 mg; however, mean body weight did not vary between each experiment. Furthermore, earlier experiments have demonstrated that whole-body Na and Cl levels, and the loss of these ions during acid-stress were not correlated with individual dry weight (Rowe, 1988). Therefore, the effects observed in these experiments are independent of body size.

Ion analysis

Analysis of whole-body ion concentrations was determined by instrumental neutron activation analysis. Individual nymphs were placed in capped vials and irradiated for 3–5 min, 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 Attec 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 RWHM (Full width at half maximum) resolution of 2.2 keV for the 1332 keV gamma ray of Co^{60}.

Statistical analysis

Seasonal effects on mean ion concentrations of nymphs exposed to either control or low pH treatments were established using a one-way analysis of variance. Differences in mean ion concentrations in nymphs exposed to control or low pH on a given date were established with Student's *t*-test.

RESULTS

Whole-body [Na] and [Cl] varied significantly ($P < 0.001$) with season following 96 hr exposure in the control (pH 6.5) treatment (Fig. 1a, b). Concentrations of these ions in nymphs were lowest in dormant nymphs (December–March). Minimum [Na] (238 μM/g) and [Cl] (179 μM/g) occurred on 27 March, while maximum [Na] (432 μg) occurred on 16 July and [Cl] (303 μM/g) on 17 May. The difference between these maxima and minima are 45 and 41%, respectively.

In nymphs exposed to low pH, Na and Cl levels also varied significantly ($P < 0.01$) with season and minimum and maximum levels occurred at the same time as those of nymphs in control treatments (Fig. 1a, b). Moreover, concentrations of these ions were consistently lower following exposure to low pH relative to the controls. The greatest decreases in [Na] and [Cl] occurred in growth phase nymphs. For example, on 27 March decreases were small (Na = 25 μM/g, 11%; Cl = 35 μM/g, 18%), while on 17 May decreases were larger (Na = 88 μM/g, 22%; Cl = 61 μM/g, 20%).

Whole-body [K] ranged from 230–271 μM/g, but did not vary significantly ($P > 0.05$) with season or pH (Fig. 1c).

Molting and mortality rates vary with season of exposure and are generally increased at low pH (Fig. 1d, e). Mortality never occurred in the control treatments. At pH 3.5, mortality was low in the winter exposures (0–6%) relative to spring and summer exposures (13 and 22%). Molting rates were more variable. In the control pH, nymphs did not molt during the dormant phase, but did molt during the growth phase (October, 5%; May, 5%; July, 13%). At pH 3.5, molting rates were low in dormant nymphs (0–5%) and high in growing nymphs (28–42%). Therefore, both molting and mortality rates at low pH are highest when ion loss has occurred.

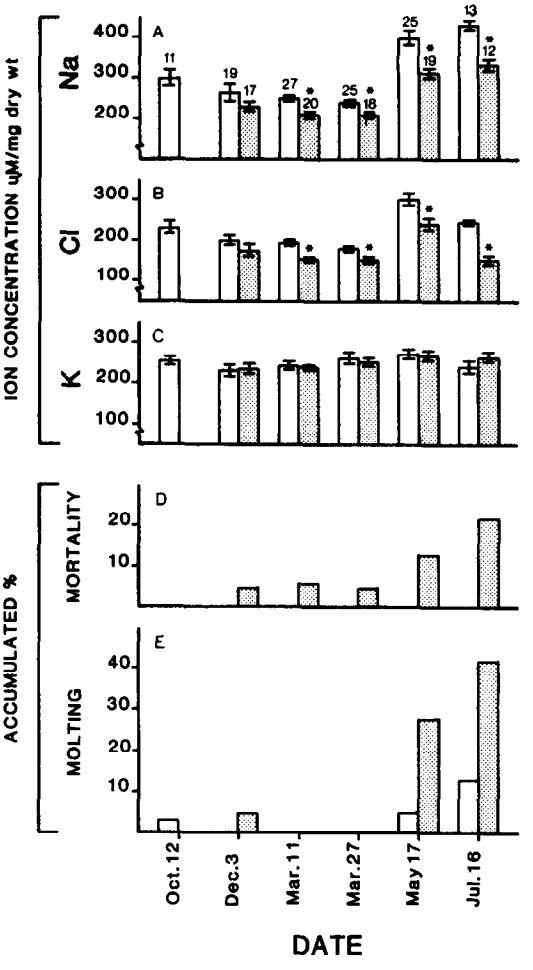

Fig. 1. Mean (±SE) whole body (A) Na, (B) Cl and (C) K concentrations, and percent (D) mortality and (E) molting following 96-hr exposure to pH 6.5 (clear bars) or pH 3.5 (shaded bars) on 5 dates. Asterisks indicate significant difference (*t*-test, $P < 0.05$) relative to pH 6.5. Number of nymphs used for calculating mean ion concentrations are given above each bar in (A).

DISCUSSION

Seasonal variation in whole-body ion levels

This study demonstrates a strong seasonal influence on whole-body Na and Cl concentrations in *S. femoratum*, that is independent of body size. The magnitude of variation was great (Na = 45%, Cl = 36%), with the lowest values occurring in dormant winter nymphs. Vangenechten *et al.* (1979) also

reported a reduction of whole-body Na/wet wt in two corixids (14 and 30%), collected in winter relative to those in spring. In contrast to Vangenechten *et al.* we measured concentrations in dry animals, therefore, changes in ion levels may reflect changes in haemolymph concentrations and/or haemolymph volume.

