Effects of aluminum, calcium and low pH on egg hatching and nymphal survival of *Cloeon triangulifer* McDunnough (Ephemeroptera: Baetidae)*

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Abstract

Laboratory experiments were conducted to assess the effects of aluminum, calcium and low pH on egg hatching and nymphal survival of the mayfly *Cloeon triangulifer*. Percent successful hatch (living nymphs breaking free of the chorion) decreased and percent partial hatch (nymphs dying attached to the chorion) increased with increasing acidity (pH 7.5–3.0). Most hatches occurring below pH 5.0 were partial hatches. Decreased time of exposure to acidic waters increased percent successful hatch and decreased percent partial hatch. Time to first hatch was not affected by pH. Eggs were incubated in acidic waters (pH 4.0 and 5.5) with additions of calcium (10 and 100 mg l^{-1}) and aluminum (100 and 500 μg l^{-1}). Aluminum decreased percent successful hatch and increased percent partial hatch and calcium increased both percent successful hatch and percent partial hatch. Time to first hatch was increased by both aluminum and calcium. The 96 h LC50 for small nymphs was pH 4.75. Addition of aluminum (100 and 500 μg l^{-1}) to acidic waters (pH 4.0 and 5.0) reduced nymphal mortality by 8–22%. Toxic effects of low pH on egg hatching and early nymphs may contribute to the absence of mayflies from acidified habitats.

Introduction

Mayflies are often reduced in abundance or absent in aquatic habitats at low pH (Weiderholm, 1984; Okland & Okland, 1986), although species such as *Leptophlebia vespertina* are tolerant of acidic conditions as low as pH 4.0. Decreased recruitment of *Ephemerella funeralis* after experimental stream acidification to pH 4.0

suggests that either eggs or small nymphs may be especially sensitive (Fiance, 1978). Successful egg hatching of *Leptophlebia cupida*, *Habrophlebia vibrans* and *Stenonema femoratum* has been recorded at pH 4.0 but significant mortality of nymphs of *Baetis flavistriga* occurred before the hatch was complete at pH 4.0 and 4.5 (Rowe *et al.*, 1988b). Effects on aquatic organisms by elevated hydrogen ion concentration may be modified by levels of aluminum (Baker & Schofield, 1982; Ormerod *et al.*, 1987; McCahon & Pascoe, 1989) and calcium (Brown, 1983). In addition to long-term low pH conditions, acid

snow melt and severe storm events may produce temporary depressions in pH (Keller, 1983); therefore, the ability of an organism to survive may depend on tolerance of a particular life stage or the duration to which it is exposed.

Our objectives were to assess, with laboratory bioassays, the sensitivity of mayfly eggs and young nymphs to low pH and to determine whether aluminum, calcium, or both, negatively or positively modified the effect of low pH. Measures of effects on eggs were hatching rates and duration of embryonic development. Lethality experiments were conducted with young nymphs. The parthenogenic mayfly *Cloeon triangulifer* was selected for our experiments because previous experience indicated eggs having a high rate of hatching success could readily be obtained and nymphs could be successfully maintained in the laboratory (Gibbs, 1977).

Materials and methods

Chemistry of test waters

Water used in bioassays (dilution water) was double-distilled, deionized water titred with dechlorinated tap water to a specific conductance of 15–17 μS cm^{-1}, typical of surface waters in New England (Haines & Akielaszek, 1983). To avoid the use of chemical buffers, stock solutions were used to change the water in each bioassay container daily. Levels of pH were monitored daily, and adjustments were made in stock solutions only, using NaOH or H_2SO_4. Aluminum was added as $Al_2(SO_4)_3$ and calcium as $CaCl_2 \cdot 2H_2O$; accordingly, mean sulfate concentrations increased with Al concentrations and mean chloride concentrations increased with Ca concentrations (Table 1).

