CONSEQUENCES OF ENVIRONMENTAL ACIDIFICATION TO THE FRESHWATER AMPHIPOD *HYALELLA AZTECA*

by

Lee C. Grapentine

A thesis submitted to the
Faculty of Graduate Studies
The University of Manitoba
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of
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ABSTRACT

The effects of acidification on the freshwater amphipod Hyalella azteca was investigated by (a) examining the role of pH in the natural distribution of the species in the Experimental Lakes Area, northwestern Ontario, and (b) determining the responses to acidification in a series of field and laboratory experiments. pH and Ca concentration were important factors affecting the presence of H. azteca in lakes. The amphipod was absent from all five lakes that had pH 5.03 - 5.40 or Ca concentration <1.24 mg/L, and present in 29 out of the 31 remaining lakes that had pH >6.0 and Ca >1.24 mg/L. In the field and laboratory experiments, amphipods contained in small enclosures and held in acidified lakes (pH 5.45, 5.66), 500-L mesocosms (pH $\leq$ 5.8) or 4-L aquaria (pH $\leq$ 5.6) showed lower ($P<0.05$) survivorship than that in corresponding neutral-pH, control treatments. Early life history stages of H. azteca had lower ($P<0.05$) tolerance of low pH than those in later stages. It is predicted that populations of H. azteca will decline in habitats where the pH remains <5.8, a condition common in a substantial proportion of lakes in eastern North America.

PART I. GENERAL INTRODUCTION

OBJECTIVES

Surface waters in many regions throughout the world are currently undergoing substantial alterations due to human activities (Stumm 1986). Whereas earlier phases of water quality impairment involved mainly acute, localized pollution of receiving waters, present impairment includes chronic, global-scale interference with hydrogeochemical cycles (Stumm 1986). The acidification of lakes and streams is an example of such a problem. Evidence linking anthropogenic emissions of sulfur and nitrogen oxides and other contaminants with decreases in pH of surface waters is extensive (see reviews by National Academy of Sciences (NAS) 1981, National Research Council of Canada (NRCC) 1981, U.S. Environmental Protection Agency (USEPA) 1984, and Martin 1986).

Aquatic ecosystems are deleteriously affected by acidification. Among the earliest and most marked responses of the biota to decreased pH is the disappearance of benthic crustaceans such as amphipods (Sutcliffe and Carrick 1973, Okland,K.A. 1980, Brehm and Meijering 1982, Stephenson and Mackie 1986), the notostracan Lepidurus arcticus (Raddum and Fjellheim 1984, Borgstrom et al. in Okland and Okland 1986), the opposum shrimp Mysis relicta (Nero and Schindler 1983) and

crayfish (Schindler and Turner 1982, Svardson in Okland and Okland 1986). Loss of these and other sensitive species such as cyprinid fish can disrupt interactions of a lake's food web and affect other organisms at a pH higher than that which is directly toxic to them (Schindler et al. 1985).

It is important to detect and measure ecological changes due to acidification in order to assess the environmental impacts of acid deposition. Establishing a direct cause and effect relationship between acidification and biological change in natural systems is difficult, however, because observations on a system before acidification are scarce. Alternative approaches are to correlate pH and distribution across a gradient of pH in lakes, and to examine responses to acidification in experimental systems.

Hyalella azteca is one of the most common and widely distributed species of amphipods in North America, and yet has only recently received attention in studies of the ecological effects of acidification (e.g. Stephenson and Mackie 1986). Hyalella azteca can dominate the zoobenthic community of the littoral zone and thus be an important component of the food web of lakes (see below). In addition, its widespread distribution and tolerance of most environmental conditions make its absence from a lake a potential indicator of ecological effects of acidification.

The overall objectives of this study were 1) to measure the responses of natural populations of H. azteca to lake acidification, and 2) if H. azteca populations decline in acidified lakes, to estimate the lowest pH that would allow their long-term survival.

These objectives were addressed using several approaches. After a

preliminary examination of the occurrence of H. azteca in the Experimental Lakes Area (ELA), a region of Precambrian Shield lakes in northwestern Ontario (see Description of study area, Part II), the relationship of pH to the natural distribution of H. azteca was examined in more detail. Two field experiments also were carried out involving the transplantation of amphipods from neutral waters into acidified lakes, and the placement of amphipods into mesocosms of artificially acidified lake water. In addition, three laboratory toxicity tests were conducted.

Part I of this thesis includes a summary of the processes and effects of lake acidification, especially those affecting benthic crustaceans, and a brief review of the ecology of H. azteca. Part II describes the relationship between pH and the distribution of H. azteca at ELA. Part III describes the acidification experiments, and Part IV states the main results and conclusions.

BACKGROUND ON LAKE ACIDIFICATION

Acidification of surface waters is a phenomenon that has been documented in several regions of the world. Lakes and streams that had near neutral pH several decades ago now measure 1 or more pH units below previous levels. Such changes can affect the structure and function of aquatic ecosystems.

Several recent reviews (Haines 1981, NAS 1981, NRCC 1981, Overrein et al. 1981, Dillon et al. 1984, USEPA 1984) discuss the chemical and

biological consequences of lake acidification. Here, causes and effects of acidification, especially those that are important to the benthic habitat, are summarized.

Causes of Acidification

Surface water acidification is the result of increased acidity of precipitation, a trend that began roughly 200 years ago (Likens et al. 1979). The burning of fossil fuels produces oxides of sulfur and nitrogen, which can be transformed in the atmosphere to sulfuric and nitric acids (Brosset 1973, Dovland and Semb 1980, NAS 1981, NRCC 1981). During this time they may be transported several thousand kilometres (Doveland and Semb 1980, NAS 1981, NRCC 1981). These pollutants can be removed from the atmosphere by direct transfer to the ground in gaseous or particulate form (dry deposition), or by water droplets (wet deposition) (Fowler 1980).

Sulfuric and nitric acids are the major constituents responsible for the acidity of precipitation (Likens et al. 1979). In addition, other pollutants such as ammonium ions, trace metals and complex organic compounds can be important contaminants (Haines 1981, NAS 1981, NRCC 1981, Overrein et al. 1981).

The extent to which acidic deposition acidifies surface waters of a region depends largely on the mineral composition of the underlying bedrock (NRCC 1981). Non-carbonate rocks and rocks resistant to dissolution through weathering (such as highly siliceous rocks) afford the lowest capacity to neutralize acids (Likens et al. 1979, NRCC 1981, Dillon et al. 1984). Lakes lying over such poorly buffered

terrain, which may include 50 - 80% of the world's freshwater (NAS 1981), are therefore the most vulnerable to acidification (Wright et al. 1980). Lakes acidified to below pH 5.0 as a result of acidic precipitation are found in southern Norway and Sweden, Britain, eastern Canada and northeastern United States (Likens et al. 1979, NAS 1981, NRCC 1981, Overrein et al. 1981, Jeffries et al. 1986, Kelso et al. 1986, Linthurst et al. 1986).

Chemical Changes

Most precipitation entering an aquatic ecosystem does so after contacting terrestrial surfaces (Overrein et al. 1981, Dillon et al. 1984). Reactions between acidic deposition and components of the watershed thus influence the composition of water that eventually enters lakes. Dissolution, cation exchange and microbial reduction reactions involving minerals, soil and vegetation can result in the net uptake of H^+, NH_4^+, and NO_3^- by and the net release of Ca^{2+}, Mg^{2+}, Al^{3+}, Mn^{2+} and Fe^{3+} from a watershed receiving acidic deposition (NRCC 1981, Overrein et al. 1981, Dillon et al. 1984). The main determinants of the acid neutralizing capacity in a region are mineral composition of the bedrock, glacial overburden, soil type and vegetation (Likens et al. 1979).

The ability of freshwaters to resist depression of pH from acidic runoff and precipitation depends primarily on the amount of geologically derived carbonate alkalinity supplied from the watershed (Stumm and Morgan 1970, NRCC 1981, Dillon et al. 1984). In lakes with low amounts of such mineral alkalinity (i.e. lakes vulnerable to

acidification), internally produced alkalinity from microbial reductions of SO_4^{2-} and NO_3^-, and exchange of H^+ for Ca^{2+} in sediments can become the main sources of neutralizing capacity (Schindler 1986). Decrease of pH in a lake occurs when the sources of alkalinity are exhausted or overwhelmed (Henriksen 1980, Schindler et al. 1986).

Depression of pH can be spatially and temporally heterogeneous thoughout a lake. During spring, large amounts of acid that accumulate in the watershed through the winter can run into streams and lakes within a short period of time (Oden and Ahl 1970, Hagen and Langeland 1973, Hultberg 1977, Jeffries et al. 1979, Hendrey et al. 1980, Nilssen 1980). This meltwater normally forms a surface layer from one to several metres thick in the lake until ice melts and full lake circulation occurs (Jeffries et al. 1979, Hendrey et al. 1980). As a result, short-term decreases of 1.0 - 2.0 pH units may occur near the surfaces of lakes while deeper layers remain near original values (NAS 1981).

The pH in the sediments of a lake can differ markedly from that of the overlying water. In a lake that was experimentally acidified to below pH 5.2 in the surface waters over eight years, a pH of 6.8 was maintained at depths greater than 1 cm below the sediment-water interface (Kelly et al. 1984). Among 15 lakes in southern Ontario the lowest pH measured in the water was 4.6, whereas the lowest pH measured 2 cm into the sediments was only 5.6 (Kelso et al. 1982). This buffering apparently results from the reduction of sulfate and nitrate by bacteria (Kelly et al. 1982) and exchange of Ca^{2+} and Mg^{2+} for H^+ in the sediment (Oliver and Kelso 1983).

Sulfate and nitrate are the principal anions deposited with H^+ in

acidic precipitation. Whereas nitrate is largely retained in the watershed surrounding a lake, sulfate concentration increases in the water of acidified lakes (Dillon et al. 1984). In experimentally acidified lakes, significant amounts of sulfate were reduced by bacteria and precipitated as iron sulfides (Schindler et al. 1980, Cook and Schindler 1983). Schindler (1985) suggested that in Precambrian Shield lakes receiving high levels of sulfate deposition, dissolved iron concentrations may be substantially reduced, causing disruptions of phosphorus and trace element cycles.

Metal concentrations in lake waters are affected by deposition from precipitation, leaching from watersheds and mobilization from sediments (NAS 1981, NRCC 1981, Dillon et al. 1984). Consequently, lakes with lower pH generally have higher metal concentrations than similar, less acidic lakes (Haines 1981). Aluminum, manganese, iron, zinc, and possibly cadmium and nickel can be leached from terrestrial soils. However only aluminum and manganese concentrations appear to be consistently elevated in acidified lakes (Wright et al. 1980, NRCC 1981, Dillon et al. 1984, LaZerte 1986). Higher than average concentrations of copper, cadmium, lead, nickel and other trace metals also have been observed in various acidified lakes, especially those located near industrial sources of sulfur and nitrogen oxide emissions (Conroy et al. 1976, Dickson 1980, Franzin and McFarlane 1980, Haines 1981).

Effects on the Benthic Community

Microbes, algae, macrophytes and invertebrates living on the

bottoms of lakes and streams make up the benthic community. Benthic invertebrates process detritus, recycle nutrients, mix sediments and serve as principal food sources for fish, waterfowl and riparian mammals (Singer 1984). Compared to other biota in aquatic ecosystems, benthic organisms show some of the earliest and most pronounced responses to acidification.

Increased accumulations of allochthonous organic matter and reduced rates of decomposition (as measured by leaf breakdown and oxygen consumption) have been observed in some acidified lakes and streams (pH < 5.0) compared to those with higher pH (Grahn et al. 1974, Hendrey et al. 1976, Friberg et al. 1980, Traaen 1980, Hildrew et al. 1984, Allard and Moreau 1986). In these studies, reduced microbial density or lower microbial activity were thought to cause slower decomposition. Aerobic bacterial populations often decline, whereas fungal density increases with acidification (Haines 1981, Laake in Overrein et al. 1981, NRCC 1981). Reduced microbial activity could affect nutrient recycling, primary production and detritivore production (Francis 1986). In contrast, in situ measurements of carbon dioxide and methane generation (products of microbial activity) in the sediments of an acidified lake showed no change over an epilimnetic pH range of 5.1 - 6.7, due presumably to the maintenance of higher pH in the sediments by sulfate and nitrate reducing bacteria (Kelly et al. 1984).

Benthic algal populations commonly proliferate in the littoral zone of acidified lakes, forming dense mats over the sediments (Grahn et al. 1974, Hendrey et al. 1976, Almer et al. 1978, Hendrey and Vertucci 1980, Stokes 1981, Schindler and Turner 1982). Usually these blooms

involve filamentous blue-green or green algae, especially Mougeotia sp. Species of Sphagnum moss have shown similar responses to acidification in Scandinavia, invading lakes and expanding to cover extensive areas of the littoral zone (Hultberg and Grahn 1976, Grahn 1977, Hendrey and Vertucci 1980). As a result, angiosperms such as Lobelia sp. and Isoetes sp., that previously dominated the biomass of macrophytes, declined (Overrein et al. 1981, Grahn 1986). Dense mats of algae and mosses could restrict nutrient and metal movements by sealing off the sediments from the overlying water (Grahn et al. 1974, Hendrey and Vertucci 1980, Stokes 1981). In addition, because of its high cation exchange capacity, Sphagnum may take up inorganic nutrients (such as Ca and K) or metals (such as Fe or Al) and release H^+, thereby further contributing to the acidification process (Hultberg and Grahn 1976, Grahn 1977, Hendrey and Vertucci 1980, NRCC 1981, Singer 1982).

It has been known for some time that fish populations decline with the acidification of lakes. Surveys in North America and Europe report the disappearance of species from lakes with pH as high as 6.0, and the elimination of all species in lakes with pH less than 5.0 (Haines 1981, NRCC 1981, Eilers et al. 1984). Failure of osmoregulation and aluminum toxicity appear to be the main causes of mortality (Baker and Schofield 1980, Muniz and Leivestad 1980). Low reproductive success and poor recruitment contribute to the decline of populations (Haines 1981, NRCC 1981, Overrein et al. 1981, Rosseland 1986). As top predators in aquatic ecosystems, losses of fish populations may have important effects on the macroinvertebrate community (see below).

Although some studies (Crisman et al. 1980, Collins et al. 1981, Kenttamies et al. 1985, Dermott et al. 1986) found no relationship between abundance or species richness and lake water pH, benthic invertebrate communities of acidified systems generally have fewer species and lower numbers of individuals compared to those in similar waters of higher pH (Sutcliffe and Carrick 1973, Grahn et al. 1974, Conroy et al. 1976, Leivestad et al. 1976, Wiederholm and Eriksson 1977, Mossberg and Nyberg 1979, Nilssen 1980, Okland and Okland 1980, Raddum 1980, Townsend et al. 1983, Raddum and Fjellheim 1984). Within all taxonomic groups (at the class or order level), species richness is reduced as pH declines (Eilers et al. 1984). The Hirudinea, Mollusca, Malacostraca and Ephemeroptera are especially poorly represented at pH less than 5.5. Groups such as the Chironomidae, Corixidae, Dytiscidae and Megaloptera include a greater number of acid tolerant species, so these taxa tend to dominate the fauna of low pH systems (Okland and Okland 1986).

The relationship between abundance of macroinvertebrates and pH is less direct than that between taxon richness and pH. With few exceptions, densities of leeches, molluscs, crustaceans and mayflies are lower in acidified lakes than those in near neutral pH lakes (see reviews of Overrein et al. 1981, Singer 1984, Okland and Okland 1986). To a lesser extent, abundance of stoneflies and caddisflies is also reduced at low pH. Oligochaetes and dipterans vary in their responses to acidification, showing no change or increasing in abundance according to some studies, and decreasing according to others. In contrast, megalopterans, hemipterans and coleopterans are more abundant in acidified lakes, often thriving at pH less than 4.5

(Overrein et al. 1981, Singer 1984, Okland and Okland 1986).

High concentrations of hydrogen ions are, in general, toxic to aquatic animals. In freshwaters, animals with exposed permeable membranes must actively take up ions in order to maintain their internal ion concentrations. With a decrease in pH, hydrogen ions increasingly compete with cations at ion uptake sites, and interfere with osmoregulation (Havas 1981, Sutcliffe 1983). Calcium regulation is especially important for crustaceans, which require it to harden their exoskeletons, and molluscs, which secrete calcium to construct their shells. Not only is calcium uptake impaired at low pH (Havas 1981, Okland and Okland 1986), calcium is also lost at a higher rate due to increased solubility (Singer 1984). Under very acidic conditions (pH 3.0 - 4.5), hydrogen ions can directly enter and acidify internal fluids (Havas 1981), and disrupt pH dependent bodily processes.

Decreased pH can affect the responses by organisms to metals through changes in metal speciation in solution or by altering biological sensitivity (Campbell and Stokes 1985). Among metals studied, aluminum appeared to be the most important, especially for fishes (Baker and Schofield 1980, Overrein et al. 1981, Campbell and Stokes 1985). Concentrations of Al one to two orders of magnitude greater than those usually observed in acidified lakes are toxic to some crustaceans (Havas and Hutchinson 1982), whereas they are apparently harmless to corixids (Witters et al. 1984). For other metals, commonly-occurring concentrations may be toxic but studies are not conclusive enough to allow generalizations (Campbell and Stokes 1985).

A decrease in pH can affect aquatic organisms indirectly by altering populations of trophically interacting species. Such alterations can stress organisms at a pH above the level that is directly toxic. In an experimentally acidified Ontario lake, loss of benthic crustaceans and minnows between pH 5.0 - 5.8 eliminated most of the prey available to lake trout, whose condition deteriorated (Schindler et al. 1985). Sawyer (1974) stated that availabilty of food organisms is the most important factor determining leech distribution. Raddum (1980) and Nilssen (1980) pointed out that molluscs and oligochaetes, which are prey of leeches, become rarer in acidified lakes, and this may partly account for disappearance of leeches from these lakes. Disappearance of predatory fish populations due to acidification may explain the increased abundance of aquatic hemipterans, coleopterans and odonates in Scandinavian lakes (Eriksson 1979, Eriksson et al. 1980, Henrickson and Oscarson 1981, Bendell 1986). Species of these acid tolerant taxa are very sensitive to predation by fish. When fish were experimentally removed from a non-acidic lake, corixids and dytiscids became more abundant (Stenson et al. 1978).

Sensitivity of Macroinvertebrates to Acidification

The sensitivity of benthic invertebrate species to acidification depends on several factors: their tolerance to the chemical conditions of acidified waters, their degree of exposure to these conditions, and their trophic relationships with other species in the ecosystem.

Morphological and physiological characteristics of an animal affect

its ability to accommodate to chemical stress. Two important characteristics appear to be the permeability of the integument and the possession of external gills, both of which are involved in osmoregulation. The greater the surface area that allows exchange of ions between the organism and the environment, the greater the stress due to low pH. Groups such as leeches, molluscs, crustaceans and mayflies, which are rare or absent in acidified waters, have permeable body walls or external gill surfaces. In contrast, species such as corixids, dytiscids and the megalopteran Sialis lutaria, which are tolerant of low pH waters, can breathe air and have tough, impermeable exoskeletons (Hendrey and Wright 1976, Vangenechten and Vanderborght 1980).

The requirement of calcium is another important characteristic affecting tolerance to low pH. Acidic water corrodes calcareous shells (Singer 1982) and retards the uptake of calcium by aquatic animals (Malley 1980, Havas 1981). Therefore, organisms requiring high amounts of calcium are more stressed by low pH than those requiring less calcium. In fact, crustaceans and molluscs are among the first animals to disappear as pH decreases in lakes (Okland and Okland 1980, Havas 1981, Schindler et al. 1985).

Tolerance of acidic conditions can vary among life cycle stages. Bell (1971) showed that in several orders of aquatic insects, the pH at which 50% of the test animals emerged successfully was 0.5 - 2.0 units lower than the pH at which 50% of the larvae survived after 30 days. Appelberg (1984) reported high mortality in early post-reproductive stages of Astacus astacus, and a drastic loss of attached eggs at low pH. Molting Orconectes virilis suffered higher

mortality at pH 4 than non-molting individuals (Malley 1980). Failure of recruitment of O. virilis was a major contributor to the decline of the population in an experimentally acidified lake by the time pH fell to pH ~ 5.0 (I.J. Davies pers. comm., Schindler et al. 1985). At molting, demand for calcium and other ions is high (Greenaway 1974, Mantel and Farmer 1983). Young crustaceans not only molt more frequently than adults, but are also proportionately more sensitive to ionic stress due to their higher surface area to volume ratios.

