THE EFFECTS OF ACIDIFICATION ON THE TRANSFORMATION OF DETRITAL

ENERGY BY THE SHREDDING CADDISFLY, *CLISTORONIA MAGNIFICA* (BANKS)

(LIMNEPHILIDAE)

by

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(Limnephilidae).

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ABSTRACT

The possible mechanisms underlying the widely reported simplification of insect communities in acidifed waters was examined. Acidification was postulated to reduce the cycling of detrital energy by inhibiting the processing of coarse particulate organic matter by invertebrates of the shredder functional group. The caddisfly, *Clistoronia magnifica* (Banks) (Limnephilidae), was used to investigate the effects of pH 4.0 to 6.4 and increased concentrations of nickel on growth and survival of this shredder.

Hypotheses examined were that low pH would reduce the transformation of detrital energy into shredder biomass by (1) reducing survival, (2) increasing maintenance costs at the expense of growth, and (3) reducing food quality through inhibition of microbial activity on the leaf litter food resource. A pH of 4 reduced larval survival immediately after hatching and during the moult from third to fourth instar. However, metabolic costs of surviving larvae were not affected by low pH. Surviving larvae developed more rapidly to a larger size at pH 4.0-5.2 than at pH 5.8-6.4. Growth enhancement of late-instar larvae at low pH did not offset the loss in biomass production that resulted from reduced survival of early-instar larvae.

Enhancement of larval growth was due to effects of low pH on the leaf litter food resource, which suggested improved nutritional quality of leaves conditioned at low pH. Conditioning of leaves at pH 4 resulted in a doubling of fungal biomass and a smaller increase in bacterial abundance as compared with leaves conditioned at pH 6. Increased microbial colonization of leaves at low pH did not alter larval ingestion rates but improved availability of microbe- and leaf-derived energy for larval growth.

Larval growth and survival were more severely affected by elevated levels of dissolved nickel than by low pH. Exposure to <100 μg Ni^{2+}/L reduced growth and survival of larvae and viability of pupae. Toxicity of nickel decreased with a decrease in pH from 6.2 to 4.1, presumably due to increased competition between Ni^{2+} and H^+ for cellular uptake sites. Nickel temporarily improved larval survival at pH 4.1 by reducing pH-induced mortality of early-instar larvae. Both nickel in solution and nickel accumulated by microbes on the leaves contributed to reduced viability of *C. magnifica*.

the Chemistry Laboratory Services (Environmental Protection Service, West Vancouver), and leaf samples were analysed by Analytical Service Laboratories (Vancouver).

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GENERAL INTRODUCTION

THE ACIDIFICATION PROBLEM

The term acid rain refers to increased hydrogen ion concentration in wet and dry deposition of atmospheric materials. Increased acidity is primarily caused by emission of sulphur and nitrogen oxides, originating from the combustion of fossil fuels in industrial regions (Gorham 1976). Acidic precipitation produces large changes in the chemical, physical, and biological properties of fresh waters, particularly in areas underlain by bedrock resistant to chemical weathering. In such waters the usually low buffering capacity is readily exhausted by acidic inputs from the atmosphere, causing a drop in pH, frequently to as low as 4. That decrease in pH is often associated with an increase in dissolved metal concentrations (Wright and Gjessing 1976).

The detrimental effects of acidification on biota of lakes and streams are well documented, particularly in southern Scandinavia and northeastern North America (Haines 1981, Hendrey *et al*. 1976, Singer 1982). However, most of the research to date has been predominantly descriptive: surveys of low pH waters have revealed strongly reduced abundance and diversity of phytoplankton (Kwiatkowski and Roff 1976), zooplankton (Roff and Kwiatkowski 1977), invertebrates (Raddum and Fjellheim 1984), and fish (Schofield 1976). That the observed changes are

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attributable to increased acidification was demonstrated

conclusively by experimental manipulation of the pH in streams

(Burton *et al*. 1980, Hall *et al*. 1980, Zischke *et al*. 1983) and

lakes (Schindler and Turner 1982). Although such studies

contribute to a general understanding of the consequences of the

acidification process, they fail to clarify the specific

mechanisms underlying the simplification of aquatic communities

under acid stress. It is not known to what extent observed

biotic changes are due to toxic effects of low pH and/or

associated trace metals, or to altered trophic relationships

resulting from these toxic effects.

OBJECTIVES

The goal of the research reported here is to establish some cause and effect relationships underlying the widely reported oligotrophication (Grahn *et al.* 1974) of acidified waters. My research focussed on the detritus-decomposer component of aquatic food webs. Organic matter has been observed to accumulate in acidified lakes (Grahn *et al.* 1974) and streams (Friberg *et al.* 1980, Hall *et al.* 1980). Inhibited breakdown of these materials could reduce the flow of detrital energy to consumer organisms. Invertebrates of the shredder functional group (Cummins 1973) transfer that energy to other trophic levels by converting coarse particulate organic matter (CPOM) into shredder biomass and fine particulate organic matter (Cummins 1974). Inhibition of the shredding process by acidification could reduce detritus turnover. Since some ecosystem models have suggested a tight coupling between heterotroph processes and primary production (McIntire and Colby 1978, O'Neill 1976), disrupted cycling of detrital energy could be important in the overall simplification of communities in acidified waters.

Specific objectives of this study are:
(1) To determine to what extent acidification affects the processing of detrital energy by shredding invertebrates;

(2) To establish which mechanisms are responsible for the
observed changes;

(3) To develop a conceptual model of how acidification alters
the flow of energy through the shredder-CPOM component of
aquatic food webs.

The limnephilid caddisfly, *Clistoronia magnifica* (Banks),
was used as a model species to investigate the effects of
acidification on the transformation of detrital energy by
shredders. *C. magnifica* was selected because it is a shredder
that is important in the diet of fish (Winterbourn 1971). *C.
magnifica* is locally abundant in lakes of British Columbia's
coastal mountains (Winterbourn 1971) and is easily reared in the
laboratory (Anderson 1978). In Chapter 1 I measured larval
growth and survival at pH 4-6.4 in the laboratory and
differentiated between direct pH effects and indirect effects
via the food resource. The effects of pH 4-6.4 on larval feeding
and the fate of ingested energy were also assessed. In Chapter 2
the effect of pH on microbial colonization of leaf litter, and
microbe-mediated changes in shredder feeding and utilization of
leaf-derived and microbe-derived energy are described. In
Chapter 3 I examined the role of metal toxicity and pH-metal
interactions as determinants of shredder growth and survival.

BIOLOGY OF THE TEST SPECIES

Clistoronia magnifica belongs to the Limnephilidae, the largest Trichoptera family in North America, which includes many shredding species. The genus *Clistoronia* has four species distributed over western montane areas from Alaska to Arizona (Wiggins 1977). The precise distribution of *C. magnifica* is not well known. There are scattered records from lakes in Oregon, Washington, and British Columbia (Anderson 1976a). Winterbourn (1971) studied its life history in Marion Lake at an elevation of 300 m in the coast mountain range near Vancouver, B.C. Egg masses were present in August and September, attached to floating macrophyte leaves (*Nuphar* and *Potamogeton*). leaves. Larvae spent several instars in submerged marginal vegetation before moving down to the sediment to disperse and to spend the winter. Most larval growth occurred from August to January, by which time larvae were in final instar. Adults emerged from early May to late June, but reproduction did not occur until August.

Winterbourn (1971) classified *C. magnifica* as a sediment feeder, but in other studies the species showed typical shredder feeding behavior (Anderson and Cummins 1979). *C. magnifica* is important in the transfer of energy from detritus to fish predators: in Marion Lake caddisflies comprised up to 74% of wet

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weight of food items in rainbow trout stomachs in the winter months, with *C. magnifica* making up 20-75% of the caddisfly total (Winterbourn 1971).

C. magnifica has been reared since 1973 at Oregon State University in Corvallis (Anderson 1978). Egg masses were obtained from that stock on two occasions (March 1982 and 1983). In addition, 50-100 egg masses were collected in Marion Lake in August of both years. Larvae were reared at Simon Fraser University in shallow pans with aerated dechlorinated tapwater at a constant temperature of 12-13°C. Stream-conditioned leaves of alder, *Alnus rubra*, were provided as food, supplemented with wheat grains, needed for complete development (Anderson and Cummins 1979). No animal protein was supplied (Anderson 1976b), as there was extensive cannibalism in the rearing trays. Reproductive diapause of the adults was eliminated by rearing under continuous long-day conditions (16 L : 8 D), allowing production of a complete generation in about 6 months (Anderson 1978). Corvallis and Marion Lake stocks were reared separately.

CHAPTER 1

DIRECT AND INDIRECT EFFECTS OF LOW PH ON THE TRANSFORMATION OF DETRITAL ENERGY BY THE SHREDDING CADDISFLY, *CLISTORONIA MAGNIFICA* (LIMNEPHILIDAE)

INTRODUCTION

Acidification of fresh waters in northwestern Europe and northeastern North America has resulted in reduced abundance and diversity of organisms at all trophic levels (Haines 1981). It is not known to what extent observed changes are due to direct toxic effects of low pH and/or associated metals, or to indirect effects resulting from altered trophic relationships. Disrupted cycling of detrital energy could be an important mechanism of community change. Accumulation of coarse particulate organic matter (CPOM) in acidified waters (Grahn et al. 1974) could reduce the flow of detrital energy to consumer organisms.

Energy contained in CPOM is extracted by the combined action of microbes and detritivorous invertebrates. Microbial colonization generally augments the nutritional value of CPOM to shredding invertebrates: colonized CPOM is ingested at higher rates (Anderson and Grafius 1975) and yields better growth (Smock and Harlowe 1983, Ward and Cummins 1979) and higher survival (Kostalos and Seymour 1976) than less colonized material. Since low pH inhibited microbial activity on leaf litter (McKinley and Vestal 1982), I postulated that low pH would reduce shredder growth by lowering food quality. In addition to such indirect effects, direct toxic effects of low pH on shredder growth and survival could further reduce the

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transfer of detrital energy. Even if shredders are tolerant of acidic conditions (Friberg *et al*. 1980, Townsend *et al*. 1983), low pH could reduce energy available for growth by increasing maintenance costs. Acid-tolerant organisms can maintain body functions (such as sodium regulation) at low pH (Havas 1980, Vangenechten and Vanderborght 1980), but presumably at higher metabolic costs.

The objective of this chapter is to test the hypotheses that increased acidity reduces the transformation of detrital energy into shredder biomass directly, by reducing survival or increasing shredder maintenance costs, and indirectly, by reducing the nutritional value of CPOM consumed by the shredder. I studied growth and survival of *C. magnifica* larvae under various pH regimes and differentiated between direct pH effects and indirect effects via the food.

METHODS

Experiments were conducted in the laboratory in dechlorinated tapwater. Some water quality characteristics are listed in Table 1-1. Larvae of *C. magnifica* were held in standing or flowing water in Plexiglas trays of 45x45 cm with a volume of about 4 L. Reagent grade H_2SO_4 was used to obtain various experimental pH regimes. Under static conditions pH levels were maintained by daily replacement of the water. In the flow-through system pH levels were maintained by continuously pumping acid from appropriate stock solutions into mixing beakers with outflows of 100 ml/min into the rearing trays. In experiments conducted under static conditions water temperature was 12.5-13.5°C. Temperature in the flow-through system was generally 10-13°C, but occasionally dropped below 10° C for as long as one week at a time. Larvae were fed leaves of alder, *Alnus rubra*, picked from one tree in late summer and air-dried. Prior to experiments, leaves were conditioned in the laboratory at 10-13°C in flowing dechlorinated tapwater with decaying alder leaves providing the microbial inoculum. Decaying leaves were collected periodically from a softwater mountain stream near Vancouver, BC (Spring Creek) and were homogenized in a Waring blender. Duration of leaf conditioning and ambient pH data are reported for each experiment. The pH values are means of daily pH measurements; the observed range of fluctuations is listed

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Table 1-1. Some chemical characteristics of Simon Fraser
University dechlorinated tapwater used in this study.

Analysis	Units	Tapwater	
		reference	acidified
pH	$-\log[H^+]$	5.9-6.4	3.9-4.5
Hardness (Ca,Mg)	mg $CaCO_3$/L	4.7	4.4
Dissolved oxygen	mg/L	7.9-10.1	7.9-10.1
Na	mg/L	0.5	0.5
Ca	mg/L	1.7	1.6
Mg	mg/L	0.1	0.1

between brackets. Wheat grains were used as a dietary supplement (Anderson 1978). Both alder and wheat were usually supplied in excess of consumption. The photoperiod was set at 16 L : 8 D.

Growth, Development, and Survival

Effects of low pH on development and survival of *C. magnifica* were studied by exposing larvae to an average pH of 4.4 (3.9-4.6), 5.8 (5.6-6.2), and 6.4 (6.3-6.9) from first instar through pupation and adult emergence. Larvae came from the Corvallis stock and were reared individually in Plexiglas cylinders, 4 cm in diameter and 3 cm high, which were placed in the Plexiglas trays (50 cylinders/tray, 1 tray/pH). The cylinders had 470 μm Nitex screen bottoms, which were raised slightly above the tray bottom to allow fecal pellets to fall through. Larvae were fed alder leaves that were conditioned for 3-4 weeks at corresponding test pH. The experiment was repeated with larvae from the Marion Lake stock exposed to pH 4.2 (3.9-4.5), 5.2 (4.8-5.3), and 6.2 (5.9-6.4) in the flow-through system. There were 4 replicate trays at each pH with 50 larvae/tray. For all subsequent experiments I used larvae from the Marion Lake stock.

I examined the effect of low pH on growth of late-instar larvae, as these stages account for more than 60% of total

biomass production (Anderson 1978). Mid-fourth instar larvae were held until pupation at pH 4.3 (3.9-4.5) and pH 6.0 (5.6-6.2) under static conditions with 2 replicates of 40 larvae at each pH. Larvae were fed alder leaves that were conditioned for 4 weeks at the corresponding test pH.

The relative importance of direct and indirect (microbe-mediated) effects of low pH on shredder growth was examined as follows. Groups of 30 larvae were exposed from late-third instar until pupation to pH 4.1 (3.8-4.6) and pH 6.3 (5.8-6.6) in the flow-through system. Larvae were reared individually in the previously described cylinders. Larvae from both groups were fed disks of alder leaves conditioned for 4 weeks at either pH 4.1 or 6.3. Disks in each treatment were replaced at least 6 times per week to minimize any pH-induced changes in the leaf microbial community during feeding. Wheat supplements were limited to 1 kernel/larva every other week, to accentuate possible differences in larval growth resulting from microbe-mediated effects of low pH on the leaf material.

Development and survival in all experiments were recorded weekly. Larval growth was expressed as the time required to complete various instars, or as the change in larval dry weight. Adult forewing length and dry weight of pupae sacrificed towards the end of adult emergence were used as additional measures of growth.

Direct effects of pH on development of eggs and survival of early-instar larvae were also examined. Egg masses were transferred within 12 h of oviposition from flight cages to Plexiglas trays at pH 6.4 (5.9-6.8) and pH 4.1 (3.9-4.7) under static conditions. Each day an equal number of newly-laid egg masses were allocated to the two treatments until each tray had a total of 10. Stage of development and egg hatch were checked twice a week. Larval survival was monitored on 5 replicates of 25 larvae at each pH for 3 weeks following emergence from the egg mass.

Ingestion

The effects of ambient pH on larval feeding were assessed gravimetrically. Third, fourth, and fifth instar larvae held at pH 4.0 and 6.2 were fed for 24-72 h (depending on instar) with disks cut from 1 or 2 alder leaves that were conditioned for 4 weeks at pH 6.4. Change in pH in the feeding units was never more than 0.5 unit after 24 h. Disks were cut in pairs according to position on the leaf and the amount of vein included. One disk of each pair was set aside in water and the other was offered to a larva. At the end of the feeding period the difference in dry weight between those disks was taken as the amount of leaf material ingested. Control disks served to

estimate the initial weight of the disks used for feeding within a 5% error. Prior to the feeding trials, larvae were maintained at experimental conditions for 2 weeks and were fed stream-conditioned alder. Colour difference between leaves collected from the stream (dark) and those conditioned in the lab (light) permitted correction for case construction during the trials; at the end of the feeding period light coloured fragments were removed from the larval case and weighed with the unconsumed portion of the leaf disk. Ingestion rates were calculated as mg dry weight of leaf material ingested/mg larval dry weight/24 h for 3 replicates of 9-15 larvae at each pH.

Fate of Ingested Energy

To determine whether low pH increased metabolic costs at the expense of growth, I examined the fate of labelled leaf carbon ingested by mid-fourth and early-fifth instar larvae that were held at different pH. Alder leaves were labelled in the laboratory by exposing terminal shoots of 3-5 year old alders to $^{14}CO_2$ in a closed system. The system consisted of a loop of Tygon tubing connecting an air pump with a CO_2-release vessel and a Plexiglas cuvette holding one shoot with 2-3 developing leaves. Labelled CO_2 was released from ^{14}C-$NaHCO_3$ (42 mCi/mmol) by injecting 2N HCl into the release vessel, followed by shaking

to drive the gas out of solution. The ^{14}C-activity in the recirculating air was monitored continuously with a recorder connected to a GM counter in the circuit. Incubation of the leaves was continued until 99% of the released ^{14}C-activity (20 μCi) had been taken up (1-2 h), resulting in an uptake of usually >10^6 dpm/1.5 cm^2 disk. That was sufficient to trace the labelled carbon following ingestion by the larvae. Generally 50-75% of the label taken up by the leaves was incorporated into the ethanol-insoluble fraction after the leaves had photosynthesized for 2-3 days, indicating labelling of refractory leaf components. Leaves were air-dried and stored for later use. Prior to experiments, leaves were conditioned for 2 weeks at pH 6.4 to enhance palatability.

The onset of egestion of radioactive food was estimated by feeding 20 early-third instar larvae on a labelled disk (>7×10^4dpm/disk) for 5 min. Larvae were transferred to unlabelled food and ^{14}C-activity in the feces was monitored for 3 h at intervals of 5-35 min. The cohesive fecal pellets were collected by filtration through Whatman no.1 filter paper. Radioactivity first appeared in the feces between 80 and 100 min following feeding on labelled alder (Fig. 1-1 A). Since gut loading times generally increase with larval size (Zimmerman and Wissing 1978), a 1-h feeding time was short enough to ensure that fourth and fifth instar larvae did not egest labelled material during feeding on the labelled food (Sorokin 1968).

The time required to void larval guts of labelled food was estimated by feeding fourth (n=20) and fifth (n=10) instar larvae for 1 h on a labelled leaf disk. The ^{14}C-activity in the feces was monitored 3,6,12,24, 30, and 48 h after transfer to an unlabelled disk. Defecation of ^{14}C was 99% complete after 24 h for fourth and after 30 h for fifth instar larvae (Fig. 1-1 B).

