NOTES

Hatching Success of Mayfly Eggs at Low pH

Locke Rowe[1], Jeffrey Hudson,[2] and Michael Berrill

Watershed Ecosystems Program, Trent University, Peterborough, Ont. K9J 7B8

Rowe, L., J. Hudson, and M. Berrill. 1988. Hatching success of mayfly eggs at low pH. Can. J. Fish Aquat. Sci. 45: 1649–1652.

Eggs of the mayflies *Leptophlebia cupida*, *Habrophlebia vibrans*, *Stenonema femoratum*, and *Baetis flavistriga* were reared in soft water at several pH levels (4.0, 4.5, 5.0, and 6.5) in the laboratory. The proportion of eggs undergoing eclosion did not vary with pH. However, in *B. flavistriga*, significant mortality of nymphs occurred before the hatch was complete at pH 4.0 (91%) and 4.5 (12%). Hatching rate of *H. vibrans* was significantly retarded at pH 4.0, 4.5, and especially at pH 5.0, but was unaffected in the other three species. The effects of elevated $[H^+]$ on these species are in agreement with their relative acid sensitivity inferred from field surveys.

Les oeufs des éphémères *Leptophlebia cupida*, *Habrophlebia vibrans*, *Stenonema femoratum* et *Baetis flavistriga* ont été élevés en laboratoire dans de l'eau douce à différents pH (4,0, 4,5, 5,0 et 6,5). La proportion d'oeufs qui ont éclos ne variait pas avec le pH. Toutefois, chez *B. flavistriga*, on a observé une importante mortalité des nymphes avant l'éclosion complète lorsque le pH était de 4,0 (91 %) et de 4,5 (12 %). L'éclosion chez *H. vibrans* était significativement retardée à pH 4,0, 4,5 et surtout à pH 5,0, mais elle n'était pas modifiée chez les trois autres espèces. Les effets d'une concentration élevée d'ions H^+ chez ces espèces sont conformes à leur sensibilité à l'acide montrée par des études sur le terrain.

Received March 24, 1987
Accepted May 25, 1988
(J9190)

Reçu le 24 mars 1987
Accepté le 25 mai 1988

A reduction in abundance and diverity of aquatic insects with decreasing pH has been well documented (Okland and Okland 1988). Fiance (1978) observed a decrease in the recruitment success of the mayfly *Ephemerella funeralis*, following the experimental acidification of a stream, which he attributed to a particular sensitivity of eggs or very early instars. However, little is known about the sensitivity of aquatic insect eggs to elevated concentrations of hydrogen ion ($[H^+]$). Studies of insect egg hatching success at low pH have thus far been limited to species that are known to be acid tolerant. van Frankenhuyzen et al. (1985) found no change in development rate or hatching success in eggs of the caddisfly *Clistoronia magnifica* at pH 6.1 relative to pH 8.1. Similar results were reported for development time and hatching success of four Odonata species at pH 3.5 (Hudson and Berrill 1986). In contrast, mayflies are generally considered sensitive to elevated $[H^+]$ (MacKay and Kersey 1985; Okland and Okland 1986), but the sensitivity of the egg stage is unknown.

To test the prediction that mayfly eggs are sensitive to elevated $[H^+]$, we measured the proportion of total hatch, the proportion of incomplete hatch, and the hatching rate of four mayfly species in soft water at various pH levels. The selected species (*Leptophlebia cupida*, *Habrophlebia vibrans*, *Stenonema femoratum*, and *Baetis flavistriga*) occur in softwater streams on the Precambrian Shield in the Haliburton region of south-central Ontario and were suspected to have various acid sensitivities.

Methods

Species and collections — Synoptic surveys of streams in Haliburton and elsewhere demonstrate that populations of *L. cupida* occur in acidic ($pH < 5.0$) and less acidic ($pH > 5.0$) streams whereas *S. femoratum*, *H. vibrans*, and *B. flavistriga* are present only in the latter (Hall and Ide 1987; R. J. Hall, Dorset, Ont., unpubl. data).