Ion levels of *S. femoratum* may be related to the growth cycle of this mayfly, with the lowest Na and Cl levels occurring in winter during the time of no growth. Although all nymphs were starved 72 hr previous to and during these experiments, dormant winter animals were presumably not feeding for some time before the experiments. Stobbart (1959, 1965) found that starvation of the mosquito larva *Aedes aegypti* resulted in shrinkage of the anal papillae, a five-fold decrease in Na exchange rates at these papillae and a reduction in haemolymph [Na]. Herzog (1987) also reports a decrease in haemolymph ion levels (Na and Cl) and an apparent decrease in haemolymph volume following long-term (10–50 days) starvation of larval *Aeshna cyanea*. The combined effect of reduced ion levels and haemolymph volume may be a mechanism to reduce the energy expended on active uptake of such ions (Potts, 1954; Herzog, 1987). We measured whole-body ion levels as a function of dry weight and, therefore, cannot assign losses to a given compartment. Nonetheless, we suspect that both reduced haemolymph volume and ion levels must have occurred, to explain the very large decrease in the ion levels of dormant nymphs.

Whole-body [K] was remarkably constant throughout the season (Fig. 1). K levels in aquatic insect haemolymph are typically a minor constituent of the haemolymph ion burden and only a small fraction of whole-body K is contained in the haemolymph (Shaw and Stobbart, 1963). Aquatic insects appear able to maintain constant haemolymph K levels even when external levels are varied more than an order of magnitude (Ramsay, 1953; Nicholls, 1983).

Effects of low pH on whole-body ion levels and mortality

Whole-body [Na] and [Cl] are consistently lower in nymphs exposed to pH 3.5 relative to those exposed to pH 6.5, and mortality is associated with this ion loss. Our results are consistent with other studies of acid-stressed aquatic insects (Vangenechten and Vanderborght, 1980; Havas and Hutchinson, 1983; Lechlietner *et al.*, 1985; Rowe, 1987). In all of these tests acute ion loss occurred only at extremely low pH levels (2.8–3.5) and short-term exposure of *S. femoratum* to pH 4.5 had no effect on whole-body [Na] or [Cl] (Rowe, 1987). In contrast, acute ion loss occurs at higher pH levels (4.5–5.5) in fish, amphibians and crustaceans (Wood and McDonald, 1982; Freda, 1986; Havas and Hutchinson, 1982, 1983). Aquatic insects appear similar to these other taxa in their response to acid stress, but are more tolerant.

Whole-body [K] was unaffected by exposure to low pH, indicating that haemolymph acidosis is not occurring here. Losses of K are typical of fish and crayfish suffering haemolymph acidosis (Lade and Brown, 1963; Wood and Rogano, 1986).

Ion loss and mortality at low pH varied with season. This strong seasonal variation in acid sensitivity has not previously been reported in an aquatic insect. Seasonal effects appear to be intimately related to, and of greater magnitude than, those due to treatment pH. During their growth phase (May–July) stressed nymphs have relatively high ion concentrations, and in acid-stressed nymphs the rates of ion loss and mortality are also relatively high. The converse is true in dormant (December–March) nymphs. It is in the latter period of this dormancy that pH depressions commonly occur, therefore, our short-term exposures suggest that *S. femoratum* will be relatively tolerant during the period of lowest pH.

Effects of low pH on molting

Molting of *S. femoratum* varied with both season and pH. In the control pH, molting occurred only during the growth phase and is correlated with high Na and Cl levels in the nymphs. Molting of a congener, *S. vicarium*, has also been reported to vary with season, independently of experimental conditions (Webb and Merritt, 1987).

When molting occurred, the rate was much higher in the low pH treatments and was associated with mortality and large losses of Na and Cl. These results are similar to those reported for another mayfly, *Leptophlebia cupida*, during acid stress (Rowe 1987). However, molting is probably a mechanism to inhibit ion loss and not a cause of mortality. Both of these mayflies respond with high rates of molting at higher pH levels (pH 4.5) without suffering mortality (Rowe, 1987). Furthermore, ion loss appears to cease by the eighth day of exposure if molting has occurred (Rowe 1987, unpublished data). Chloride cells of most aquatic insects are embedded in the integument (Komnick, 1977) and such insects may molt to increase the number of chloride cells during ionic stress (Wichard *et al.*, 1973). Increases in the chloride cell density during chronic acid stress, have recently been demonstrated in the grills of fish (Leino and McCormick, 1984). If *S. femoratum* is able to compensate for ion loss by molting, then longer term experiments may demonstrate that nymphs are less sensitive during the growth phase, in contrast to the short term exposures reported here.

CONCLUSIONS

Whole-body [Na] and [Cl], molting rate and mortality rate vary with season in both acid stressed and unstressed nymphs. This variation appears to be related to the growth cycle and is independent of body-size and experimental temperature. At the time of year when severe pH depressions are common, *S. femoratum* is in a non-growing dormant phase and is tolerant of short term (4-day) exposure to low pH (pH 3.5) relative to growing nymphs. Ion loss, mortality and molting are greatest during the growth phase. Low pH-induced molting may be a compensatory mechanism for ion loss. Winter dormancy is common in aquatic insects, therefore, furture experiments concerning acid stress in such insects should consider the possibility of a strong seasonal effect on acid sensitivity.

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