To ensure maximal feeding on labelled disks, larvae were not fed for 24-48 h before experiments. Following 1 h of feeding on a labelled disk, each larva was rinsed and transferred to a tightly-stoppered 30 ml vial containing an unlabelled leaf disk in 10 ml of dechlorinated tapwater. Each larva was transferred to fresh water after 2,4,6,8,16, and 24 h. Larvae were removed from the vials after 24 h (fourth instar) or 30 h (fifth instar). Larvae were rinsed and radioactivity remaining in the body was determined. Body counts were corrected for uptake on external surfaces by subtracting radioactivity accumulated by dead larvae that were exposed for 1 h to labelled disks (generally 2-3 times background levels). During each time interval respired $^{14}CO_2$ in the vial's airspace was absorbed by a glass fiber filter (GF/A, 2.1 cm) that had been soaked in 0.1 ml hyamine hydroxide and inserted inside the rubber stopper (Hargrave 1970). After the larva had been removed the water was acidified by injecting 0.2 ml 2N H_2SO_4 through the stopper and the vial was shaken for 1 h, which removed >99% of respired $^{14}CO_2$ remaining in the water. Glass fiber filters were then removed and radioactivity was counted. Fecal particles were

Fig. 1-1. A. Time course of ^{14}C appearing in the feces of third
instar C. magnifica larvae following 5 min of feeding on a
labelled alder leaf.
B. Clearance of ^{14}C from the guts of fourth and fifth
instar larvae during 48 h of feeding on an unlabelled alder
leaf. Δdpm/larva expressed as the change in % of ^{14}C in the
particulate fraction.
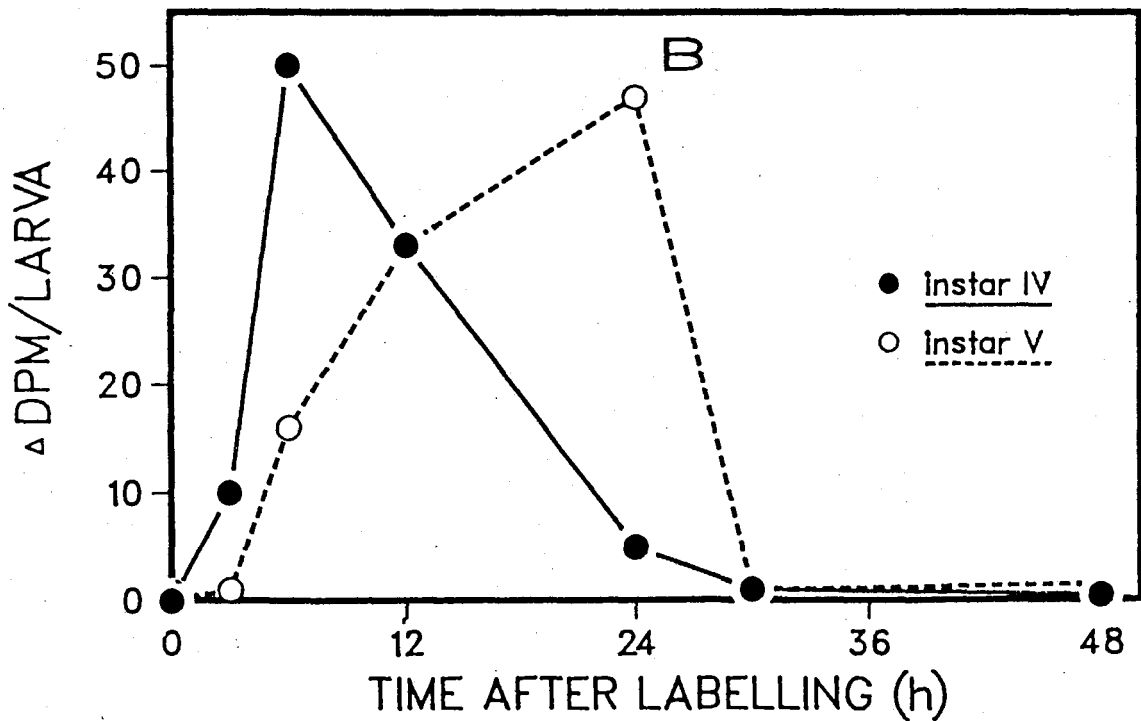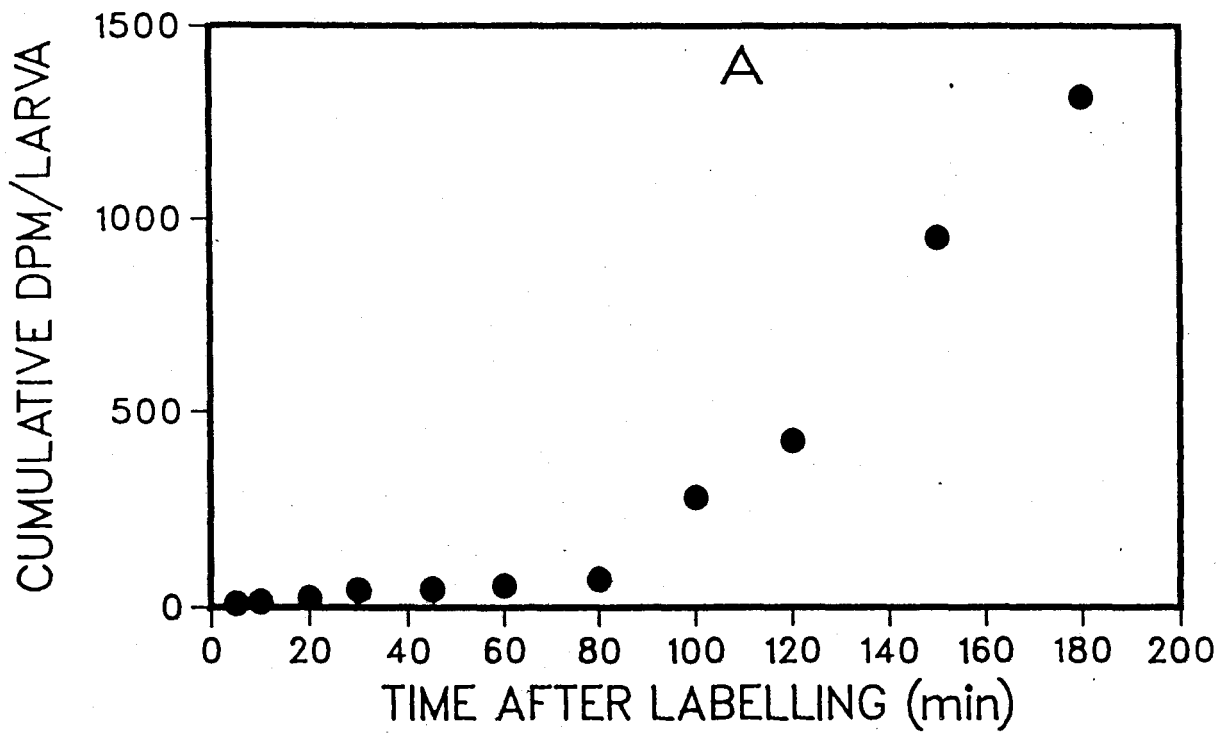
filtered from the acidified water and radioactivity on the filters (Whatman no.1) was determined.

Radioactivity in glass fiber filters (respired), particulate fraction (egested), and solution (excretion), summed over the 24 or 30-h assimilation period, were added to body counts (net production) to yield total ingested ^{14}C. All fractions were expressed as percentage of total ingested ^{14}C for each larva. Assimilation was calculated as the sum of respiration, excretion, and net production. Fate of ingested carbon was studied using fourth instar larvae held at pH 4.0, 5.2, and 6.4 (3 replicates of 9 larvae/pH) and fifth instar larvae held at pH 4.0 and 6.4 (10 larvae/pH). Change in pH in the vials was never more than 0.3 units after 8 h. Prior to experiments, larvae were kept at experimental conditions for at least 1 week. Variability in energy utilization due to possible differences between leaves was minimized by cutting all disks for one experiment from the same leaf.

Larvae, leaf disks, and filters with particulate matter were combusted in a Packard Tricarb combuster. Glass fiber filters were counted directly in 10 ml scintillation cocktail (Scintiverse II) with a few drops of glacial acetic acid to reduce chemiluminescence. Aqueous samples were counted directly in 18 ml of Scintiverse II. Samples were counted on a Beckman LSC-8000 scintillation counter with internal standard quench correction. The activity in vials was corrected for counting

efficiency and background.

Statistical Analysis

Parametric comparison of treatment effects were conducted using Student's t-test or analysis of variance (ANOVA). Differences between treatment means were determined using Duncan's multiple range test. Normality of the data was checked graphically or by using the Kolmogorov-Smirnov goodness of fit test. An arcsine transformation was performed on percentages. In case of heterogeneity of variances (Bartlett's test), data were analysed with the nonparametric Kruskal-Wallis test, followed by *a posteriori* comparison of means based on rank sums (Dunn 1964). Significance was accepted at the 95% confidence level ($p \leq 0.05$).

RESULTS

Exposure of *C. magnifica* larvae to low pH did not result in the postulated growth reduction. On the contrary, larvae from both the Corvallis and Marion Lake stock developed faster (Table 1-2) and to a larger size (Table 1-3, Fig. 1-2) at low pH than at higher pH levels. Development time from first instar until adult emergence was 10-15 days shorter at pH<5.2 than at pH>5.8 ($p < 0.01$), primarily due to differences in development rate in the fifth instar ($p < 0.05$) (Table 1-2, one-way ANOVA) . Larvae reared at pH 4.2-5.2 produced significantly larger adults ($p < 0.01$) and heavier pupae ($p < 0.05$) than larvae reared at pH 5.8-6.4 (Table 1-3, one-way ANOVA). Late-instar larvae reared at pH 4.3 were heavier than larvae at pH 6.0 after 7 and 9 weeks of exposure ($p < 0.05$, *t*-test, Fig. 1-2), pupated two weeks earlier (Fig. 1-2 inset), and produced heavier pupae (i.e. 29.1±0.9 (SE) mg at pH 4.3 (n=36) as opposed to 24.6±1.3 mg (n=19) at pH 6.0, $p < 0.01$, *t*-test).

Acceleration of larval growth from third instar until pupation resulted primarily from indirect effects of low pH on the leaf litter. Larval development was significantly affected by leaf conditioning pH ($p < 0.05$) but not by rearing pH ($p > 0.15$)(Table 1-4, two-way ANOVA). Larvae held at pH 6.3 and fed leaves conditioned at pH 6.3 developed substantially slower

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Table 1-2. Duration of developmental stages of *C. magnifica* in relation to rearing pH. Mean no. of days ($\bar{X}$) with SE and sample size (n).[1]

pH	Early-first instar until fifth instar			Fifth instar until pupation			Pupal instar until adult eclosion			Early-first instar until adult eclosion		
	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n
Part I[2]												
4.4	74.2	2.7	27	40.8 a	1.7	25	21.5	1.8	12	134.8 a	1.7	9
5.8	77.1	1.4	40	47.1 b	1.4	36	21.9	0.6	17	150.5 b	2.9	17
6.4	76.8	1.8	37	47.3 b	2.1	28	20.9	1.3	9	147.5 b	3.6	9
Part II[3]												
4.2										134.4 a	4.8	48
5.2										133.0 a	2.1	67
6.2										144.0 b	1.8	47

[1]Means within a column followed by different letters are significantly different ($p \leq 0.05$).
[2]Larvae from Corvallis stock, reared individually (static).
[3]Larvae from Marion Lake stock, reared together (flow-through).

Table 1-3. Winglength (mm) and pupal dry weight (mg) of *C. magnifica* (both sexes) held at various pH levels from first instar until pupation. Mean ($\bar{X}$) with SE and sample size (n).[1]

Rearing pH	Winglength			Pupal weight		
	$\bar{X}$	SE	n	$\bar{X}$	SE	n
Part I[2]						
4.4	199.4 a	1.3	17	30.5 a	1.4	12
5.8	191.7 b	1.7	17	27.0 b	1.0	16
6.4	190.2 b	2.1	22	27.0 b	1.0	17
Part II[3]						
4.2				32.9 a	1.0	23
5.2				31.4 a	1.0	27
6.2				27.7 b	0.9	23

[1]Means within a column followed by different letters are significantly different (p≤0.05).
[2]Larvae from Corvallis stock, reared individually (static).
[3]Larvae from Marion Lake stock, reared together (flow-through).

Fig. 1-2. Growth and pupation of C. magnifica larvae exposed to
pH 4.3 and 6.0 from mid-fourth instar until pupation.
Means ± SE. * indicates a significant difference between pH
treatments at $p < 0.05$.
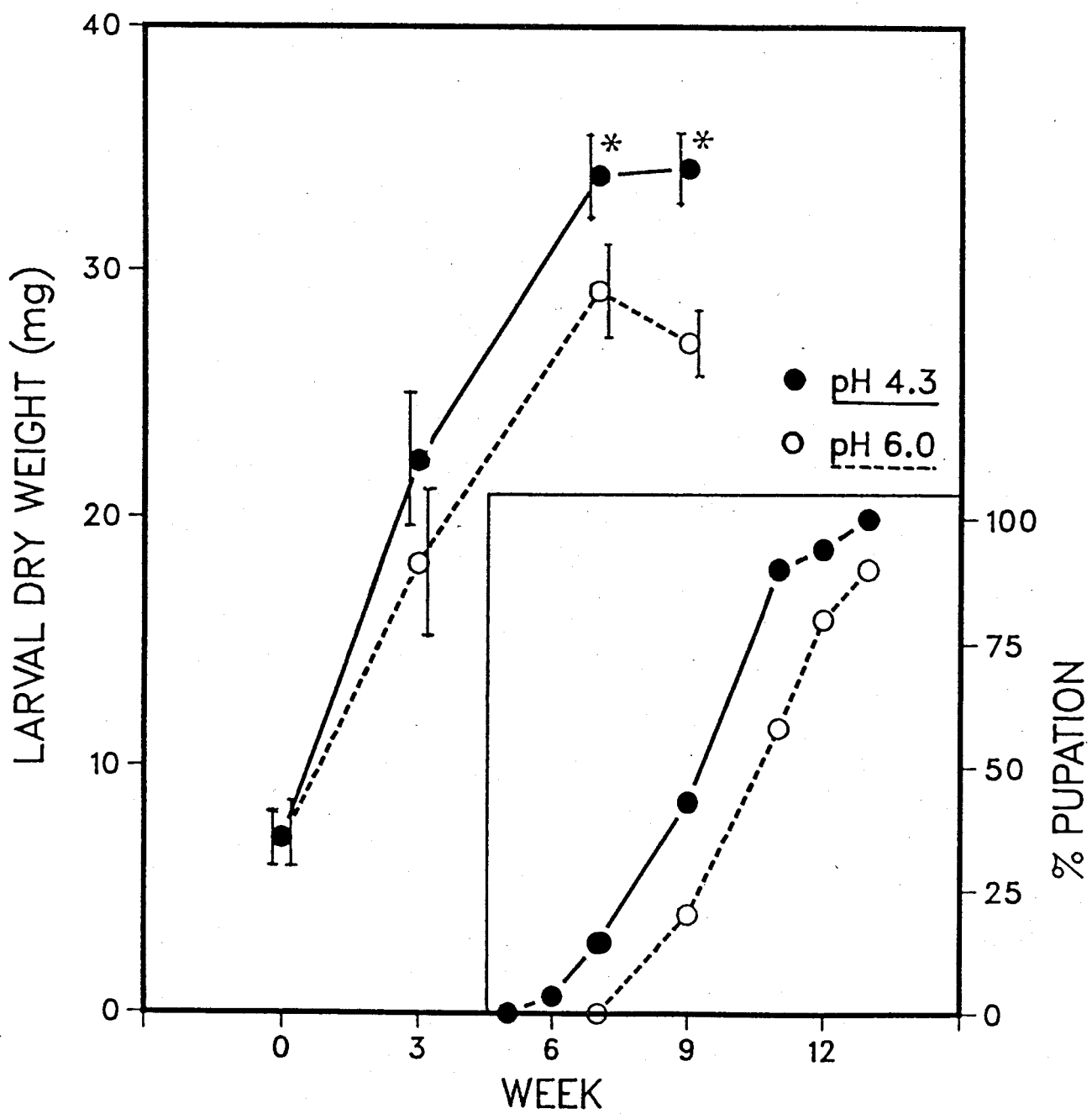
(20-30 days) than larvae in the other treatments. When fed leaves conditioned at pH 4.1, larvae at pH 6.3 developed just as fast as larvae at pH 4.1 fed leaves conditioned at pH 4.1. That growth acceleration was largely eliminated when larvae at pH 4.1 were fed leaves conditioned at pH 6.3. Similar trends were apparent in the duration of the third plus fourth larval stage and of the fifth stage. Pupal weights were low (mean: 14 mg) and did not reflect any effects of the above treatments.

Low pH had no apparent effect on development and hatching of eggs (Table 1-5). In both pH treatments the same time elapsed from oviposition to early-eyed stage (larval eyes just beginning to show) and late-eyed stage (eyes fully developed) ($p>0.50$, t-test). The difference in time until first hatch (2 days later at pH 4.1 than at pH 6.4) was not significant ($p<0.08$). The proportion of eggs remaining in the mass after completion of hatching was highly variable between egg masses (2-50%), but was on average not different between treatments ($p>0.50$) . Survival of early-instar larvae was significantly reduced by low pH within one week of exposure and was on average 23% lower at pH 4.1 than at pH 6.4 (Fig. 1-3, $p<0.01$, t-test).

There was also a marked negative effect of low pH on survival of late-instar larvae (Fig. 1-4 A). Mean survival time of the 50 individually reared larvae in the first experiment was significantly lower at pH 4.4 than at pH 5.8 and 6.4 ($p<0.01$, Kruskal-Wallis, using survival time as the ranked observation).

Table 1-4. Duration of developmental stages of *C. magnifica* reared at pH 4.1 and 6.3. Larvae at each rearing pH (rear pH) were fed alder leaves conditioned at either pH (cond pH). Mean no. of days ($\bar{X}$) with SE and sample size (n).[1]

Rear pH	Cond pH	Early-third instar until fifth instar			Fifth instar until pupation			Early-third instar until pupation		
		$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n
6.3	6.3	100.0 a	8.2	18	101.4 a	4.8	16	197.0 a	6.9	16
6.3	4.1	82.5 ab	4.9	20	87.4 b	4.8	17	169.0 b	6.8	17
4.1	4.1	79.6 b	4.9	25	91.8 ab	3.5	22	170.0 bc	5.9	22
4.1	6.3	89.9 ab	8.1	17	98.6 ab	4.1	13	186.9 ac	9.1	13

[1]Means within a column followed by different letters are significantly different ($p \leq 0.05$).

Table 1-5. Effect of pH on development and hatching of eggs of *C. magnifica* exposed to test pH within 12 h of deposition. Mean no. of days ($\bar{X}$) to reach indicated stages with SE for n egg masses.

pH	Early-eyed stage			Late-eyed stage			First hatch			% unhatched		
	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n
4.1	11.0	0.9	3	14.4	0.3	10	19.3	0.5	10	16.8	6.3	10
6.1	11.5	0.8	4	14.1	0.3	7	17.6	0.8	7	15.5	7.9	7

Fig. 1-3. Survival of early-instar larvae of C. magnifica at pH
4.1 and pH 6.4 immediately following hatching of egg masses
held at corresponding pH levels. Means ± SE. * indicates a
significant difference between pH treatments at p<0.01.

29-b
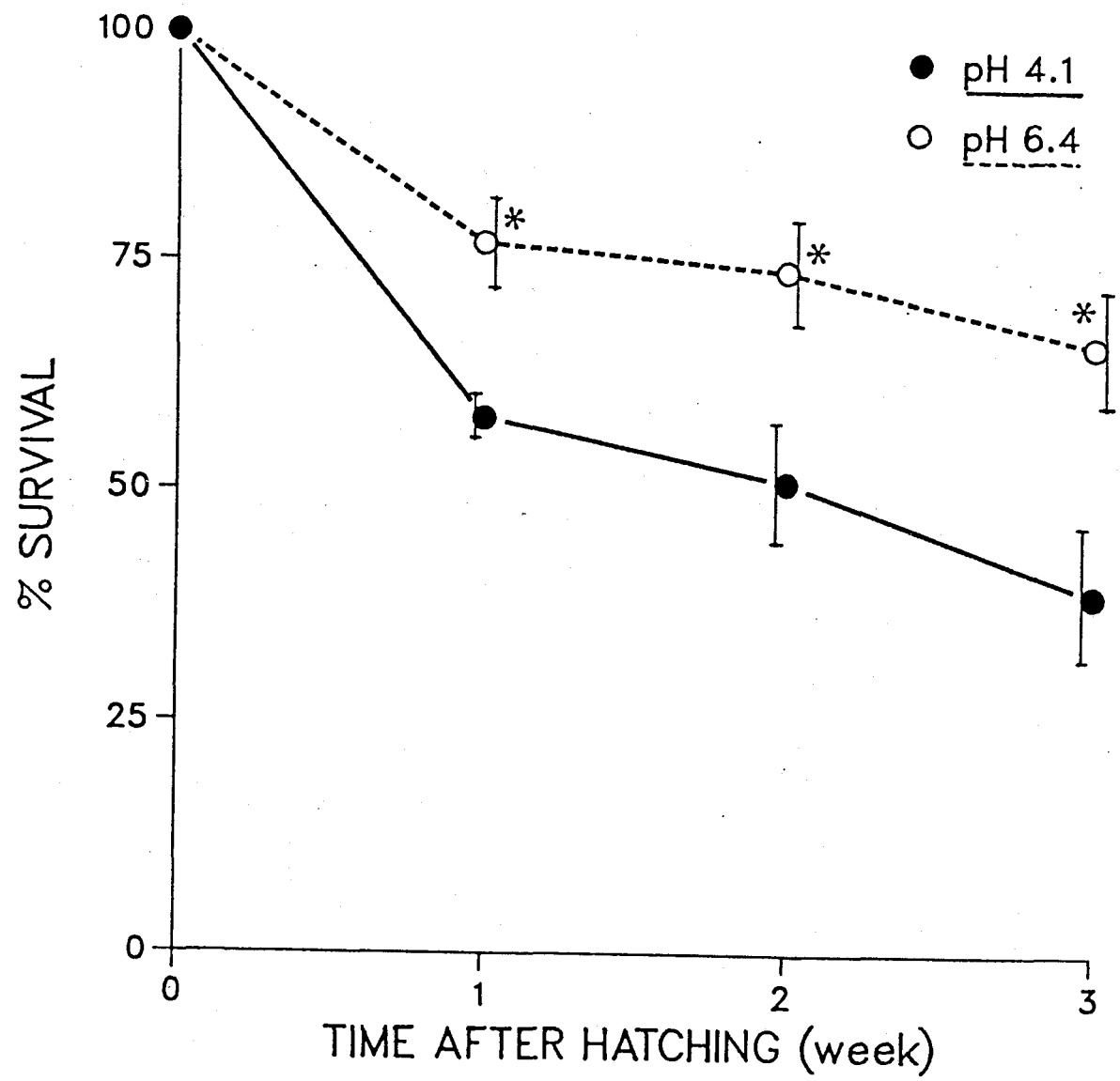
That difference was largely due to a drop in larval survival from 94% to 60% between weeks 3 and 6 at pH 4.4 (Fig. 1-4 A), coinciding with the third to fourth instar-moult (Fig. 1-4 B). Moulted fourth instar larvae frequently died inside their cases after casting off the old skin but before hardening of the new integument. That phenomenon was not observed during other moults. A similar but less pronounced drop in survival during moulting (from 76% in week 3 to 42 % in week 9) was observed at pH 4.2 in the second experiment (Fig. 1-4 C), but mean survival time was not significantly lower at pH 4.2 than at higher pH levels ($p > 0.10$, Kruskal-Wallis).

Ingestion rates were significantly affected by instar ($p < 0.01$) and the interaction between instar and pH ($p < 0.01$) (Table 1-6, two-way ANOVA). Ambient pH did not affect ingestion of alder leaves by third and fifth instar larvae, but fourth instar larvae ingested significantly more at pH 4.0 than at pH 6.2.

There were no pH-dependent differences in the proportion of labelled leaf carbon egested, respired, or excreted by fourth instar larvae (Fig. 1-5, two-way ANOVA). The percentage of ingested ^{14}C egested increased curvilinearly with time until reaching a plateau between 8 and 12 h, reflecting cessation of defecation. Most egestion (96%) occurred between 2 and 16 h.

Radioactivity remaining in the water after filtration was assumed to be dissolved organic compounds from larval excretion

Fig.1-4. A. Survival of C. magnifica larvae from first instar
    until adult emergence reared individually at pH 4.4, 5.8,
    and 6.4 (Corvallis stock).
    B. Number of survivors at pH 4.4 in first through third
    instar (I-III), fourth instar (IV), fifth instar (V), pupal
    stage (P), and adult stage (A) in the same experiment.
    C. Survival of C. magnifica larvae reared in groups of 50
    at pH 4.2, 5.2, and 6.2 (Marion Lake stock).
    Shaded areas indicate period of third to fourth instar
    moult in each experiment.