With the exception of *S. femoratum*, all eggs used in this study were collected in the field and all four collection sites mentioned below are located in south-central Ontario. *Leptophlebia cupida* that were ovipositing on the surface of Plastic Lake outflow (pH 4.9–5.9; LaZerte 1984) were collected on May 6, 1986, and placed in petri dishes where they then oviposited. *Habrophebia vibrans* were collected in a similar fashion from Costello Creek (pH 6.2–6.5; Hall and Ide 1987) on June 13, 1986. To collect *B. flavistriga* eggs, which are oviposited on underwater surfaces, we placed clean rocks in areas of Jacksons Creek (pH 7.2–8.0) where females had been

[1]Present address: Department of Zoology, University of British Columbia, Vancouver, B.C. V6T 2A9.

[2]Present address: Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1.

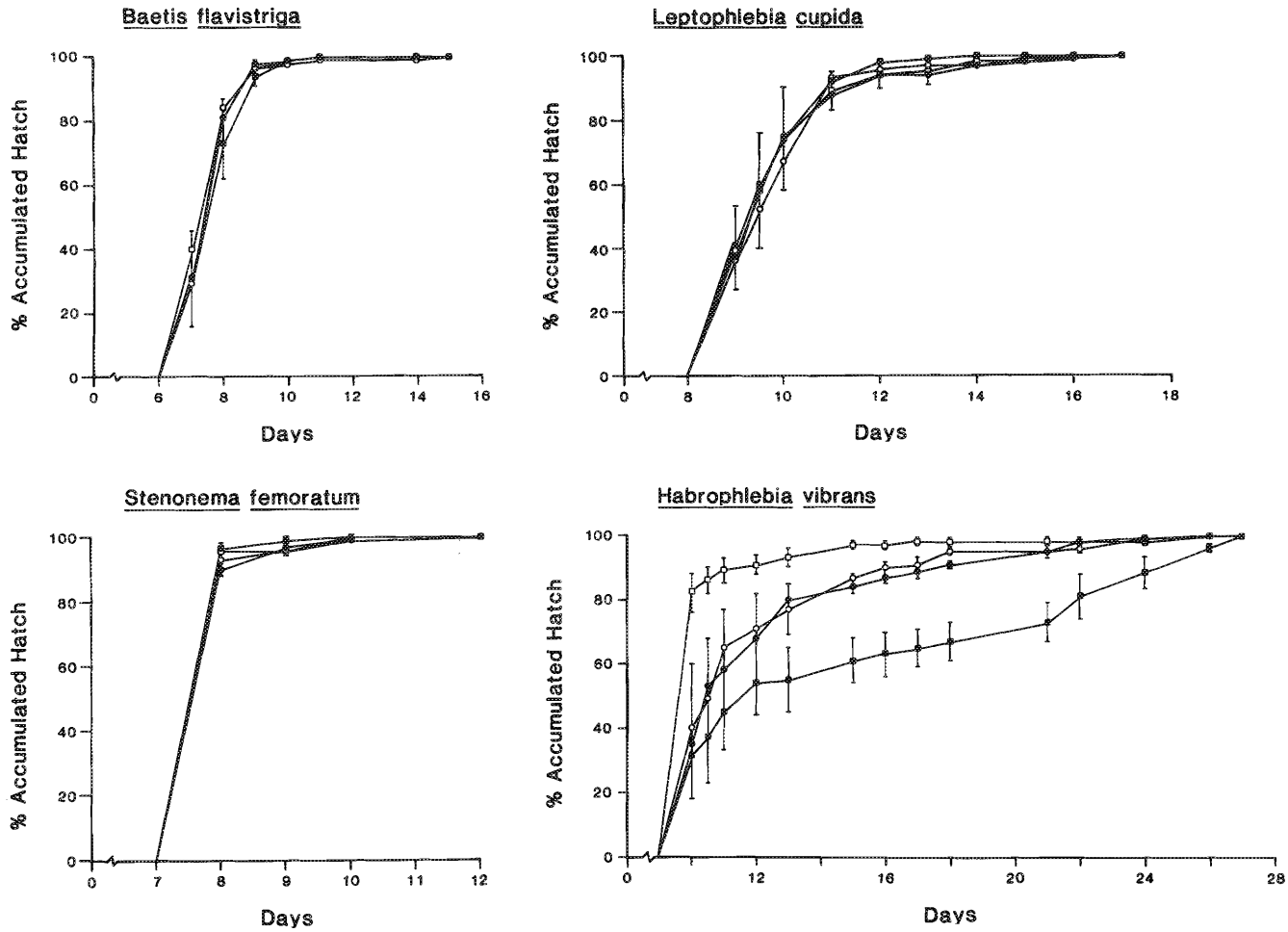

FIG. 1. Mean (± SE) proportion of total hatch accumulated over time for eggs of four mayfly species exposed to pH 4.0 (●), 4.5 (○), 5.0 (■), and 6.5 (□).