Inflection point alkalinity was calculated using the Gran procedure (Stumm & Morgan, 1981). For cation determination, a 60 ml aliquot of water was acidified with 1.5 ml 1 : 1 HNO_3 and analyzed with a Perkin-Elmer model 703 atomic absorption spectrophotometer. Sodium and potassium were analyzed by air-acetylene flame,

calcium and magnesium by nitrous oxide -acetylene flame, and aluminum (total acid-reactive aluminum) by graphite furnace. Aluminum was also speciated into labile and non-labile monomeric fractions by passing a 60 ml aliquot through a cation exchange column (Dowex 50W-X8, Na form; procedure modified from Rosseland & Skogheim, 1982) and subsequently analysed as above. Anion (chloride, sulfate, nitrate, and fluoride) samples were filtered (0.45 μm Nucleopore) and frozen until analysed with a Dionex System 2110i ion chromatograph.

Egg bioassays

Eggs were obtained from adults reared from field-collected nymphs. Nymphs were collected from a pond with a pH of 7.5 (Ca^{2+} = 13.2 mg l^{-1}; Mg^{2+} = 4.4 mg l^{-1}; Cl = 2.8 mg l^{-1}; SO_4^{2-} = 6.5 mg l^{-1}; total Al = 49 μg l^{-1}; specific conductance = 710 μg l^{-1}) and transported to the laboratory in chilled containers where they were maintained in aerated glass dishes at 21 °C with a 16 h light : 8 h dark photoperiod. Leaf litter and associated organic sediment from the collection pond were provided as substrate and nourishment. Mature nymphs were placed under emergence cages. Subimagoes emerged from late afternoon through early evening, molted to the imaginal form in about 24 h, and were ready to oviposit 24 h later. Eggs were obtained by holding the mayfly by the wings with forceps and touching the tip of the abdomen to the surface of water in a petri dish; 150–1500 eggs were extruded by each mayfly. Eggs produced filamentous attachments to the substrate within 24 h of oviposition.

Eggs were incubated in test waters under static conditions in 60 × 15 mm petri dishes. A minimum of 100 eggs were used in each replicate and a minimum of three replicates were used in each experiment. Successfully hatched, partially hatched and dead eggs were counted and removed daily. In a successful hatch, a living nymph broke completely free from the chorion. In a partial hatch, the chorion was split to some degree but the nymph died without freeing itself. Eggs in

Table 1. Composition of dilution water adjusted for pH, aluminum, and calcium.

pH	Nominal Aluminum (µg l⁻¹)	Nominal Calcium (mg l⁻¹)	Na⁺ (mg l⁻¹)	K⁺ (mg l⁻¹)	Ca²⁺ (mg l⁻¹)	Mg²⁺ (mg l⁻¹)	Total Al (µg l⁻¹)	Non-labile Al (µg l⁻¹)	Labile Al (µg l⁻¹)	F⁻ (mg l⁻¹)	Cl⁻ (mg l⁻¹)	NO₃⁻ (mg l⁻¹)	SO₄²⁻ (mg l⁻¹)
4.0	0	0	0.28	0.04	1.13	0.06	6	0	6	0.23	1.95	0	4.27
4.5	0	0	1.11	0.08	1.14	0.06	1	0	1	0.25	2.02	0	8.65
5.0	0	0	1.14	0.07	1.07	0.06	0	0	0	0.25	2.02	0	2.79
5.5	0	0	1.26	0.03	1.09	0.06	0	0	0	0.21	1.84	0	8.50
4.0	100	0	0.82	0.03	1.09	0.06	137	70	67	0.08	2.02	0	5.19
4.5	100	0	1.02	0.04	1.08	0.06	138	64	74	0.23	1.88	0	10.18
5.0	100	0	1.42	0.03	1.12	0.06	164	55	109	0.23	1.88	0	9.41
5.5	100	0	2.42	0.03	1.11	0.06	121	53	68	0.23	1.91	0	4.76
4.0	500	0	1.17	0.07	1.00	0.08	547	264	283	0.21	1.84	0	9.61
4.5	500	0	1.40	0.08	1.09	0.07	568	262	306	0.21	1.88	0	9.22
5.0	500	0	1.58	0.06	1.09	0.06	551	297	254	0.21	1.84	0	10.47
5.5	500	0	2.26	0.07	1.15	0.06	576	206	370	0.19	1.88	0	7.97
4.0	0	10	0.86	0.12	12	0.09	8	0	8	0.21	21.10	0	3.51
4.0	0	100	1.31	0.09	106	0.35	4	0	4	0.25	180.43	0	4.08
5.5	0	10	0.80	0.05	14	0.08	0	0	0	0.21	24.65	0	1.54
5.5	0	100	2.60	0.08	100	0.32	0	0	0	0.17	178.83	0	2.11
4.0	100	10	1.81	0.11	12	0.06	142	69	73	0.15	19.50	0	6.53
4.0	100	100	1.94	0.08	103	0.34	89	60	29	0.25	193.12	0	5.38
5.5	100	10	1.67	0.05	12	0.07	128	66	62	0.21	20.85	0	3.46
5.5	100	100	4.64	0.14	101	0.33	83	60	23	0.19	181.52	0	4.61
4.0	500	10	1.33	0.06	14	0.07	604	242	362	0.17	25.43	0	8.21
4.0	500	100	2.32	0.07	103	0.34	444	230	214	0.10	181.21	0	9.99
5.5	500	10	2.51	0.05	14	0.08	385	90	295	0.17	25.43	0	5.14
5.5	500	100	2.59	0.26	99	0.32	277	44	233	0.15	181.84	0	5.09