Acidic conditions in a lake can vary both temporally and spatially (see above). Thus exposure to low pH depends on when and where an organism is active. Acidification is often episodic, occurring most intensely during spring snow melt and peak rainfall events (Gjessing et al. 1976, Jeffries et al. 1979, Nilssen 1980). Macroinvertebrates that are active or in sensitive life history stages during these periods experience greater exposure to toxic conditions than those that are inactive or in less sensitive stages.

Because acidic meltwater entering lakes tends to remain in the upper layers of thermally stratified lakes, inhabitants of deep, profundal regions may escape extreme acidic conditions of the littoral zone when full lake circulation is restricted. Fauna living in lake sediments also may be insulated from acidic water due to the buffering reactions of sulfate and nitrate reducing microbes. This may explain smaller differences in the composition of the infauna of acidified lakes with various pH's, compared to the more exposed epifauna (Collins et al. 1981).

Sensitivity of a species to lake acidification is also related to the nature of its trophic interactions. Changes in populations of

prey, predator or competitor organisms were important factors in the responses of corixids and dytiscids (Henrickson and Oscarson 1981), zooplankton (Nilssen et al. 1984), and cyprinids and salmonids (Schindler et al. 1985) in acidified lakes.

Pertinence to Hyalella azteca

The changes in an acidified lake that are of greatest importance to a benthic crustacean such as Hyalella azteca include:

1. a decrease in pH, which can interfere with the maintenance and uptake of essential cations,
2. a potential increase in concentration of metals,
3. a possible change in quality of food supply (algae, macrophytes and associated microbes), and
4. a loss of predatory fishes, although this may occur at a pH lower than that tolerated by crustaceans.

Compared to other biota, benthic crustaceans are relatively sensitive to acidification. A decrease in pH to below 5.5 is likely to severely impair their ability to survive because of their permeable external gills and high requirement for calcium.

ECOLOGY OF *HYALELLA AZTECA*

Hyalella azteca is one of the most common and widely distributed freshwater amphipods of North America (Bousfield 1958, Pennak 1978). Eurytopic and easily dispersed, it is found in almost all permanent freshwaters that reach summer temperatures over 10^{o} C (Bousfield 1958). Amphipods are important components of aquatic food webs. They convert plant and animal detritus into animal protein, and they are consumed by a variety of predatory invertebrates and vertebrates (Bousfield 1970). The general biology of *H. azteca* has been reviewed by de March (1981). Therefore, only a summary of the ecology of *H. azteca* appears here.

The genus *Hyalella* (Talitridae) is predominantly neotropical and the only freshwater representative of the primarily marine and semi-terrestrial superfamily Talitroidea (Bousfield 1958, Bulycheva in de March 1981). *Hyalella* is monotypic over most of North America, whereas in South America, over 50 species have been described (Bousfield, pers. comm.). The geographical distribution of *H. azteca* extends from roughly the treeline in northern Canada south to Guatemala and the Caribbean islands, and from the Atlantic to the Pacific , including coastal islands (Bousfield 1970, 1973, de March 1981). It has been collected from lakes, ponds, marshes, estuaries, rivers, streams and ditches (Bousfield 1958).

Hyalella azteca is a good swimmer and is also able to "walk" short distances over dry land (de March 1981). The species is capable of dispersing long distances in the feathers of waterfowl (Swanson 1984)

and possibly in the fur of mammals (Peck 1975). The mating system of H. azteca facilitates dispersal (Bousfield pers. comm.). The mating procedure includes a precopulatory "carrying" phase (amplexus), during which the superior male grasps the usually smaller female by the anterior body and carries her around until her ovipositional molt, when insemination can occur (Strong 1973). Dispersing amphipods in amplexus are thus more likely to establish populations in new habitats than individuals dispersing singly.

Like other amphipods, H. azteca lives in association with aquatic vascular plants, attached algae, allochthonous leaves and twigs, and other organic bottom debris (Embody 1912, Wienert 1950, Biette 1969, Strong 1972, de March 1981). H. azteca tends not to burrow into sediments (Wienert 1950), is most abundant in shallow water (Hargrave 1970a, Mathias 1971) and is negatively phototropic (Wienert 1950). Although tolerant of a wide range of chemical and physical conditions, highest densities are found in warm, lentic, productive waters of high ionic strength and neutral pH (Wienert 1950, de March 1981). Densities can be very high, over 10,000 m^{-2} in the prairie pothole lakes of southern Manitoba (de March 1981).

Dead plant and animal matter, algae and macrophytes are the chief sources of food for H. azteca (Embody 1912, Wienert 1950). Bacteria, fungi and algae growing on ingested detritus are important sources of nutrients (Hargrave 1970b). In general, H. azteca is omnivorous and can use almost any organic substrate as food (Bousfield 1970).

Hyalella is heavily preyed upon by fishes, especially trouts, perch, pike and sunfish (Embody 1912, Cooper 1965, Bousfield 1970, Strong 1972). Cooper (1965) determined that yellow perch feed

selectively on the largest individuals of a population. Invertebrates such as dragonfly nymphs, water scorpions, dytiscids, notonectids, larval megalopterans and belostomatids also feed on amphipods (Embody 1912, Bousfield 1970). As well, waterfowl consume them in substantial numbers (Wienert 1950).

Several parasites use H. azteca as an intermediate host, including acanthocephalans (Podesta and Holmes 1970, Bethel and Holmes 1977, Rice and Mitchell 1980, Uznanski and Nickol 1980), nematodes (Wong and Anderson 1982), trematodes (Cannon 1971, Hazen and Esch 1977) and cestodes (McLaughlin 1975). Infestation by some of these parasites causes changes in certain behavioral traits that increase the chances of the amphipod being consumed by the definitive host (de March 1981).

Populations of H. azteca produce from one to as many as five generations per year (de March 1981). In colder climates, growth and development is slower and animals become inactive over winter (Cooper 1965, Mathias 1971, Strong 1972). Reproductive activity is initiated and maintained by a photoperiod with more than 12 hours light (de March 1977) and temperatures greater than 16 - 20^{o} C (Cooper 1965, Strong 1972, de March 1977). In southern Manitoba, these conditions occur roughly three months after spring equinox (de March 1981). Reproduction is sexual. Males and females pair and copulate at regular intervals, as often as every 8 days during the summer (de March 1977). Following insemination, up to 30 eggs develop and hatch within an abdominal brood pouch (de March 1978). The young continue to be carried until the next mating, at which time the female molts, releases the young, and ovulates another clutch of eggs to be fertilized by the male (Strong 1973).

The young amphipods molt about 8 times before becoming sexually mature (Geisler 1944, Strong 1972, de March 1981). Rates of egestion of food, growth, egg development, molting, and production are all directly related to temperature (Embody 1912, Geisler 1944, Bove'e 1950, Cooper 1965, Mathias 1971, Hargrave 1972, de March 1978, Lindeman and Momot 1983). Growth rates also may be affected by food supply, such as benthic algal production (Hargrave 1970).

Overwintering adults produce only one or a few broods of progeny in the spring before dying (Cooper 1965). At temperate latitudes (45 - 50^{o}N), this new generation can mature in about 30 days and begin producing a second cohort of amphipods, which will make up the overwintering population (de March 1981). Essentially none of the adults recruited from the spring reproduction survives the summer (Embody 1912, Cooper 1965, Strong 1972). Reproduction ceases in response to decreased daylight length and temperature (Cooper 1965, Strong 1972, de March 1977), and this occurs approximately at fall equinox in southern Manitoba (de March 1977). Although growth rate drops off, final sizes of the overwintering amphipods are larger than the sizes of summer amphipods (Cooper 1965, Strong 1972, de March 1978).

In summary, Hyalella azteca is a mobile species which is highly adaptable to environmental conditions. Availability of organic substrate appears to determine its distribution within a lake. Temperature and photoperiod are the major environmental factors affecting growth and development, and its chief source of mortality is predation by fishes.

PART II. THE ROLE OF pH IN THE DISTRIBUTION OF *HYALELLA AZTECA* IN THE EXPERIMENTAL LAKES AREA

INTRODUCTION

Although a study of the distribution of a species may not indicate the effects of a single factor as clearly as in an experiment, it can provide information on community and ecosystem-level effects, and long-term responses that are difficult to obtain from organisms isolated from their environment. A demonstration that pH is an important factor controlling the distribution of *Hyalella azteca* implies detrimental consequences of lake acidification.

Hyalella azteca is tolerant of a broad range of environmental conditions. Until recently, only mean summer water temperature < 10°C was known to restrict the distribution of this species. However, Stephenson and Mackie (1986) have now shown that pH < 5.6 limits its distribution in southern Ontario waters.

The natural distributions of other amphipods and benthic crustaceans also are limited by pH. The amphipods *Gammarus fossarum*, *G. lacustris*, *G. roeseli* and *Pontoporeia affinis* are rare or absent in waters with pH < 6.0 (Dadswell 1974, Okland,K.A. 1980, Brehm and Meijering 1982, Matthias 1983, Raddum and Fjellheim 1984, Schrimpff and Foeckler 1985), and *G. pulex* becomes rare at pH < 5.5 (Sutcliffe and Carrick 1973, Brehm and Meijering 1982, Otto and Svensson 1983).

Other malacostracans such as Mysis relicta, Astacus astacus and Orconectes propinquus, and the notostracan Lepidurus arcticus are rare or absent at pH < 5.5 - 6.0 (Dadswell 1974, Raddum and Fjellheim 1984, Berrill et al. 1985, Borgstrom et al. in Okland and Okland 1986, Lund in Okland and Okland 1986, Svardson in Okland and Okland 1986).

In 1984, a preliminary survey of lakes in the Experimental Lakes Area (ELA), northwestern Ontario, suggested that pH also may affect the distribution of H. azteca. Thirteen out of 15 lakes sampled contained populations of H. azteca. Only lakes 223 and 302S did not have H. azteca, and both of these were being experimentally acidified by additions of sulfuric acid (Schindler 1985, Schindler et al. 1985). Lakes 223 and 302S had mean epilimnion pH's during the ice-free season of 5.4 and 5.6, respectively, whereas the other 13 lakes had pH's >6.0. In addition, H. azteca was present in lake 302S in 1983, when the mean pH was 5.9. Its disappearance by 1984 provided further circumstantial evidence of a negative effect of low pH on populations of H. azteca. Therefore, in 1985, a broader survey was undertaken. Thirty lakes having pH's ranging from 5.0 to 8.8 were sampled to determine more precisely the effects of pH on the distribution of natural populations of H. azteca.

Calcium, magnesium, temperature and oxygen can affect the distribution of freshwater amphipods (Sutcliffe and Carrick 1973, Dadswell 1974, Okland and Okland 1985, Schrimpff and Foeckler 1985). Temperature and oxygen concentrations in the littoral zones of lakes at ELA, the favored habitat of H. azteca, were known to be satisfactory. However, concentrations of calcium and magnesium probably were limiting. Surface waters at ELA are among the most

dilute in the world, having an average calcium concentration of 1.6 - 2.0 mg/L and an average magnesium concentration of 0.9 mg/L (Armstrong and Schindler 1971, Beamish et al 1976). These concentrations are near or below the lowest ones at which Gammarus fossarum, G. lacustris, G. pulex and G. roeseli are found in Europe (Sutcliffe and Carrick 1973, Okland,K.A. 1980, Brehm and Meijering 1982, Matthias 1983, Schrimpff and Foeckler 1985). The lowest calcium concentration at which de March (1981) stated H. azteca is found was 7 mg/L.

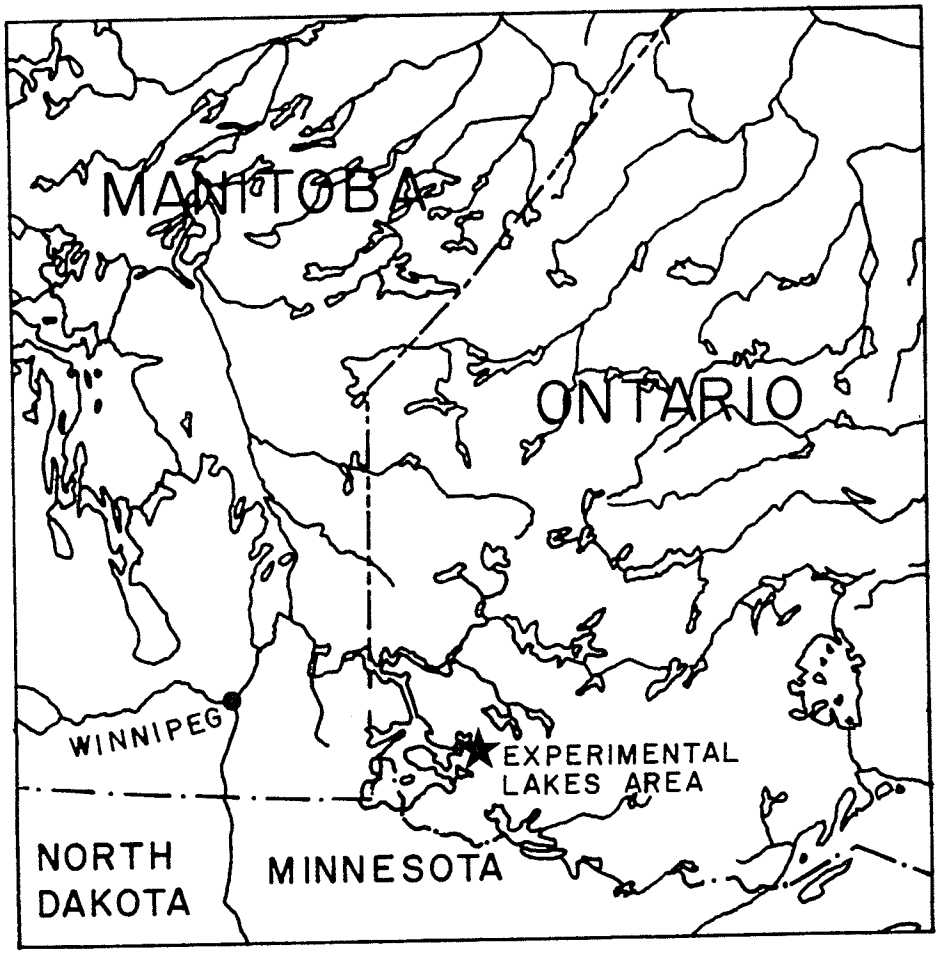
The objective of this study was to determine the effects of various concentrations of calcium, magnesium and hydrogen ion on the distribution of H. azteca at ELA.

DESCRIPTION OF STUDY AREA

ELA is located in northwestern Ontario ($93^{o}30'$ - $94^{o}00'$ W, $49^{o}30'$ - $49^{o}45'$ N) (Fig.1). A series of background studies published in the Journal of the Fisheries Research Board of Canada in 1971 (28:123-301) provides a detailed limnological characterization of ELA. Therefore, only a summary of the relevant geological, chemical and biological conditions appears here.

The lakes of ELA lie on the southwestern part of the Precambrian Shield, about 200 km east of the Shield - Ordovician limestone boundary (Brunskill and Schindler 1971). The area is underlain by acid granodiorite bedrock, and overlain in some areas by thin glacial drift of quartz, plagioclase and K-feldspar sand and gravel (Brunskill

Figure 1. Location of the Experimental Lakes Area, at $93^{o}30'$ - $94^{o}00'$ W, $49^{o}30'$ - $49^{o}45'$ N.


MANITOBA
ONTARIO
WINNIPEG
EXPERIMENTAL
LAKES AREA
NORTH
DAKOTA
MINNESOTA

and Schindler 1971). These materials show low rates of chemical weathering (Brunskill et al. 1971). Terrestrial vegetation is typical of a boreal subclimax forest, with the dominant tree species being jackpine, black spruce, trembling aspen and white birch (Brunskill and Schindler 1971).

Over 1000 lakes are located in the vicinity of ELA. Cleugh and Hauser (1971) numbered them and provided a map with a list of coordinates for their locations. Surface areas ranged from <1 - 1300 ha and maximum depths 2 - >117 m, although the majority of lakes surveyed were <50 ha in area and <10 m in depth (Cleugh and Hauser 1971). In the present study, 30 lakes were sampled, having surface areas of 0.5 - 1007 ha (median = 12.2 ha) and maximum depths of 1 - >117 m.

Lake waters of ELA are, in addition to being very dilute, similar to each other in chemical composition (Beamish et al. 1976). Table 1 lists the average concentrations of major chemical constituents of ELA lakes. Average concentrations of major cations are approximately 2.0, 0.9, 0.6 and 0.3 mg/L for Ca, Na, Mg and K, respectively (Beamish et al. 1976). Average pH is about 6.5, and alkalinity is 40 - 80 ueq/L for most lakes (Schindler and Ruszczynski 1983). Nutrient and heavy metal concentrations are also low compared to lakes in other regions of the world. Prokopowich (1979) reported N:P ratios of 20:1 to 50:1 and C:N ratios of 2:1 to 7:1 by weight. Distributions by weight of various fractions of C and N were : dissolved organic > suspended > dissolved inorganic, and for P it was: dissolved organic $\simeq$ suspended > dissolved inorganic.

Table 1. Chemical composition of lakes in the Experimental Lakes Area - mean values of measurements for lakes sampled in various surveys.

Chemical Constituent (unit)	Concentration	Reference
Ca (mg/L)	1.6, 2.0	1, 2, 3
Na (mg/L)	0.9	1, 2
Mg (mg/L)	0.6, 0.9	1, 2
K (mg/L)	0.3, 0.4	1, 2
pH	6.5	2, 3
alkalinity (ueq/L)	60	4
SO_4 (mg/L)	3, 4	1, 2
Cl (mg/L)	0.8, 1.4	1, 2
conductivity (umho/cm)	19	1, 2
DOC (umol/L)	755	2
NH_3-N (ug/L)	27	2
NO_3-N (ug/L)	8, <20	1, 2
NO_2-N (ug/L)	3	2
TDN (ug/L)	177, 266	1, 2
susp-N (ug/L)	101	2
PO_4-P (ug/L)	<1, 6	1, 2
TDP (ug/L)	7, 11	1, 2
susp-P (ug/L)	5	2
Mn (ug/L)	3	2
total Fe (ug/L)	50	1
Pb (ug/L)	<1	2
Cu (ug/L)	2	2
Ni (ug/L)	<3	2
Cd (ug/L)	<0.1	2
Zn (ug/L)	<0.1	2

1. Armstrong and Schindler 1971 (<_40 lakes)
2. Beamish et al. 1976 (104 lakes)
3. Schindler and Ruszczynski 1983 (101 lakes)
4. Schindler et al. 1986 (101 lakes)

Studies of primary production, phytoplankton composition and diatom assemblages in sediments show ELA lakes to be low in productivity (Schindler and Holmgren 1971, Stockner 1971). Macrophytes are scarce in the littoral zones (Brunskill and Schindler 1971). Hamilton (1971) sampled the sublittoral and profundal zoobenthic communities in 15 lakes. Faunal composition in a lake was related to dissolved oxygen concentration and temperature of bottom water (which are largely determined by morphometric features). Larger and deeper lakes were dominated by the amphipod Pontoporeia affinis and the mollusc Pisidium conventus, whereas smaller lakes (mean depth <10 m) were dominated by chironomids and chaoborids.

Several lakes in ELA have been used in whole lake experiments. Lakes 226NE, 226SW, 302, 303, 304 and 261 received nutrient additions between 1973 and 1980. Lake 227 has been continuously fertilized since 1969 with H_3PO_4 and $NaNO_3$. Acidification experiments have involved lakes 114, 223, 302S and 302N since 1976. Presently, L302N receives additions of HNO_3, whereas lakes 114, 223 and 302S receive H_2SO_4. Schindler (1985) discussed the effects of these perturbations on chemical conditions of the lakes.