31-b
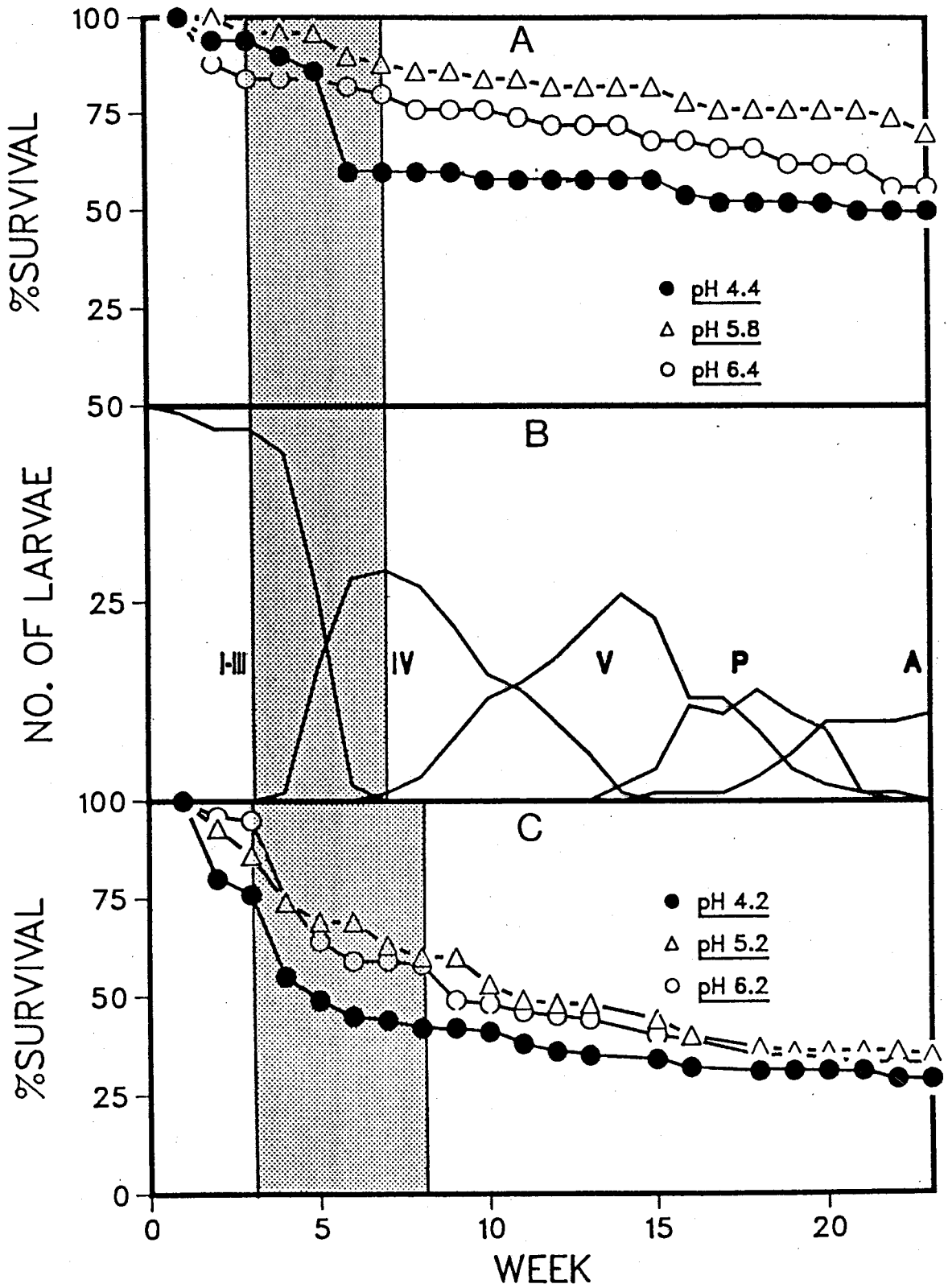
Table 1-6. Ingestion of alder leaves (means ± SE)
by third, fourth, and fifth instar larvae of *C. magnifica*
held at pH 4.0 and 6.2. Leaves were conditioned for
2 weeks at pH 6.4.[1]

Instar	pH	Dry weight larva (mg)	Ingestion (mg/mg/day)
III	4.0	1.7±0.26	0.36±0.02 a
	6.2	1.8±0.23	0.36±0.02 a
IV	4.0	4.9±0.39	0.38±0.03 a
	6.2	7.6±1.32	0.24±0.02 b
V	4.0	17.7±2.27	0.26±0.01 b
	6.2	16.7±4.22	0.28±0.02 b

[1]Means within and between instar followed by different
letters are significantly different ($p \leq 0.05$).

and leaching from feces. Experiments with labelled feces indicated that 4.5% of total ^{14}C in feces leached within 1 h, reaching a maximum of 7% after 24 h, independent of ambient pH. This leaching of soluble ^{14}C indicated that excretion in Fig. 1-5 represented an overestimate. As fecal particles remained in the water for 2-8 h, actual excretion of ^{14}C was approximated by subtracting 5% of egested ^{14}C from the total activity in the water. Corrected estimates are shown in Table 1-7. Of the total ^{14}C ingested by fourth instar larvae, an average of 61% was egested, 28% was transformed into shredder biomass (net production), 7% was lost to respiration and 3.5% to excretion. Assimilation efficiency averaged 39%, independent of pH ($p > 0.50$, one-way ANOVA). Likewise, pH had no significant effect on the partitioning of energy by fifth instar larvae (Table 1-7, $p > 0.50$, t-test).

Fig. 1-5. Comparison of ^{14}C-labelled material egested, respired, and excreted by fourth instar C. magnifica larvae held at various pH levels for 24 h following feeding on unlabelled alder leaves.

34-b
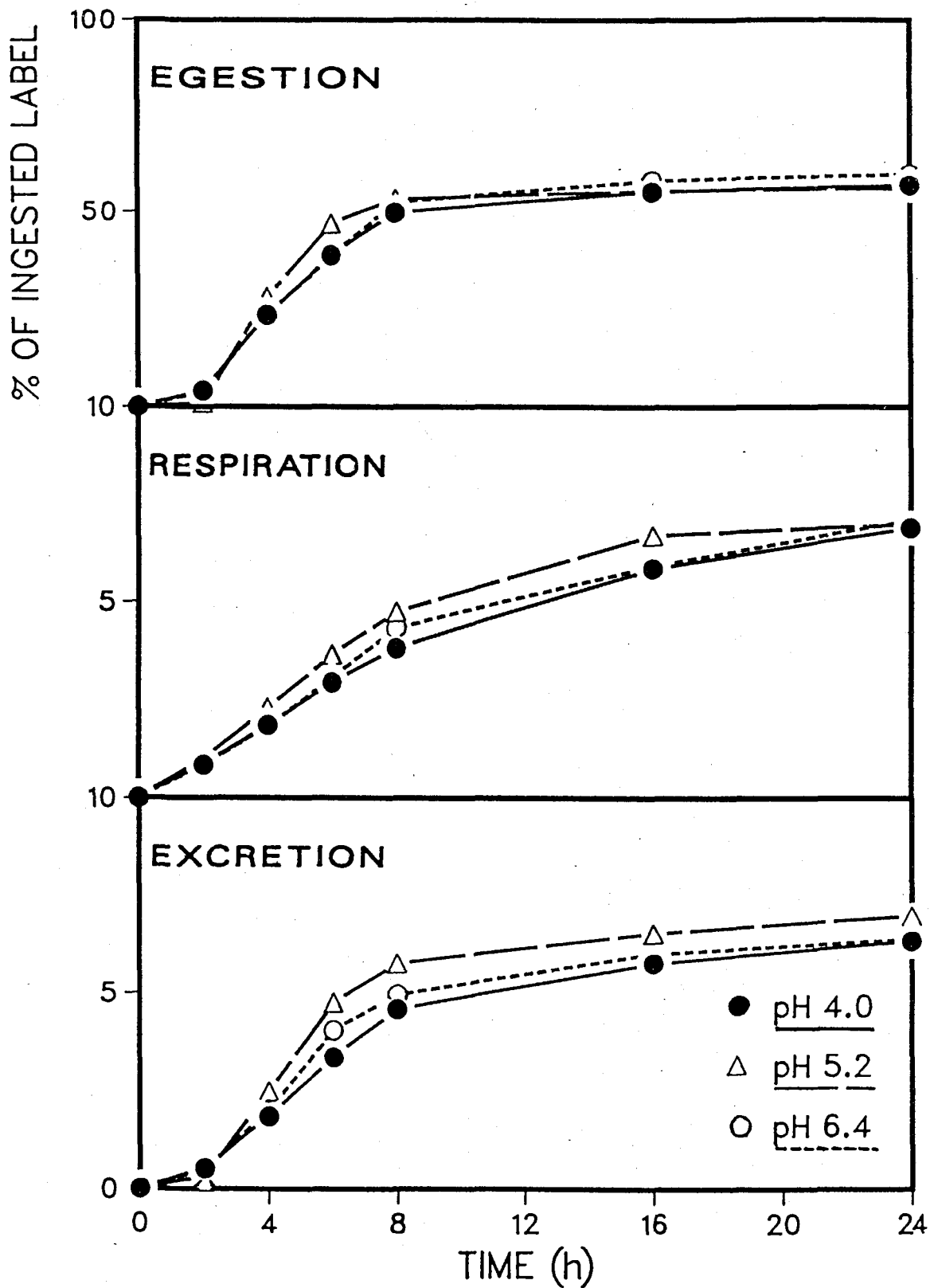
Table 1-7. Fate of ^{14}C ingested by larvae of *C. magnifica* held at pH 4.0, 5.2, and 6.4 for 24 h (fourth instar) or 30 h (fifth instar) following feeding on labelled alder leaves.

Instar	pH	Percentage of ingested ^{14}C (mean ± SE)				
		Egested	Produced	Respired	Excreted	Assimilated[1]
IV	4.0	61.5±2.1	28.0±0.2	6.9±0.3	3.3±0.3	38.5±2.5
	5.2	59.0±3.1	28.8±2.1	6.9±0.4	4.1±0.4	41.0±2.5
	6.4	62.9±2.8	26.9±0.2	6.4±0.4	3.3±0.4	37.2±2.4
V	4.0	62.6±4.8	27.0±4.8	5.7±1.0	3.8±0.4	37.3±4.8
	6.4	60.0±2.8	30.4±2.8	5.6±0.3	3.9±0.3	39.9±2.8

[1]Assimilated ^{14}C is the sum of the fractions used for growth (produced), respired, and excreted.

DISCUSSION

Results of experiments with larvae of the shredding caddisfly, *Clistoronia magnifica*, did not support the hypothesis that low pH reduces shredder growth. Larvae developed faster to a larger size at pH 4.0-5.2 than at pH 5.8-6.4 under a variety of experimental conditions (Table 1-2, 1-3, Fig. 1-2). Larvae from the laboratory-reared stock (Corvallis) showed a similar growth response to low pH as larvae from field-collected egg masses (Marion Lake).

Enhanced growth at low pH did not result from higher ingestion rates. Low pH did not significantly alter ingestion of alder leaves by *C. magnifica* larvae. Higher ingestion rates of fourth instar larvae at pH 4.0 (Table 1-6) were attributable to smaller larval size in that treatment. Larvae in the pH 4 trials were smaller than larvae in the pH 6 trials (about 5 and 8 mg respectively), because only small larvae were available from the rearing stock when the pH 4 trials were conducted. Larvae of 5 and 8 mg were estimated to ingest 0.37 and 0.25 mg/mg/day respectively (Grafius 1977, Fig.19-c), which corresponds to the difference in ingestion rates observed between pH 4.0 and 6.2.

Low pH did not increase metabolic costs of fourth and fifth instar larvae. The same proportion of ingested detrital carbon was respired and used for growth by larvae held at pH 4.0 as by

larvae held at higher pH (Table 1-7). Absolute metabolic losses may have been underestimated, as transfer of larvae to unlabelled food would lower the specific activity of respired and excreted carbon (Conover and Francis 1973), but relative losses in the three pH treatments presumably remained the same. These data suggest that late-instar larvae did not experience metabolic stress at pH 4.0. Acid-tolerance of fourth instar larvae was also demonstrated in a 12-day toxicity test, resulting in mortality of 100% at pH 2.0, 35% at pH 2.5, 5% at pH 3.0, and 0% at pH 3.5 (n=30). A similar tolerance of low pH has been observed for certain other species of the Limnephilidae (Havas and Hutchinson 1982, Mackay and Kersey 1985).

Enhanced growth at low pH was apparently due to the indirect effects of low pH on the food resource. Growth of late-instar larvae was enhanced at pH 6.3 when they were fed leaves conditioned at pH 4.1 and was reduced when larvae held at pH 4.1 were fed leaves conditioned at pH 6.3 (Table 1-4). Growth enhancement was not reflected in pupal weights, which were low. Low pupal weights and slow larval development relative to other experiments (Table 1-2, 1-3) were presumably due to insufficient wheat supplements and frequent falling of the rearing temperature below 10°C. Better growth on leaves conditioned at low pH infers improved nutritional quality or palatability of those leaves. That increase is probably microbe-mediated, since microbial conditioning generally enhances nutritional value and palatability of leaf litter to shredders (see Anderson and

37

Sedell 1979). In particular, colonization by aquatic fungi enhances feeding and growth of shredders (Arsuffi and Suberkropp 1984, Barlocher and Kendrick 1973). A shift from bacterial decomposers to fungi has been observed at low pH (Traaen 1980) and might account for enhanced growth of larvae feeding on leaves conditioned at low pH.

The ^{14}C-activity in the particulate fraction was assumed to represent material not assimilated during gut passage. Regurgitation of undigested food did not contribute to particulate ^{14}C, since ^{14}C-activity was consistently low prior to the egestion of labelled feces (Fig. 1-1 A). The contribution of assimilated label incorporated into fecal pellets (Johannes and Satomi 1967) was probably insignificant, considering that an assimilation efficiency of about 40% is rather high. Grafius (1977) reported an assimilation efficiency of 25% for *C. magnifica* fed on alder leaves. My estimate could be higher because labelling with $^{14}CO_2$ necessitated the use of young leaves, which are generally more nutritious than senescent leaves (Scriber and Slansky 1981). Non-homogeneous distribution of ^{14}C among leaf constituents could also result in high assimilation estimates. That was apparently not a serious problem, given that my estimate lies within the range of 20-40% reported for other shredders feeding on alder (e.g. Nilsson 1974, Prus 1971).

The effect of not feeding the larvae before measuring the fate of ingested label on observed assimilation efficiencies is difficult to assess. Unfed larvae ingested labelled leaf material at a higher rate than fed larvae. If high ingestion rates persisted thoughout the 24 h of feeding on unlabelled alder, faster gut passage of labelled food could lower assimilation efficiencies. Alternatively, reduced food availability may result in increased digestive enzyme activity and more efficient assimilation (Hassett and Landry 1983). I feel that food deprivation was short enough to have a minimal effect on the fate of ingested ^{14}C.

Sensitivity of aquatic invertebrates to low pH is associated with disturbance of ionic regulation (Havas and Hutchinson 1983). High sensitivity of early-instar larvae (Fig. 1-3) has also been observed for other invertebrates (Fiance 1978) and could be related to a large surface to volume ratio. Reduced survival of early-instar larvae was not evident in Fig. 1-4 A, possibly because larvae for that experiment were selected from batches held at test pH for several days. Survival of larger larvae was reduced primarily during the moult from third to fourth instar (Fig. 1-4). During moulting, low pH might interfere with phenolic tanning of the new integument, or larvae may not be able to buffer the sudden influx of acid water taken up to aid shedding of the old cuticle (Havas 1980). In either case it is not clear why *C. magnifica* larvae are sensitive only during that particular moult. Death of moulting larvae was noted

only when larvae were reared individually (Fig. 1-4 A); when reared together, cannibalism obscured actual cause of death and had an overriding effect on survival (Fig. 1-4 C).

I assessed the net effect of accelerated growth and reduced survival on the transformation of detrital energy into shredder biomass by comparing production at pH 4.4 and 6.4 in the first growth experiment. Production was calculated by using Ricker's (1958) method: Production = Growth rate x Mean Biomass. Since I did not measure change in larval weights in that experiment, the calculations were performed using mean instar weights. The calculated cumulative production of larvae reared at pH 4.4 was only 80% of the biomass produced at pH 6.4, due to mortality during moulting (Fig. 1-6, curves A and B). But when enhanced growth was simulated by introducing a 20% weight differential in the fifth instar (Fig. 1-2), estimated production of the two groups was similar (Fig. 1-6 curves A and C). Apparently the effect of decreased survival during moulting on production was offset by increased growth of late-instar larvae. However, when an additional mortality of 23% was introduced in the first instar (Fig. 1-3), production of the acid-exposed population reached only 70% of that of the control group (Fig. 1-6 curve D), despite better growth in the fifth instar. Although hypothetical, these calculations emphasize the potential importance of pH-induced mortality of newly-hatched larvae in lowering invertebrate production in an acid environment.

Fig.1-6. Cumulative biomass production (mg dry weight/tray) of
    C. magnifica reared at pH 4.4 and 6.4.
    A. Production at pH 6.4
    B. Production at pH 4.4 not including enhanced growth
    C. Production at pH 4.4 including enhanced growth
    D. Production at pH 4.4 including enhanced growth and
    early-instar mortality.
    Enhanced growth was simulated by introducing a 20% weight
    increase of fifth instar larvae.

41-b
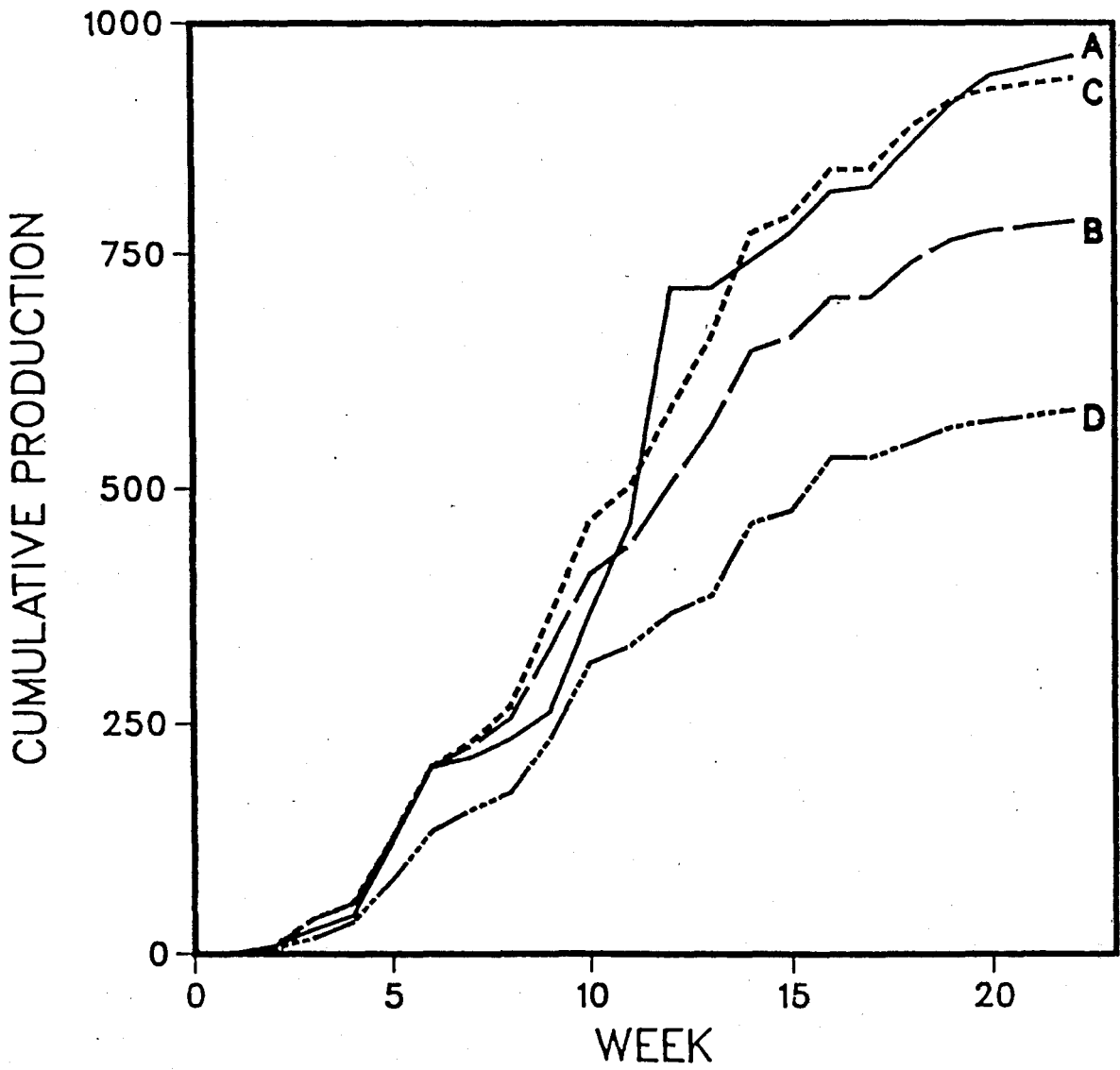
Direct effects of low pH on shredder growth and feeding were not detectable in laboratory studies with *C. magnifica*. Indirect effects enhanced growth of late-instar larvae, probably through a microbe-mediated increase in food quality. My data suggest that low pH may nevertheless lower shredder biomass production by reducing larval survival, particularly in early instars. Reduced production at low pH implies reduced transfer of detrital energy to predators, but applicability of these laboratory data to the field needs further investigation. The basis of enhanced shredder growth needs to be established, and whether the growth response also occurs under field conditions. Furthermore, the effects of increased metal concentrations on microbial conditioning and shredder performance and interactive effects between metals and low pH need to be considered.