which no developing embryo could be detected and the egg content appeared vacuolated were considered dead. 'Days to first hatch' was used as a measure of the duration of embryonic development (Sweeney & Vannote, 1984).

Experiment A

To determine the effect of pH on egg hatching and duration of embryonic development, newly oviposited eggs were immediately exposed to test waters of pH 3.0, 4.0, 5.0, and 6.0. Controls consisted of dilution water adjusted to pH 7.5, that of the pond water from which the mayflies were collected.

Experiment B

The effects of delayed exposure to low pH were determined with a second group of eggs incubated at pH 7.5 for 7 d or until reaching the 'eyed' stage, indicating that hatching was imminent. The eggs were then exposed to the same pH levels as in Experiment A.

Experiment C

To examine effects of aluminum on egg hatching within the critical pH range determined in Experiment A, freshly oviposited eggs were incubated at pH 4.0, 4.5, 5.0, and 5.5 with Al concentrations of 0 (control), 100, and 500 μg l^{-1}.

Experiment D

Possible modifying effects of aluminum and calcium were examined by immediately exposing eggs at pH 4.0 and 5.5 with Al concentrations of 0 (control), 100, and 500 μg l^{-1} and Ca concentrations of 0 (control), 10, and 100 mg l^{-1}.

Nymphal bioassays

Small (1–3 mm) field-collected nymphs were acclimated to dilution water for 24 h without food. Aerated, static 96 h bioassays were conducted in 300 cc glass culture dishes. Ten nymphs per replicate and three replicates per treatment were used except for experiments with aluminum, where two replicates per treatment were used.

Numbers of nymphs remaining alive in each dish and presence or absence of successful molting were recorded every 4 h until 12 h, and every 12 h thereafter. Dead individuals were identified by lack of response to tactile stimulus.

Experiment E

The effect of pH alone was examined with bioassays at pH 3.0, 4.0, 5.0, 6.0, and 7.5 (control).

Experiment F

The effects of aluminum and pH were examined with bioassays at pH 4.0 and 5.5 at Al concentrations of 0 (control), 100, and 500 μg l^{-1}.

Analyses of test waters during Experiments C, D, and F are shown in Table 1.

Statistical analysis

All eggs and nymphs, as well as pH, Al, and Ca treatments were randomly assigned to dishes in each experiment. Differences between treatments in egg hatching and larval mortality were assessed using analysis of variance (ANOVA or GLM), and Duncan's multiple range test (DMRC) was used to compare means. One-way ANOVA (GLM) was used in Experiments A, B, and E, two-way ANOVA in Experiments B and C and three-way ANOVA in Experiment D. The arcsine root transformation was used to normalize variance when data were in the form of percentages. The 96 h LC50 of pH for nymphs was calculated using the POLO program (Russell et al., 1977).