METHODS

Selection of Lakes

Lakes representing a wide range of pH's and Ca and Mg

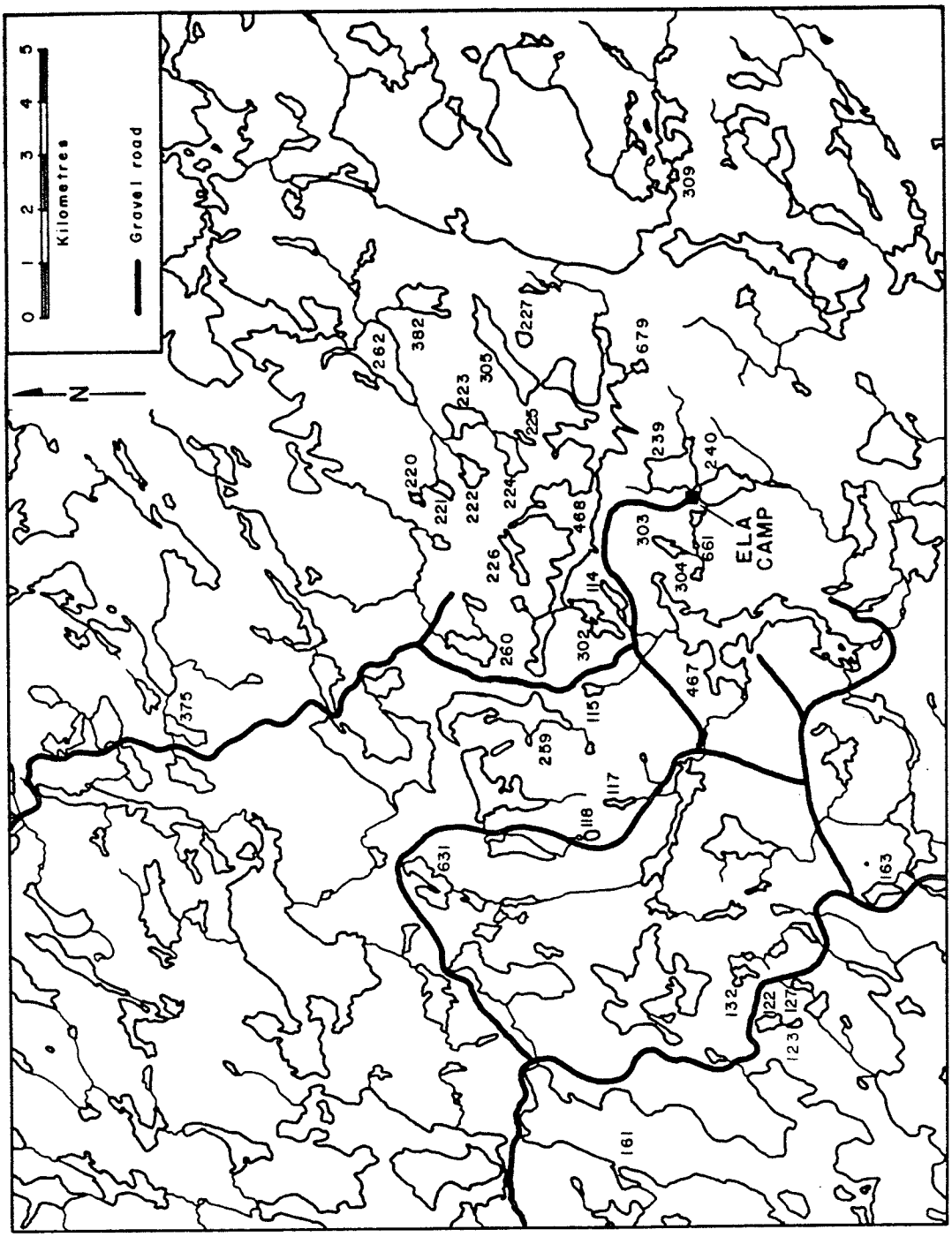
concentrations were sampled to detect limiting effects on H. azteca distribution. Water chemistry data from previous surveys (Beamish et al. 1976, Schindler and Ruszczynski 1983) were used to select as many lakes as possible having pH's of <6.0, alkalinities <30 ueq/L and Ca concentrations of <1.5 or >3.0 mg/L. Magnesium concentration was correlated with concentration of Ca. Only readily accessible lakes were included. Another set of accessible lakes having pH >6.0 or from 1.5 - 3.0 mg Ca/L was then added, resulting in a total of 30 lakes (Fig. 2).

Sampling Procedures

Lakes were sampled in August and September 1985, a period of late summer stagnation for ELA lakes (Schindler and Ruszczynski 1983). Four samples of water from the littoral zone of each lake were collected. Two 60-ml glass BOD bottles, pre-rinsed three times with distilled water and twice with lake water, were filled for duplicate pH measurements. Two 125-ml polyethylene bottles, similarily pre-rinsed, were filled for Ca and Mg determinations. All water samples were taken from 0.3 - 0.5 m below the surface in water about 1 m in depth. Care was taken to exclude from the samples gas bubbles and sediments disturbed from the lake bottom. Water samples were kept cool with crushed ice until they were processed in the laboratory.

Preliminary collections of H. azteca in ELA lakes showed that highest densities occurred in substrates rich in organic matter such as allochthonous debris (leaves, twigs, bark), macrophytes and emergent vegetation at depths <1 m. Therefore, all sampling for

Figure 2. Location of lakes in the Experimental Lakes Area sampled for Hyalella azteca. Numbers correspond to those given by Cleugh and Hauser (1971). Lakes 240, 259, 262, 382, 467 and 468 were sampled in 1984. All others were sampled in 1985.


0 1 2 3 4 5
Kilometres
Gravel road
N
ELA CAMP
309
679
227
262
382
223
305
225
220
221
222
224
239
240
468
226
303
304
661
114
260
302
467
115
259
117
118
631
375
163
132
122
127
123
161

amphipods was confined to such habitats. Sampling was standardized as follows: a triangular dipnet was scraped once over suitable substrate in five different locations along the lakeshore. Contents from each sweep were emptied into a sorting pan and examined for amphipods. Hyalella azteca specimens were usually identified with a magnifying glass on location, but some were preserved in ethanol for later verification under a microscope. Presence or absence of H. azteca in each net sweep was recorded, as well as whether there were >10 or <10 individuals per sweep. If no H. azteca were collected after five sweeps, a further five areas were sampled. Up to 30 min was spent sampling for amphipods. Specimens of H. azteca, if present, were usually found within the first 5 min of searching.

Analyses

Water samples for pH determination were allowed to reach room temperature before measurement. Within 4.5 h of sample collection, pH was measured with a Radiometer PHM4 pH meter and either an Orion Research 910200 electrode or Radiometer GK2401C combined electrode, calibrated with Fisher pH 4.00 and 7.00 buffers. During measurement, exchange of CO_2 between the sample and the atmosphere was minimized with a bored rubber stopper fitted around the electrode. This provided an airtight seal when the electrode was placed in the BOD bottle. pH was recorded only when readings stabilized. Previous testing with replicate samples of water from L239 showed the precision of this method to be at least $\pm$ 0.05 pH units.

Samples for Ca and Mg analyses were stored at $5^{o}C$ within 4 h of

collection, filtered through Whatman GF/C filters and acidified within 24 h. Concentrations of Ca and Mg were determined by atomic absorption (Stainton et al. 1977) within 2 wk of sample collection.

Relative abundance of H. azteca in a lake was ranked using three categories: a) "absent" if no individuals were found in 10 out of 10 net sweeps; b) "low" if individuals were found in $\leq$ 3 out of 5 sweeps, or if individuals were found in 4 out of 5 sweeps but averaged less than 10 per sweep; and c) "high" if 5 out 5 net sweeps contained individuals, or if individuals were present in 4 out of 5 sweeps but averaged greater than 10 individuals per sweep.

RESULTS

Measurements of duplicate pH samples from lakes usually differed by <0.05 pH units, which is within the range of the measurement error. Lakes ranged in pH from 5.0 to 8.8, with a median of 6.6 (Table 2). Concentrations of calcium were between 1.1 and 3.5 mg/L, and those of magnesium between 0.44 and 1.1 mg/L. The ranges were similar to those expected from previous measurements made in the lakes.

Hyalella azteca was absent from 7 lakes and present in 23. Among the lakes having H. azteca, relative abundance was ranked as "low" in 5 and "high" in all others.

Examination of relative abundance and the chemical conditions of the lakes suggested that epilimnion concentrations of calcium and pH affect amphipod distribution. No H. azteca were found in lakes with

Table 2. Concentration of calcium and magnesium, pH, and relative abundance of Hyalella azteca in 30 lakes at ELA.

Lake	Calcium (mg/L)	Magnesium (mg/L)	pH	Relative abundance*
114	1.28	0.55	6.11	1
115	1.54	0.63	6.27	1
117	1.59	0.64	6.55	0
118	1.19	0.55	7.23	0
122	1.63	0.53	6.42	1
123	1.48	0.56	6.44	2
127	1.38	0.54	6.32	2
132	1.38	0.47	6.60	2
161	2.95	0.72	7.53	2
163	3.16	0.92	6.01	2
220	1.14	0.52	6.63	0
221	1.83	0.70	6.41	2
222	3.54	0.78	7.09	2
223	2.56	0.60	5.38	0
224	1.77	0.44	7.23	2
225	1.05	0.57	5.03	0
226	1.78	0.60	6.85	2
227	1.83	0.64	6.71	2
239	3.01	0.96	7.01	2
260	2.37	0.71	7.07	2
302N	1.95	0.68	6.33	1
302S	2.22	0.67	5.40	0
303	1.60	0.63	8.82	2
304	1.73	0.74	6.83	1
305	2.27	0.66	7.15	2
309	2.45	0.78	6.86	2
375	1.73	0.69	6.89	2
631	3.45	0.82	7.49	2
661	1.64	0.72	6.44	0
679	3.36	1.09	6.39	2

* 0 = absent, 1 = low, 2 = high. See text for explanation of categories.

pH's <6.0 or calcium concentrations <1.28 mg/L. Lakes in which relative abundance was ranked low had pH's <6.9 and calcium concentrations <2.0 mg/L. Fig. 3 illustrates the relationship between relative abundance, pH and calcium concentration. The lower calcium limit for presence (1.24 mg/L) is suggested as the average of the lowest concentration at which H. azteca was found, and the next lowest lake calcium concentration recorded. The lower pH limit for presence (pH 5.7) is suggested as the average of the lowest pH at which H. azteca was found and the next lowest lake pH recorded. As calcium concentration and pH increase, lakes tended to be ranked as having high relative abundances of H. azteca. However, two anomalies to this trend are evident at about 1.60 mg Ca/L and pH 6.5. These lakes, L117 and L661, had no H. azteca although pH and calcium concentrations were above the suggested lower tolerance levels. Possible reasons for the absence of H. azteca from these lakes are discussed below.

Concentration of magnesium at ELA does not appear to limit relative abundance of H. azteca (Table 2). A weak tendency towards an increase in the number of lakes having high relative abundances with increasing pH and Mg concentrations is evident, but the two lakes with the lowest magnesium concentrations both had high relative abundances of amphipods (Fig. 4). Concentration of magnesium plus calcium (a rough measure of hardness) plotted against pH did not improve discrimination among the three abundance categories over calcium versus pH (Fig. 5).

Figure 3. pH, calcium concentration and relative abundances of Hyalella azteca in ELA lakes. 0 = absent from lake; 1 = low relative abundance; 2 = high relative abundance. (See text for explanation of categories.) Lines indicate suggested lower tolerance limits.

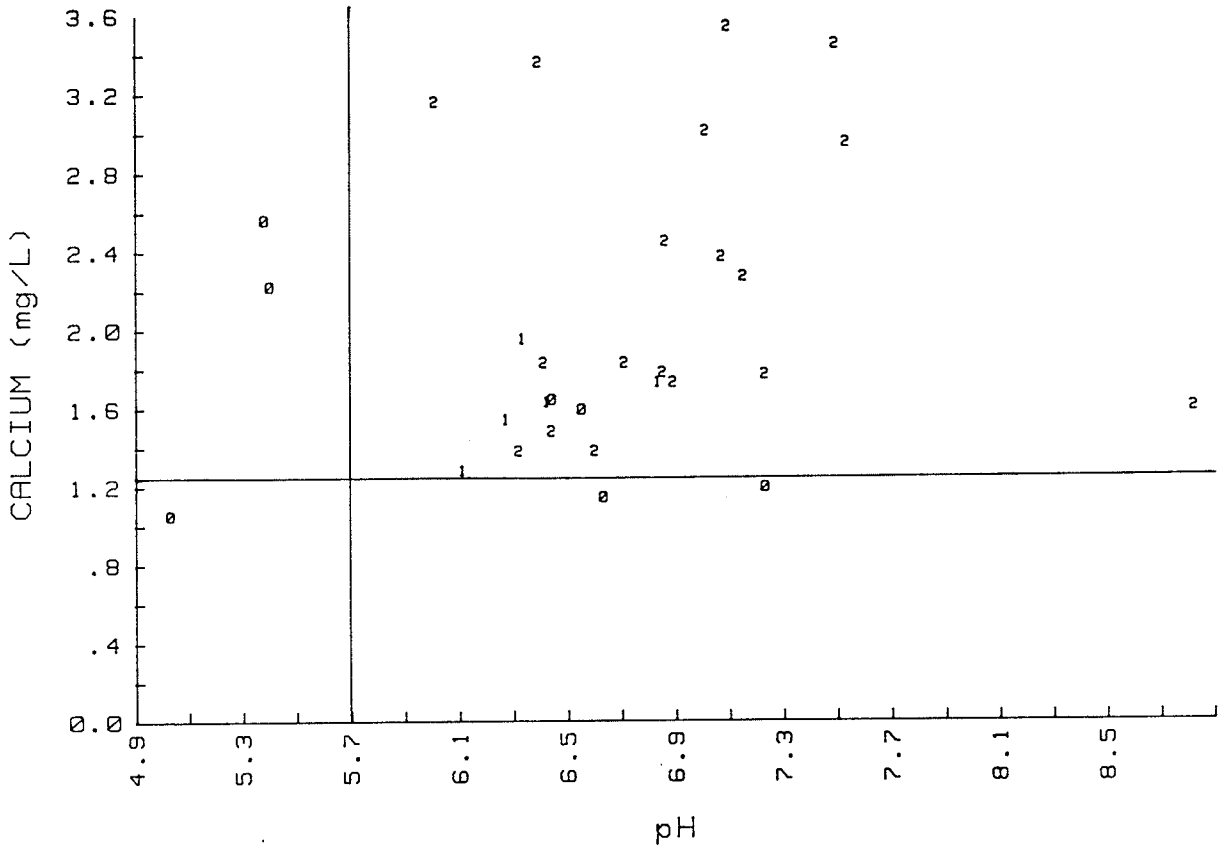

CALCIUM (mg/L)
3.6
3.2
2.8
2.4
2.0
1.6
1.2
.8
.4
0.0
4.9
5.3
5.7
6.1
6.5
6.9
7.3
7.7
8.1
8.5
pH

Figure 4. pH, magnesium concentration and relative abundances of Hyalella azteca in ELA lakes. 0 = absent from lake; 1 = low relative abundance; 2 = high relative abundance. (See text for explanation of categories.)

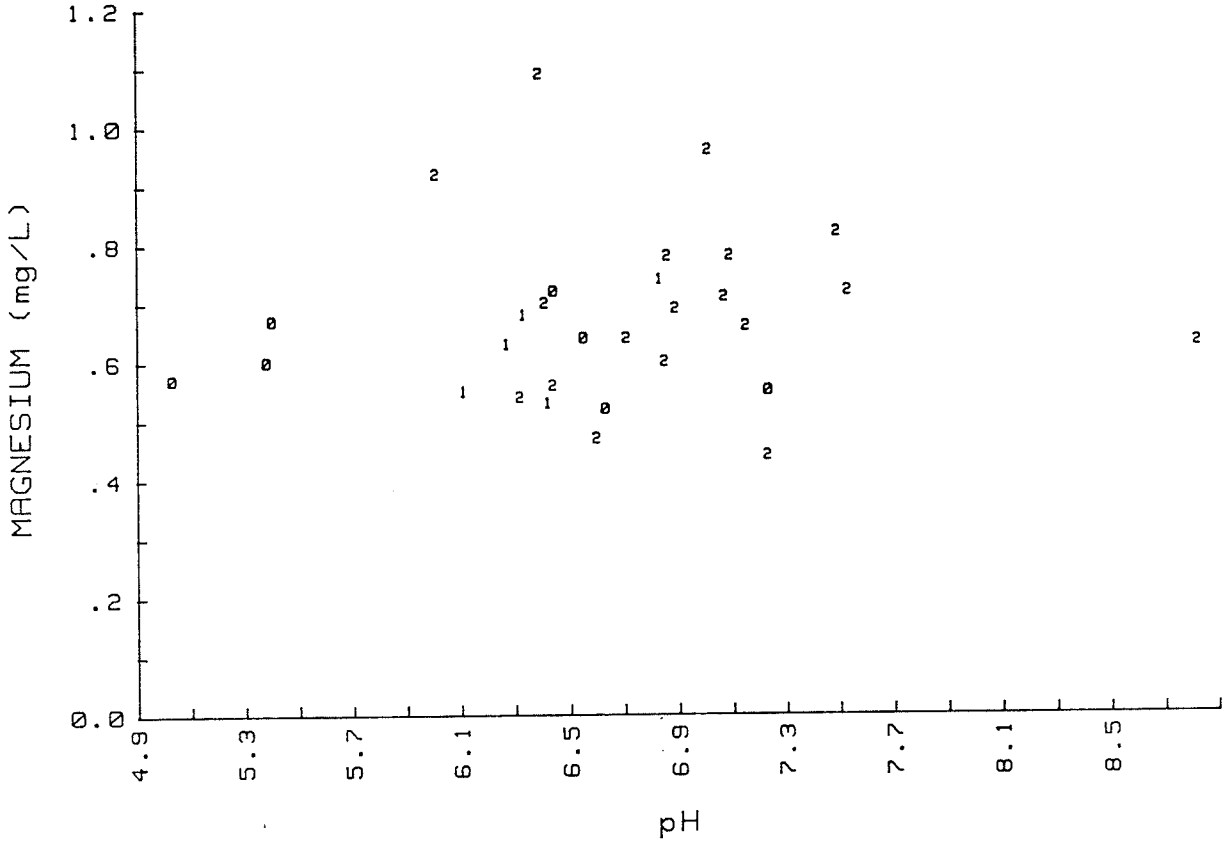
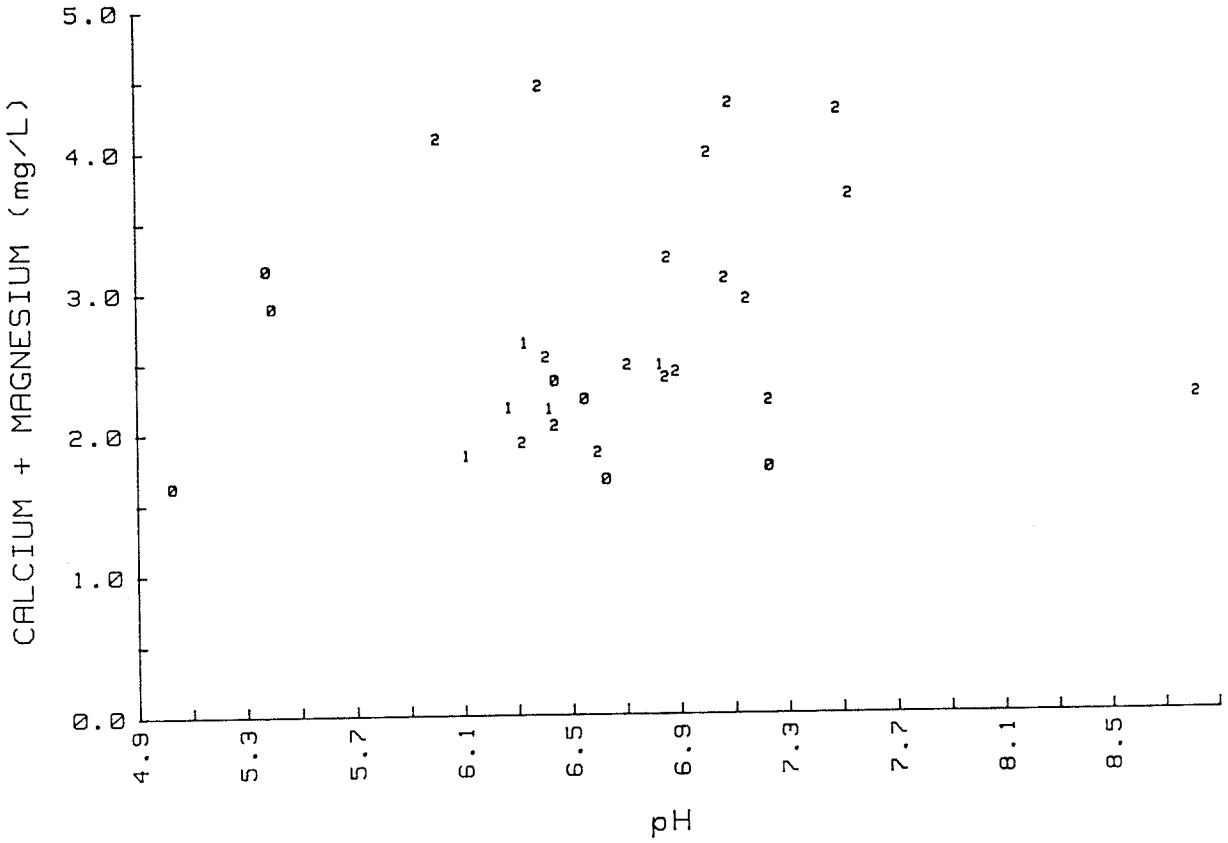

MAGNESIUM (mg/L)
1.2
1.0
.8
.6
.4
.2
0.0
4.9
5.3
5.7
6.1
6.5
6.9
7.3
7.7
8.1
8.5
pH

Figure 5. pH, Ca + Mg concentrations and relative abundances of Hyalella azteca in ELA lakes. 0 = absent from lakes; 1 = low relative abundance; 2 = high relative abundance. (See text for explanation of categories.)