CHAPTER 2

MICROBE-MEDIATED EFFECTS OF LOW PH ON THE FATE OF DETRITAL ENERGY INGESTED BY THE SHREDDING CADDISFLY, *CLISTORONIA MAGNIFICA* (LIMNEPHILIDAE)

INTRODUCTION

Accumulation of organic matter has been suggested to contribute to the oligotrophication of acidified waters (Grahn et al. 1974) by reducing the cycling of detrital energy. Decreased rates of organic matter breakdown appear primarily attributable to reduced activity of microbial decomposers in low pH waters (Kelly et al. 1984, McKinley and Vestal 1982, Traaen 1980). Although shredders often dominate invertebrate communities in acid waters (Mackay and Kersey 1985), I postulated in Chapter 1 that inhibition of microbial activity on coarse particulate organic matter would reduce growth of shredders by reducing the nutritional quality of their food supply. However, larvae of the shredding caddisfly, *Clistoronia magnifica* (Banks), reared at pH 4.2-5.2 developed more rapidly to a larger size than those reared at pH 5.8-6.4. Growth enhancement resulted from indirect effects of low pH via the food resource. I postulated that conditioning at low pH improved nutritional quality of leaf litter, possibly as a result of increased abundance of fungi.

There is some evidence that acidification increases abundance of fungal decomposers in lakes and streams (see Singer 1982). Fungal colonization enhances nutritional value of leaf litter, because fungal biomass contributes directly to shredder

growth (Barlocher and Kendrick 1973, Rossi and Fano 1979), while
degradative activity of fungi enhances palatability of leaf
litter (Arsuffi and Suberkropp 1984, Suberkropp *et al*. 1983) and
availability of leaf-derived energy (Barlocher 1982, 1983). In
this chapter I examined microbe-mediated changes in food quality
as a possible basis for enhanced growth of *C. magnifica* under
acid conditions. I related pH-induced changes in the abundance
of fungi and bacteria on leaf litter to changes in ingestion
rates and assimilation of leaf- and microbe-derived energy by
late-instar *C. magnifica* larvae.

METHODS

Conditioning of Leaves

All experiments were conducted in dechlorinated tapwater.
Quality of this water is described in Table 1-1. Full-grown
leaves of alder, *Alnus rubra*, were picked from the tree and
air-dried. Leaves were soaked for at least 48 h and then
conditioned at pH 4.0-4.5 and pH 5.8-6.4. Details of the
conditioning procedure are given in Chapter 1. Leaves were
conditioned in tanks with recirculating water or in trays with a
flow-through of 100 ml/min. Possible accumulation of leaf
leachates in the tanks was minimized by replacing the water
every other week. Leaves in the tanks were suspended parallel to
direction of flow to maximize exposure of both surfaces. In the
trays leaves were incubated as loose packs. Conditioning pH was
maintained as described in Chapter 1. The pH values reported for
each experiment represent means of daily measurements.

Microbial Abundance

Microbial abundance on leaves incubated in the tanks at pH 4.1 and 6.3 was examined by scanning electron microscopy (SEM) in two replicate experiments. A total of 15 disks (1.5 cm²) were cut from 5 leaves after incubation for 3 weeks at each pH level. Disk were fixed in paraformaldehyde (2%) and osmium tetraoxide (2%), dehydrated in acetone, and dried in a critical point dryer. Dried disks were mounted, coated with gold *in vacuo*, and examined with a Perkin-Elmer Autoscan SEM. Examination was restricted to the ventral leaf surface where most microbial growth occurred. I photographed 4-5 areas at 100x along a predetermined transect on 3-4 disks that were randomly selected from the 15 disks at each pH. Fungal abundance was estimated by measuring total length of hyphae on the photographs.

In a second experiment I quantified bacterial and fungal abundance on leaves conditioned at pH 4.0 and 6.2 as a function of conditioning time. Leaves were soaked for 6 days and then conditioned in the flow-through trays. Bacterial abundance and fungal biomass were estimated after 0, 2, 4, and 6 weeks of conditioning. On each sampling occasion, 40 disks (1.5 cm²) were cut from 10 leaves at each pH. Disks were fixed for 30 min in 2% paraformaldehyde and homogenized (8 min at the highest setting of a Virtis model 23 homogenizer) in 40 ml of filter-sterilized (0.20 μm Millipore) sodium pyrophosphate (0.01M) as deflocculating agent. From each homogenate 10 aliquots of 1 ml were taken. Five were used to prepare slides for bacterial enumeration using acridine orange staining, as adapted for leaf

litter by Suberkropp and Klug (1976). Fungi in the other 5 aliquots were stained with aniline blue (Newell and Statzell-Tallman 1982). All slides were examined with a Zeiss Standard WL microscope fitted with an epifluorescence light condenser and UV source (HBO-50 high pressure mercury lamp). From each of the bacterial slides 15-25 randomly selected fields of 2500 μm^2 each were counted. Length and diameter of fungal fragments in each slide were measured in 36 fields of 17671 μm^2 each. Fungal volume was calculated [equation 4 of Baath and Soderstrom (1979)] and converted into fungal biomass using a dry mass density of 0.30 mg/cm^3, an average value that was obtained from data of Newell and Statzell-Tallman (1982). Bacterial numbers and fungal biomass per field were converted to number of bacteria and mg mycelium per disk, using appropriate conversion factors for area of field, effective filter area, quantity of homogenate filtered, and dilution. These methods yield at least relative comparisons between pH treatments.

Microbial Fatty Acids

Changes in the relative abundance of fungi, bacteria, and other microorganisms associated with detritus have been successfully inferred from analysis of fatty acids (Bobbie and

White 1980). I assessed the relative contribution of fungi and bacteria to the composition of the microbial community on alder leaves by determining microbial fatty acid composition. Leaves were incubated for 4 weeks at pH 4.1 and 6.2 in the flow-through trays, after which 200 disks (1.5 cm²) were cut from 5 leaves in each pH treatment. Disks were fixed in 2% paraformaldehyde and sonicated at a frequency which removed about 50-75% of the microbes with minimum disruption of plant cells (determined by sonicating ^{14}C-labelled disks with ^{3}H-labelled microbes). Microbes dislodged by the ultrasound treatment were recovered by filtration on 0.20 μm Millipore filters. Lipids were extracted (Bligh and Dyer 1959) and transesterified to methylesters using boron fluoride-methanol (Morrison and Smith 1964). Esters were extracted with hexane and purified on a 2 cm long column of silicagel, 1.5 cm in diameter. The purified fractions were analysed on a Hewlett-Packard 5880 gas chromatograph equipped with a flame ionization detector and a 25-m fused silica capillary column (DB-1, 0.2 mm diameter). The oven temperature was programmed at 15°C/min from 40 to 180°C and 2°C/min from 180-240°C. Esters were identified by comparing retention times with standards and whenever possible by gas chromatographic-mass spectral analysis (GC-MS). Concentrations were expressed as equivalents of an internal standard (methyltridecanoate) added as acid prior to esterification. Concentrations were corrected for fatty acids released from plant cells during sonication by subtracting acids resulting from sonication of leaves without

microflora. Efficiency of the esterification, extraction, and
purification procedures were checked with a standard mixture of
bacterial fatty acids. There were 3 replicate incubations at
each pH.

Ingestion.

Microbe-mediated effects of low pH on ingestion of alder
leaves were studied in relation to conditioning time, using the
paired leaf disk technique described in Chapter 1. Alder leaves
were conditioned for 0, 2, 3, 4, or 5 weeks in the tanks at pH
4.3 and 6.0 and fed to fourth instar *C. magnifica* larvae that
were held at pH 6.4 (3 replicates of 9-11 larvae per treatment).
Larvae were held for 1 week before the experiment on leaves
conditioned for the same duration and at the same pH as leaves
used in the feeding trials.

Fate of Ingested Energy

The fate of energy derived from microbial biomass
(hereafter referred to as microbe-derived or microbial energy)
was initially studied by labelling microbes on alder leaves

after conditioning for 3 weeks at pH 4.1 or 6.4. Microbes were labelled by incubating leaf disks for 48 h with D[U-]^{14}C-glucose (200 mCi/mmol, 10 μCi per 5 disks in 10 ml dechlorinated tapwater). Labelled disks were fed for 1 h to 3 replicates of 6 fourth instar *C. magnifica* larvae held at pH 6.4. After feeding on labelled disks larvae were transferred to unlabelled alder and allowed to clear their guts for 24 h. Fate of ingested microbial carbon was assessed as in Chapter 1. Larvae were transferred to fresh water every 8 h to maintain the pH at 5.8-6.4.

I subsequently studied the effect of conditioning time and conditioning pH on the fate of microbe- and leaf-derived energy ingested simultaneously. Young alder leaves were labelled with ^{14}C as described in Chapter 1 and conditioned for 2,3,4, or 5 weeks at pH 4.1 or 6.4 in the flow-through trays. Microbes were labelled by incubating leaf disks for 96 h with D[1-^{3}H]glucose (3.8 Ci/mmol, 20 μCi per 5 disks in 10 ml dechlorinated tapwater). Dual-labelled disks were fed for 1 h to 3 replicates of 8 fifth instar larvae held at pH 6.4. Assimilation of ^{14}C and ^{3}H was followed during 30 h of feeding on unlabelled alder. Larvae were transferred to fresh water 6, 12, and 24 h following feeding on the labelled disks. Experiments with feces from labelled larvae indicated that leaching from fecal pellets amounted to 4.7±2.1% (n=10) and 7.6±3.8% (n=10) of fecal ^{14}C and ^{3}H respectively. These values were used to correct excretion estimates for soluble label derived from the feces. Uptake of

^{14}C and ^{3}H by sterilized disks was always <5% of uptake by unsterilized disks.

The fraction of leaf-derived ^{14}C incorporated into microbial cells during conditioning of labelled alder leaves was estimated as follows. ^{14}C-labelled leaves were conditioned at pH 4.1 and 6.4 for 4 weeks. Microbes on 10 disks from each pH treatment were labelled with ^{3}H-glucose, dislodged by sonication (15 s at 50 Watts on a Biosonik-II sonicator with a BP-II probe), and collected on a 0.20 μm Millipore filter. The ratio of ^{3}H and ^{14}C on these filters provided an estimate of the proportion of ^{14}C taken up by microbes on the leaf disk. The proportion of total ^{3}H present on the filter was an estimate of the removal efficiency of the sonication procedure, after correction for adsorbed label (i.e. uptake by conditioned but sterilized disks). ^{14}C on the filter, corrected for release of particulate ^{14}C from the leaf disk during sonication (as estimated from sonication of unconditioned ^{14}C-labelled disks), was assumed to be of microbial origin and was divided by the removal efficiency to calculate total microbial ^{14}C. My data indicate that during 4 weeks of conditioning <5% of ^{14}C present in a leaf disk was incorporated into microbial biomass, causing a negligible overestimate of the assimilation efficiency of leaf-derived carbon.

Ingestion and radioisotope experiments were conducted in dechlorinated tapwater at 12.5-13.5°C under a 16 L : 8 D

photoperiod. Larvae were held at experimental conditions for at
least 1 week before experimentation and were first generation
laboratory-reared offspring from egg masses collected in Marion
Lake, B.C.

Statistical Analysis

Statistical analysis of the data was performed as described
in Chapter 1. Heterogeneity of variances of fungal hyphae
lengths was corrected by square-root transformation.

RESULTS

SEM indicated an abundant growth of fungi and bacteria on alder leaves after 3 weeks of conditioning. Bacteria were mostly rod-shaped, up to 1.5 μm long and 0.5 μm wide. Despite large variability between disks, fungal hyphae (mean diameter 3.5 μm, range 1.5-5 μm) were about four times more abundant on leaves conditioned at pH 4.1 than on leaves conditioned at pH 6.3 ($p<0.01$, one-way ANOVA, Table 2-1).

Bacterial density and fungal biomass were significantly affected by the duration and pH of conditioning and their interaction ($p\leq0.01$, two-way ANOVA, Fig. 2-1). Bacterial density increased with conditioning time and was slightly higher at pH 4.0 than at pH 6.2 after 4 weeks of conditioning. Fungal biomass increased for 4 weeks and then remained approximately 1.5-fold higher at pH 4.0 than at pH 6.2.

Twenty microbial methylesters were distinguished on the basis of retention times, but only 12 were positively identified by GC-MS (Table 2-2). Leaves conditioned at pH 4.1 contained higher levels of most fatty acids than did leaves conditioned at pH 6.0, but not all differences were statistically significant (t-test, Table 2-2).

Table 2-1: Fungal abundance on alder leaves after 3 weeks of conditioning at pH 4.1 and pH 6.3.

Exp. no.	Conditioning pH	Length (mm) of fungal hyphae/leaf disk (mean ± SE)[1]	No. of disks
1	4.1	4082±305 a	4
	6.3	1774±302 b	4
2	4.1	2406±544 a	3
	6.3	665±158 b	3

[1]Means within an experiment followed by different letters are significantly different ($p \leq 0.05$).

Fig. 2-1. Fungal biomass (mg/disk) and bacterial abundance
    ($\times 10^5$/disk) on alder leaves conditioned at pH 4.0 and 6.2
    in relation to conditioning time. Means ± SE. * indicates a
    significant difference between pH treatments at p≤0.05.

56-b
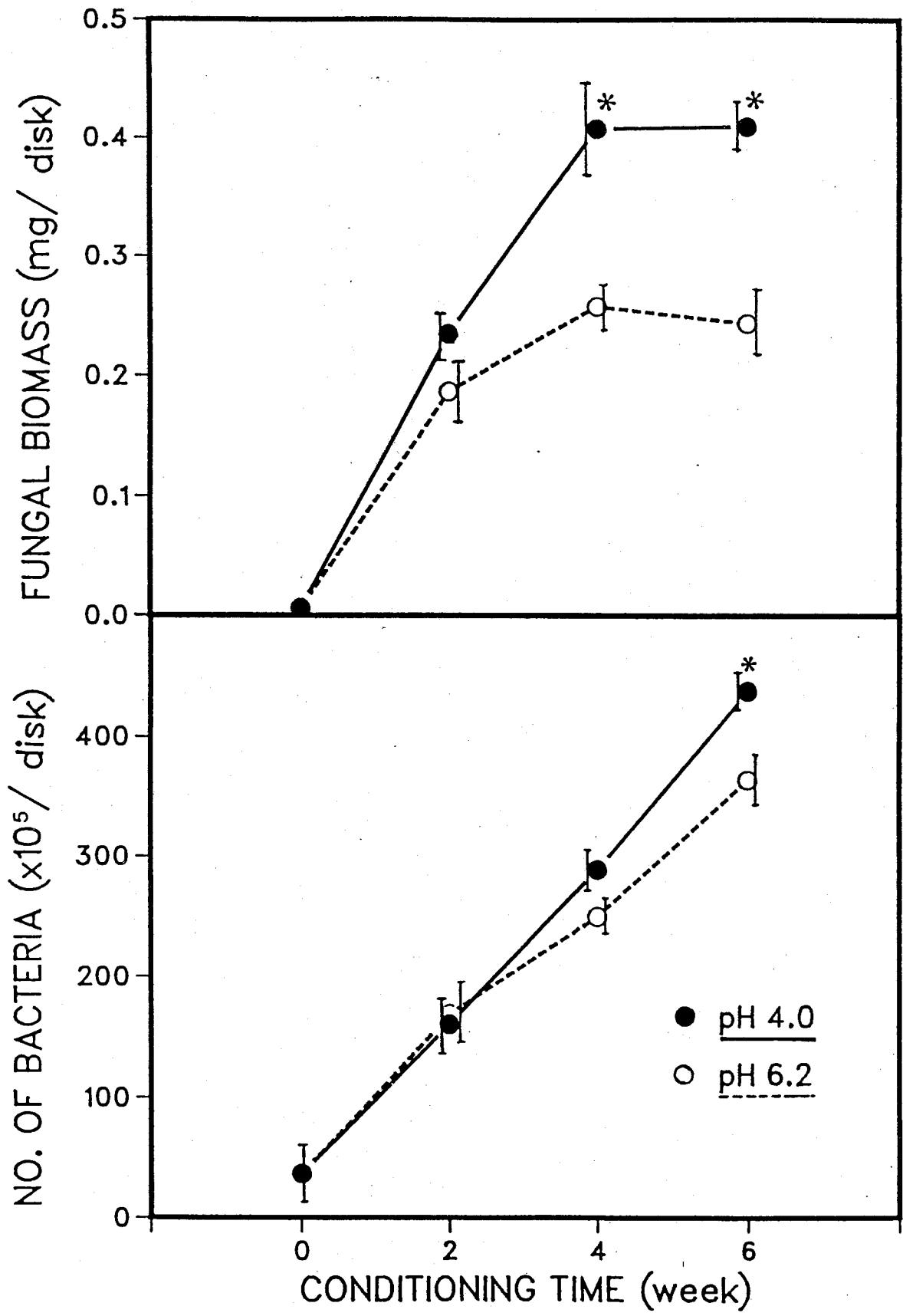
Table 2-2. Fatty acids derived from microbes on alder leaves conditioned for 4 weeks at pH 4.1 and 6.2.

Methylester[2]	μg/ml (mean ± SE)[1] of fatty acids	
	pH 4.1	pH 6.2
11:0	6.2± 0.8 a	2.9± 0.9 b
12:0	7.2± 1.3	5.9± 0.6
14:0	14.0± 4.9	11.6± 1.7
15:0	8.6± 3.2	8.6± 2.5
16:0	177±10.2	152±13.4
17:0	5.1± 1.0	3.4± 1.4
19:0	2.7± 0.4	3.0± 0.3
16:1	25.5±10.9	23.1± 7.4
18:1ω9	27.0±10.5	39.0±17.0
18:1ω7[3]	16.1± 1.7 a	6.5±0.01 b
18:2	183±14.6 a	132±11.7 b
18:3ω3	171±41.6	135±31.4
total polyenoics-	354±56.3	268±94.7
3 OH 12:0[3]	0.5±0.04 a	0.1± 0.2 b
2 OH 14:0[3]	0.4± 0.2	0.2± 0.2
3 OH 14:0[3]	0.3± 0.2	0.0
2 OH 16:0[3]	3.5±0.04 a	1.7± 0.7 b
total hydroxy-	4.8± 0.4 a	2.0± 1.1 b
a 15:0[3]	3.3± 0.6	2.7± 1.3
a 17:0	3.7± 2.8	3.4± 1.4
total branched-	7.0± 2.2	6.0± 2.6
Δ17:0[3]	6.4± 4.8	3.2± 0.1
Δ19:0[3]	0.9±0.01 a	0.6±0.07 b
total cyclopropane-	7.3± 2.7	3.8± 0.2

----

[1]Means on a line followed by different letters are significantly different (p≤0.05, n=3).
[2]Fatty acids are designated as the no. of carbon atoms:no. of double bonds, ω indicates position of the double bond.
[3]No positive identification by GC-MS.

Ingestion of alder leaves by *C. magnifica* was not affected by the pH at which the leaves were conditioned (p>0.50) (Fig. 2-2, two-way ANOVA). Ingestion rates increased significantly with conditioning time (p<0.01), with no interaction between time and pH (p>0.50).

Conditioning pH significantly affected the % of ingested microbial carbon that was egested, transformed into shredder biomass, and assimilated by *C. magnifica* (p<0.01, one-way ANOVA, Table 2-3). Efficiency of assimilation of microbial energy from leaves conditioned for 3 weeks at pH 4.1 was about 9% higher than from leaves conditioned at pH 6.4, due to higher net production and lower egestion. The proportion of label lost to respiration and excretion was the same in both treatments.