Results

Effects of pH and duration of exposure on eggs (Experiments A and B)

Immediate exposure of eggs to decreasing pH resulted in decreasing ($p < 0.01$) percent successful hatch and increasing ($p < 0.01$) percent partial hatch (Table 2). At pH 3.0 many eggs that did not develop deteriorated rapidly and were floating at the water surface within 24 h; many of the surviv-

Table 2. Mean percent successful hatch ± SE, partial hatch ± SE and days to first successful hatch ± SE of *C. triangulifer* eggs exposed to pH 3.0, 4.0, 5.0, 6.0 and 7.5 (control). Eggs were either immediately exposed after oviposition or exposure was delayed for 7 d while eggs were held at pH 7.5.

pH	% successful hatch		% partial hatch		Days to 1st hatch
	Imm. exp.	Del. exp.	Imm. exp.	Del. exp.	Imm. exp.
3.0	0 ± 0a*	0 ± 0a	42.3 ± 6.6a	69.0 ± 4.4a	No successful hatch
4.0	4.9 ± 2.8b	18.3 ± 12.0b	49.7 ± 8.3a	34.7 ± 14.9b	8.0 ± 0a
5.0	31.2 ± 7.7c	45.0 ± 9.0c	40.3 ± 12.5b	0 ± 0c	8.0 ± 0a
6.0	30.1 ± 7.2c	43.0 ± 3.5c	5.3 ± 1.2c	0 ± 0c	9.0 ± 1.0a
7.5	42.0 ± 5.2c	68.0 ± 2.9d	0.7 ± 0.3c	0 ± 0c	8.3 ± 0.3a

* Means within a column followed by the same letter are not significantly different ($p > 0.05$, DMRC).

ing eggs failed to develop attachment fibers. At pH 4.0, early embryonic development appeared normal but many eggs failed to develop attachment fibers. Days to first successful hatch did not vary among pH levels (Table 2).

Eggs with delayed exposure to decreasing pH also showed decreasing ($p < 0.01$) percent successful hatch and increasing ($p < 0.05$) percent partial hatch (Table 2). Decrease in time of exposure increased ($p < 0.01$) percent successful hatch and decreased ($p < 0.05$) percent partial hatch.
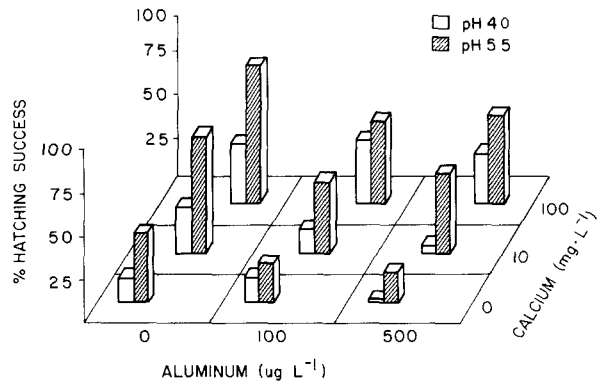

Fig. 1. Mean percent successful hatch of *C. triangulifer* eggs exposed to acidic waters with calcium and aluminum.

Table 3. Mean percent successful hatch, percent partial hatch, and days to first hatch of *C. triangulifer* eggs exposed to pH 4.0 and 5.5 with Ca and Al. Mean values ± SE for each factor, pooling data across remaining factors.