CALCIUM + MAGNESIUM (mg/L)
5.0
4.0
3.0
2.0
1.0
0.0
4.9
5.3
5.7
6.1
6.5
6.9
7.3
7.7
8.1
8.5
pH

DISCUSSION

The limits of geographical distribution of a freshwater species depend on historical events linked with its ability to cross barriers, its physiological requirements, and its behavior patterns (Macan 1961). The ubiquitous distribution of H. azteca in North America and its apparent ease of dispersal suggest that geographical barriers do not restrict this species from colonizing lakes. Therefore, absence from a lake implies that H. azteca cannot survive the environmental conditions extant in that lake.

Until recently, conditions that control the distribution of H. azteca were not known. According to Wienert (1950), the availability of suitable organic substrate was the most important habitat requirement, a condition which affects distribution within a lake, but not likely among lakes. Except in northern regions (where mean summer water temperatures are < 10°C), other physical and chemical conditions in most unpolluted lakes do not normally reach levels that are intolerable to this hardy amphipod. Behavioral factors such as competition and predation appear unlikely to control presence of H. azteca in lakes. For example, Biette (1969) concluded that H. azteca and Gammarus lacustris probably avoided competition in the littoral zone of West Blue Lake, Manitoba, by selecting different microhabitats. The fact that H. azteca is eurytopic and often dominant in shallow-water benthic communities further reduces the likelihood that competition limits distribution among lakes. Similarly, predation probably does not prevent the survival of H.

azteca in lakes. While predation may be important in regulating abundance and size structure of benthic invertebrate populations, structurally complex habitats such as patches of aquatic plants and dead vegetation provide refuges from predators as well as food and substrate, thereby allowing coexistence of prey and predators (Macan 1977, Crowder and Cooper 1982).

Results from this study indicate that chemical conditions can become limiting in some lakes. Hyalella azteca is absent from all ELA lakes having pH <5.7 and calcium concentration <1.24 mg/L. Its absence from lakes 117 and 661 may be due to peculiarities of these lakes. The pH of L661 was 6.44 when measured in August 1985 (this study). However in May 1985, pH was 5.35 (Schindler, unpubl. data), which is below the inferred lower limit of tolerance. Substantial pH fluctuations occurred in L661 when water temperature exceeded 15^{o}C (probably due to variation in sulfate reduction and oxidation [Schindler, pers. comm.]), whereas in 20 other lakes for which pH was measured from May to October, seasonal variation was generally much less and did not involve depressions below 5.7 (Schindler, unpublished data). Lake 117 is chemically anomalous because it has a high concentration of dissolved organic carbon (DOC) (1531 umol C/L), which is double the average for ELA (Beamish et al. 1976). Organic anions can bind ionic calcium (Ohle 1955a in Wetzel 1975, Stumm and Morgan 1970), thereby making it unavailable for uptake by aquatic organisms. Because the analytical method used in this study measures total calcium and does not distinguish between free ions and calcium bound to dissolved organic anions, the amount of calcium actually available to animals in a lake with a high concentrations of DOC may be

considerably less than that indicated by measurements of total calcium (M. Stainton, pers. comm.). Aho (1966) reported a negative correlation between the number of species of molluscs in some lakes of southern Finland and water color (a measure of the concentration of humic substances). Despite equal total hardnesses, the number of available calcium ions were greater in colorless waters than in those that are rich in humic substances.

A lower tolerance limit of pH 5.7 is close to that determined by Stephenson and Mackie (1986) for H. azteca in southern Ontario. Out of 79 lakes sampled, H. azteca was absent from lakes having negative epilimnetic summertime alkalinities (corresponding to pH <5.6) and present in all but four lakes having positive alkalinities. Of these four lakes, two had prior negative alkalinities and one had low pH (5.83), alkalinity (0.031 meq/L) and calcium concentration (1.42 mg/L). Therefore, these lakes were vulnerable to short-term acidification below pH 5.6.

Dermott (1985) reported that H. azteca was abundant in the littoral zone of a poorly buffered Ontario Shield lake in which surface water pH was temporarily depressed to as low as 4.7 during spring runoff. Such pH depression due to pulses of acidic meltwater tend to occur only in the upper layers of lakes (see Part I), and in fact, the lowest pH in the top 2 cm of sediments recorded from a series of lakes, including the above one, was 5.8 (Dermott 1985). Higher pH in and near sediments is not uncommon in acidified lakes (Kelso et al. 1982, Oliver and Kelso 1983, Kelly et al. 1984), and may insulate benthic fauna from extreme pH of the water column (Collins et al. 1981, Dermott 1985), especially during temporary pH depressions. In

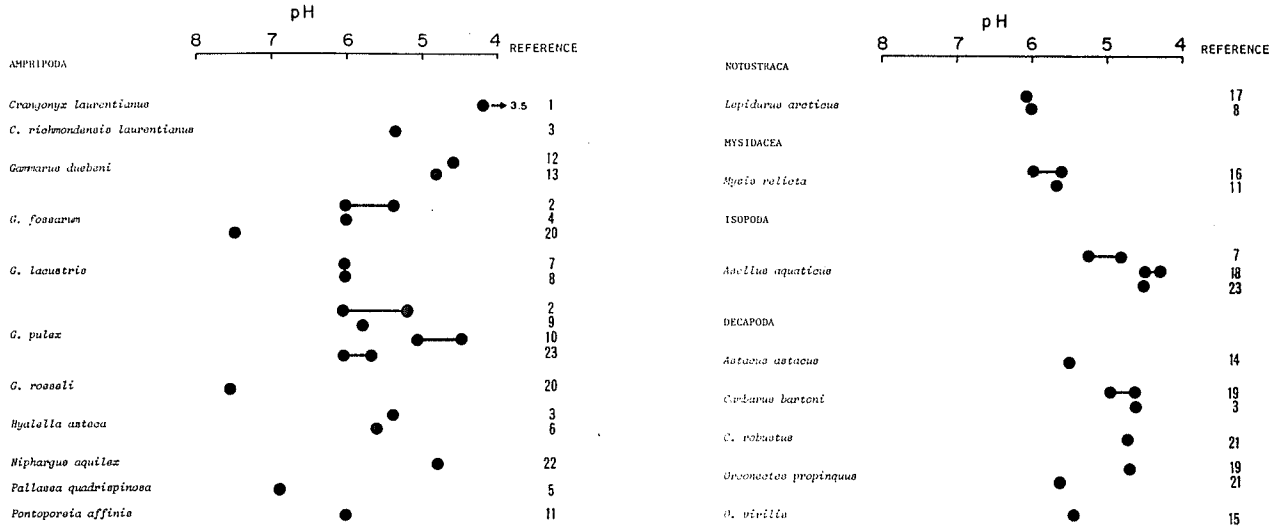
addition, water temperatures during spring runoff would be low (~4°C). Stephenson and Mackie (1986) determined that at 4°C the 10-d LC50 of H^+ to H. azteca was pH 4.5. Thus, amphipods probably could survive a short-term depression of pH during spring runoff.

Populations of other species of amphipods decline or disappear from lakes and streams with pH decreases over the range of 6.0 to 5.0 (Fig. 6). In Norway, G. lacustris is not found in lakes with pH <6.0 (Okland, K.A. 1980, Raddum and Fjellheim 1984). However, elevation above sea level, temperature and total hardness are factors important in mediating tolerance to low pH. For example, in warm lakewaters (>18°C), at low elevation (<440 m ASL), and total hardness <0.5°dH (which corresponds to ~2.7 mg Ca^{2+}/L and ~0.6mg Mg^{2+}/L), G. lacustris is rare at pH <6.5 (Okland and Okland 1985). In Sweden, Germany and the British Isles, G. fossarum, G. pulex and G. roeseli are rarely found at pH < 5.8 - 6.0 (Sutcliffe and Carrick 1973, Brehm and Meijering 1982, Matthias 1983, Otto and Svensson 1983, Schrimpff and Foeckler 1985). However, Pinkster et al. (in Sutcliffe and Carrick 1973) and Pinkster (in Sutcliffe and Carrick 1973) reported that G. pulex occurs at pH 4.5 - 5.0 in France.

Some species of amphipods are tolerant of and apparently favor acidic conditions. Gammarus duebeni is found in lakes with pH's as low as 4.6 (Hynes 1954, Sutcliffe and Carrick 1973). Sutcliffe (1967, 1983) suggested that when the concentration of Na^+ is sufficiently high, pH does not limit distribution of G. duebeni. Abundance of Niphargus aquilex in some southern English streams increases as pH decreases, replacing G. pulex in the most acid sites (pH $\geq$ 4.8) (Townsend et al. 1983). In North America, an analogous situation may

Figure 6. Lower limits of pH for natural distributions of benthic crustaceans. Single dots indicate the lowest pH measured in habitats from which the species was regularly collected. Connected dots indicate the lower limits of pH between which the specie was occasionally collected.

LOWER LIMITS OF pH FOR THE NATURAL RANGES OF BENTHIC CRUSTACEANS



1. Bousfield (pers. comm.)
2. Brehm and Meijering 1982
3. Dermott 1985
4. Matthias 1983
5. Kenttämies et al. 1985
6. Stephenson and Mackie 1986
7. Økland 1980
8. Raddum and Fjellheim 1984
9. Sutcliffe and Carrick 1973
10. Pinkster et al., and Pinkster 1972 in Sutcliffe and Carrick 1973
11. Dadswell 1974
12. Sutcliffe 1967
13. Hynes 1954
14. Lund 1969 and Svärdson 1974 in Økland and Økland 1986
15. Schindler and Turner 1982
16. Nero and Schindler 1983, Schindler et al. 1985
17. Borgström et al. in Økland and Økland 1986
18. Mossberg and Nyberg 1979
19. Collins et al. 1981
20. Schrimpff and Foeckler 1985
21. Berrill et al. 1985
22. Townsend et al. 1983
23. Otto and Svensson 1983

exist with Crangonyx laurentianus. This acid tolerant species has been collected from waters with pH as low as 3.5 (Bousfield, pers. comm.). In this study, Crangonyx sp. was present in 5 out of 7 lakes in which H. azteca was absent (including the most acidic lakes), 5 out of 5 lakes in which H. azteca was low in relative abundance, and 5 out of 18 lakes in which H. azteca was high in relative abundance. Although Crangonyx specimens were not always identified to species, C. laurentianus is the only other species of amphipod collected from the littoral zones of lakes in ELA.

The amphipods Pontoporeia affinis and Pallasea quadrispinosa are stenothermic, glacial relicts distributed in North America and Europe, respectively (Hutchinson 1967, Dadswell 1974). Both are intolerant of warm water, low oxygen concentrations and low pH. Pontoporeia affinis was absent from lakes with pH <6.0 (Dadswell 1974), and P. quadrispinosa though present in only 3 out 55 lakes surveyed, was absent from lakes with pH <6.9 (Kenttamies et al. 1985).

It may be possible to explain differences in tolerance to low pH based on osmoregulatory ability. As pH declines, maintenance of internal ionic concentrations becomes increasingly difficult (see PART I). Thus, animals better able to osmoregulate should be more tolerant of low pH. Nimmo (1968) determined that H. azteca was better able to retain certain ions than G. lacustris. Hyalella azteca also appears to be more tolerant of acidic conditions (Fig.6). Bousfield (pers. comm.) suggests that tolerance may be a function of phyletic antiquity. Groups such as the Crangonyx section of the Gammaridae, whose distant ancestral types migrated from marine to freshwaters, would have had the longest time to evolve the osmoregulatory ability

to cope with dilute and ionically variable conditions. On the other hand, groups such as Pontoporeia, whose immediate ancestral types are estuarine and marine, are expected to be stenohaline and less tolerant of low pH.

Species of other groups of benthic crustaceans also tend to be rare or absent at pH <5.5 - 5.0, with some exceptions. The notostracan L. arcticus, the mysid M. relicta and decapods A. astacus and O. propinquus are not present in waters with pH <5.5 - 6.0 (Dadswell 1974, Nero and Schindler 1983, Raddum and Fjellheim 1984, Berrill et al. 1985, Borgstrom et al. in Okland and Okland 1986, Lund in Okland and Okland 1986, Svardson in Okland and Okland 1986). Collins et al. (1981) reported finding a single O. propinquus in a lake with pH 4.7, but the population was probably in the process of disappearing. In contrast, the isopod Asellus aquaticus and decapods Cambarus bartoni and C. robustus can tolerate pH <5.0 (Mossberg and Nyberg 1979, Okland, K.A. 1980, Collins et al. 1981, Otto and Svensson 1983, Berrill et al. 1985).

Stephenson and Mackie (1986) and Mills and Schindler (1986) have suggested that H. azteca would be useful in monitoring the early effects of cultural acidification of surface waters. The results of this study support its use as an indicator of lake acidification because pH appears to be the main factor that determines presence of H. azteca in North American lakes. In addition to being responsive to a decrease in pH, H. azteca is easily collected from lakes and readily identified. It is the most common freshwater amphipod in North America (Bousfield 1958) and so widespread in distribution that its absence from a lake can be considered indicative of conditions that

prevent long-term survival. Its tolerance of a wide range of natural conditions allows the conclusion that conditions prohibiting survival are probably anthropogenic in origin.

PART III. EFFECTS OF EXPERIMENTAL ACIDIFICATION ON *HYALELLA AZTECA*

INTRODUCTION

Hyalella azteca is one of the most widely distributed amphipods in North America (see Part I). The only conditions that restrict this species from temperate lakes are low concentrations of calcium, and more importantly, low pH (Stephenson and Mackie 1986; this study, Part II). Although such information on distribution suggests that lake acidification is likely to affect populations of *H. azteca*, it does not give direct causal evidence for effects due to low pH. Natural populations are exposed to all physical, chemical and biological factors of their environment, which complicates the analysis of responses to a single factor. Therefore, experiments were conducted with two main purposes: (a) to show that pH directly affects the ability of *H. azteca* to survive, and (b) to determine the lowest pH that allows long-term survival.

Specific objectives, methods and results are described for five experiments on the effects of environmental acidification on *H. azteca*. In these experiments, amphipods held either in groups or individually in small enclosures, were placed into acidified lakes, acidified mesocosms situated in a neutral-pH lake, or acidified vessels in a laboratory. Survivorship was measured under various concentrations of H^+, in some cases within different size classes or

within populations originating from waters of different chemical backgrounds. Table 3 summarizes the conditions under which the five experiments were conducted.

METHODS

Transplantion Experiment

Preliminary sampling of 15 lakes in the Experimental Lakes Area (ELA) during late spring 1984 showed that Hyalella azteca was present in all lakes except L223 and L302S. Both of these lakes received additions of sulfuric acid to decrease pH, and their mean epilimnetic pH's during the ice-free period in 1984 were 5.40 (L223) and 5.60 (L302S). The other 13 lakes were unacidified and had pH >6.0 (M. Turner, Freshwater Institute, pers. comm.). Hyalella azteca was present in L302S in 1983, when the mean open water pH of the epilimnion was 5.90.

The disappearance of H. azteca from L302S between 1983 and 1984, and its presence in non-acidified lakes of ELA (Part II), suggests that populations may be affected by decreased pH. In order to determine whether absence from the acidified lakes was due to low pH, groups of amphipods collected from unacidified L239 were placed in small enclosures and tranferred into L223 and L302S; into L302N, a lake acidified with nitric acid and having a mean epilimnetic pH of 6.32; L382, a lake with neutral pH; and back into L239, to assess the

Table 3. Summary of conditions under which five experiments were conducted on the effects of acidification on Hyalella azteca.

Experiment	Mean pH of Treatments *	Medium	Amphipod Enclosure
transplantation	5.45,5.66,6.32, 6.92,7.07	lake	345-ml acrylic tube
bag acidification	5.62,5.80, 6.12,7.18	500-L bag of L239 water	22-ml vial
acute toxicity A	4.50,4.78,5.18, 5.67,7.38	4-L jar of L239 water	22-ml vial
acute toxicity B	5.15,5.65,7.36	4-L jar of L239 water	22-ml vial
chronic toxicity	5.51,5.70, 5.99,7.44	4-L jar of L239 water	22-ml vial

Experiment	Source of Amphipods	N	Size Classes	Acid	Temperature (^{o}C)	Duration (d)
transplantation	L239	675	no selection	H_2SO_4, HNO_3	10-27	36
bag acidification	L239	240	adult, juvenile, newborn	HCl	14-20	26
acute toxicity A	LaSalle River	100	adult, juvenile	HCl	23	5
acute toxicity B	L239, LaSalle River	90	adult, juvenile	HCl	23	5
chronic toxicity	L239	426	newborn	HCl	23	30

* These pH's are the grand means of pH measurements taken during the experiment for all treatment replicates. Mean pH's of individual treatment replicates are given in the Results.

effects of containment in the enclosures. Each of the five lakes received nine groups of amphipods, three in each of three different sites along the shorelines (Fig. 7). Sites within a lake were selected to be as widely separated as possible, while also containing substrate in which H. azteca are found. Each experimental population consisted of 15 individuals of various sizes.

On 2-4 August 1984, approximately 1000 H. azteca were collected with a 200-um mesh sieve from the littoral zone of L239 and held in pails of lake water. From this sample, 675 individuals were transferred to 45 petri dishes of lake water, without regard to sex, so that each dish contained 15 amphipods with similar proportions of large, medium and small individuals. These groups were then randomly placed into 45 plastic, 345-ml enclosures (Fig. 8), which were previously assigned to the 45 sites used. Each enclosure contained ~2 g of dried allochthonous organic matter (leaves, twigs, bark), which provided cover and a substrate for feeding. Within 24 h of removal from pails, amphipods contained in enclosures were placed into their assigned sites in lakes. Enclosures were anchored on the sediment, under about 1 m of water. Between 17 and 19 August, each enclosure received approximately 0.25 ml of commercial fish food and alfalfa powder to supplement the food supply.

Eight or nine times over a period of 36 days, the contents of each enclosure were emptied into a white sorting pan and the living amphipods, including any offspring, were counted. Following this, detritus and amphipods were replaced in the enclosures and returned to the lake. Water temperature was measured at each site 10-20 cm below the water surface during each counting. Duplicate samples of water

Figure 7. Transplantation experiment: experimental design. Groups of Hyalella azteca collected from L239 were placed in small enclosures and held in L239 and 4 other lakes. pH shown for each lake is the mean of epilimnion samples taken from all three sites periodically during the experiment.