Conditioning time and pH significantly affected the fate of microbe-derived ^{3}H, with a significant interaction (Fig. 2-3, two-way ANOVA, p-values are included in the figure). Assimilation of microbial energy derived from leaves conditioned at either pH increased with time, reaching the highest efficiency after 4 weeks. Microbial energy obtained from leaves conditioned at pH 4.1 was assimilated with a higher efficiency than from leaves conditioned at pH 6.4. The magnitude of this difference was a function of conditioning time, ranging from 0% in week 2 to 15% in week 4. Utilization of microbial energy for larval growth (production) followed a similar pattern, which was reflected in mirror image by the change in egested ^{3}H. There was

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Fig. 2-2. Ingestion rates (mg leaf/mg larva dry weight/day) of
    fourth instar C. magnifica larvae that were held at pH 6.2
    and fed with alder leaves conditioned at pH 4.3 and 6.0.
    Means ± SE.
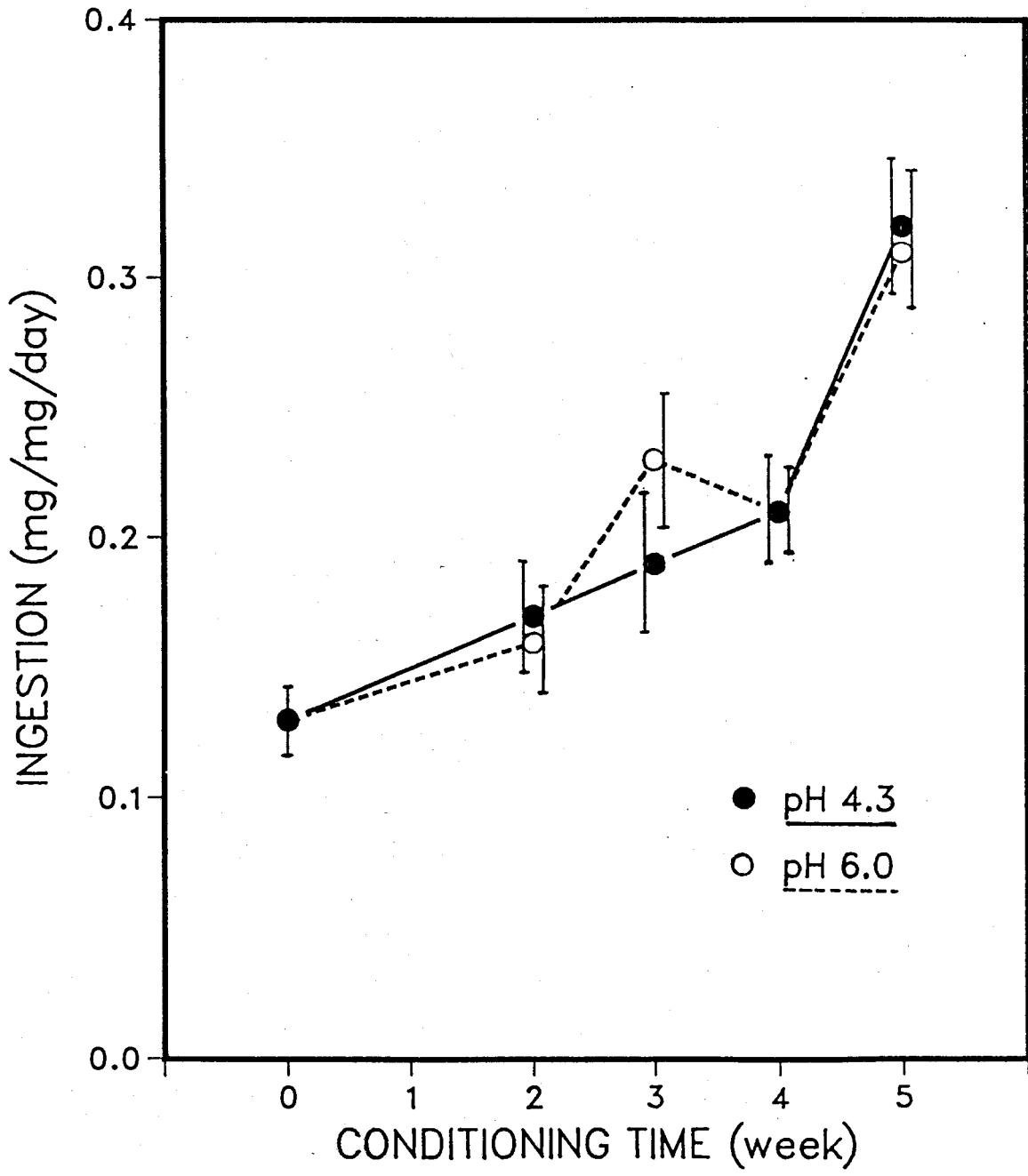
Table 2-3. Fate of microbial carbon obtained from alder leaves by fourth instar larvae of *C. magnifica* held at pH 6.4. Leaves were conditioned for 3 weeks at pH 4.1 and 6.4.

pH	Percentage of ingested ^{14}C (mean ± SE)[1]				
	Egested	Produced	Respired	Excreted	Assimilated[2]
4.1	9.1±5.5 a	72.2±3.3 a	15.8±0.9	2.9±0.7	91.6±3.9 a
6.4	17.1±2.0 b	64.9±1.6 b	15.5±0.3	2.3±0.1	82.7±1.4 b

[1]Means within a column followed by diferent letters are significantly different (p≤0.05).
[2]Assimilated fraction is the sum of ^{14}C used for growth (produced), respired, and excreted.

Fig. 2-3. Fate of microbe-derived energy (^{3}H) ingested by fifth
    instar larvae of C. magnifica fed on alder leaves
    conditioned at pH 4.1 and 6.4.
    P-values are indicated for the effects of pH, conditioning
    time, and their interaction. * indicates a significant
    difference between pH treatments at $p \leq 0.05$.
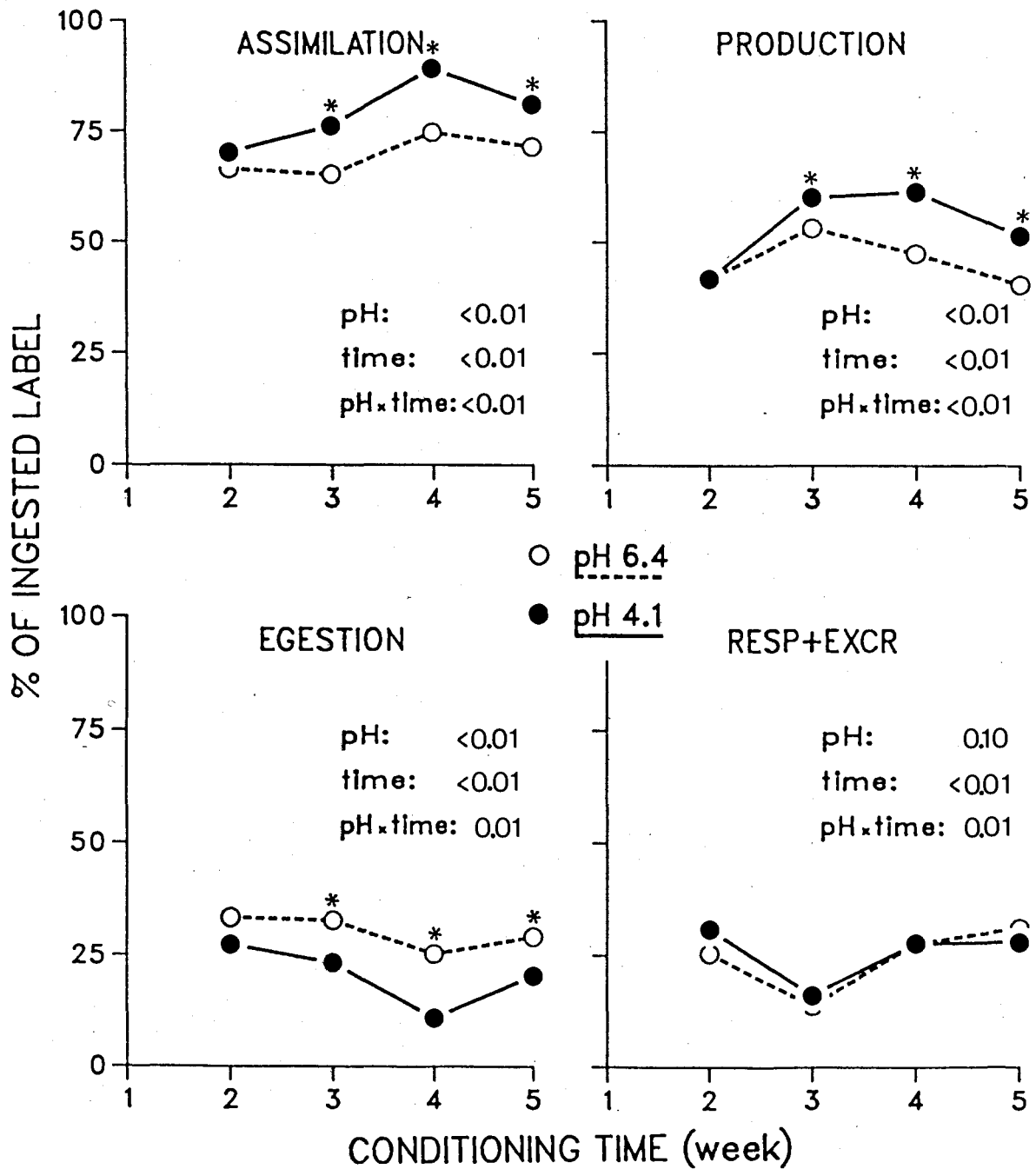
Fig. 2-4. Fate of leaf-derived energy (^{14}C) ingested by fifth instar larvae of _C. magnifica_ fed on alder leaves conditioned at pH 4.1 and 6.4.
P-values are indicated for the effects of pH, conditioning time, and their interaction. * indicates a significant difference between pH treatments at $p \leq 0.05$.
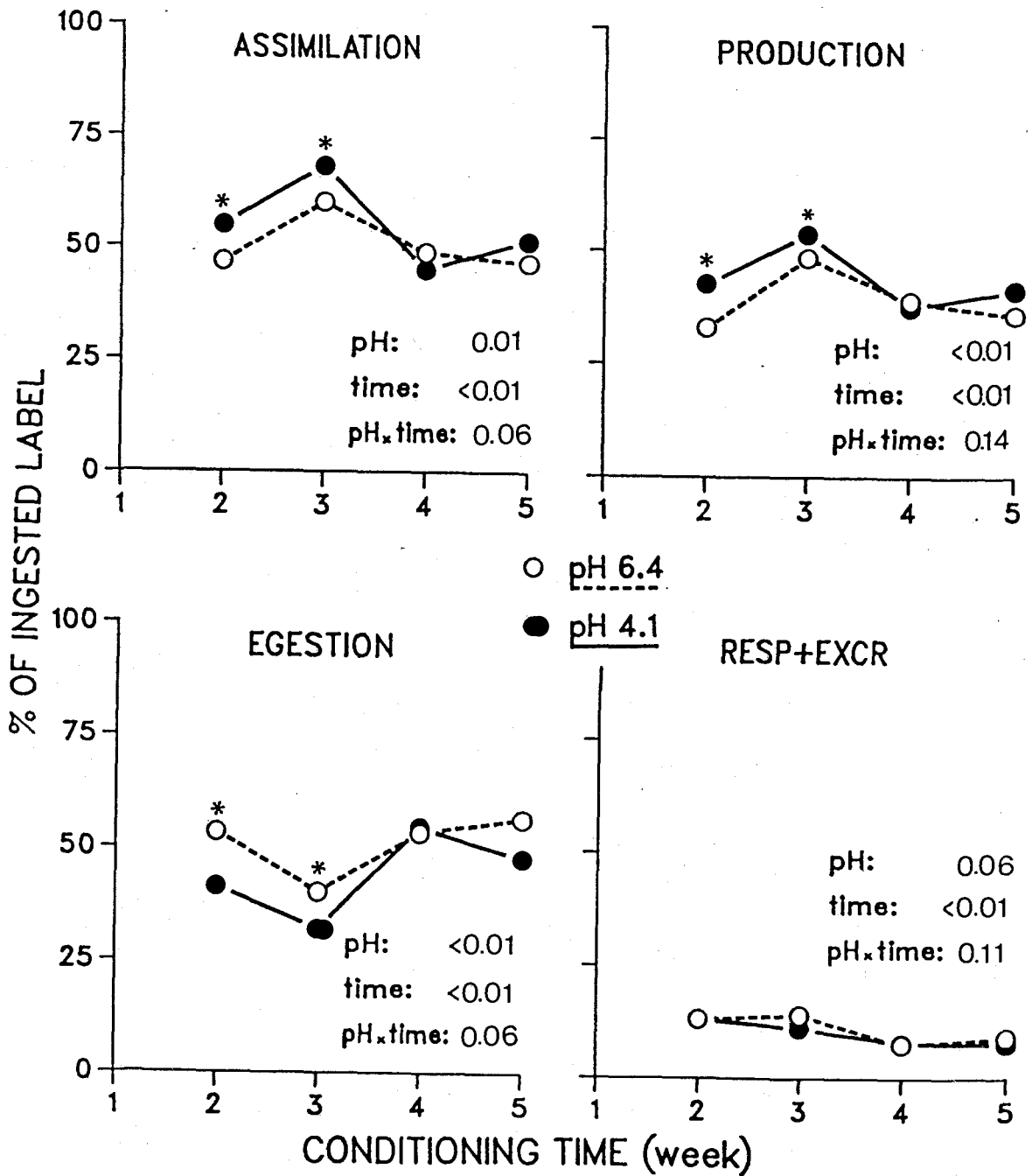
no effect of pH on the proportion of ^3H in the water, which was assumed to represent metabolic waste products (i.e. excretion plus respiration).

Fate of leaf-derived ^{14}C was significantly affected by conditioning time and pH with a limited interaction (Fig. 2-4, tow-way ANOVA, p-values are included in the figure). Assimilation of leaf-derived energy did not increase with time, but was higher in week 3 than in other weeks. Leaf material conditioned at pH 4.1 for 2 and 3 weeks was assimilated with a 5-10% higher efficiency than leaves conditioned at pH 6.4. Assimilation was on average 5% higher for leaves conditioned at pH 4.1, as was the case for the proportion of ingested ^{14}C used for larval growth. There was no effect of pH on the proportion of carbon respired, excreted, or of the two fractions combined.

DISCUSSION

Microbial abundance data demonstrate that conditioning of
alder leaves at different pH resulted in differential microbial
colonization, thereby providing a possible basis for
microbe-mediated effects of low pH on shredder growth.
Conditioning of alder leaves at pH 4 resulted in a 1.5-3.5 fold
increase of fungi and a smaller increase of bacteria as compared
with leaves conditioned at pH 6 (Table 2-1, Fig. 2-1).

Assessment of these changes in microbial community
structure by analysis of fatty acids was not conclusive (Table
2-2). A 1.3-fold increase in polyenoic acids, which are found in
fungi and not in bacteria (Bobbie and White 1980), suggests
increased fungal biomass. Higher levels of some typical
bacterial fatty acids, such as *cis*-vaccenic acid (C18:1ω7), a
product of anaerobic bacterial metabolism, suggest increased
bacterial biomass as well, but other measures of bacterial
biomass, such as anteiso-branched acids, did not confirm this.
Fatty acid composition did not indicate a shift from bacterial
decomposers to fungi on leaf material conditioned at low pH
(Table 2-4). Inhibition of procaryotic growth and stimulation of
eucaryotic growth at low pH would decrease the first 5 ratios
listed in Table 2-4 and increase the last ratio (Bobbie and
White 1980). The observed increase in the latter was not

Table 2-4. Ratios (mean ± SE)[1] of bacterial and fungal
fatty acids on alder leaves conditioned for 4 weeks at pH 4.1
and 6.2, as calculated from Table 2-2.

Fatty acid ratio[2]	pH 4.1	pH 6.2
branched/15:0+17:0	0.51 ±0.00	0.47±0.06
cyclopropane/16:0	0.043±0.018 a	0.025±0.001 b
hydroxy/16:0	0.027±0.001 a	0.014±0.005 b
total bacterial/16:0[3]	0.108±0.005 a	0.064±0.002 b
18:1ω7/18:1ω9	0.83 ±0.28 a	0.23 ±0.10 b
polyenoics/16:0	2.03 ±0.43	1.73 ±0.20

[1]Means on a line followed by different letters are
 significantly different (p≤0.05).
[2]Fatty acids are designated as in Table 2.
[3]Total bacterial acids=branched+cyclopropane+hydroxy.

significant (p>0.10, t-test), whereas the 4 other ratios tended to be higher in the pH 4 samples, suggesting increased bacterial abundance. Thus, fatty acid analysis suggested increased abundance of both fungi and bacteria on alder leaves conditioned at low pH. However, observed changes in fatty acid composition should be interpreted with caution. Bacterial fatty acids were present in low concentrations and their mass spectra were generally too contaminated with unknown fragments to allow unequivocal identification. Furthermore, sonication of leaf disks without aquatic microbiota yielded relatively high concentrations of 16 and 18 carbon saturated and unsaturated acids, indicating a substantial contribution of leaf-derived acids. That contribution was subtracted from fatty acid concentrations in the pH-treatments, but could not be accurately quantified, because conditioned disks probably released more plant-derived fatty acids during sonication than unconditioned disks.

Ingestion of alder by *C. magnifica* increased with conditioning time (Fig. 2-2). Conditioning of leaf litter generally results in a complex of biochemical and physical changes largely mediated by microbial colonization. Softening of the leaf tissue, increased microbial biomass, higher leaf nitrogen content, and partial decomposition of refractory leaf components all appear to contribute to enhanced shredder feeding (Barlocher and Kendrick 1975, Kaushik and Hynes 1971, Lawson *et al*. 1984). Differential microbial colonization of leaves

conditioned at different pH, however, did not result in differential ingestion rates (Fig. 2-2). Differences between leaves in palatability prior to conditioning may have masked differences in ingestion due to conditioning pH. That is not likely, given that half of one leaf conditioned for 5 weeks at pH 4.3 was ingested at the same rate as the other half conditioned at pH 6.0 (0.21 mg/mg/day in both treatments, n=15 fifth instar larvae). Enhanced growth of larvae at low pH thus cannot be explained by greater palatability and corresponding higher ingestion of alder leaves conditioned at low pH.

Results of experiments with labelled microbes supported the hypothesis that conditioning at low pH increased food quality. Fourth and fifth instar larvae that were fed leaves conditioned at pH 4.1 assimilated microbial energy with a 10-15% higher efficiency than those fed leaves conditioned at pH 6.4. The resultant energy gain was associated with increased production of larval tissue. That phenomenon was observed on 2 occasions (Table 2-3, Fig. 2-3), using different isotopes, different instars, and microbial inocula collected at different times.

A 10-15% increase in assimilation of microbial energy cannot account for the increase in larval growth at low pH. Assuming that microbial biomass constitutes 10% of leaf dry weight (Barlocher and Kendrick 1981), and that microbes contain twice as much assimilable material per unit weight as leaves (Table 2-3 vs Table 1-7), 20% of energy assimilated by C.

magnifica would be of microbial origin. Detritivorous aquatic invertebrates generally derive <30% of the total energy demand from microbial biomass (Cammen 1980, Findlay *et al.* 1984, Lawson *et al.* 1984). The remaining energy requirement is presumably met by assimilation of the leaf matrix. I therefore hypothesized that increased availability of leaf energy, due to increased microbial activity, contributed to enhancement of larval growth at low pH.

Increased assimilation of microbe-derived energy was possibly associated with a more efficient assimilation of leaf-derived energy (Fig. 2-4). Energy derived from leaves conditioned at pH 4.1 was assimilated with a 5-10% higher efficiency than energy obtained from leaves conditioned at pH 6.4. However, large variability of the estimates precluded a conclusive demonstration. Assimilation estimates of leaf-derived ^{14}C were much more variable than estimates of microbe-derived ^{3}H (coefficients of variation were 20±8% and 8±3.8% respectively, n=8). Larger variability probably arose from differences between leaves in factors governing leaf quality other than microbial conditioning, such as nutritional status of the leaf prior to conditioning, leaf softness (Herbst 1982), and leaf thickness (Sutcliffe *et al.* 1981). None of those variables was controlled in my experiments. Leaves in week 3, for example, were softer than leaves used in other weeks, which may have contributed to the high assimilation efficiencies observed in that week. Large variability between leaves may have obscured microbe-induced

differences in assimilation of leaf-derived energy in weeks 4 and 5, but also makes the observed differences in weeks 2 and 3 suspect. To assess whether those differences are attributable to conditioning pH, between-leaf variation in assimilation should be examined in more detail.

Only a slight increase in availability of leaf energy would be sufficient to account for the growth increase of *C. magnifica* observed in Chapter 1. A 5-10% increase in assimilation would result in 14-28% more energy useable for growth (Table 2-5). Coupled with a 10% increase in assimilation of microbial energy (Table 2-3) and a doubling of microbial biomass (Fig. 2-1), 35-50% more energy would be available for growth of larvae feeding on alder leaves conditioned at low pH. Such an energy gain would be adequate to account for the weight increase of about 20% observed at low pH (Fig. 1-2).

The increase in assimilation of microbial energy reflects the observed increase in fungal biomass. The concomitant increase in bacterial abundance probably contributed less to enhancement of shredder growth, because bacteria are generally less important in shredder nutrition than fungi (e.g. Barlocher and Kendrick 1981). The nutritional importance of fungi to *C. magnifica* is further demonstrated by the observation that this species can complete its development on a diet of axenic fungal cultures (Hanson *et al.* 1984).

Table 2-5. Illustration of hypothetical gain in potential
growth of *C. magnifica* larvae fed on leaves conditioned
at pH 4 as compared with pH 6. In relative energy units. [1]

pH	Energy source	Increase in AE (%)	Ingested energy	Assimi- lated energy	Energy used for growth	% Gain in growth
6	Leaf	0	100	40	30	
	Microbes	0	10	8	6	
					--	
					36	
4	Leaf	5	100	45	35	
	Microbes	0	10	8	6	
					--	
					41	14
4	Leaf	10	100	50	40	
	Microbes	0	10	8	6	
					--	
					46	28
4	Leaf	5	100	45	35	
	Microbes[2]	10	20	18	14	
					--	
					49	36
4	Leaf	10	100	50	40	
	Microbes[2]	10	20	18	14	
					--	
					54	50

[1]Assimilation efficiencies (AE) and growth efficiencies are
 based on Table 2-3 (microbes) and Fig. 2-4 (leaf).
[2]including doubling of microbial biomass.

Increased availability of leaf-derived energy is presumably the result of additional modification of the leaf substrate by microbial enzymes. Most shredders are unable to digest structural polysaccharides (Martin *et al*. 1981, Monk 1976), unless aided by cellulolytic enzymes of microbial origin (Barlocher 1982, Winterbourn 1982). Fungi are the major source of these enzymes (Suberkropp and Klug 1976, 1980). Low pH may increase the production of cellulases by favoring fungal colonization, or may increase cellulase activity (Sinsabaugh *et al*. 1981).

Enhancement of growth occurred primarily in late instars (Table 1-2), which suggests increased availability of carbohydrates. Growth of the final instar of *C. magnifica* is limited by a carbohydrate source for synthesis of storage lipids needed during the pupal stage (Hanson *et al*. 1983). Leaves conditioned at pH 4.1 may provide more carbohydrates than leaves conditioned at pH 6.4, due to increased fungal biomass and additional modification of the leaf substrate by fungal enzymes. However, qualitatively important nutrients associated with microbial biomass, such as vitamins, amino acids, or essential fatty acids (Table 1), could also contribute to better growth at low pH.

The increase in fungal biomass was due to proliferation of higher fungi. The incidence of aseptate hyphae of 1.5-3.5 μm width was the same in samples of both pH treatments, suggesting

no change in occurrence of lower fungi (Phycomycetes). The incidence of 3.5-5.0 μm wide septate hyphae doubled at lower pH, indicating greater abundance of higher fungi (Hyphomycetes). That group includes the aquatic hyphomycetes, saprophytes with leaf-degrading ability (Suberkropp and Klug 1980), which are readily utilized by caddisfly shredders (Suberkropp *et al*. 1983) and which grow best at pH 4-5 (Rosset and Barlocher 1985).

Increased fungal colonization of leaf litter at low pH infers higher rates of leaf breakdown. In this regard the laboratory data contradict field observations of reduced leaf litter breakdown under acid conditions (McKinley and Vestal 1982, Mackay and Kersey 1985). It is possible that low pH enhances fungal colonization of leaf litter, but that in the field pH effects are confounded by factors that influence activity of detrital microorganisms and that covary with pH, such as calcium content of the water and increased metal concentrations.