(1) pH	% successful hatch	% partial hatch	Days to 1st hatch
4.0	17.8 ± 2.6a*	32.5 ± 2.2a	11.5 ± 1.0a
5.5	42.4 ± 4.1b	13.1 ± 2.3b	10.4 ± 0.8a
(2) Al (μg l^{-1})			
0	40.2 ± 5.5a	20.7 ± 4.4a	8.3 ± 0.3a
100	27.3 ± 3.4b	21.5 ± 2.7ab	12.1 ± 1.0b
500	22.8 ± 5.3b	26.3 ± 3.5b	12.2 ± 1.4b
(3) Ca (mg l^{-1})			
0	17.6 ± 3.9a	15.9 ± 2.6a	8.6 ± 0.2a
10	30.2 ± 4.8b	29.8 ± 3.9b	14.9 ± 1.3b
100	42.1 ± 4.9c	22.8 ± 3.4c	9.0 ± 0.7a

* Means followed by the same letter within each column of (1), (2), or (3) are not significantly different ($p > 0.05$, DMRC).
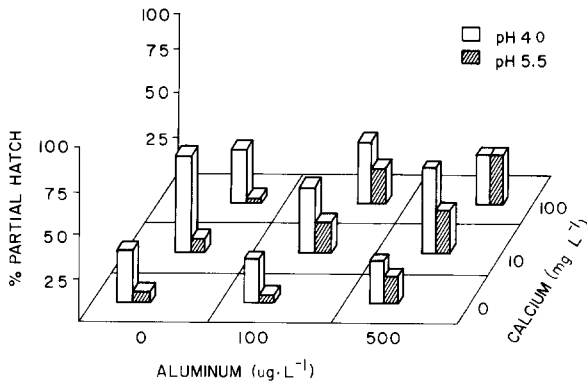
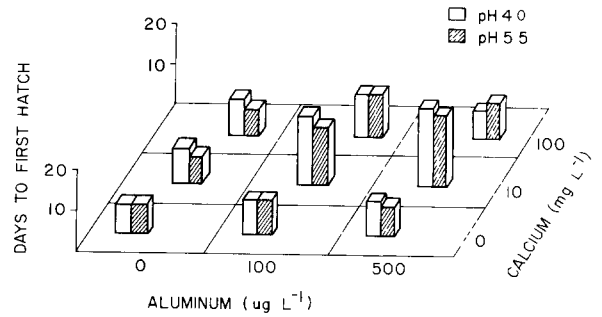Fig. 2. Mean percent partial hatch of C. triangulifer eggs exposed to acidic waters with calcium and aluminum.

Fig. 3. Mean days to first hatch for C. triangulifer eggs exposed to acidic waters with calcium and aluminum.

Effects of pH, aluminum and calcium on eggs (Experiments C and D)

Increased aluminum alone in acidic waters (Experiment C) did not affect percent successful or partial hatch, or days to first hatch ($p > 0.05$). In contrast, in Experiment D, increased Al ($p < 0.05$) and decreased pH ($p < 0.001$) resulted in decreased percent successful hatch. However, increased Ca showed increased percent successful hatch ($p < 0.001$, Table 3, Fig. 1). Percent partial hatch was increased by increased Al ($p < 0.05$) and Ca ($p < 0.001$) and decreased pH ($p < 0.001$) and an Al × pH interaction was observed ($p < 0.001$, Table 3, Fig. 2).