TRANSPLANTATION EXPERIMENT

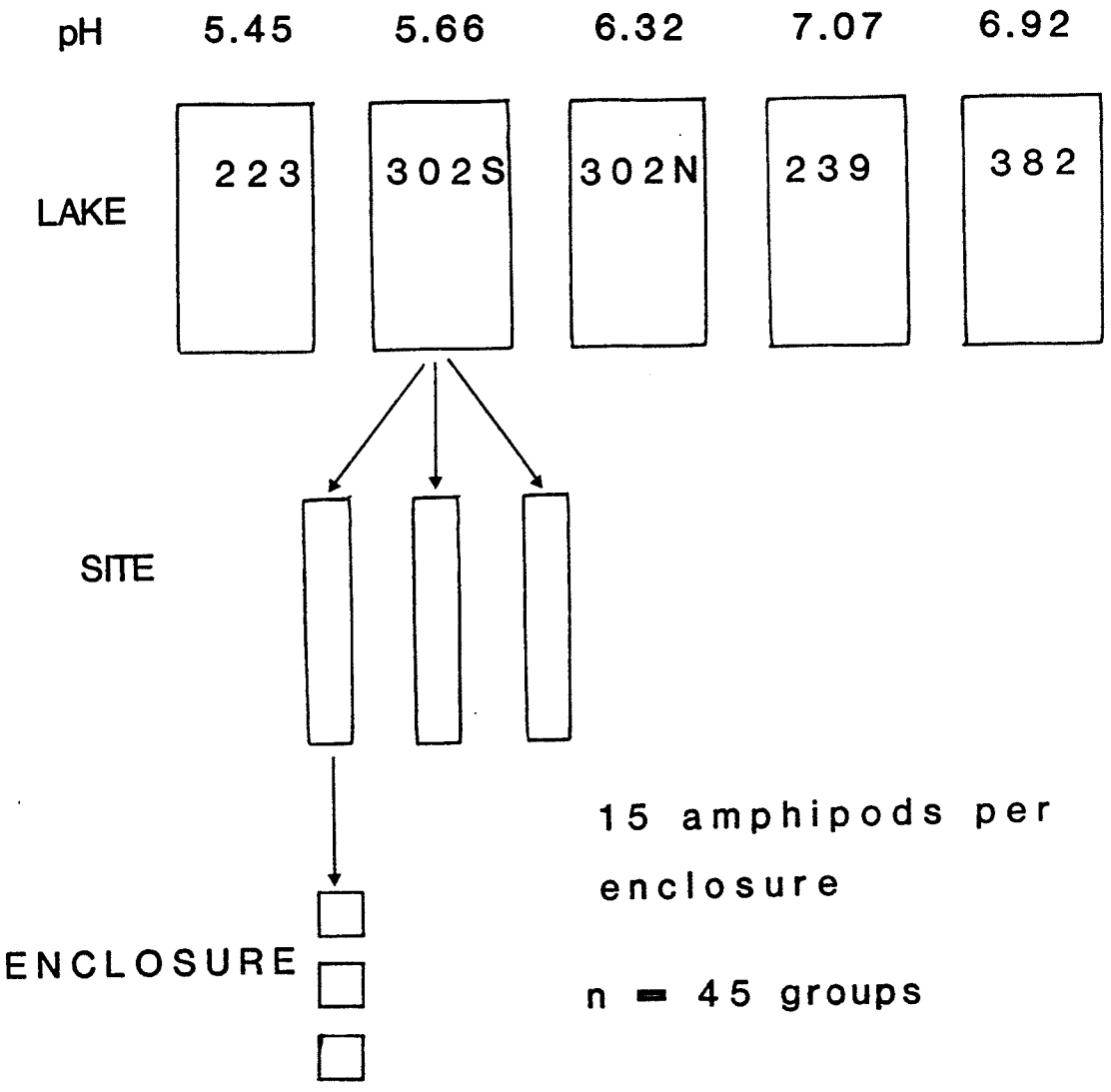
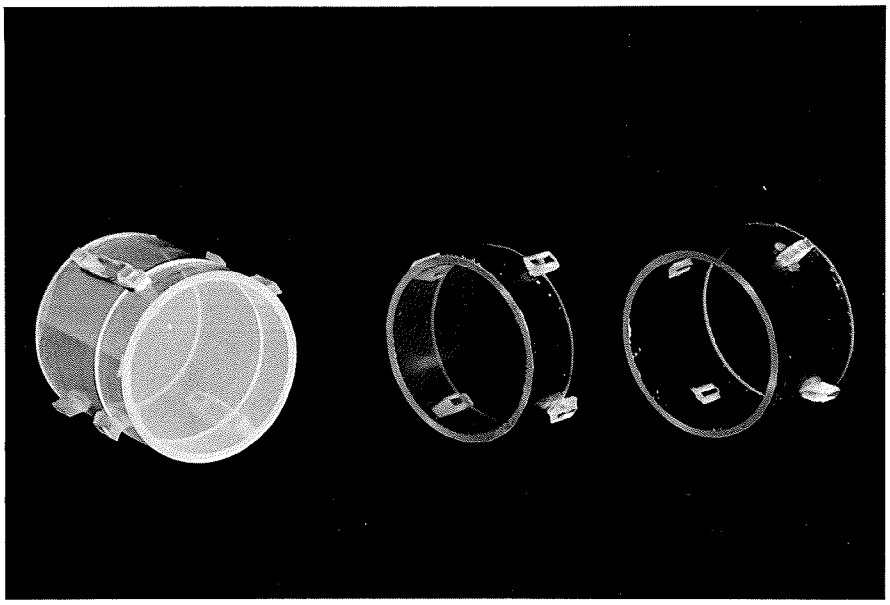


Figure 8. Enclosures for H. azteca used in the transplantation experiment. Fitted together, the two sections of clear, acrylic tube (7.6 cm diam., 0.32 cm wall) are 7.6 cm in length. Bonded to each end is 200-um mesh screen.



were collected from each site in 60-ml glass BOD bottles, three or four times during the experiment. Within 5 h after sampling, pH was measured with either a Radiometer pH Meter 29 and Fisher combination polymer body-gel filled electrode, or an Orion Research Model 801 pH meter with 910200 electrode.

Survivorship (i.e. the number or proportion of individuals surviving) after 19 days was compared among sites within lakes, and among lakes by ANOVA and multiple comparison of means using Fisher's protected least significant difference (LSD) test (Steel and Torrie 1980).

Bag Acidification Experiment

The purpose of the bag acidification experiment was to examine survivorship in lake water of pH 5.6 - 6.3, a range which is hypothesized to include the lower tolerance limit of H. azteca. Amphipods were placed singly into modified vials and held in eight bags or "limnocorrals" of acidified lake water situated in L239 (Fig. 9). The mean pH's of the acidified treatments were 6.12, 5.80 and 5.62 (= 0.76, 1.58 and 2.40 umol H^+/L), whereas the mean pH of the control treatment was 7.18 (= 0.66 umol H^+/L). Treatments were replicated twice (Fig. 10). Each group of amphipods placed into treatments consisted of three distinct size classes in order to assess the effect of size on the ability of an individual to survive acidic conditions. Smaller and younger amphipods probably experience greater stress than larger amphipods under low pH for several reasons. Younger crustaceans grow and molt at a faster rate than older ones

Figure 9. Limnocorrals used to contain lake water in the bag acidification experiment, situated in L239, Experimental Lakes Area.

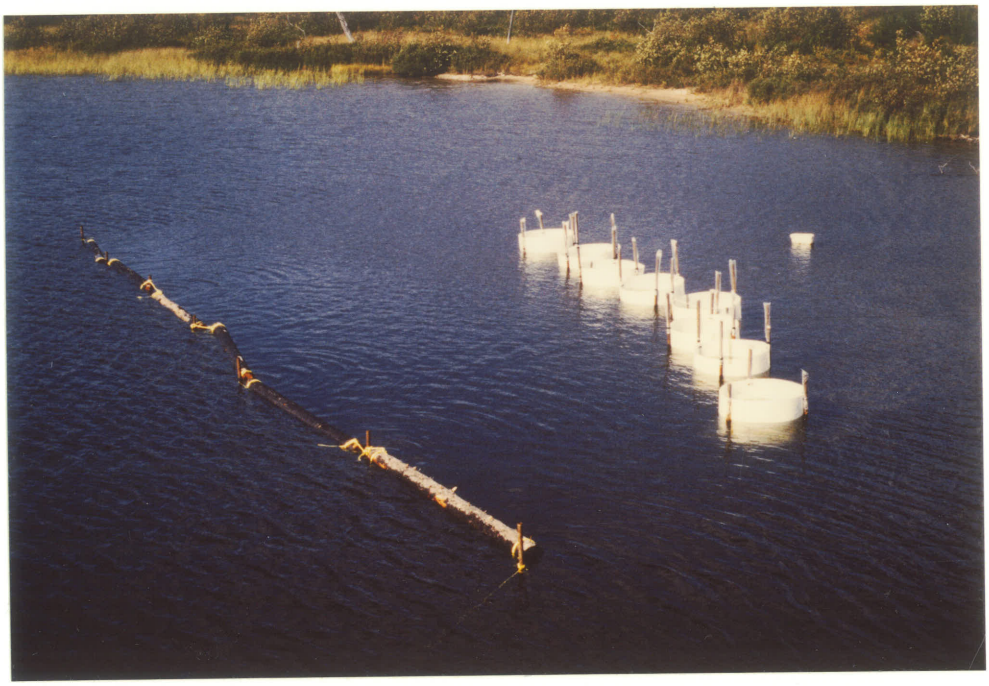
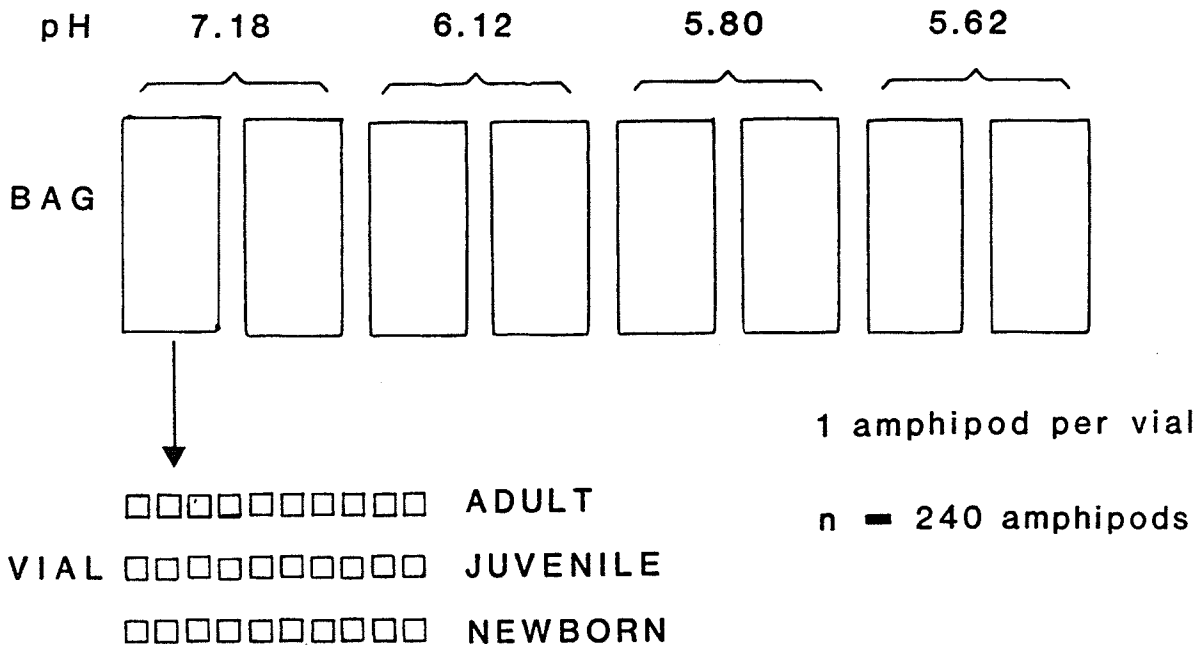


Figure 10. Bag acidification experiment: experimental design. H. azteca of 3 size classes were placed into modified vials and held in bags of acidified lake water. pH shown for each treatment is the mean of all measurements from both replicate bags made during the experiment.

BAG ACIDIFICATION



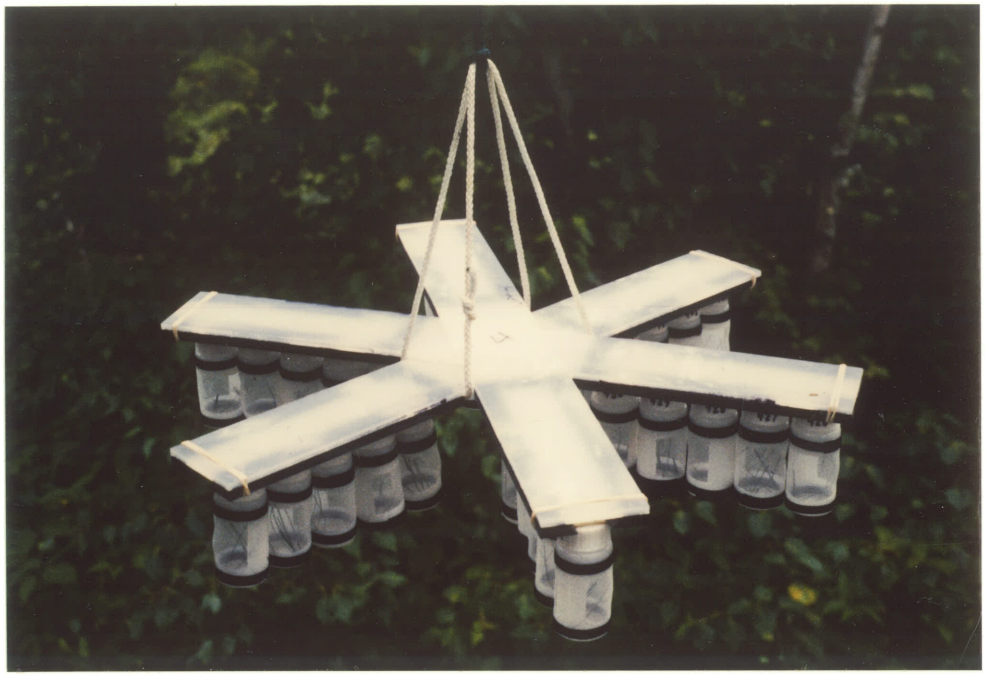
(Buikema and Benfield 1979). They also have greater body surface area to volume ratios. Because high concentrations of H^+ can interfere with the uptake of cations such as Na^+ and Ca^{2+} (Sutcliffe 1983) and crustaceans require Ca^{2+} to harden their cuticles after each molt (Stevenson 1985), faster molting and smaller individuals should find it more difficult to maintain internal stores of Ca^{2+} and consequently be more sensitive to acidic conditions.

The limnocorrals of lake water were 1-m diameter transparent polyethylene bags placed inside and supported by rigid, polyethylene tubes (1 m diam., 1 m high). The tubes were bolted to metal posts anchored into the sediments near the east inflow of L239 (see fig. 9 in Brunskill and Schindler 1971) at a depth of ~0.75 m (Fig. 9). Bags were filled with lake water on 16 August, resulting in volumes ranging from 525-550 L. The pH of six of the eight bags was adjusted with dilute solutions of hydrochloric acid and sodium hydroxide to reach intended H^+ concentrations of 1.00, 2.00 and 3.00 umol/L. After additions of acid and base solutions, water in the bags was stirred. pH of water from the bags was measured in 60-ml glass BOD bottles at room temperature with a Radiometer PHM pH meter, and either an Orion Research 910200 electrode or a Radiometer GK2401C electrode.

The enclosures were 22-ml polyethylene vials with 17-cm^2, 200-um mesh windows. Each vial contained a few plastic fibres from a fish spawning mat to serve as a substrate, and several flakes of commercial fish food, which were replaced as consumed. The 30 vials for each treatment fitted into an acrylic holder (Fig. 11), which was suspended ~20 cm below the surface in the limnocorral.

On 22 August 1985, sufficient numbers of amphipods to supply the

Figure 11. Modified vials in acrylic holders used to individually contain H. azteca in the bag acidification experiment.



experiment were collected from L239. Five adult (total body length ~ > 5 mm) and ten juvenile (total body length ~ 3 mm) individuals were placed randomly and separately into 15 enclosures per limnocorral. In addition to the above 15 amphipods, five gravid females were put individually into vials and randomly placed in each bag on 24 August. Offspring of these amphipods were put into vials in the same bag as their mothers as they were released from brood pouches, until each bag contained 10 "newborn" (total body length < 1 mm) amphipods, for a total of 30 amphipods of three size classes.

Approximately every 3 days, each amphipod was examined and its time of death recorded. Water temperature was measured periodically, and pH every 1 - 2 days. The experiment was terminated after 26 days.

Survivorship (and 95% confidence intervals) of all size classes within bags was estimated by the product-limit (P-L) method described by Lee (1980). This method requires no assumptions on the statistical distribution of survival times, is suitable for small sample sizes, allows unequal times of placement of individuals into treatments, and can accommodate censored data (i.e. observations of individuals not followed until death). The estimates of survivorship for day 26 were compared by ANOVA, and regressed against mean concentration of H^+ in bags.

P-L estimates of survivorship functions also were made for each size class within bags (= treatment replicates). Tests for differences in survivorship between size classes with treatments were made using a K-sample generalized Kruskal-Wallis H-test (Lee 1980). This non-parametric test is similar to a group of Wilcoxon-type tests, which are appropriate for samples with censored survival data (Lee

1980, Pyke and Thompson 1986), but the Kruskal-Wallis H-test allows use of more than two samples.

Acute Toxicity Test A

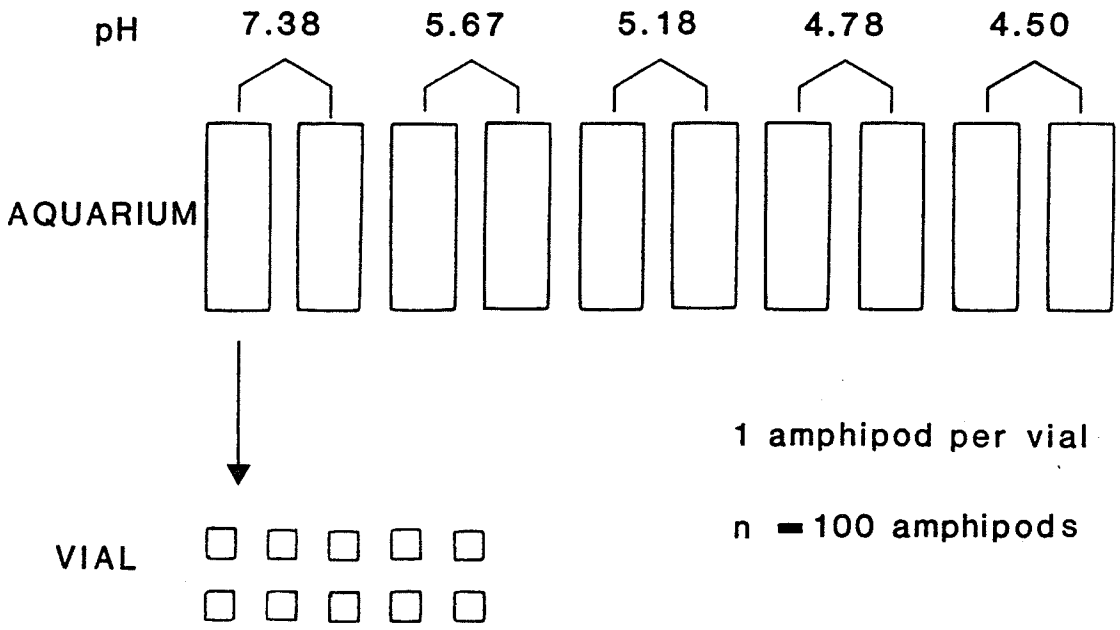
Responses of organisms to acutely lethal concentrations of substances are often measured in toxicological studies. A commonly estimated parameter is the concentration of a substance that is lethal to 50% of the test population (LC50) after a specific exposure time (e.g. 96 h). Compared to long-term experiments with sublethal or chronically lethal concentrations, acute toxicity tests are usually easier to carry out and replicate, and provide faster results.