Is the observed increase of fungi in my study an artifact of leaf conditioning in the laboratory? My fungal biomass estimates and observed fungal colonization pattern corresponded to field observations (Barlocher and Kendrick 1981, Chamier *et al*. 1984), which suggest that my laboratory observations may be representative of natural conditions. Furthermore, increased incidence of fungal decomposers has been observed under field conditions (see Singer 1982), although these observations

generally lack a quantitative basis.

In conclusion, enhanced growth of *C. magnifica* larvae at low pH in the laboratory was attributable to the effects of pH on microbial colonization of the leaf litter. Increased fungal colonization of leaves at low pH improved availability of microbe- and leaf-derived energy for growth of larvae at pH levels that did not adversely their performance. Now that the basis of enhanced shredder growth is known, a field experiment is needed to establish whether the observed shredder-CPOM-microbe interactions also occur in acidified waters. In particular, modifying effects of increased metals concentrations need to be studied.

CHAPTER 3

INTERACTIVE EFFECTS OF LOW PH AND NICKEL ON GROWTH AND SURVIVAL OF THE SHREDDING CADDISFLY, *CLISTORONIA MAGNIFICA* (LIMNEPHILIDAE)

INTRODUCTION

In waters subjected to acidic precipitation a decrease in
pH is often associated with an increase in certain dissolved
metals (NRCC 1981a). This increase has been attributed to
industrial contamination of precipitation (Wright and Gjessing
1976) and to leaching from watershed soils (Malmer 1976) and
lake sediments (Schindler *et al*. 1980). Organisms in acidified
waters could be affected by low pH, the toxic effects of metals,
or by the interaction between metals and low pH. In previous
chapters I assessed the direct and indirect (microbe-mediated)
effects of low pH on growth and survival of the shredding
caddisfly, *Clistoronia magnifica*. In this chapter I investigate
how *C. magnifica* is affected by the interaction between low pH
and nickel.

Dissolved metals are generally believed to be more toxic to
aquatic organisms under acid conditions (e.g. Spry *et al*. 1980),
but several studies have reported decreased toxicity (e.g.
Borgmann 1983). The relationship between pH and metal toxicity
is not simple. The pH may influence metal toxicity directly, by
affecting metal uptake sites, or indirectly, by determining
chemical speciation of the metal (Peterson *et al*. 1984). In
using nickel, a metal without a strong pH-dependent speciation,
some of these complexities were minimized. In soft waters and at

the pH levels used in this study, nickel should occur predominantly as the divalent cation (Ni^{2+}) (Richter and Theis 1980). Nickel is also one of the metals associated with freshwater acidification, primarily in areas affected by deposition of atmospheric pollutants (NRCC 1981a). Elevated levels of nickel have been recorded in acidic precipitation in Scandinavia and North America (Haines 1981). Deposition of atmospheric nickel (Gorham 1976) and leaching from lake sediments under acidic conditions (Stokes and Szokalo 1977) contribute to elevated nickel concentrations in the water. Those concentrations range from below 10 μg/L in uncontaminated surface waters to 1000 μg/L near smelting operations, although levels seldom exceed 100 μg/L (NRCC 1981b).

The objective of this chapter is to investigate the effect of low pH on metal toxicity and to assess the potential effects of pH-metal interactions on the transformation of detrital energy into shredder biomass, using nickel as a model. Growth and survival of *C. magnifica* larvae were first studied in a range of pH-nickel combinations similar to those reported in contaminated Canadian Shield lakes (NRCC 1981b). I then studied growth and survival of larvae exposed to a concentration representative of moderately-contaminated waters (<100 μg/L) and assessed the importance of direct effects of nickel in solution and indirect effects via the leaf litter.

METHODS

Experiments were conducted in dechlorinated tapwater.
Background levels of some dissolved metals are reported in Table
3-1. Other water quality characteristics are given in Table 1-1.
C. magnifica larvae were reared in flowing water (100 ml/min) in
Plexiglas trays (Chapter 1). Reagent grade H_2SO_4 was pumped from
stock solutions into mixing beakers to obtain various pH
regimes. Outflows from the beakers received nickel pumped from
appropriate stock solutions prepared by dissolving reagent grade
$NiCl_2.6H_2O$ in glass-distilled water. Water temperature was
generally maintained at 10-13°C, but occasionally dropped below
10°C. Larvae were fed leaves of alder, *Alnus rubra*, supplemented
with wheat grains. Alder leaves were conditioned as described in
Chapter 1. Experiments were conducted under a 16 L : 8 D
photoperiod.

Toxic effects of nickel on *C. magnifica* were studied by
exposing larvae from first instar until pupation to nickel
concentrations of 55 (range 30-90), 215 (range 145-260), and 700
(range 645-800) μg Ni^{2+}/L. Tapwater with no nickel added served
as the control. Mean concentrations and ranges were derived by
measuring nickel in the test water every other week (3
samples/treatment) using graphite furnace atomic absorption
(detection limit 20 μg Ni^{2+}/L). Removal of suspended solids by

Table 3-1. Background levels of some metals in tapwater used in the experiments. Concentrations were measured by Inductively Coupled Plasma Emission (n=4)

Metal	Concentration of dissolved metal (μg/l)	
	Reference (pH 6.2)	Acidified (pH 4.0)
Fe	288	352
Mn	26	36
Al	<50	<50
Ni	<20	<20
Pb	<20	<20
Zn	4	4
Cu	<5	6
Cr	<5	<5
Cd	<2	<2

filtration (0.45 μm Millipore filters, APHA 1980) did not reduce the measured nickel concentration, indicating that all nickel was in dissolved form. Selection of exposure levels was based on toxicity data reported for *C. magnifica* by Nebeker *et al.* (1984).

The interactive effects of nickel and pH on larval growth and survival were studied by conducting each nickel exposure at mean pH levels of 4.1 (range 3.9-4.3), 5.5 (range 4.9-5.7), and 6.2 (5.9-6.4). There were 2 replicates for each nickel-pH combination with 50 larvae per replicate. Larvae were fed once a week with whole alder leaves conditioned for 4 weeks at pH 6.0-6.5. Larval development and survival were recorded weekly for the first 5 weeks and thereafter every other week. Pupae were sacrificed and dry weights determined when 75% of the surviving larvae had pupated. The experiment was terminated after 19 weeks.

The effects of low levels of nickel on growth and survival of *C. magnifica* were studied by exposing larvae from early-fourth instar until pupation to a concentration of 75 (range 70-100) μg Ni^{2+}/L. Larvae were reared individually in Plexiglas cylinders (Chapter 1) (18 cylinders/tray, 1 tray/treatment) and were held at pH 4.1 (range 3.9-4.5) and 6.2 (range 5.9-6.4). Larvae were fed disks of alder leaves conditioned for 4 weeks at a pH that corresponded to the rearing pH. Leaves were conditioned in water with no added nickel or in water with 75 μg Ni^{2+}/L. To assess the importance of nickel in

solution and nickel associated with the leaf litter, there were 4 nickel treatments at each pH: (1) no nickel in the water and larvae fed leaves conditioned without nickel (control), (2) no nickel in the water and larvae fed leaves conditioned in the presence of nickel, (3) nickel in the water and larvae fed leaves conditioned without nickel, and (4) nickel in the water and larvae fed leaves conditioned in the presence of nickel. Leaf disks in all treatments were replaced daily. The experiment was terminated after 30 weeks when most larvae had pupated. Development and survival of larvae were recorded weekly. Larval growth was expressed as the time required to reach the pupal stage. Accumulation of nickel in the leaf litter during conditioning was measured by graphite furnace atomic absorption (detection limit: 0.05 $\mu g \cdot Ni^{2+}/g$) after nitric-perchloric acid digestion of the leaf samples.

Statistical analysis of the data was performed as described in Chapter 1. Survival data were analysed using contingency tables based on the G-statistic (G-test).

RESULTS

Nickel had a significant effect on mean survival time within each pH treatment when compared with the control (no nickel added) (p<0.01, Kruskal Wallis, Table 3-2). The effect of nickel varied with pH (Fig. 3-1). Mean survival time of larvae was reduced at pH 6.2 when larvae were exposed to 215 and 700 μg Ni^{2+}/L and at pH 5.5 only by 700 μg Ni^{2+}/L. Mean survival at pH 4.1 was reduced at 700 μg Ni^{2+}/L, but increased at 215 μg Ni^{2+}/L. Likewise, pH had a significant effect on mean survival time within each nickel exposure (p<0.01, Table 3-2). In water with no nickel added, pH did not significantly affect mean survival time (p>0.10). In water with 55 μg Ni^{2+}/L, mean survival was higher at pH 5.5 than at pH 4.1 and 6.2. Mean survival at 215 μg Ni^{2+}/L was considerably higher at pH 4.1 and 5.5 than at pH 6.2, while survival at 700 μg Ni^{2+}/L was higher at pH 4.1 than at pH 5.5 and 6.2.

Nickel improved survival of early-instar larvae at pH 4.1. With no nickel added larval survival in the first 2 weeks of exposure was significantly lower at pH 4.1 than at pH 5.5 and 6.2 (Fig. 3-2, week 2, p<0.05, G-test). That drop in early-instar survival did not occur when 215 or 700 μg Ni^{2+}/L were added to the test water (Fig. 3-1). Survival in the first 2 weeks at 215 and 700 μg Ni^{2+}/L was significantly higher than at

Fig. 3-1. The effect of various nickel exposures (μg/L) on survival of C. magnifica larvae from first instar until pupation at pH 4.1, 5.5, and 6.2.
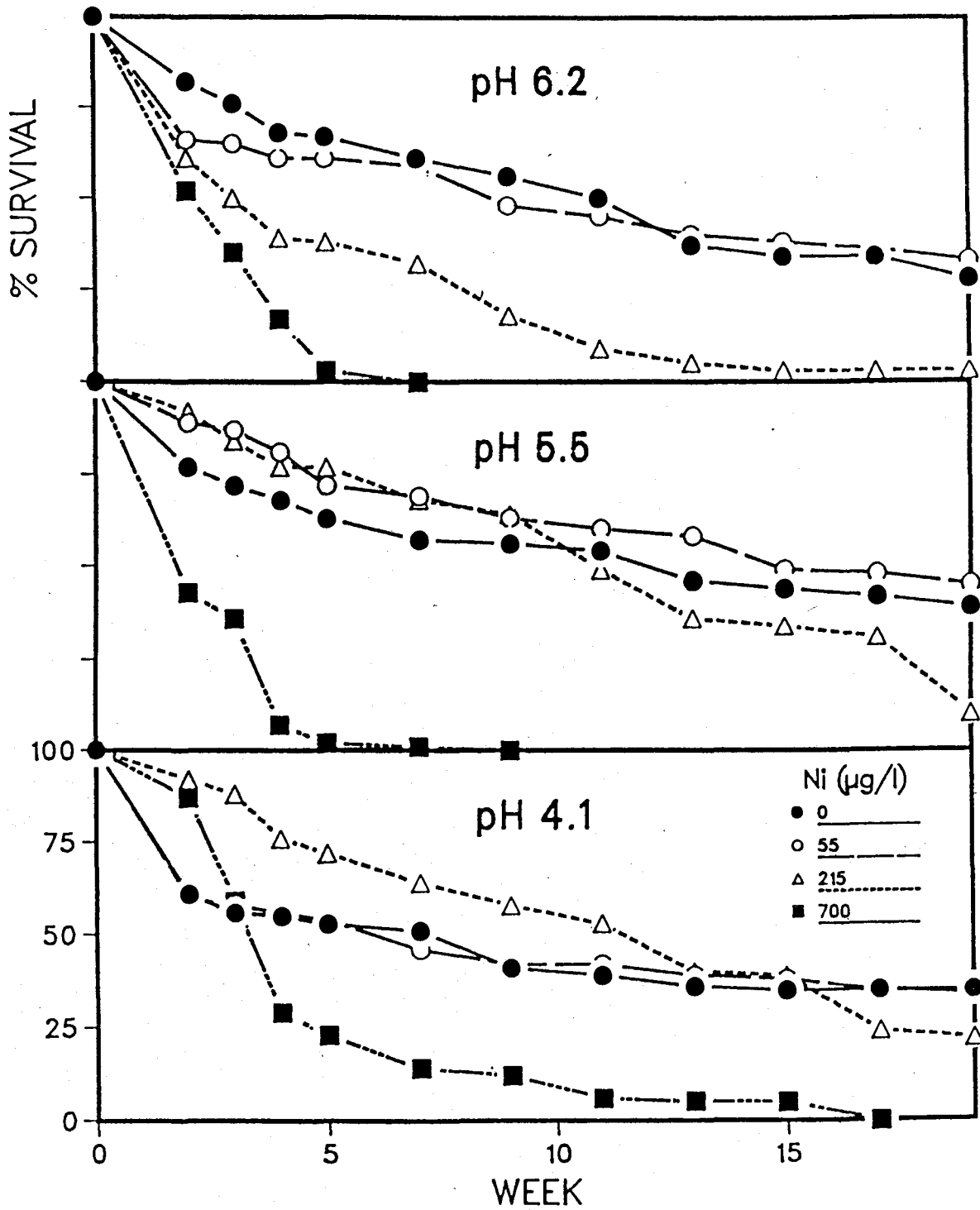
Fig. 3-2. The effect of pH on survival of C. magnifica larvae exposed from first instar until pupation to 0 (no nickel added), 55, 215, and 700 µg Ni^{2+}/L.

83-b
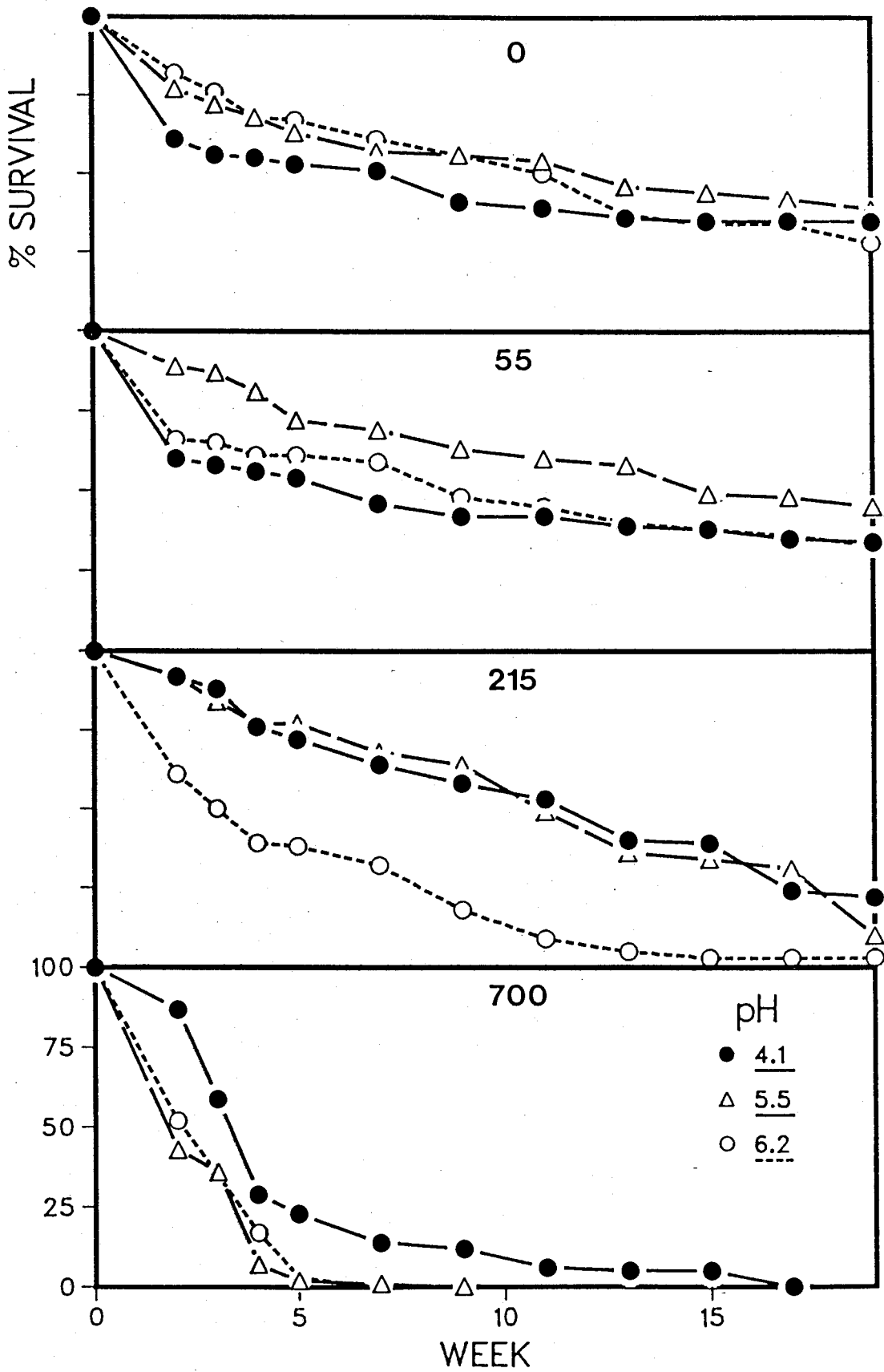
Table 3-2. Survival time of *C. magnifica* larvae held from first instar until pupation at pH 4.1, 5.5, and 6.2 at various nickel exposures (n=100).

[Ni^{2+}] (μg/L)	Survival time in weeks (mean±SE)[1]		
	pH 4.1	pH 5.5	pH 6.2
0	9.87±0.80 a	11.80±0.78 a	11.02±0.68 a
55	9.87±0.81 a	13.40±0.72 a	10.79±0.78 a
215	11.78±0.65 b	11.81±0.63 a	5.69±0.45 b
700	5.05±0.36 c	3.00±0.14 b	3.10±0.13 c

[1]Different letters within a column indicate significantly different means (p≤0.05). Underscored means within a row are not significantly different (p>0.05).

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0 and 55 μg Ni^{2+}/L (p<0.01) and did not differ from survival at pH 6.2 in water with no added nickel (p>0.50) (G-test). Improved early-instar survival persisted only 2 weeks at 700 μg Ni^{2+}/L and 15 weeks at 215 μg Ni^{2+}/L. This ameliorating effect of nickel was not observed at pH 5.5 or 6.2.

Survival at the end of the experiment was lower at 700 and 215 μg Ni^{2+}/L than at 0 and 55 μg Ni^{2+}/L under all pH conditions (Fig. 3-1, week 19, p<0.05, G-test). At 700 μg Ni^{2+}/L few larvae reached pupation, as most larvae died before the fourth instar (Fig. 3-3). Mortality at 215 μg Ni^{2+}/L occurred before and during the fourth instar at pH 6.2, whereas at pH 4.1 and 5.5 significant mortality occurred only in late instars (Fig. 3-3, Fig. 3-1). Larvae at 215 and 700 μg Ni^{2+}/L typically died with their brown discoloured abdomen protruding from the posterior case opening. These symptoms were never observed in the 0 and 55 μg Ni^{2+}/L. Larval development in water with 55 μg Ni^{2+}/L generally proceeded as in water with no added nickel. Pupation at 0 and 55 μg Ni^{2+}/L started about 2 weeks earlier at pH 4.1 than at pH 5.5 and 6.2 (Fig. 3-3).

Pupae in the 215 and 700 μg Ni^{2+}/L treatments were mostly nonviable. Larvae often sealed their case, but died as prepupae or pupae. The few larvae that sealed the case at 700 μg Ni^{2+}/L at pH 4.1 (n=5) and at 215 μg Ni^{2+}/L at pH 5.5 (n=10) and pH 6.2 (n=3) did not develop beyond the prepupal stage. At pH 4.1 and 215 μg Ni^{2+}/L only 33% of the sealed cases (n=22) contained

Fig. 3-3. Development of C. magnifica larvae exposed from first
instar until pupation to 0, 55, 215, and 700 μg Ni^{2+}/L at
pH 4.1, 5.5, and 6.2.