Days to first hatch showed increase ($p < 0.001$) with Al, Ca and Al × Ca interaction (Table 3, Fig. 3). Only the moderate level of Ca
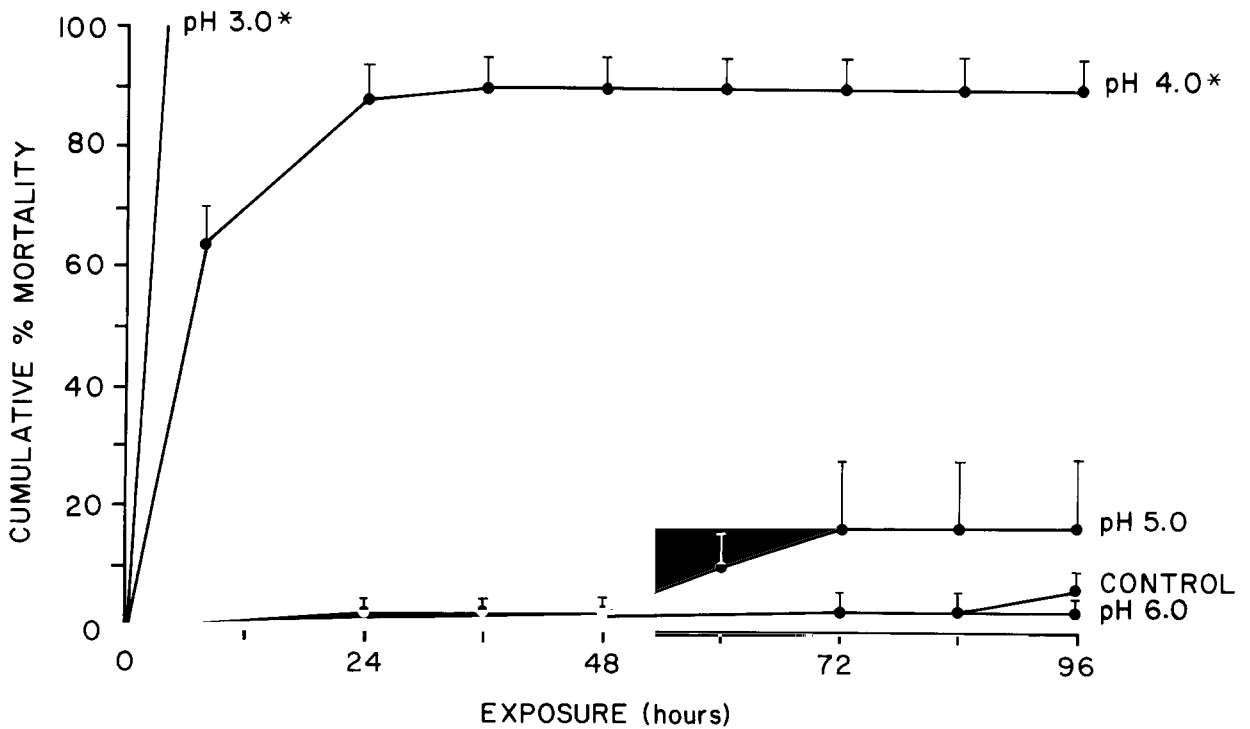

Fig. 4. Cumulative percent mortality ± SE of C. triangulifer nymphs exposed to test waters for 96 h. * indicates significant difference ($p < 0.01$).

(10 mg l^{-1}) increased days to first hatch. Difference in pH did not affect days to first hatch ($p > 0.05$).

Effects of pH on nymphs (Experiment E)

Decreased pH reduced ($p < 0.01$) survival of nymphs (Fig. 4). At pH 3.0, there was 100% mortality after 4 h and at pH 4.0 90% mortality after 36 h. There were no differences in mortality at pH 5.0 to pH 7.5. Surviving nymphs in all treatments molted successfully. The calculated 96 h LC50 was pH 4.74 ($n = 150$, slope = 1.03 $\pm$ 0.13, 95% CL = 4.51 $\leq$ 4.74 $\leq$ 4.94). Accordingly, subsequent experiments were performed within the pH 4.0–5.5 range.

Effect of pH and aluminum on nymphs (Experiment F)

There was an 8–22% lower mortality of nymphs exposed to acidic waters with Al than of nymphs exposed to acidic waters without Al (Table 4). No statistical analysis was attempted with these data as there were only two replicates per treatment. Surviving nymphs in all treatments molted successfully.

Discussion

Gibbs (1977) reported 86% successful hatching of eggs in pond water from which *C. triangulifer*

Table 4. Mean ($n = 2$) percent mortality of *C. triangulifer* nymphs exposed to pH 4.0 and 5.0 at aluminum concentrations of 0, 100 and 500 μg l^{-1} for 96 h.

Al (μg l^{-1})	pH	
	4.0	5.0
0	28.6	15.5
100	8.3	5.6
500	6.2	7.1

was collected. Thus exposure of *C. triangulifer* eggs to such dilute test waters may have reduced successful hatching in control (Table 2) as well as experimental treatments.