Although acute toxicity tests may not provide appropriate estimates of long-term (natural) tolerance limits to a contaminant, they still produce quantitative measurements of effects. Sloof et al. (1986) found that, within the same species, acute toxicity (estimated as LC50 or median effective concentration [EC50]) was related to chronic toxicity (estimated as No Observable Effects Concentration) for 164 chemicals (r=0.89). Acute responses to the same contaminant among different organisms, or responses to different contaminants by the same species or genetic strain can be compared.

Survivorship was examined for 5 days in groups of laboratory cultured H. azteca from the LaSalle River, Manitoba ($49^{o}45'N$, $97^{o}10'W$) held individually in 22-ml enclosures and placed into 4-L glass aquaria of lake water acidified to H^{+} concentrations of 2.14, 6.61, 16.6 and 31.6 umol/L (= pH 5.67, 5.18, 4.78 and 4.50). The unacidified control treatment had pH = 7.38 (Fig. 12). These treatments were

Figure 12. Acute toxicity test A: experimental design. *H. azteca* were placed into modified vials and held in aquaria of acidified lake water. pH shown for each treatment is the mean of all measurements from both replicate aquaria made during the experiment.

ACUTE TOXICITY TEST A



replicated twice.

The experimental amphipods came from a LaSalle River culture established three months previously, and maintained at the Freshwater Institute according to de March (1981). Water for the culture came from L239. Water lost due to evaporation was replaced with distilled, de-ionized laboratory water.

Amphipods were individually enclosed in the same modified polyethylene vials as described for the bag acidification experiment. Ten vials were submerged per 4-L aquarium (Fig. 13). Experimental water was collected from L239, less than 2 months before the experiment and filtered through a 70-um mesh net. Chemical characteristics of L239 are given in Table 4.

pH was adjusted with weak solutions of hydrochloric acid and sodium hydroxide to reach intended H^+ concentrations of 2.50, 7.50, 17.5 and 32.5 umol/L. After additions of acid or base, water in the aquaria was stirred. Measurements of pH were made two to four times daily in 60-ml glass BOD bottles of water siphoned from each aquarium. Within 1.5 h of collection, pH was measured with a Radiometer pH meter model 62 and Radiometer GK2401C combined electrode. Earlier measurements on replicate samples showed a precision of $\pm$ 0.02 pH units, and measurements of standard pH 6.00 and 5.00 HCl and de-ionized water solutions showed accuracy was $\pm$ 0.02 pH units. After pH measurement, water in BOD bottles was returned to its original aquarium.

Each aquarium was aerated with filtered air and a plastic bubbler. Water temperature was kept at 23°C and daylength was 16 h with fluorescent light producing irradiance of 100-200 uE/(m^2s), under which normal growth and reproduction should occur (de March 1981).

Figure 13. Modified vials in 4-L aquaria used to hold *H. azteca* in laboratory toxicity experiments.

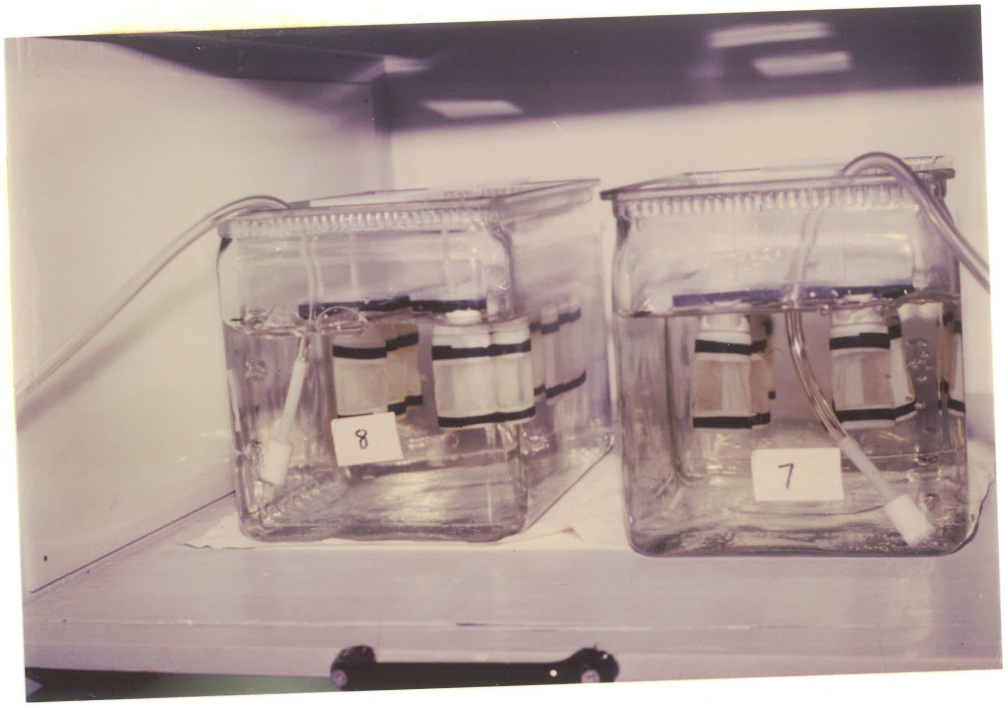

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Table 4. Chemical characteristics of Lake 239 (G.Linsey and M.Turner, unpubl. data). Concentrations are means at 1 m depth for 1983 - 1985.

	ueq/L	mg/L				
pH	Alkalinity	Ca	Mg	Na	K	SO_4
6.82	151	3.17	0.96	1.51	0.84	5.94

mg/L	ug/L				
Cl	NO_3-N	NH_3-N	TDN	susp-N	total N
0.61	18	22	266	54.6	320

ug/L					
TDP	susp-P	total P	Chl a	total Fe	total Mn
3.7	4.3	8.1	1.4	65.9	8.7

Amphipods collected from the culture were randomly placed into treatments on 13 May 1986. They were examined after 3, 9, 18, and then every 12 h thereafter for 5 days, and recorded as live or dead (no movement when disturbed). After death or after five days, amphipods were preserved in 70% ethanol. All specimens were aged by counting the number of antennal segments and measuring head lengths (Cooper 1965, Strong 1972). The number of segments on one of each of the first and second pairs of antennae were counted, and head length was measured as the straight line distance from the rostrum to the dorsal-posterior margin of the head.

Survivorship was analyzed by several methods. ANOVA was done for survivorship at 96 h, and mean survivorship in treatments was compared by Fisher's protected LSD procedure. Linear regressions were calculated for 96-h survivorship against mean concentration of H^+ and mean pH of aquaria. Ninety-six hour LC50 was estimated by the following method. Survivorship data were transformed into logits, where logit P = ln(P/[1-P]), and P = proportion of individuals dead at time of observation (Ashton 1972), and regressed against time to obtain mortality rates in each aquarium (de March 1979). From these regressions, the expected time at which 50% of the amphipods would die was estimated for each aquarium, logarithm transformed and regressed against aquarium pH. The 96-h LC50 was the pH when time equals four days.

The effect of size or age on survival time should be more easily detected among 80 individuals than among 10. Therefore, individual survival times of amphipods within each aquarium were divided by the mean survival time of the ten amphipods in the aquarium. The

resultant "relative survival times" of all aquaria, except the unacidified ones, were pooled and regressed against head length, and against number of antennal segments.

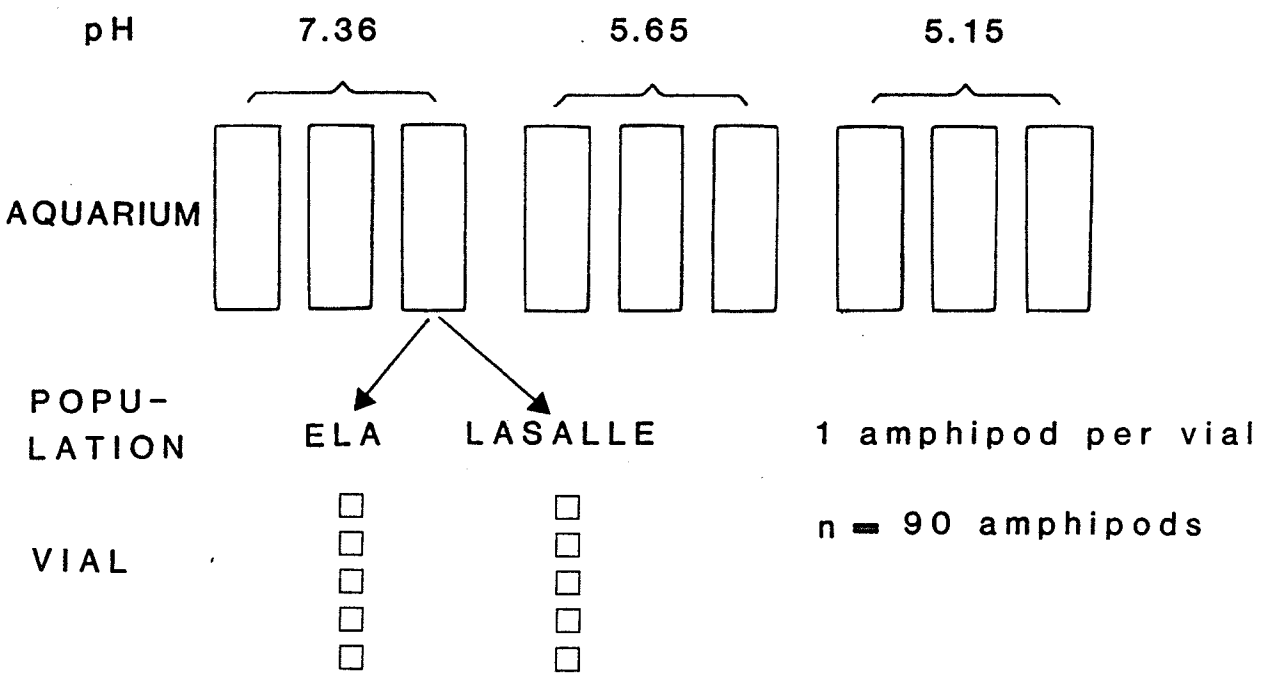
Acute Toxicity Test B

A second toxicity test involving acutely lethal concentrations of H^+ was conducted in order to compare the responses to acidified lake water (L239) of H. azteca from two different populations - a laboratory culture established from the LaSalle River, Manitoba, and amphipods from L239, ELA. Any difference in responses between these two populations would have to be considered when comparing results from studies involving LaSalle River H. azteca (acute toxicity test A), and ELA H. azteca (natural distribution, other experimental data). Lake water in ELA is roughly one to two orders of magnitude more dilute than the LaSalle River. Mean concentration of calcium in the LaSalle River is 60 mg/L (D. Williamson, Department of Environment and Workplace Health and Safety, Manitoba, pers. comm.) compared to 2 mg/L for ELA waters (Beamish et al. 1976). Amphipods that are adapted to low concentrations of major ions should be better able to regulate internal concentrations, and consequently be more tolerant of the osmotic stress of acidic conditions, than amphipods that are adapted to harder waters.

Survivorship was examined for 5 days in groups of amphipods held at three pH levels - 7.36 (control), 5.65 (2.24 umol H^+/L) and 5.15 (7.08 umol H^+/L). Treatments had three replicates (Fig. 14). Amphipods were placed in small plastic vials, and 10 vials were placed in each

Figure 14. Acute toxicity test B: experimental design. H. azteca from the LaSalle River, Manitoba and L239, ELA were placed into modified vials and held in aquaria of acidified lake water. pH shown for each treatment is the mean of all measurements from the three replicate aquaria made during the experiment.

ACUTE TOXICITY TEST B



aquarium. Half the amphipods were from the LaSalle River and the other half were from ELA.

Amphipods originating from L239 came from two sources. Twenty-nine were collected one day before the start of the experiment, and 16 were laboratory-grown offspring of amphipods collected 7 wk (11 June 1986) prior to the experiment. All 45 LaSalle River H. azteca were from a culture established 6 mo previously and maintained according to de March (1981), except that culture water came from L239, and water lost by evaporation was replaced with distilled, de-ionized water.

On 31 July 1986, five amphipods from L239 and five from the LaSalle River culture were randomly placed into each aquarium containing 4-L of filtered (70 um) water collected 1 wk previously from L239 (Table 4). Amphipods were held individually in the same 22-ml polyethylene vials as described for the bag acidification experiment.

The pH of jars was adjusted and measured, and test conditions were maintained as described for acute toxicity test A. Targets for acidified treatments were 2.50 umol H^+/L (pH 5.60) and 7.50 umol H^+/L (pH 5.12).

Amphipods were examined twelve times over 5 days, after 3, 9, 18, and then every 12 h thereafter. Amphipods showing no movement when disturbed were recorded as dead and were preserved in 70% ethanol. After 5 days survivors were also preserved. Specimens were aged by measuring head length (see acute toxicity test A).

Survivorship of amphipods was tested for differences between populations and between treatments with a K-sample generalized Kruskal-Wallis H-test (Lee 1980). Rates of mortality and 96-h LC50's were estimated as described for acute toxicity test A. The effect of

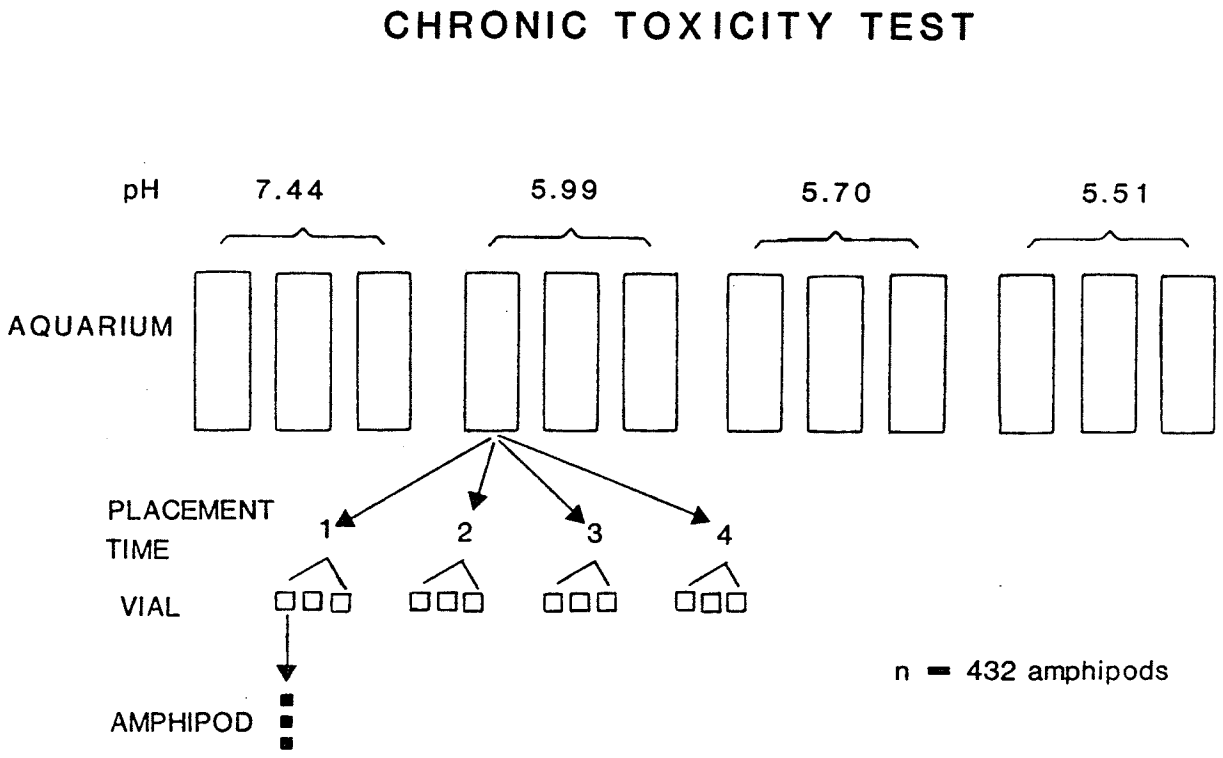
size on survival time in acidified treatments was also determined as for acute toxicity test A.

Chronic Toxicity Test

It is widely recognized that contaminants may be lethal under long-term exposure at concentrations substantially lower than those that are lethal under shorter-term exposure. Sensitivity also can vary among life stages of an organism (see Part II). Crustaceans are especially vulnerable immediately after hatching because of their relatively high rate of metabolism and growth, and small size (Buikema and Benfield 1979). The objective of this experiment was to examine the effects of moderately reduced pH (i.e. pH hypothesized to be sublethal in the short-term and lethal in the long-term) on survivorship of H. azteca exposed from the time of release from their brood pouch until death. The mean pH's treatments were 5.51, 5.70, 5.99 and 7.44, with three replicates of each treatment (Fig. 15). In order to collect and place into treatment all amphipods, four separate placement times were required.

Hyalella azteca for the chronic toxicity test were collected from L239 on 11 June 1986, transported to Winnipeg, and held in plastic pails with coarse detritus at 23°C. Gravid females were isolated in beakers and checked at least every 3 days for offspring released from brood pouches. Between 19 and 30 June, a total of 426 amphipods <3 days old were placed into 12 aquaria, three randomly assigned amphipods per enclosure (as described for the acute toxicity tests) and 12 enclosures per aquarium (Fig. 15), except for one of the

Figure 15. Chronic toxicity test: experimental design. Three *H. azteca* < 3 days old were placed in modified vials and held in aquaria of acidified lake water. Placements were carried out on 4 separate dates, ~3 days apart. pH shown for each treatment is the mean of all measurements from the three replicate aquaria made during the experiment.


CHRONIC TOXICITY TEST
pH
7.44
5.99
5.70
5.51
AQUARIUM
PLACEMENT TIME
1
2
3
4
VIAL
AMPHIPOD
n = 432 amphipods

control aquaria, which received 30 instead of 36 animals. Four placement times, 3 or 4 days apart, were required. At each placement time, three vials containing nine amphipods were put into each 4-L glass aquarium of filtered (70-um mesh) water collected from L239 <2 months before the experiment.

Treatments of 1, 2 and 3 umol H^+/L were established before addition of amphipods, and aquaria were maintained as described for the acute toxicity tests. Halfway through the experiment (2 wk after start), 1 L of water was removed from each aquarium and replaced with fresh lake water acidified to the target treatment pH. After the experiment, 125 ml of water was taken from each aquarium for measurement of calcium concentration (Stainton et al. 1977).

Amphipods were examined every 3 days until nearly all individuals placed into acidified treatments died. Time of death for each individual was recorded. Differences in survivorship among treatments within time replicates were analysed by K-sample generalized Kruskal-Wallis H-tests (Lee 1980).

RESULTS

Transplantation Experiment

Mean pH's of lake epilimnia during the experiment were 5.45 (L223), 5.66 (L302S), 6.32 (302N), 6.92 (L382) and 7.07 (L239). Ninety-five percent confidence intervals for the variation in pH among sites

within lakes, and of sites through time, were within 0.1 pH units of the lake mean in all lakes (Fig. 16). Water temperature was 10-27^{o}C, and was > 20^{o} during the first 20 days of the experiment. Differences between lakes were less than ~2^{o} on any given day.

Survivorship in enclosures placed in L302N, L382 and L239 was similar, and higher than survivorship in L302S and L223 (Fig. 17). Initial increases in the number of amphipods alive occurred in some enclosures due to reproduction. Survivorship changed little after 20 days. Analysis of variance of survivorship in enclosures on day 19 showed that variation among lakes was highly significant ($P<0.001$), but variation among sites within lakes was not significant (Table 5). There were no significant differences in the number of survivors per enclosure among L302N, L239 and L382, whereas mean survivorship was significantly ($P<0.05$) lower in L302S. Furthermore, survivorship in L223 was significantly lower than in L302S. After 35 days, survivorship averaged between 10 - 12 individuals per enclosure in the lakes that were not acidified with sulfuric acid (L302N, L239, L382). However, in the lakes that were acidified with sulfuric acid, only an average of five amphipods per enclosure remained in L302S, and in L223 fewer than one amphipod per enclosure survived.

Differences in mean number of survivors among lakes are accounted for by differences in concentration of H^{+}. Figure 18 shows an inverse, linear relationship between mean number of survivors per enclosure on day 19 and mean concentration of H^{+} of lake water. (For L239, the numbers of survivors in enclosures on day 19 were not counted and so were interpolated from survivorship curves and used to calculate the mean for the lake.) Although there were differences in

Figure 16. Transplantation experiment: pH of sites where enclosures containing H. azteca were placed in lakes.