TABLE 1. Mean (SE) proportion of total hatch (first row), mean (SE) proportion of total hatch that was incomplete (second row), and total number of eggs exposed at each pH (third row).

Species	pH 4.0	pH 4.5	pH 5.0	pH 6.5
L. cupida	0.86 (0.036)	0.90 (0.014)	0.78 (0.044)	0.84 (0.046)
	0.00	0.00	0.00	0.00
	289	304	233	240
H. vibrans	0.89 (0.030)	0.88 (0.033)	0.70 (0.107)	0.89 (0.020)
	0.00	0.00	0.00	0.00
	481	352	410	388
S. femoratum	0.94 (0.015)	0.97 (0.006)	0.94 (0.015)	0.90 (0.015)
	0.03 (0.016)	0.01 (0.006)	0.00	0.00
	258	433	411	437
B. flavistriga	0.80 (0.040)	0.90 (0.040)	0.83 (0.042)	0.85 (0.060)
	0.91 (0.018)	0.12 (0.013)	0.01 (0.003)	0.00
	478	528	503	613

observed ovipositing. The following day (June 23, 1986), ovipositing females and their eggs were collected from these rocks. Final intar *S. femoratum* were collected in late May from Thompsons' Creek (pH 7.2–8.0) and reared to imago in the laboratory. Imagoes were then force copulated (Huff and McCafferty 1974) on May 23, 1986, and these inseminated females were placed in a petri dish to oviposit.

Exposures — Water for the exposures was collected from Paint Lake in Haliburton Co., filtered (1 μm), and decarbonated prior to use. The chemical composition (millimolar) of this water was Ca^{2+} = 0.10, Na^{+} = 0.10, Cl^{-} = 0.09, K^{+} = 0.02, Mg^{2+} = 0.06, SO_4^{2-} = 0.08, and Al = 0.001. Experimental treatments were pH 4.0, 4.5, 5.0, and 6.5 which were established and maintained with 0.1 M H_2SO_4 and 0.1 M

KOH. pH varied no more than 0.2 unit over the exposure period. Exposures were conducted in modified petri dishes partially submerged in 300-mL fingerbowls (J. Hudson, unpubl. data). The petri dish was fitted with a Plexiglas chamber that fills with water through an egg-retaining mesh at its base. Eggs rested on this 20-μm nitex mesh so that aerated water from the fingerbowl could circulate over them. Four replicates at each pH were used, for a total of 16 fingerbowls for each species. The exposures were conducted at room temperature (21–26°C) under 15 h/d incandescent light.

Exposures of eggs began within 32 h of collection and all exposures for a given species were initiated on the same day. Eggs from at least three females of each species were pooled, mixed, and placed in treatments in quantities varying from 61 to 179 per replicate. Fingerbowls were monitored for hatches and pH at least once every 2 d and more often during times of rapid hatching. Hatched eggs and their nymphs were removed and discarded.

Three questions were addressed: does exposure of eggs to elevated $[H^+]$ affect the proportion of total hatch, the proportion of incomplete hatches, and the rate at which eggs hatch. Total hatch was considered as the proportion of eggs that had undergone eclosion, characterized by a longitudinal fissure in the chorion. In some cases, nymphs died before freeing themselves from this fissure; in such cases the hatch was considered incomplete. Because of possible container (petri dish) effects, observations on the timing of individual egg hatches could not be treated as independent data. As an independent measure of hatching rate, the proportions of total hatch were summed over the duration of the experiment. This method yields one datum per container that describes the area under each hatch rate curve (Fig. 1). A rapid hatch rate would have a high sum (e.g. over a 4-d experiment, $0 + 0.75 + 0.95 + 1.0 = 2.7$) relative to a slower hatch rate ($0 + 0.25 + 0.75 + 1.0 = 2.0$). For all three variables described above, the differences between treatments were assessed using a nonparametric analysis of variance test (Kruskal–Wallis).