Our results consistently indicated that decreasing pH decreased successful hatching and increased partial hatching in *C. triangulifer* (Experiments A, B, C and D). All hatches that occurred at pH 3.0 were partial hatches. There were high levels of partial hatches and low levels of successful hatches at pH 4.0 but between pH 5.0 and 7.5 the percent successful hatch remained consistently high. This suggested that hatching of *C. triangulifer* was impaired below pH 5.0. This impairment may have resulted from the direct effect of acidity on the developing embryo, or the nymph as it attempted to break free of the chorion; acidity may also have acted indirectly by affecting the hatching process.

Evidence for a direct effect of low pH on developing embryos of *C. triangulifer* was the increased successful hatching and decreased partial hatching when time of exposure of eggs to acidic conditions was delayed and short-term rather than immediate and long-term (Experiment B). This suggested a direct effect on the embryo related to time of exposure. If egg membranes protect the developing embryo from acidic conditions, the internal pH of eggs would be expected to be different from that of the surrounding water; this hypothesis has not been tested for mayfly eggs.

High levels of partial hatching occurred at low pH, with nymphs dying within the egg or still attached to the chorion by the caudal filaments. This phenomenon was also observed at pH 4.0 and 4.5 in eggs of *Baetis flavistriga* but not in eggs of *Leptophlebia cupida*, *Habrophlebia vibrans* or *Stenonema femoratum* (Rowe *et al.*, 1988b). In *B. flavistriga*, significant mortality of nymphs occurred before the hatch was complete at pH 4.0 (91%) and 4.5 (12%). We believe that the 'incomplete hatch' of Rowe *et al.* (1988b) is equivalent to the 'partial hatch' of this study. Partial hatching has also been reported in fish eggs, with 69% of salmon and 17% of sea-run brown trout dying with the head still attached to chorion at pH 4.1 (Johansson *et al.*, 1977). This hatching failure in

fish eggs may have resulted from the inactivation of a choriolytic enzyme at reduced pH (Runn et al., 1977; Peterson et al., 1980). Hatching enzymes have been identified in insects (Hinton, 1981) but their presence or function in C. triangulifer eggs is unknown.

Reduced successful hatching can also be related to disruption of the mechanical aspects of hatching. In amphibians (Gosner & Black, 1957; Pough, 1976; Dunson & Connell, 1982) the perivitelline space is reduced at low pH leaving little room for muscular movements of the embryo; hatching failure or partial hatching may occur.

Our evidence of possible mechanical disruption of the hatching process was limited to the observation that the egg attachment fibers appeared less abundant at pH 3.0 and 4.0 than at pH 5.0–7.5. This may have contributed to partial hatching by reducing the stability of the substrate from which the nymphs must extricate themselves during hatching.

When levels of both Ca and Al were varied in acidic waters, increased concentrations of Al appeared detrimental, as successful hatching was decreased and partial hatching increased (Experiment D). Also, there was an Al × pH interaction which resulted in increased partial hatch at the higher pH (5.5) level. Elevated Ca, however, increased both successful and partial hatches. Calcium additions may have had an ameliorating affect on H^+ stress on C. triangulifer eggs by decreasing membrane permeability, which could decrease the rate of internal ion loss. Calcium has been shown to modify the effect of Al at low pH in fish. Brown (1983) reported no or low survival of brown trout fry with 0.25 mg l^{-1} Ca and almost complete survival with 2 mg l^{-1} Ca after 16 d at pH's of 4.5–5.4 with 250 μg l^{-1} Al. Mortality data for Baetis rhodani indicate that nymphs collected from high Ca sites were less sensitive to acidic water with Al than those from low Ca sites (McCahon & Pascoe, 1989).

In contrast to the results with pH alone (Experiment A), moderate levels of Ca (10 mg l^{-1}) increased number of days to first hatch; in addition, days to first hatch was higher at 500 μg l^{-1} than at 100 μg l^{-1} Al (Experi-

ment D). We have no explanation for this. These experiments were conducted using eggs from overwintering nymphs and Sweeney & Vannote (1984) suggest that eggs from this generation hatch with less synchrony than eggs produced by the summer generations. It is uncertain if this was the case in this instance.