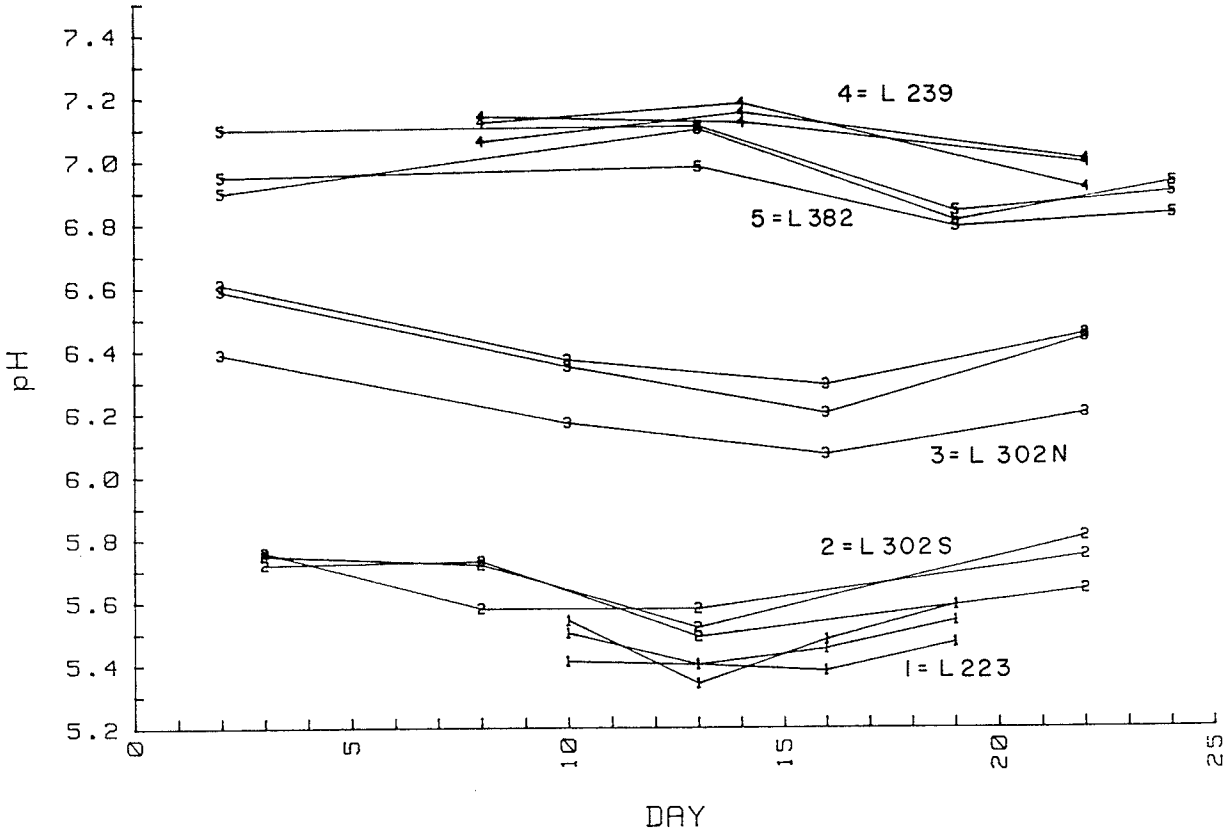

7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
pH
0
5
10
15
20
25
DAY
4= L 239
5=L 382
3= L 302N
2=L 302S
1= L 223

Figure 17. Transplantation experiment: mean number of surviving H. azteca per enclosure in lakes. 95% confidence intervals are shown only for the last two observations in each lake.

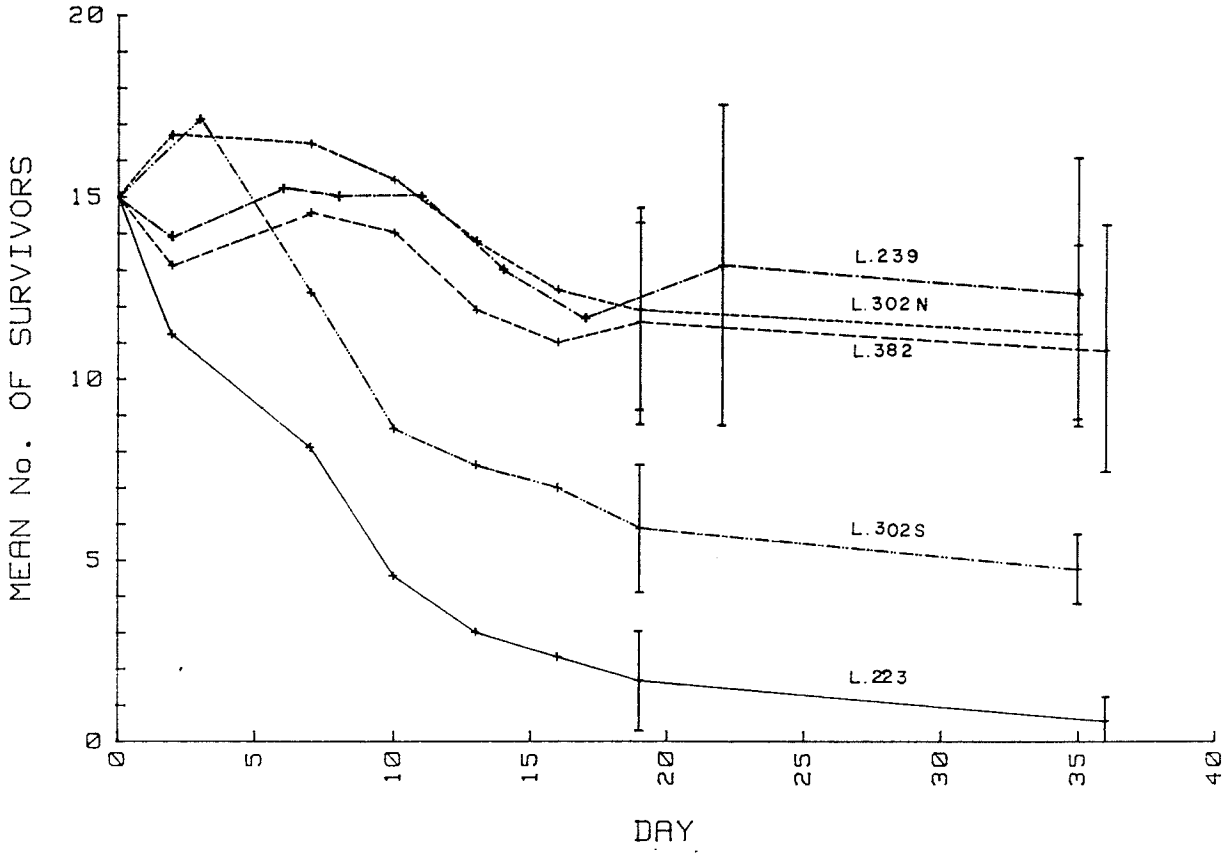

MEAN No. OF SURVIVORS
DAY
L.239
L.302N
L.382
L.302S
L.223
0
5
10
15
20
25
30
35
40

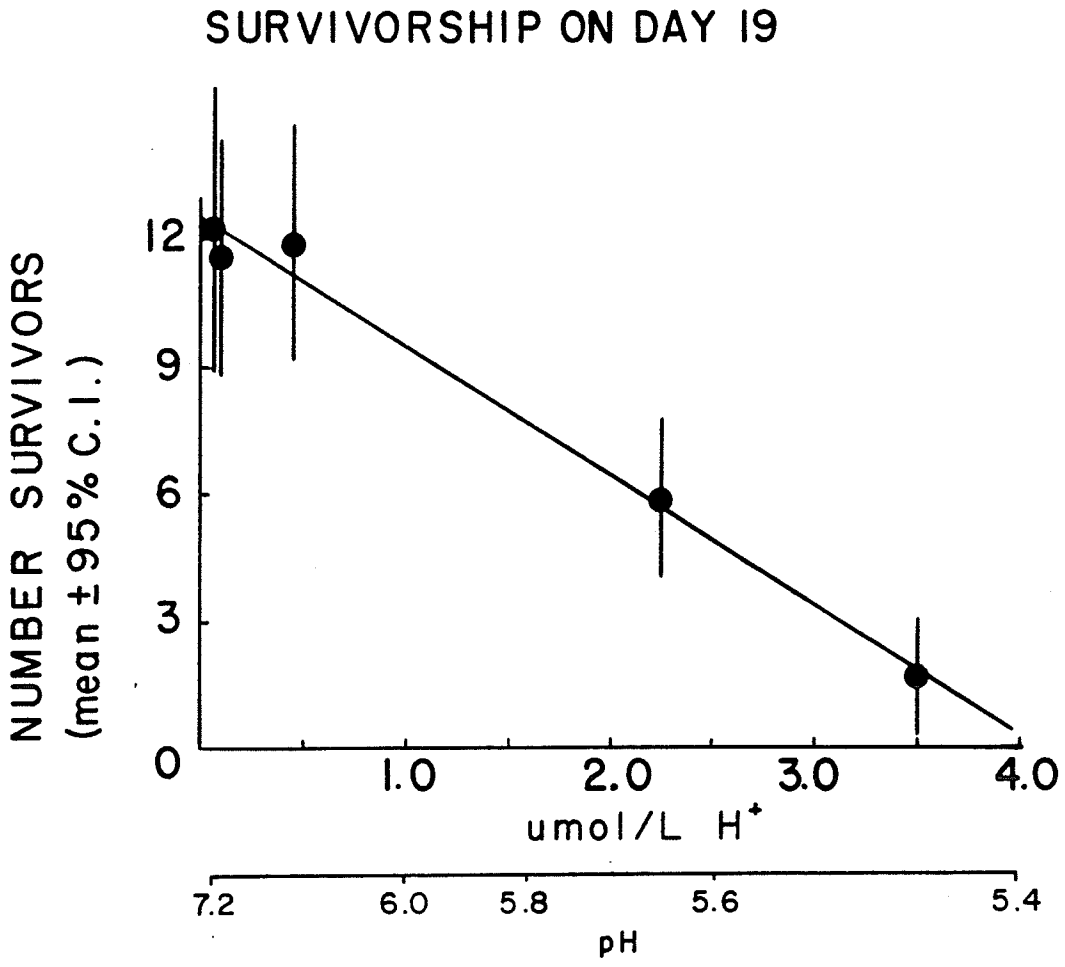
Table 5. Transplantation experiment: ANOVA of survivorship of H. azteca in lakes on DAY 19 and multiple comparison of means. Means underscored with the same line are not significantly different at alpha = 0.05.

Source of Variation	df	SS	MS	F	P
Lakes	4	794.88	198.72	26.71	< 0.001
Sites within lake (experimental error)	10	74.39	7.44	0.64	not significant
Enclosures (sampling error)	30	348.86	11.63		
Total	44	1218.13			

Comparison of Means (least significant difference, alpha = 0.05)

Lake	223	302S	382	302N	239
Mean Survivorship (day 19)	1.67	5.88	11.56	11.89	12.24

Figure 18. Transplantation experiment: relationship between mean number of survivors of H. azteca per enclosure in lakes on DAY 19 and mean concentration of hydrogen ion.


SURVIVORSHIP ON DAY 19
NUMBER SURVIVORS
(mean ± 95% C.I.)
12
9
6
3
0
1.0
2.0
3.0
4.0
umol/L H+
7.2
6.0
5.8
5.6
5.4
pH

concentrations of major ions among lakes in addition to H^+ (D.W.Schindler, unpubl. data), none was related to differences in the mean number of survivors.

Bag Acidification

Acidification of lake water in bags produced mean time weighted pH's (followed by bag number in parentheses) of 6.06 (2), 6.17 (7), 5.76 (5), 5.84 (1), 5.61 (3) and 5.62 (4), with 95% confidence intervals for the variation about these means equal to ± 0.06 pH units (Fig. 19). The pH of the control treatment replicates gradually increased from an initial pH of 7.00 to ~7.25 during the experiment, resulting in mean pH of 7.17 (bag 6) and 7.19 (bag 8). Water temperature in the bags ranged from 14 to 20°C.

Estimated survivorship functions of all amphipods in bags (i.e. size classes pooled) showed little difference among treatments until after 15 days (Fig. 20). At day 26, a marked decrease in survivorship is apparent as pH of treatment decreases. ANOVA of estimated survivorship at 26 days indicated no difference among treatments, but a regression of survivorship against mean concentration of H^+ was significant ($P<0.05$) (Fig. 21). Survivorship functions of treatment replicates agree reasonably well for all pH levels except pH 6.06 and 6.17 (bags 2,7)(Fig. 20,21). Survivorship in bag 2 was much lower than that in the bag 7. This condition was due to high mortality in newborn amphipods immediately after placement into treatment (see Fig. 22, pH 6.12 and Discussion), and was probably caused by handling. Therefore, data from Bag 2 can be considered biased. If survivorship

Figure 19: Bag acidification experiment: pH of bags. Intended treatments were: unacidified = bags 6,8; pH 6.00 = bags 2,7; pH 5.70 = bags 1,5; pH 5.52 = bags 3,4.

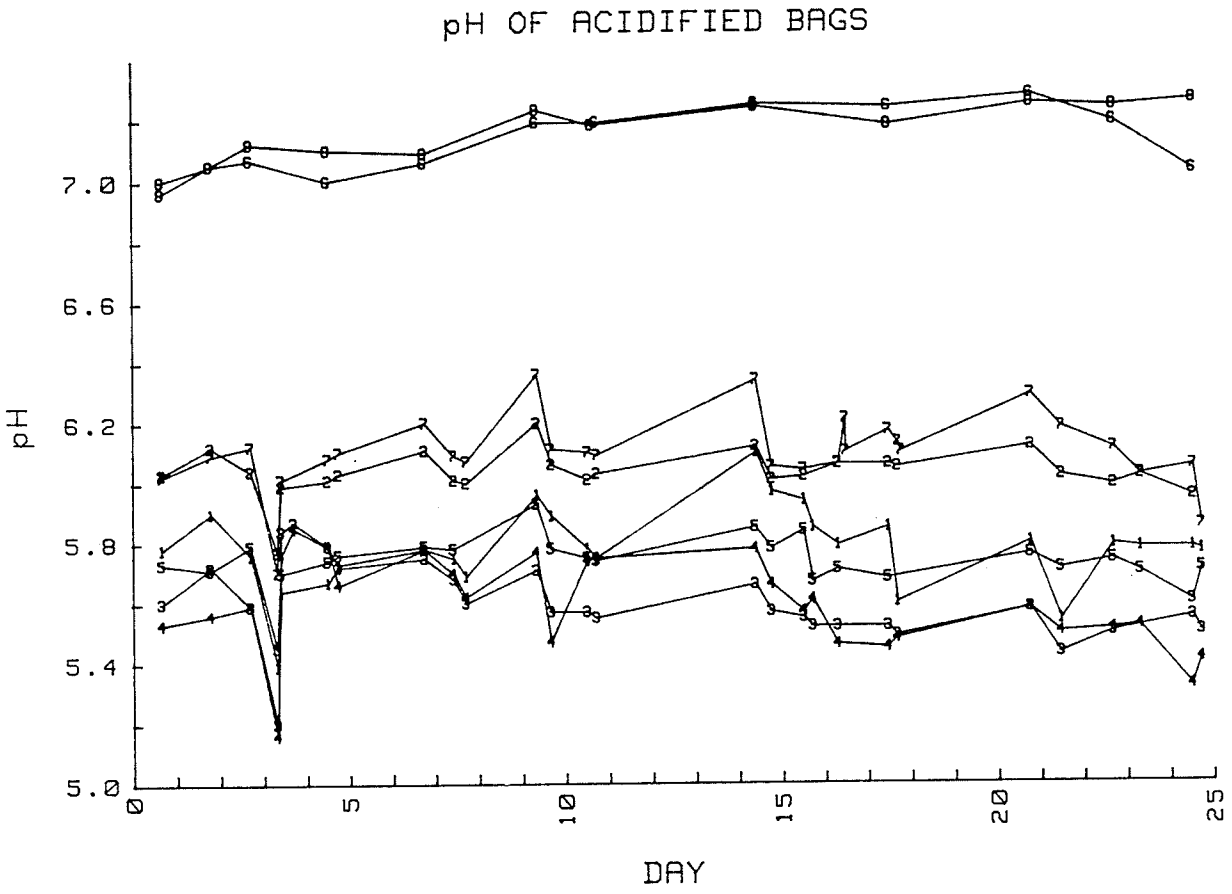

pH OF ACIDIFIED BAGS
pH
7.0
6.6
6.2
5.8
5.4
5.0
0
5
10
15
20
25
DAY

Figure 20. Bag acidification experiment: estimated survivorship functions for H. azteca at various pH's.
$\hat{S}(t)$ = (number of individuals in the bag that survived longer than time t)/(total number of individuals in the bag).