NO. OF LARVAE

WEEK

pH 4.1

pH 5.5

pH 6.2

I–III instar
IV instar
V instar
Pupae

86–b
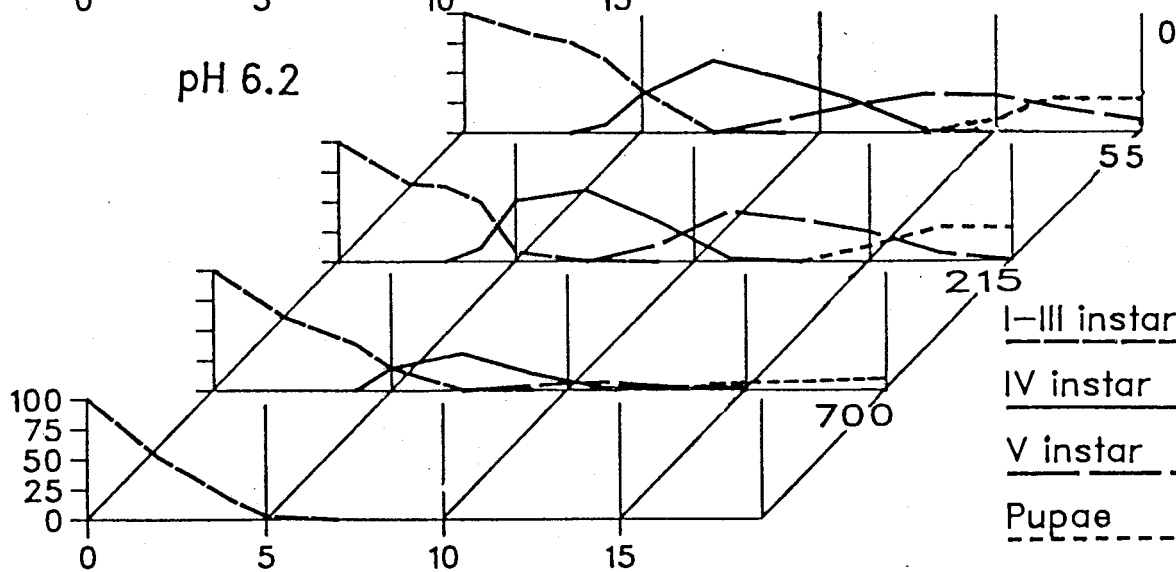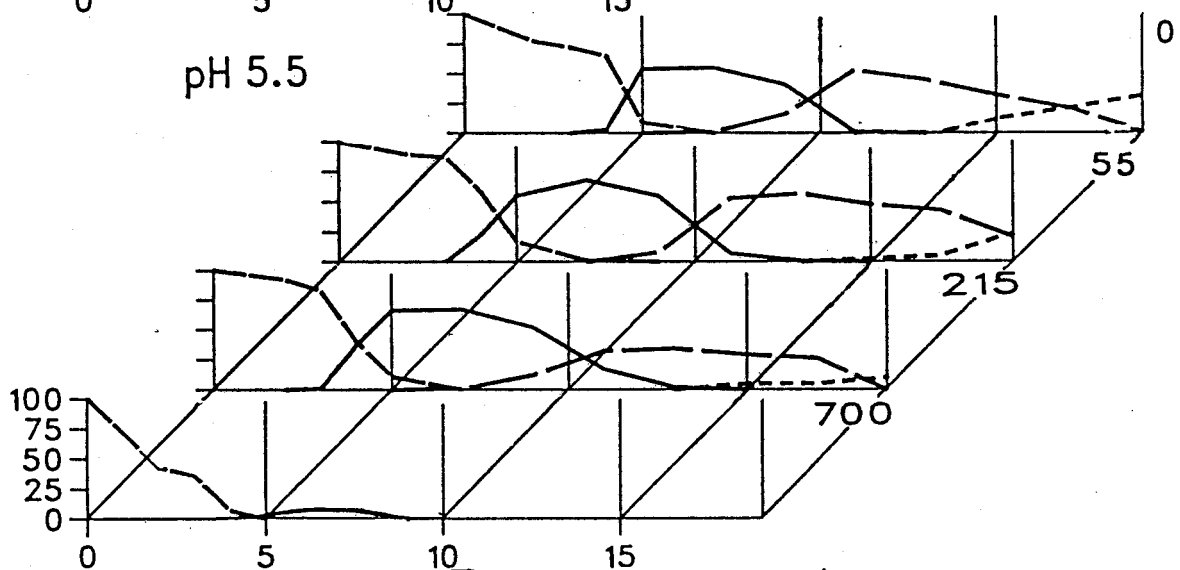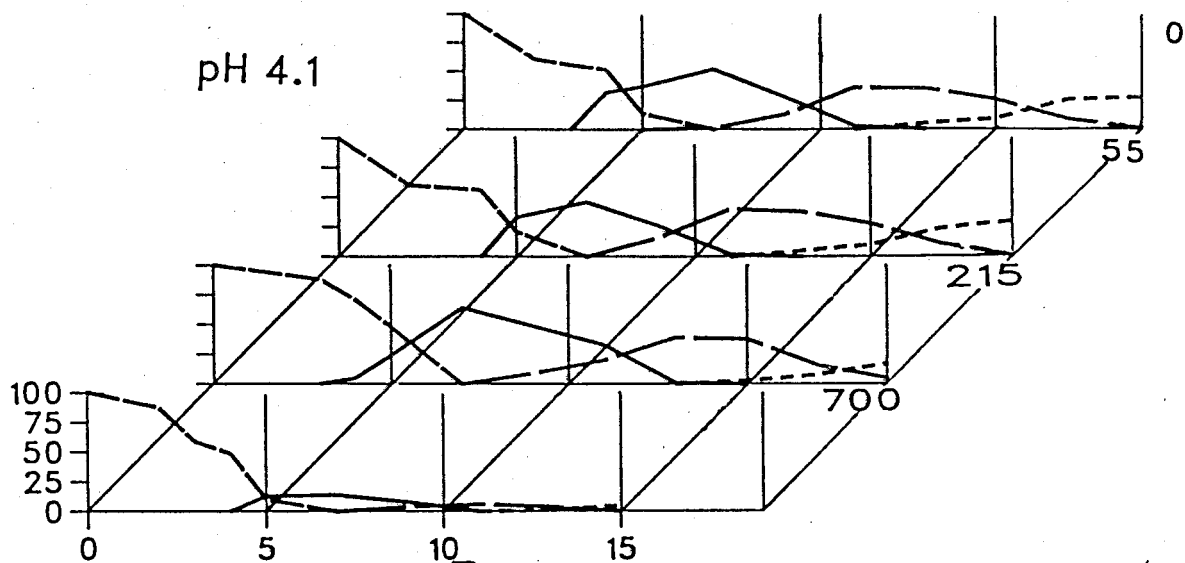
living pupae. Nickel at 55 μg/L significantly reduced the total number of living pupae at pH 6.2 ($p<0.01$) and 5.5 ($p<0.05$), but not at pH 4.1 ($p>0.50$) (Table 3-3, G-test). Dry weight of living pupae was significantly affected by pH ($p<0.05$) and nickel ($p<0.01$) (Table 3-3, two-way ANOVA). In water with no nickel pupae were generally heavier at pH 4.1 than at pH 5.5 and 6.2, whereas at 55 μg Ni^{2+}/L pupal weights did not vary with pH and were lower than in water with no nickel.

Alder leaves exposed to 75 μg Ni^{2+}/L during conditioning accumulated nickel in a pH-dependent fashion (Table 3-4). The amount of accumulated nickel increased significantly with time and was higher at pH 6.2 than at pH 4.0 ($p<0.01$, two-way ANOVA). Microbially-colonized leaves (conditioned for 3 weeks) at either pH accumulated significantly more nickel than uncolonized leaves (not conditioned) when exposed for 24 h to 75 μg Ni^{2+}/L of nickel ($p<0.01$) (Table 3-5, two-way ANOVA).

Exposure of late-instar larvae to 75 μg Ni^{2+}/L significantly increased the time to pupation (Table 3-6, three-way ANOVA). Larvae developed about 30 days faster at pH 4.0 than at pH 6.2 in the absence of nickel. That difference in development time was eliminated when larvae at pH 4.0 were exposed to nickel in both the water and the leaves. Larvae in that treatment developed just as fast as larvae held at pH 6.2 in the absence of nickel. Development was slowest when larvae at pH 6.2 were exposed to nickel in both the water and the leaves.

Table 3-3. Survival and dry weight of *C. magnifica* pupae from larvae exposed from first instar until pupation to various nickel concentrations at pH 4.1, 5.5, and 6.2. Sample size in brackets.

[Ni^{2+}] µg/L	Total no. of living pupae			Dry weight of living pupae (Mean ± SE)[1]		
	pH 4.1	5.5	6.2	pH 4.1	pH 5.5	pH 6.2
0	28	27	27	31.8±1.9 a (10)	26.8±1.4 b (10)	27.9±2.9 ab (10)
55	25	16	10	22.4±1.2 b (9)	22.2±2.1 b (15)	23.5±1.5 b (10)
215	7	0	0			
700	0	0	0			

[1]Means within and between dry weight columns followed by different letters are significantly different at p≤0.05.

Table 3-4. Uptake of Ni^{2+} by alder leaves exposed to
75 μg Ni^{2+}/L during 3 weeks of conditioning at
pH 4.1 and 6.2 (n=3).

Exposure time	Uptake of Ni^{2+} in μg/g dry weight (mean±SE)[1]	
	pH 4.0	pH 6.2
1 week	80.6±1.3 b	25.8±4.6 a
2 weeks	130.0±5.0 c	238.0±8.0 e
3 weeks	190.5±3.5 d	343.0±5.0 f

[1]Means within and between columns followed by different
 letters are significantly different at p≤0.05.

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Table 3-5. Uptake of Ni^{2+} by alder leaves exposed for 24 h to 75 μg Ni^{2+}/L at pH 4.0 and 6.2. Leaves were unconditioned (not exposed to microbes) or conditioned for 3 weeks (n=3).

leaves	Uptake of Ni^{2+} in $\mu g/g$ dry weight (mean±SE)[1]	
	pH 4.0	pH 6.2
unconditioned	24.3±1.7 a	24.0±0.1 a
conditioned	56.1±3.2 b	55.1±4.3 b

[1]Means within and between columns followed by different letters are significantly different at $p \leq 0.05$.

Table 3-6. Development and survival of *C. magnifica* larvae exposed to 75 μg Ni^{2+}/L from early-fourth instar until pupation at pH 4.0 and 6.2.

Treatment:[1]			Days to reach pupation (mean±SE)[2]		% survival	
Ni^{2+} in water	Ni^{2+} in leaves	No. of replicates	pH 4.0	pH 6.2	pH 4.0	6.2
−	−	14 10	137±8.2 a	167±8.2 b	100	95
−	+	12 13	161±5.9 b	172±3.8 bc	89	94
+	−	16 12	159±4.2 b	187±4.8 cd	95	89
+	+	12 10	167±5.2 b	>204±3.1 d[3]	75	67

[1]Means within and between columns followed by different letters are significantly different at p≤0.05.
[2]Larvae were held in water with (+) or without (−) nickel and were fed leaves that were conditioned in water with (+) or without (−) nickel.
[3]Represents an underestimate; 3 larvae had not yet pupated when the experiment was terminated.

Nickel in the water only and nickel in the leaves only both significantly delayed larval development relative to the control (no nickel). However, ingestion of nickel-exposed leaves delayed development at pH 4.0, but not at pH 6.2. Survival of larvae exposed to nickel in both the water and the leaves was significantly lower than in the other treatments at both pH levels ($p < 0.05$, G-test).

DISCUSSION

These experiments suggest that growth and survival of the shredding caddisfly, *C. magnifica*, were more severely affected by an increase in dissolved nickel than by decreased pH. Low pH reduced survival of early-instar larvae (Fig. 3-1), but did not significantly reduce mean larval survival time (Table 3-2). A similar drop in early-instar survival was observed in Figs. 1-3 and 1-4 C. Growth rates of surviving larvae were enhanced at pH 4.1, as was evident from higher pupal weights (Table 3-3), faster development of late-instar larvae (Table 3-6), and earlier pupation (Fig. 3-3). These observations also agree with earlier experiments (Fig. 1-2, Table 1-2 and 1-3). By contrast, dissolved nickel reduced survival and development of larvae and pupae, even at concentrations of <100 μg Ni^{2+}/L (Table 3-3, 3-6).

Nickel appeared more toxic to *C. magnifica* in these experiments than reported by Nebeker *et al*. (1984). In that study nickel had no effect on survival of fourth and fifth instar larvae at 690 μg/L, but reduced adult emergence at 144 μg/L and prevented reproduction at 250 μg/L. Higher toxicity in my experiment is partially attributable to greater sensitivity of early-instar larvae, since most mortality occurred prior to the fourth instar (Fig. 3-3). However, exposure to <100 μg

Ni^{2+}/L reduced survival of late-instar larvae (Table 3-6) and pupae (Table 3-3). Since elevated concentrations of the hardness cations, Ca^{2+} and Mg^{2+}, reduce metal toxicity in general (Pagenkopf 1983), a higher water hardness (Nebeker et $al.$ 1984) (54 mg $CaCO_3/L$ as compared with 5 mg $CaCO_3/L$) may have reduced nickel toxicity. However, detailed comparison is not possible considering the small sample size (n=10) and lack of replication by Nebeker et $al.$ (1984).

A decrease in pH is believed to increase metal toxicity in general by increasing the occurrence of the free (hydrated) ionic metal species, the biologically available and most toxic form (Borgmann 1983). However, nickel toxicity clearly decreased from pH 6.2 to 4.0, at least at 215 and 700 μg Ni^{2+}/L (Table 3-2, Fig. 3-2). The difference in nickel toxicity cannot be explained by possible differences in the concentration of Ni^{2+} between pH treatments. Inorganic complexation of free ionic nickel was insignificant, given the low hardness of the test water (Richter and Theis 1980). Humic substances released by the leaves and excretory products of the larvae may have removed Ni^{2+} from solution (Gamble and Schnitzer 1973, Fish and Morel 1983). However, organic complexation of metals decreases with decreasing pH (O'Shea and Mancy 1978; Guy and Chakrabarti 1976) and would thus increase availability and toxicity of Ni^{2+} at low pH.

The decrease in nickel toxicity with decreasing pH is partially attributable to improvement of larval survival at pH 4.1 (Fig. 3-1, Table 3-2). Havas (1985) observed a similar effect of aluminium on survival of *Daphnia magna* at pH 4.5. Mortality at low pH was associated with a net loss of Na^+, which was reduced by the addition of aluminium. Likewise, nickel could reduce H^+ toxicity by reducing net Na^+ loss at low pH. The mechanism of this protective effect has not been elucidated. However, polyvalent metal ions could mimic calcium, which protects against net Na^+ loss at low pH by decreasing the rate of Na^+ loss and increasing the rate of Na^+ uptake (Havas et al. 1984). The ameliorating effect of nickel may have little ecological significance, because it was not reflected in the final number of surviving larvae (Fig. 3-1, week 19) and occurred only at the higher nickel levels.

Reduction of H^+ toxicity by nickel cannot explain why nickel was less toxic at pH 5.5 than at pH 6.5 (Fig. 3-2), because pH-induced mortality occurred only at pH 4.1. A second mechanism contributing to decreasing toxicity with decreasing pH could be competition between Ni^{2+} and H^+ for the same cellular uptake sites (Pagenkopf 1983). Increased protonization of anionic binding sites on cell membranes at low pH would reduce surface complexation and uptake of metal ions. This is supported by the work of Havas (1985), who reported a reduced uptake of aluminium by *D. magna* at pH 4.5 and 5.0 as compared with pH 6.5. However, increased competition between H^+ and Ni^{2+} at low pH

does not explain why larval survival at 55 μg Ni^{2+}/L was higher at pH 5.5 than at pH 4.1 and 6.2. The reason for that difference is not clear.

Most of the accumulation of Ni^{2+} by leaves conditioned for 3 weeks in water with 75 μg Ni^{2+}/L was attributed to uptake of dissolved nickel by microbes associated with the leaf material. Background levels of nickel in alder leaves was about 3.5 μg/g dry weight. Adsorption to the leaf surface did occur, as was demonstrated by exposing leaves without microbes for 24 h to 75 μg Ni^{2+}/L, but accounted for <10% of the nickel accumulated by leaves during 3 weeks of conditioning (Table 3-5). That microbes on leaves conditioned at pH 4.0 accumulated less nickel than microbes on leaves conditioned at pH 6.2 (Table 3-4) could be due to increased competition between H$^+$ and Ni^{2+} for uptake sites on microbial cells. However, possible nickel-induced differences in microbial abundance between pH treatments cannot be excluded.

Ingestion of dilute dissolved nickel concentrated by leaf microbes contributed to reduced growth and survival of $C.$ $magnifica$ larvae at both pH 4.0 and 6.0 (Table 3-6). Ambient pH affected the relative importance of nickel in the water and nickel associated with the leaves, but a logical explanation for the observed pattern is not evident. It is not clear why ingestion of nickel-exposed leaves by larvae in water with no nickel delayed growth at pH 4.0 and not at pH 6.2, despite much

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higher levels of nickel in the leaves at pH 6.2. Differential leaching of nickel from the leaves at pH 4.0 and 6.2 did not occur, because leaves conditioned for 3 weeks at either pH had similar leaching rates (about 9% of total accumulated nickel in 24 h) when held at a pH that corresponded to the conditioning pH. Leaching was calculated to result in a negligible increase in the dissolved nickel concentration ($<1 \mu g/L$). When larvae were exposed to nickel in the water only, effects of dissolved nickel were confounded by the ingestion of nickel taken up by the leaf material prior to consumption. That was minimized by replacing the leaves once a day, but some uptake did undoubtedly take place (Table 3-5). Since the significance of metal uptake from the food by aquatic organisms varies with the particular metal and the test species (Luoma 1983), satisfactory interpretation of Table 3-6 is not likely without data on nickel body burdens of the larvae in the various treatments.

Nickel was used as a model to investigate the interaction between pH and metal toxicity. Decreasing toxicity with decreasing pH may apply to pH-metal interactions in general. This hypothesis is supported by laboratory experiments demonstrating decreasing toxicity with decreasing pH when changes in metal speciation were eliminated or accounted for, using a variety of metals (i.e. aluminium, copper, cadmium, and zinc) and organisms of several trophic levels (Borgmann 1983, Cusimano *et al.* 1984, Havas 1985, Peterson *et al.* 1984). My results indicate that such pH-metal interactions occur at pH

levels and metal concentrations representative of waters subjected to acidic precipitation. However, under field conditions the effect of pH on toxicity of metals could be overridden by changes in metal speciation occasioned by a decrease in pH. In soft waters availability and toxicity of dissolved metals could be determined predominantly by complexation with humic materials (Davis and Leckie 1978). Decreased complexation at lower pH would counteract the observed effect of pH on metal toxicity.

GENERAL DISCUSSION

Productivity of aquatic ecosystems is dependent on retention and efficient regeneration of nutrients. Decreased rates of organic matter decomposition (Traaen 1980) have been suggested to contribute to the oligotrophication of acidified waters (Grahn *et al*. 1974) by reducing the cycling of detrital energy. Reduced shredding and inhibited microbial degradation could both contribute to lower breakdown rates of CPOM. In preceding chapters I examined the effects of acidification on the utilization of leaf litter by a shredder. I shall now relate my laboratory observations to the observed response of aquatic communities to acidification.

Field observations suggest that increased accumulation of CPOM in low pH waters is not the result of reduced shredder abundance. Several studies have documented the persistence of the shredder functional group under low pH conditions. Shredders were not affected by experimental acidification of a stream (Hall *et al*. 1980) and often dominate invertebrate communities in low pH waters (Friberg *et al*. 1980, Mackay and Kersey 1985). Shredders may even increase in density with decreasing pH (Townsend *et al*. 1983). It has been suggested that the ability of many shredders to tolerate low pH has evolved as an adaptation to locally acid conditions, as may occur in decaying organic substrate (Mackay and Kersey 1985). Shredding taxa of the Plecoptera and Trichoptera appear particularly tolerant of low pH (Burton *et al*. 1980, Hall *et al*. 1980, Mackay and Kersey 1985). The response of *C. magnifica* to low pH observed in this

study may thus well be representative of other shredder species. Production of this species in the laboratory was reduced at low pH due to increased mortality during early larval stages. However, pH-induced mortality of early-instar larvae may not have a determining influence on generation survival under field conditions.

Laboratory experiments with nickel and *C. magnifica* suggest that elevated concentrations of dissolved metals may have more severe effects on shredder growth and survival than low pH. Nickel reduced viability of *C. magnifica* at levels that could occur in acidified waters. Accumulation of dissolved nickel by leaf microbes contributed to this reduction. A similar reduction was observed for the shredder *Gammarus pulex* fed cadmium-contaminated fungi (Duddridge and Wainwright 1980). Uptake of metals by detrital microbes could be a significant mechanism of metal concentration, aiding the transfer of dilute metals from the water column to shredding invertebrates. However, meaningful extrapolation of toxic effects observed in the laboratory to field conditions is not possible, given the general lack of data on bioavailability of metals in acidified waters. Most studies report total metal concentrations only, which overestimate potential toxic effects on biota, because removal of free metal ions by adsorption to suspended particulates and complexation with organic and inorganic ligands will reduce availability of the metal to the biota. In most waters metals are predominantly associated with suspended solids

(Davis and Leckie 1978), while the dissolved fraction may be dominated by nontoxic organic complexes (Driscoll et al 1980). To the best of my knowledge, toxic effects of dissolved metals on shredding invertebrates in acidified waters have not been conclusively demonstrated.

Decreased decomposition in acidic waters appears primarily attributable to reduced microbial activity. That is supported by observations of reduced breakdown of substrates that are not readily utilized by invertebrates (Hildrew et al. 1984, Traaen 1980) and of reduced microbial metabolic activity on leaf litter (McKinley and Vestal 1982, Traaen 1980) and in lake sediments (Baker et al. 1982, Kelly et al. 1984). Reduced microbial activity at low pH contradicts the increase in microbial abundance on leaf litter observed in this study. It is possible that low pH enhances microbial (in particular fungal) colonization of leaf litter, but that in the field pH effects are confounded by the effects of increased metal concentrations. Most metals associated with freshwater acidification are toxic to aquatic microbiota (Babich and Stotzky 1983), but toxicity is often measured in culture media with strong metal complexing ability and thus cannot be applied to metal concentrations in acidified waters. The few studies conducted in soft waters, however, indicate that dissolved metals tend to inhibit microbial metabolic activity at levels usually exceeding those encountered in acidified waters (Abel and Barlocher 1984, Albright et al. 1972, Baker et al. 1983, Giesy and Drawer 1978).

However, dissolved metals might reduce microbial decomposition indirectly by lowering availability of nutrients. Aluminium, for example, precipitates dissolved orthophosphate (Dickson 1978), a nutrient which limits leaf litter decomposition (Elwood *et al*. 1981). Effects of dissolved metals may thus contribute to differences between field and laboratory observations. Field observations of reduced decomposition in low pH waters are furthermore confounded by factors that covary with pH, such as calcium content of the water, which also influences activity of detrital microorganisms (Chamier and Dixon 1982, Rosset and Barlocher 1985, Egglishaw 1968).

A comprehensive overview of how acidification affects the cycling of detrital energy through the shredder-microbe-CPOM component of aquatic food webs cannot yet be synthesized. My laboratory data illustrate some potential effects of pH and dissolved metals and emphasize the complexity of interactions between the two. Effects of low pH and nickel on *C. magnifica* were beneficial or harmful, depending on the stage of larval development, and usually counteracted one another. Survival of early-instar larvae was reduced by low pH, but addition of nickel to the test water mitigated that mortality. Low pH enhanced growth of late-instar larvae, but their growth and survival was reduced by exposure to dissolved nickel, despite the ameliorating effect of low pH on nickel toxicity. The outcome of such interactions would depend on the timing and magnitude of the changes in pH and dissolved metal

concentrations.