Whatever the cause, increased number of days to first hatch effectively increased experimental exposure times for C. triangulifer eggs. We have demonstrated that longer exposures to acidic waters decreased successful hatch and increased partial hatch (Experiment B). Therefore, detrimental effects of H^+ elevations were exacerbated by increased days to first hatch. This phenomenon, combined with the lack of any Al effects observed in Experiment C, leads us to conclude that Al has little or no independent toxicity to C. triangulifer eggs.

Small nymphs showed 100% mortality after 4 h at pH 3.0 and 90% mortality after 36 h at pH 4.0 (Experiment E). This susceptibility may have contributed to the low rate of successful hatch and the high rate of partial hatch at pH 3.0 and 4.0 by directly affecting the nymphs after the chorion was ruptured.

The 96 h LC50 of pH 4.74 indicated that C. triangulifer was slightly less tolerant of low pH than Ephemerella subvaria, which Bell & Nebeker (1969) found to be the least tolerant mayfly (LC50 = pH 4.65) in their experiments. Tartar et al. (1982) found three species of Baetisca to be more tolerant than C. triangulifer with 96-h LC50's of pH 3.6 for B. lacustris, pH 3.5 for B. carolina and pH 3.2 for B. berneri. Mortalities of C. triangulifer nymphs were comparable to those observed by U. Matthias (cited in Okland & Okland, 1986) for Baetis spp. nymphs at similar pH's.

Disruption of ionic regulation in acid-stressed mayflies has been indicated by a loss of wholebody sodium and chloride and a correlated mortality (Rowe et al., 1988a, and 1989). Our observations that acid stressed nymphs continued to molt successfully agree with those of Rowe et al. (1988a) who reported that molting was more frequent in low pH treatments and was associated

with mortality and large losses of sodium and chloride ions. Rowe et al. (1988a, and 1989) suggest that molting is probably a mechanism to inhibit ion loss and not a cause of mortality and may be associated with the increase in number of chloride cells during ionic stress.

Small nymphs such as we used in our experiments are reported to be more sensitive to low pH than larger nymphs (Allard & Moreau 1987). However, Rowe et al. (1989) found that nymphs within the size range of 2–14 mg dry wt showed no size related ion losses at pH 3.5.

Aluminum at 100 and 500 μg l^{-1} appeared to ameliorate the effect of pH on mortality of nymphs of C. triangulifer at pH 4.0 and 5.0 (Experiment F). Although Al toxicity to fish has frequently been reported at low pH (see review by McCahon et al., 1987) ameliorating effects of Al on H$^+$ stress in brook trout fry (Salvelinus fontinalis) at pH 4.0 have also been reported by Schofield (1977) and in brown trout by Muniz & Leivestad (1980). Insects, including C. triangulifer, appear to be less sensitive than fish to Al toxicity. Increased gill mucus production, which has been observed in fish, was not found in mayfly nymphs (Baetis rhodani, Ecdyonurus venosus) exposed to an Al concentration of 350 μg l^{-1} at pH 5.0 (McCahon et al., 1987). Cook & Haney (1984) found that of the nine species tested (five caddisfly species, two mayfly species, a stonefly, and a beetle) Al additions caused increased mortality only in the stonefly Nemoura nigratta and the caddisflies of the genus Macronema.

Evidence of significant reduction of successful hatching and survival of nymphs below pH 5.0 suggested that C. triangulifer is one of the more sensitive mayflies for which data are available. Other mayflies showing comparable sensitivity, Baetis lapponicus, B. macani (Okland & Okland, 1986) and B. flavistriga (Rowe et al., 1988b), are also members of the family Baetidae, indicating that this taxon may be especially vulnerable to habitat acidification. Our observations that C. triangulifer eggs and small nymphs are tolerant of short-term exposures to levels of aluminum occurring in Maine surface waters suggest that this species could survive episodic Al increases such as those following snowmelt in streams. However, extended exposures of previously acid-stressed eggs could cause a detrimental Al × pH interaction, resulting in C. triangulifer recruitment reduction or failure. These negative effects are most pronounced in low ionic-strength water and can be ameliorated to a certain extent by the addition of calcium.

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