Results and Discussion

No significant difference ($p > 0.1$) in the proportion of total hatch (eclosion) occurred across pH treatments in any of the four species (Table 1). However, the proportion of incomplete hatches was significantly increased ($p < 0.001$) at low pH in *B. flavistriga* (Table 1). Incomplete hatches accounted for 91 and 12% of total hatch at pH 4.0 and 4.5, respectively. Rare and insignificant incomplete hatches were also observed at low pH in *S. femoratum* (3% at pH 4.0; 1% at pH 4.5). No incomplete hatch occurred in these species at pH 6.5 or in either *L. cupida* or *H. vibrans* at any pH. Nymphs from these incomplete hatches were assumed dead; their body had discolored to an opaque white and had not completely freed themselves from the shell. A similar inability of larvae to free themselves from hatched eggs has been observed in acid-stressed Atlantic salmon (*Salmo salar*) (Daye and Garside 1977).

Hatching rates of *L. cupida*, *S. femoratum*, and *B. flavistriga* were not significantly different across treatments ($p > 0.05$). However, *H. vibrans* responded unusually. Although total hatch remained unaffected by exposure to pH < 6.5, it was delayed by exposure to pH 4.0, 4.5, and particularly pH 5.0 ($p < 0.01$). The greater sensitivity to pH 5.0 remains unexplained. *Habrophebia vibrans* had the longest hatching period at all pH's of these four species and therefore the longest exposure to elevated $[H^+]$. The temperatures at which these exposures were conducted (21–26°C) are higher than those to which eggs would be exposed in the field. Since development rates of mayfly eggs decrease at low temperature (Howe 1967), the egg stage would be of a longer duration in the field than those reported here. Therefore, it is possible that hatching rates of all of these species may be affected by exposure to elevated $[H^+]$ at the lower field temperatures. Decreased hatch rates may result from either decreaed embryonic development rates or impairment of the hatching enzyme, as has been suggested in other taxa (Peterson et al. 1980; Freda 1986).

The deleterious effects of exposure to elevated $[H^+]$, observed here on the egg stages of *B. flavistriga* and *H. vibrans*, are in agreement with the acid sensitivity of these species and genera inferred from field surveys (Hall and Ide 1987; MacKay and Kersey 1985; Raddum and Fjellheim 1984). While we did not attempt to monitor the mortality rates of nymphs following complete hatch, the occurrence of incomplete hatching at low pH may reflect a particular sensitivity of early instar nymphs (Fiance 1978; Allard and Moreau 1987). *Stenonema femoratum* is, according to field surveys (R. J. Hall, unpubl. data), a relatively sensitive species; however, despite the small number of incomplete hatches, our data do not indicate sensitivity, and later stage nymphs also survive exposure to low pH (Rowe 1987). Further laboratory studies comparing early with later stages are required to determine whether early stages of aquatic insects are relatively sensitive to elevated $[H^+]$.

In conclusion, these results with mayflies add to a growing list of taxa where the egg stage is impaired at realistic low pH levels. Effects of H^+ on development or hatching of eggs have also been demonstrated at pH 4.0–5.5 in fish (Peterson et al. 1980), pH 3.5–5.0 in amphibians (Freda 1986), pH 5.0–5.5 in molluscans (Servos et al. 1985), and pH 4.5–5.5 in crayfish (Berrill et al. 1985).

Future research with acid-sensitive insects should therefore include the egg stage and early instars. Experiments including increased aluminum concentrations, common to acid streams, may also demonstrate greater H^+ toxicity at higher pH levels.

Acknowledgements

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of the Environment to M.B. We thank Ron Hall for providing species identifications and unpublished data, Lois Hollett for her illustrations, Dolph Schluter for statistical advice, and G. G. E. Scudder for support of the senoir author during the preparation of this manuscript.

References

ALLARD, M., AND G. MOREAU. 1987. Effects of experimental acidification on a lotic macroinvertebrate community. Hydrobiologia 144: 37–49.

BERRILL, M., L. J. HOLLETT, A. MARGOSIAN, AND J. HUDSON. 1985. Variation in tolerance to low environmental pH by the crayfish *Orconectes rusticus*, *O. propinquus* and *Cambarus robustus*. Can. J. Zool. 62: 2360–2363.

DAYE, P. G., AND E. T. GARSIDE. 1977. Lower lethal levels of pH for embryos and alevins of Atlantic salmon *Salmo salar* L. Can. J. Zool. 55: 1504–1508.

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

FREDA, J. 1986. The influence of acidic pond water on amphibians: a review. Water Air Soil Pollut. 30: 439–450.