Applicability of these interactions to field conditions is difficult to assess without more detailed data on chemical and biological changes in waters that are being acidified. Knowledge of the response of shredders and detrital microorganisms to acidification is mostly based on studies of waters that were naturally acidic or experimentally acidified. Such data may not be a good indication of biotic changes that occur in waters subjected to anthropogenic acidification. The biota of these waters are exposed to episodic fluctuations in various chemical properties of the water (e.g. pH, alkalinity, metals, nutrients), whereas the biota in naturally acidic systems are presumably well-adapted through natural selection to the relatively stable prevailing chemical conditions. Experimental acidification typically does not allow for the modifying effects of the surrounding watershed on water chemistry (e.g. leaching of metals and nutrients). Even though the sequence of events in acidified waters may not be fully understood, the end result of acidification is clear and should be all that is needed to justify mitigative action.

LITERATURE CITED

Abel, T.H., and F. Barlocher. 1984. Effects of cadmium on
    aquatic hyphomycetes. Appl. Env. Microb. 48: 245-251.

Albright, L.J., J.W. Wentworth, and E.M. Wilson. 1972. Technique
    for measuring metallic salt effects upon the indigenous
    heterotrophic microflora of a natural water. Water Res. 6:
    1589-1596.

Anderson, N.H. 1976a. The distribution and biology of the Oregon
    Trichoptera. Agric. Exp. Station Techn. Bull. 134, Oregon
    State University, Corvallis.

Anderson, N.H. 1976b. Carnivory by an aquatic detritivore,
    Clistoronia magnifica (Trichoptera: Limnephilidae).
    Ecology 57: 1081-1085.

Anderson, N.H. 1978. Continuous rearing of the caddisfly
    Clistoronia magnifica (Trichoptera: Limnephilidae). p.
    317-329. Proc. 2nd Int. Symp. on Trichoptera. W. Junk, The
    Hague.

Anderson, N.H., and K.W. Cummins. 1979. Influences of diet on
    the life histories of aquatic insects. J. Fish. Res. Board
    Canada 36: 335-342.

Anderson, N.H., and E.F. Grafius. 1975. Utilization and
    processing of allochthonous material by stream
    Trichoptera. Verh. Intern. Verein. Limnol. 19: 3083-3088.

Anderson, N.H., and J.R. Sedell. 1979. Detritus processing by
    macroinvertebrates in stream ecosystems. Ann. Rev.
    Entomol. 24: 351-377.

APHA. 1980. Standard methods for the examination of water and
    waste water. American Public Health Association, Wash.
    D.C.

Arsuffi, T.F., and K. Suberkropp. 1984. Leaf processing
    capabilities of aquatic hyphomycetes: interspecific
    differences and influence on shredder feeding preferences.
    Oikos 42: 144-154.

Baath, E., and B. Soderstrom. 1979. The significance of hyphal
    diameter in calculation of fungal biovolume. Oikos 33:
    11-14.

Babich, H., and G. Stotzky. 1983. Influence of chemical
    speciation on the toxicity of heavy metals to the
    microbes. p. 1-46. *In* Nriagu, J. O. [ed.]. Aquatic

toxicology. Wiley, N.Y.

Baker, M.D., W.E. Inniss, C.I. Mayfield, and P.T.S. Wong. 1982. Effect of pH on the growth and activity of heterotrophic sediment microorganisms. Chemosphere 11: 973-983.

Baker, M.D., W.E. Inniss, C.I. Mayfield, and P.T.S. Wong. 1983. Effect of acidification, metals, and metalloids on sediment microorganisms. Water Res. 17: 925-930.

Barlocher, F. 1982. The contribution of fungal enzymes to the digestion of leaves by *Gammarus fossarum* Koch (Amphipoda). Oecologia 52: 1-4.

Barlocher, F. 1983. Seasonal variation of standing crop and digestibility of CPOM in a Swiss Jura stream. Ecology 64: 1266-1272.

Barlocher, F., and B. Kendrick. 1973. Fungi in the diet of *Gammarus psuedolimnaeus* (Amphipoda). Oikos 24: 295-300.

Barlocher, F., and B. Kendrick. 1975. Leaf conditioning by microorganisms. Oecologia 20: 359-362.

Barlocher, F., and B. Kendrick. 1981. Role of aquatic hyphomycetes in the trophic structure of streams. p. 743-760. *In* Wicklow, D.T., and G.C. Carroll [eds]. The fungal community, its organization and role in the ecosystem. Marcel Dekker Inc., NY.

Bligh, E.G., and W.L. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Bobbie, R.J., and D.C. White. 1980. Characterization of benthic microbial community structure by high resolution gas chromatography of fatty acid methyl esters. Appl. Env. Microbiol. 39: 1212-1222.

Borgmann, U. 1983. Metal speciation and toxicity of free metal ions to aquatic biota. p. 47-72. *In* Nriagu, J.O. [ed.]. Aquatic toxicology. Wiley, N.Y.

Burton, T.M., R.M. Stanford, and J.W. Allan. 1982. The effects of acidification on stream ecosystems. p. 209-235. *In* D'Itri, F.M. [ed.]. Acid precipitation: effects on ecological systems. Ann Arbor Science, Ann Arbor, Mi.

Cammen, L.M. 1980. The significance of microbial carbon in the nutrition of the deposit feeding polychaete *Nereis succinea*. Mar. Biol. 61: 9-20.

Chamier, A., and P.A. Dixon. 1982. Pectinases in leaf

degradation by aquatic hyphomycetes: the enzymes and leaf maceration. J. Gen. Microbiol. 128: 2469-2483.

Chamier, A., P.A. Dixon, and S.A. Archer. 1984. The spatial distribution of fungi on decomposing alder leaves in a freshwater stream. Oecologia 64: 92-103.

Conover, R.J., and V. Francis. 1973. The use of radioactive isotopes to measure the transfer of materials in aquatic food chains. Mar. Biol. 18: 272-283.

Cummins, K.W. 1973. Trophic relations of aquatic insects. Ann. Rev. Appl. Entomol. 18: 183-206.

Cummins, K.W. 1974. Structure and function of stream ecosystems. Bioscience 24: 631-641.

Cusimano, F.R., D. Brakke, and G.A. Chapman. 1984. Effects of acid water on the toxicities of cadmium, copper, and zinc to trout. 11th Ann. Aquatic Toxicity Workshop, Vancouver, B.C. (Abstract).

Davis, J.A., and J.O. Leckie. 1978. Effect of adsorbed complexing ligands on trace metal uptake by hydrous oxides. Environ. Sci. Technol. 12: 1309-1315.

Dickson, W. 1978. Some effects of the acidification of Swedish lakes. Verh. Intern. Verein. Limnol. 20: 851-856.

Driscoll, C.T., J.P. Baker, J.J. Bisogni, and C.L. Schofield. 1980. Effects of aluminium speciation on fish in dilute acidified waters. Nature 284: 161-164.

Duddridge, J.E., and M. Wainwright. 1980. Heavy metal accumulation by aquatic fungi and reduction in viability of *Gammarus pulex* fed Cd^{2+}-contaminated mycelium. Water Res. 14: 1605-1611.

Dunn, O.J. 1964. Multiple comparisons using rank sums. Technometrics 6: 241-252.

Egglishaw, H.J. 1968. The quantitative relationship between bottom fauna and plant detritus in streams of different calcium concentrations. J. Appl. Ecol. 5: 731-740.

Elwood, J.W., J.D. Newbold, A.F. Trimble, and R.W. Stark. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62: 146-158.

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

Findlay, S., J.L. Meyer, and P.J. Smith. 1984. Significance of bacterial biomass in the nutrition of a freshwater isopod (*Lircues sp*). Oecologia 63: 38-42.

Fish, W., and F.M.M. Morel. 1983. Characterization of organic copper-complexing agents released by *Daphnia magna*. Can. J. Fish. Aquat. Sci. 40: 1270-1277.

Friberg, F., C. Otto, and B.S. Svensson. 1980. Effects of acidification on the dynamics of allochthonous leaf material and benthic invertebrate communities in running water. p. 304-305. *In* D. Drablos and A. Tollan, [ed.]. Ecological Impact of Acid Precipitation. Proc. Int. Conf., Sandefjord, Norway.

Gamble, D.S., and M. Schnitzer. 1973. The chemistry of fulvic acid and its reactions with metal ions. p. 265-305. *In* Singer, P.C. [ed.]. Trace metals and metal-organic interactions in natural waters. Ann Arbor Science, Ann Arbor, Mi.

Giesy, J.P., and E. Drawer. 1978. Cadmium inhibition of leaf decomposition in an aquatic microcosm. Chemosphere 6: 467-475.

Gorham, E. 1976. Acid precipitation and its influence upon aquatic ecosystems-an overview. Water, Air, Soil Pollut. 6: 437-481.

Grafius, E.J. 1977. Bioenergetics and strategies of some Trichoptera in processing and utilizing allochthonous materials. Ph.D. Thesis, Oregon State University, Corvallis, Ore.

Grahn, O., H. Hultberg, and L. Landner. 1974. Oligotrophication: a self-accelerating process in lakes subjected to excessive supply of acid substances. Ambio 3: 93-94.

Guy, R.D., and C.L. Chakrabarti. 1976. Studies of metal-organic interactions in model systems pertaining to natural waters. Can. J. Chem. 54: 2600-2611.

Haines, T.A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. Trans. Am. Fish. Soc. 110: 669-707.

Hall, R.J., G.E. Likens, S.B. Fiance, and G.R. Hendrey. 1980. Experimental acidification of a stream in the Hubbard brook experiment forest, New Hampshire. Ecology 61: 976-989.

Hanson, B.J., K.W. Cummins, A.S. Cargill, and R.R. Lowry. 1983. Dietary effects on lipid and fatty acid composition of

Clistoronia magnifica (Trichoptera: Limnephilidae).
Freshwater Invert. Biol. 2:2-15.

Hanson, B.J., A.S. Cargill, and K.W. Cummins. 1984. Role of
fungi in the nutrition of *Clistoronia magnifica*
(Trichoptera: Limnephilidae). 32nd Ann. Meet. North Am.
Benthol. Soc., Raleigh, NC. (Abstract).

Hargrave, B.T. 1970. The utilization of benthic microflora by
Hyelella azteca (Amphipoda). J. Anim. Ecol. 39: 421-437.

Hassett, R.P., and M.R. Landry. 1983. Effects of food-level
acclimation on digestive enzyme activities and feeding
behavior of *Calanus pacificus*. Mar. Biol. 75: 47-55.

Havas, M. 1980. Physiological response of aquatic animals to low
pH. p. 49-67. *In* Singer, R. [ed.]. Effects of acidic
precipitation on benthos. Proc. Reg. Symp. on Benthic
Biology, N. Am. Benthol. Soc., Hamilton, N.Y.

Havas, M., and T.C. Hutchinson. 1982. Aquatic invertebrates from
the Smoking Hills, N.W.T.: effect of pH and metals on
mortality. Can. J. Fish. Aquat. Sci. 39: 890-903.

Havas, M., and T.C. Hutchinson. 1983. Effects of low pH on the
chemical composition of aquatic invertebrates from tundra
pools at the Smoking Hills, N.W.T. Can. J. Zool. 61:
241-249.

Havas, M., T.C. Hutchinson, and G.E. Likens. 1984. Effects of
low pH on sodium regulation in two species of Daphnia.
Can. J. Zool. 62: 1965-1970.

Havas, M. 1985. Aluminum uptake and toxicity to *Daphnia magna* in
soft water at low pH. In preparation.

Hendrey, G.R., K. Baalsrud, T.S. Traaen, M. Laake, and G.
Raddum. 1976. Acid precipitation: some hydrobiological
changes. Ambio 5: 224-227.

Herbst, G.N. 1982. Effect of leaf type on the consumption rates
of aquatic detritivores. Hydrobiologia 89: 77-87.

Hildrew, A.G., C.R. Townsend, J. Francis, and K. Finch. 1984.
Cellulolytic decomposition in streams of contrasting pH
and its relationship with invertebrate community
structure. Freshwater Biol. 14: 323-328.

Johannes, R.E., and M. Satomi. 1967. Measuring organic matter
retained by aquatic invertebrates. J. Fish. Res. Board
Canada 24: 2467-2471.

Kaushik, N.K., and H.B.N. Hynes. 1971. The fate of the dead

leaves that fall into streams. Arch. Hydrobiol. 68: 465-515.

Kelly, C.A., J.W.M. Rudd, A. Furutani, and D.W. Schindler. 1984. Effects of lake acidification on rates of organic matter decomposition in sediments. Limnol. Oceanogr. 29: 687-694.

Kostalos, M., and R.L. Seymour. 1976. Role of microbially enriched detritus in the nutrition of *Gammarus minus* (Amphipoda). Oikos 27: 512-516.

Kwiatkowski, R.E., and J.C. Roff. 1976. Effects of acidity on the phytoplankton and primary productivity of selected northern Ontario lakes. Can. J. Bot. 54: 2546-2561.

Lawson, D.L., M.J. Klug, and R.W. Merritt. 1984. The influence of the physical, chemical, and microbiological characteristics of decomposing leaves on the growth of the detritivore *Tipula abdominalis* (Diptera: Tipulidae). Can. J. Zool. 62: 2339-2343.

Luoma, S.N. 1983. Bioavailability of trace metals to aquatic organisms- a review. Sci. Total Env. 28: 1-22.

Mackay, R.J., and K. Kersey. 1985. A preliminary study of aquatic insect communities in acid streams near Dorset, Ontario. Hydrobiologia 122: 3-11.

Malmer, N. 1976. Acid precipitation: chemical changes in the soil. Ambio 5: 231-224.

Martin, M.M., J.J. Kukor, J.S. Martin, D.L. Lawson , and R.W. Merritt. 1981. Digestive enzymes of larvae of three species of caddisflies (Trichoptera). Ins. Biochem. 11: 501-505.

McIntire, C.D., and J.A. Colby. 1978. A hierarchical model of lotic ecosytems. Ecol. Monogr. 48: 167-190.

McKinley, V.L., and L.R. Vestal. 1982. Effects of acid on plant litter decomposition in an arctic lake. Appl. Env. Microbiol. 43: 1188-1195.

Monk, D.C. 1976. The distribution of cellulase in freshwater invertebrates of different feeding habits. Freshwater Biol. 6: 471-475.

Morrison, W.R., and L.M. Smith. 1964. Preparation of fatty acid methylesters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5: 600-608.

Nebeker, A.V., C. Savonen, R.J. Baker, and J.K. McCrady. 1984. Effects of copper, nickel, and zinc on the life cycle of

the caddisfly *Clistoronia magnifica* (Limnephilidae).
Environ. Toxicol. Chem. 3: 645-649.

Newell, S.Y., and A. Statzell-Talman. 1982. Factors for
    conversion of fungal biovolume values to biomass, carbon
    and nitrogen: variation with mycelial ages, growth
    conditions and strains of fungi from a salt marsh. Oikos
    39: 261-268.

Nilsson, L.M. 1974. Energy budget of a laboratory population of
    Gammarus pulex (Amphipoda). Oikos 25: 35-42.

NRCC 1981a. Acidification in the Canadian aquatic environment.
    NRCC no. 18475, National Research Council of Canada,
    Ottawa.

NRCC 1981b. Effects of nickel in the Canadian environment. NRCC
    no. 18568, National Research Council of Canada, Ottawa.

O'Neill, R.V. 1976. Ecosystem persistence and heterotrophic
    regulation. Ecology 59: 1244-1253.

O'Shea, T.A., and K.H. Mancy. 1978. The effect of pH and
    hardness metal ions on the competitive interaction between
    trace metal ions and inorganic and organic complexing
    agents found in natural waters. Water Res. 12: 703-711.

Pagenkopf, G.K. 1983. Gill surface interaction model for trace
    metal toxicity to fishes: role of complexation, pH, and
    water hardness. Environ. Sci. Technol. 17: 342-352.

Peterson, H.G., F.P. Healey, and R. Wagemann. 1984. Metal
    toxicity to algae: a highly pH dependent phenomenon. Can.
    J. Fish. Aquat. Sci. 41: 974-979.

Prus, T. 1971. The assimilation efficiency of *Asellus aquaticus*
    (Crustacea: Isopoda). Freshwater Biol. 1: 287-305.

Raddum, G.C., and A. Fjellheim. 1984. Acidification and early
    warning organisms in freshwater in western Norway. Verh.
    Intern. Verein. Limnol. 22: 1973-1980.

Richter, R.O., and T.L. Theis. 1980. Nickel speciation in a
    soil/water system. p. 189-202. *In* Nriagu, J.O. [ed.].
    Nickel in the environment. Wiley, N.Y.

Ricker, W. 1958. Handbook of computations for biological
    statistics of fish populations. Bull. Fish. Res. Board
    Canada 119.

Roff, J.C., and R.E. Kwiatkowski. 1977. Zooplankton and
    zoobenthos communities of selected northern Ontario lakes
    of different acidities. Can. J. Zool. 55: 899-911.

Rosset, J., and F. Barlocher. 1985. Aquatic hyphomycetes: influence of pH, Ca^{2+} and HCO_3^- on growth in vitro. Trans. Brit. Mycol. Soc. 84: 137-145.

Rossi, L., and A.E. Fano. 1979. Role of fungi in the trophic niche of the congeneric detritivorous *Asellus aquaticus* and *A. coxalis* (Isopoda). Oikos 32: 380-385.

Schindler, D.W., R. Hesslein, and R.D. Wagemann. 1980. Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake. Can. J. Fish. Aquat. Sci. 37: 373-377.

Schindler, D.W., and M.A. Turner. 1982. Biological, chemical and physical responses of lakes to experimental acidification. Water, Air, Soil Pollut. 18: 259-271.

Schofield, C.L. 1976. Acid precipitation: effects on fish. Ambio 5: 228-230.

Scriber, J.M., and F. Slansky. 1981. The nutritional ecology of immature insects. Ann. Rev. of Entomol. 26: 183-211.

Singer, R. 1982. Effects of acidic precipitation on benthos. p. 329-367. *In* D'Itri, F.M. [ed.]. Acid precipitation effects on ecological systems. Ann Arbor Science, Ann Arbor, Mi.

Sinsabaugh, R.L., E.F. Benfield, and A.E. Linkins. 1981. Cellulase activity associated with the decomposition of leaf litter in a woodland stream. Oikos 36: 184-190.

Smock, L.A., and K.L. Harlowe. 1983. Utilization and processing of freshwater wetland macrophytes by the detritivore *Asellus forbesi*. Ecology 64: 1556-1565.

Sorokin, J.I. 1968. The use of ^{14}C in the study of nutrition of aquatic animals. Mitt. Intern. Verein. Limnol. 16: 1-41.

Spry, D.J., C.M. Wood, and P.V. Hodson. 1981. The effects of environmental acid on freshwater fish with particular reference to the softwater lakes in Ontario and the modifying effects of heavy metals. A literature review. Can. Techn. Rep. Fish. Aquat. Sci. no. 999.

Stokes, P.M., and A.M. Szokalo. 1977. Sediment-water interchange of copper and nickel in experimental aquaria. Water Pollut. Res. Can. 12: 157-177.

Suberkropp, K., T.F. Arsuffi, and J.P. Anderson. 1983. Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. Appl. Env. Microbiol. 46: 237-244.

Suberkropp, K., and M.J. Klug. 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57: 707-719.

Suberkropp, K., and M.J. Klug. 1980. The maceration of deciduous leaf litter by aquatic hyphomycetes. Can. J. Bot. 58: 1025-1031.

Sutcliffe, D.W., T.R. Carrick, and L.M. Willoughby. 1981. Effects of diet, body size, age and temperature on growth rates in the amphipod *Gammarus pulex*. Freshwater Biol. 11: 183-214.

Townsend, C.R., A.G. Hildrew, and J. Francis. 1983. Community structure in some southern English streams: the influence of physicochemical factors. Freshwater Biol. 13: 521-544.

Traaen, T.S. 1980. Effects of acidity on decomposition of organic matter in aquatic environments. p. 340-341. *In* D. Drablos, and A. Tollan [eds]. Ecological Impact of Acid Precipitation. Proc. Int. Conf., SNSF, Norway.

Vangenechten, J.H.D., and O.L.J. Vanderborght. 1980. Effect of acid pH on sodium and chloride balance in an inhabitant of acid fresh waters: the waterbug *Corixa punctata* (Illig.)(Insecta: Hemiptera). p. 342-343. *In* Drablos, D., and A. Tollan [ed.].Ecological Impact of Acidic Precipitation. Proc. Int. Conf., SNSF, Norway.

Ward, G.M., K.W. Cummins. 1979. Effects of food quality on growth of a stream detritivore, *Paratendipes albimanus* (Meigen) (Diptera: Chironomidae). Ecology 60: 57-64.

Wiggins, G.B. 1977. Larvae of the North American caddisfly genera. University of Toronto Press, Toronto.

Winterbourn, M.J. 1971. The life histories and trophic relationships of the Trichoptera of Marion lake, British Columbia. Can. J. Zool. 49: 623-635.

Winterbourn, M.J. 1982. Food Utilization by a stream detritivore, *Zelandopsyche ingens* (Trichoptera: Oeconesidae). Int. Revue ges. Hydrobiol. 67: 209-222.

Wright, R.F., and E.T. Gjessing. 1976. Acid precipitation: changes in the chemical composition of lakes. Ambio 5: 219-223.

Zimmerman, M.C., and T.E. Wissing. 1978. Effects of temperature on gut loading and gut clearing times of the burrowing mayfly, *Hexagenia limbata*. Freshwater Biol. 8: 269-277.

Zischke, J.A., J.W. Arthur, K.J. Nordlie, R.O. Hermanutz, D.A.

Standen, and T.P. Henry. 1983. Acidification effects on macroinvertebrates and fathead minnows (*Pimephales promelas*) in outdoor experimental channels. Water Res. 17: 47-63.