HALL, R. J., AND 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.

HOWE, R. W. 1967. Temperature effects on embryonic development in insects. Annu. Rev. Entomol. 12: 15–42.

HUDSON, J., AND M. BERRILL. 1986. Tolerance of low pH by the eggs of Odonata (dragonflies and damselflies). Hydrobiologia 140: 21–25.
HUFF, B. L., AND W. P. MCCAFFERTY. 1974. Parthenogenesis and reproductive biology in four species of the mayfly genus *Stenonema*. Wasmann J. Biol. 32: 247–254.
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.
MACKAY, R. J., AND K. E. KERSEY. 1985. A preliminary study of aquatic insect communities and leaf decomposition in acid streams near Dorset, Ontario. Hydrobiologia 122: 3–11.
OKLAND, J., AND K. A. OKLAND. 1986. The effects of acid deposition on benthic animals in lakes and streams. Experimentia 42: 471–486.
PETERSON, R. H., P. G. DAYE, AND J. L. METCALFE. 1980. Inhibition of Atlantic salmon (*Salmo salar*) hatching at low pH. Can. J. Fish. Aquat. Sci. 37: 770–774.
RADDUM, G. G., AND A. FJELLHEIM. 1984. Acidification and early warning organisms in freshwater in western Norway. Verh. Int. Ver. Limnol. 22: 1973–1980.
ROWE, L. 1987. Inter and intra-specific variation in the response of the mayflies *Stenonema femoratum* and *Leptophlebia cupida* to short-term exposures at low pH. Thesis, Trent University, Peterborough, Ont.
SERVOS, M. R., J. B. ROOKE, AND G. L. MACKIE. 1985. Reproduction of selected Mollusca in some low alkalinity lakes in south-central Ontario. Can. J. Zool. 63: 511–515.
VAN FRANKENHUYZEN, K., G. H. GEEN, AND 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.

Computer-Driven Motor Control with Positional Memory for Mechanical Microscope Stage

Kenneth S. Hiltz[1] and Jonathan G. Dean[2]

Department of Electrical Engineering, Technical University of Nova Scotia, P.O. Box 1000, Halifax, N.S. B3J 2X4

Peter Schwinghamer[3]

Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, Nfld. A1C 5X1

and Donald J. Belliveau

Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2

Hiltz, K. S., J. G. Dean, P. Schwinghamer, and D. J. Belliveau. 1988. Computer-driven motor control with positional memory for mechanical microscope stage. Can. J. Fish. Aquat. Sci. 45: 1652–1656.

A computerized control system for the *X–Y* mechanical stage of an inverted microscope was constructed using relatively inexpensive electrical components and requiring minimal modification to the stage. The control consists of two linear stepper motors, one for each axis, driven by commands from an IBM-compatible PC via a serial communications port and a simply constructed electrical controller. Menu-driven software regulates the motions of the steppers and allows keyboard input of data on the type and number of particles in settling chambers or on microscope slides. Frame locations of particles are stored automatically to facilitate reexamination of samples. The system allows samples to be removed and replaced while retaining the ability to relocate particles of interest.

On a construit un système de commande informatisé pour le platine à chariot selon les axes *X–Y* d'un microscope inversé en utilisant des composantes électriques relativement peu coûteuses et n'exigeant qu'une modification minime au platine. Le système se compose de deux moteurs pas-à-pas linéaires, un pour chaque axe, actionnés par des commandes provenant d'un ordinateur personnel compatible avec IBM via une porte de communications en série et un régulateur électrique de construction simple. Un logiciel piloté par menu règle les mouvements des moteurs pas-à-pas et permet l'introduction par clavier des données sur le type et le nombre de particules dans les chambres de sédimentation ou sur les lames de microscope. La localisation des particules dans le cadre est emmagasinée automatiquement pour faciliter le réexamen des échantillons. Le système permet d'enlever les échantillons et de les remplacer tout en permettant de relocaliser les particules présentant un intérêt.

Received October 28, 1987
Accepted May 25, 1988
(J9471)

Reçu le 28 octobre 1987
Accepté le 25 mai 1988

[1]Present address: Instrumar Limited, P.O. Box 13246, St. John's, Nfld. A1B 4A5.
[2]Present address: 290 East River Road, No. 15, Rochester, NY 14623, USA.
[3]Author to whom correspondence should be addressed.