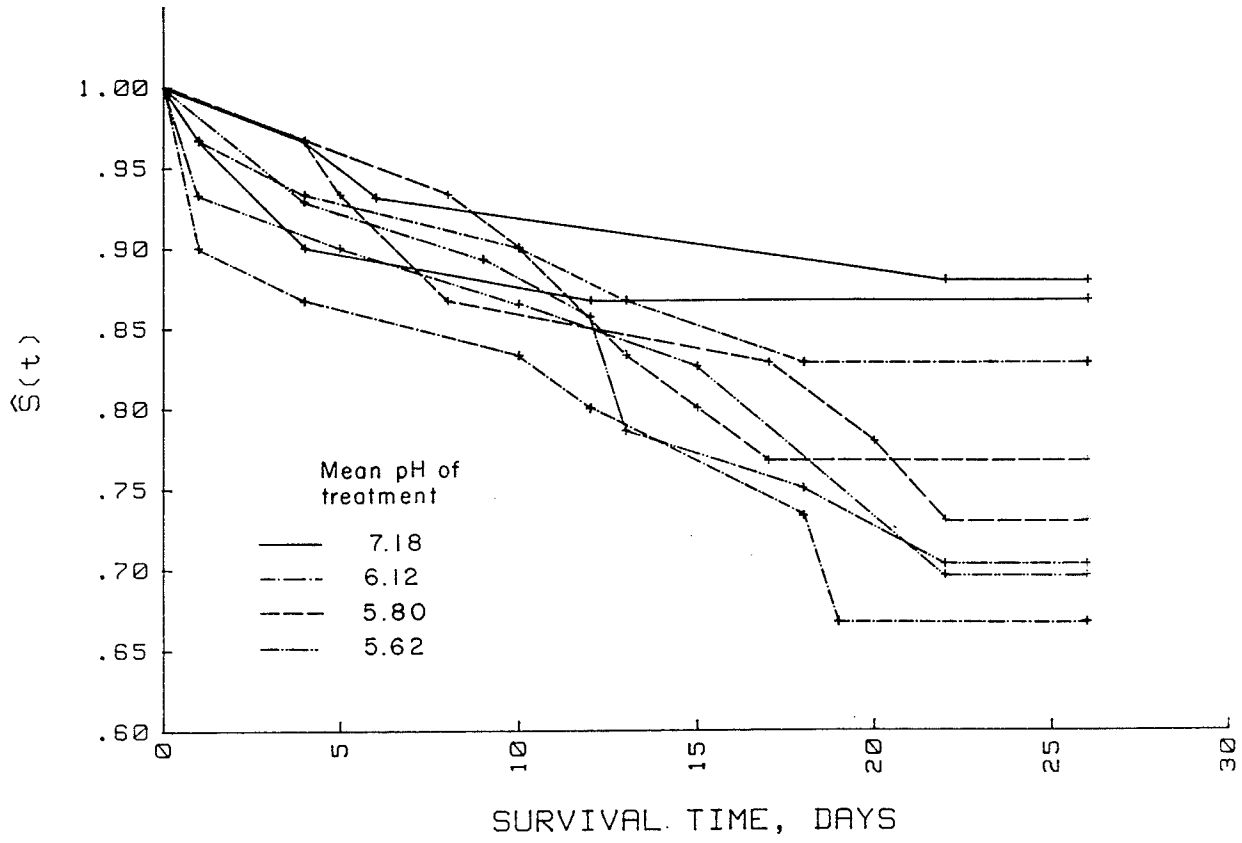
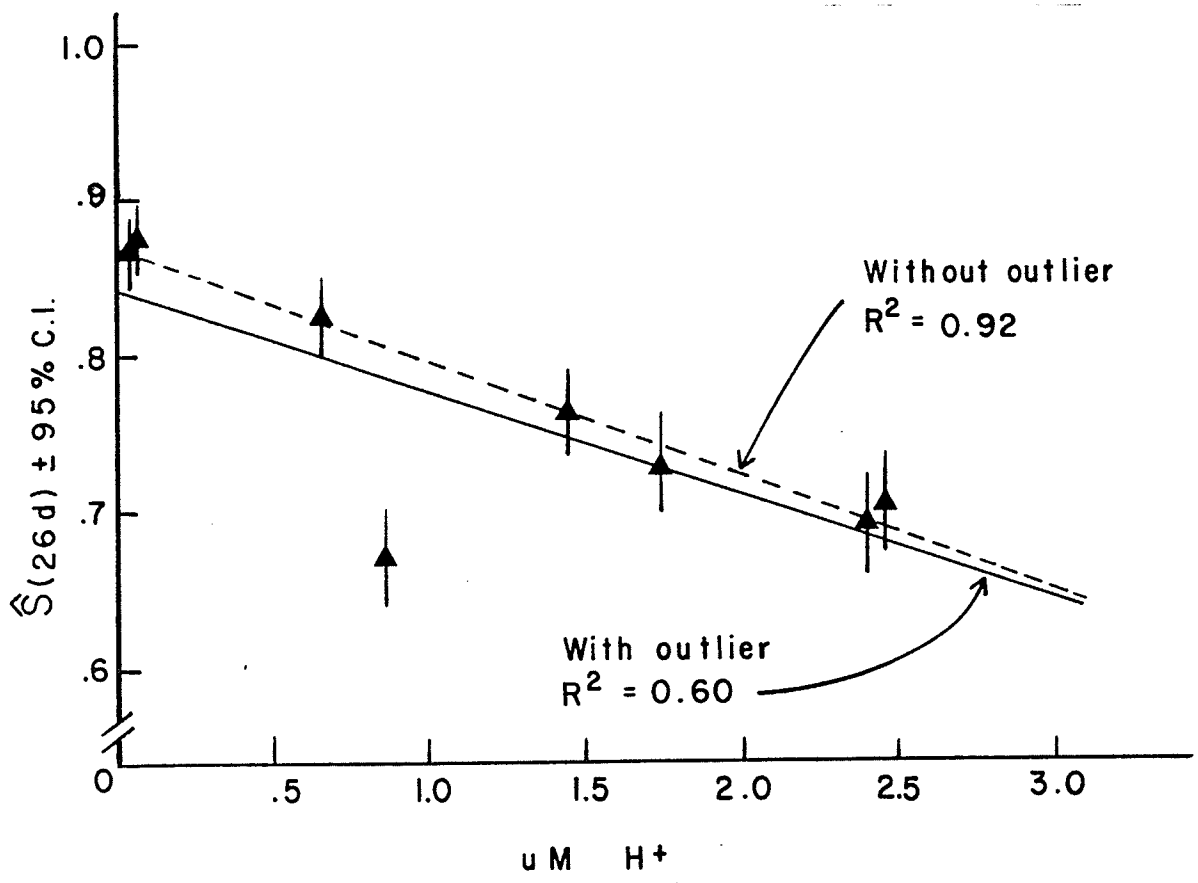

1.00
.95
.90
.85
.80
.75
.70
.65
.60
Ŝ(t)
Mean pH of treatment
7.18
6.12
5.80
5.62
0
5
10
15
20
25
30
SURVIVAL TIME, DAYS

Figure 21. Bag acidification experiment: relationship between estimated survivorship of H. azteca on DAY 26 and mean hydrogen ion concentration of bag. Linear regressions are for data with and without outlier (bag 2) observation.


1.0
.9
.8
.7
.6
0
$\hat{S}$(26d) ± 95% C.I.
Without outlier
R^2 = 0.92
With outlier
R^2 = 0.60
.5
1.0
1.5
2.0
2.5
3.0
u M H+

on day 26 in this replicate of the pH 6.12 treatment is estimated as a missing value, and the ANOVA is recalculated, the effects of pH on survivorship are very significant ($P<0.01$) (Table 6). The regression of survivorship at 26 days against H^+ concentration without data from Bag 2 is also very significant ($P<0.01$) (Fig. 21).

All size classes of amphipods survived equally well under unacidified conditions (Fig. 22). Newborn amphipod survivorship was significantly lower than that of adults at pH 6.12 ($P<0.025$), pH 5.80 ($P<0.05$) and pH 5.62 ($P<0.005$). Juveniles survived as well or better than adults in all treatments, except at pH 5.60.

Acute Toxicity A

Mean pH's of aquaria were 7.37, 7.39, 5.70, 5.65, 5.21, 5.15, 4.78, 4.77, 4.51 and 4.50 (Fig. 23). The average 95% confidence interval of these means was $\pm$ 0.06 pH units.

Survivorship of H. azteca in aquaria is shown in Fig. 24. Although differences between treatment replicates are evident at pH 5.67, 5.18 and 4.78, ANOVA of survivorship at 96 h indicates a significant ($P<0.025$) effect of pH (Table 7). Survivorship in all acidified treatments was significantly lower ($P<0.05$) than that of the control treatment, and survivorship at pH 5.67 was significantly higher than that at pH 4.50. Over the range of 0 - 32.5 umol H^+/L, survivorship at 96 h decreased non-linearly on the arithmetric scale (Fig. 25A), but on the negative-logarithm scale of pH it increased in a linear manner ($r^2 = 0.87$, $P<0.001$) (Fig. 25B).

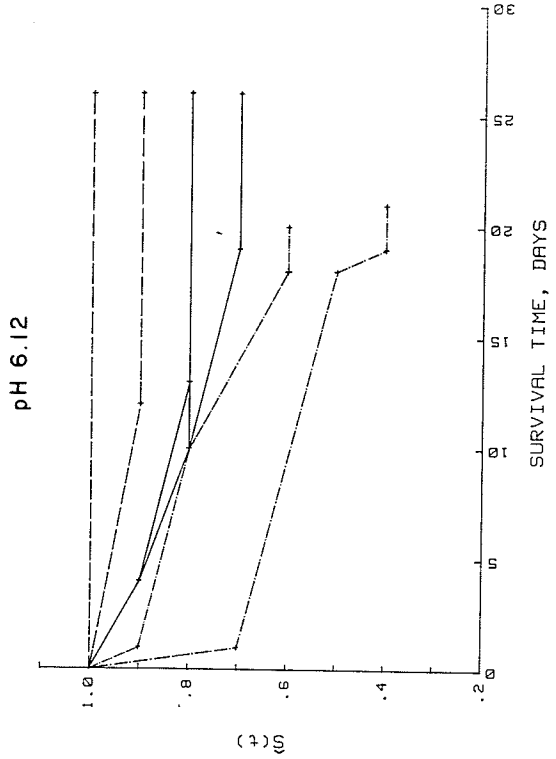
Mortality rates of amphipods in aquaria (determined as the slopes

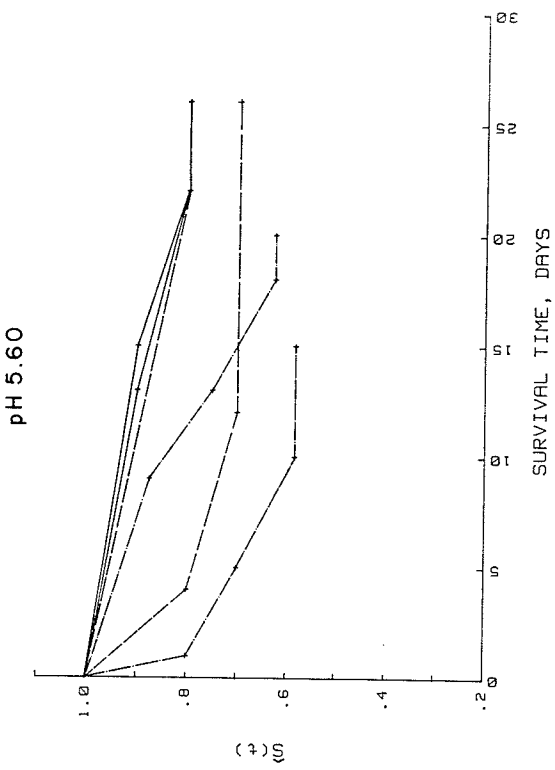
Table 6. Bag acidification experiment: Recalculated ANOVA for survivorship of Hyalella azteca at various pH's on DAY 26 with the observation from Bag 2 discarded and estimated as a missing value.

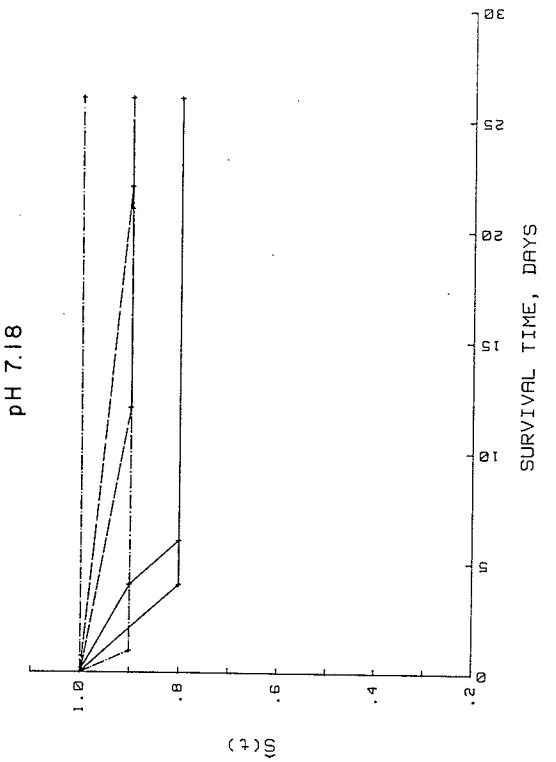
Source of Variation	df	SS	MS	F	P
pH	3	.03811[+]	.01270	39.64	< 0.01
Error	4-1	.0009615	.0003205		
Total	6-1	.03908			

[+] upward bias in SS(pH) = .00307; unbiased SS(pH) = .035042

Figure 22. Bag acidification experiment: survivorship functions of size classes of H. azteca within treatment replicates. $\hat{S}(t)$ = (number of individuals in the size class within the bag that survived longer than time t)/(total number of individuals in the size class in the bag). (3 size classes X 2 bags per treatment = 6 functions per treatment ——— = adults (n=10); – – – – = juveniles (n=10); —·—·—·— = newborns (n=10).


pH 6.12
S(t)
SURVIVAL TIME, DAYS


pH 5.60
S(t)
SURVIVAL TIME, DAYS


pH 7.18
S(t)
SURVIVAL TIME, DAYS

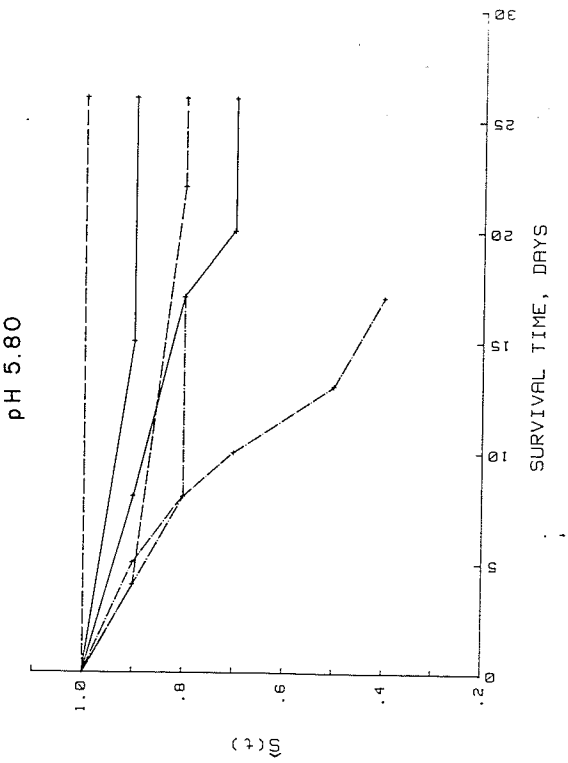

pH 5.80
S(t)
SURVIVAL TIME, DAYS

Figure 23. Acute toxicity test A: pH of aquaria. Intended treatments were: unacidified = aquaria 7,9; pH 5.60 = aquaria 5,8; pH 5.12 = aquaria 2,4; pH 4.76 = aquaria 3,6; pH 4.49 = aquaria 0,1.

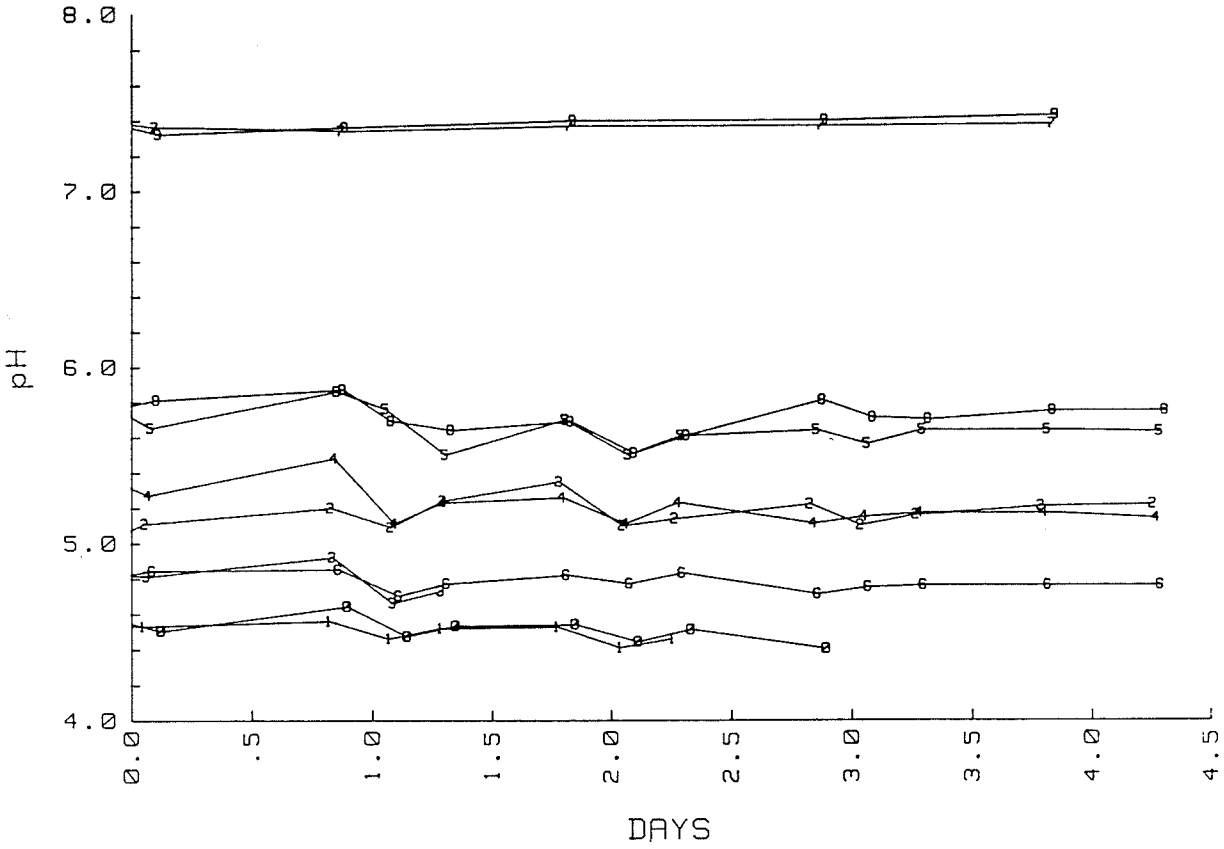

8.0
7.0
6.0
5.0
4.0
pH
0.0
.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
DAYS

Figure 24. Acute toxicity test A: survivorship of H. azteca in aquaria at various pH's. n=10 amphipods per aquarium.

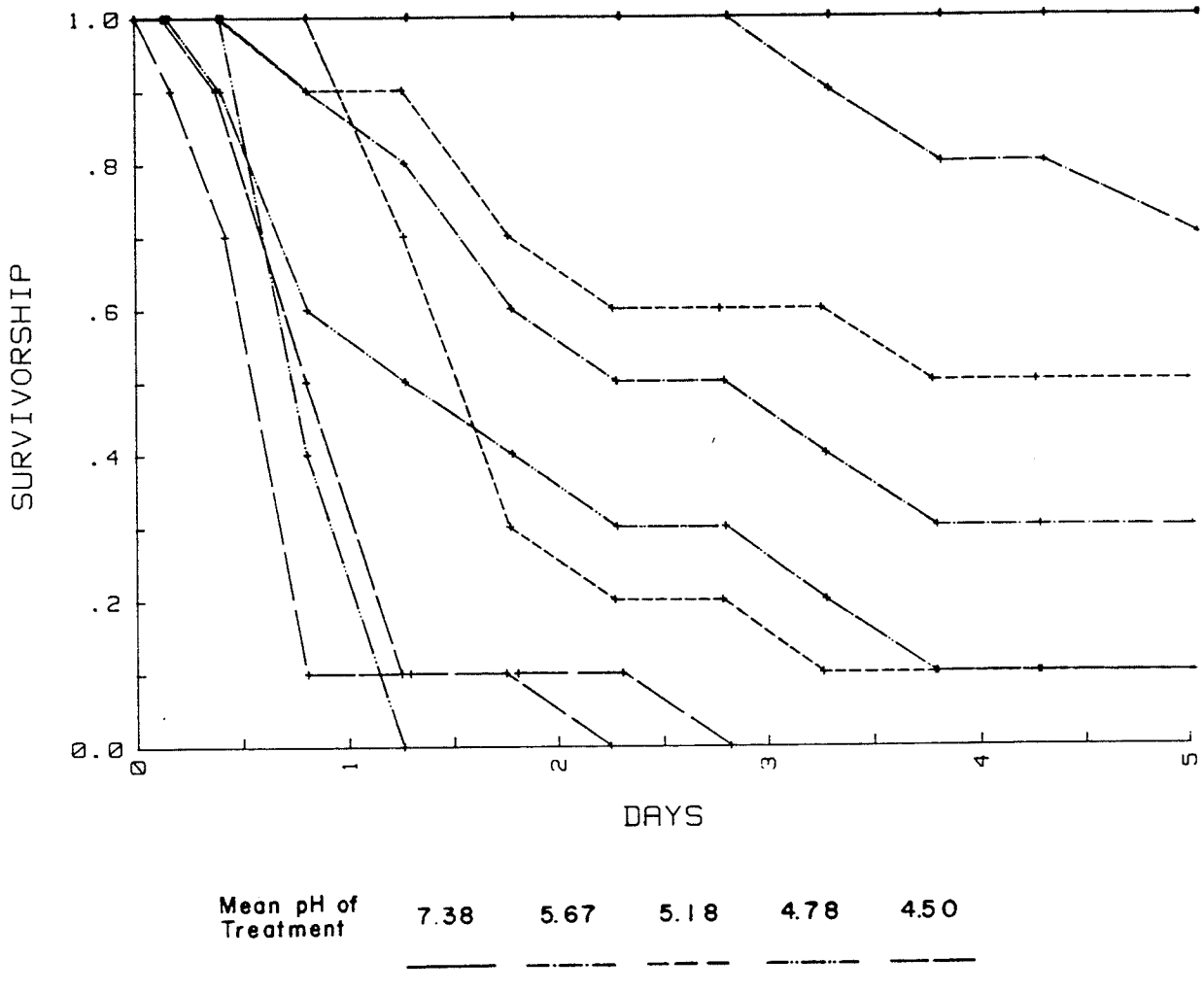

1.0
.8
.6
.4
.2
0.0
SURVIVORSHIP
0
1
2
3
4
5
DAYS
Mean pH of Treatment
7.38
5.67
5.18
4.78
4.50

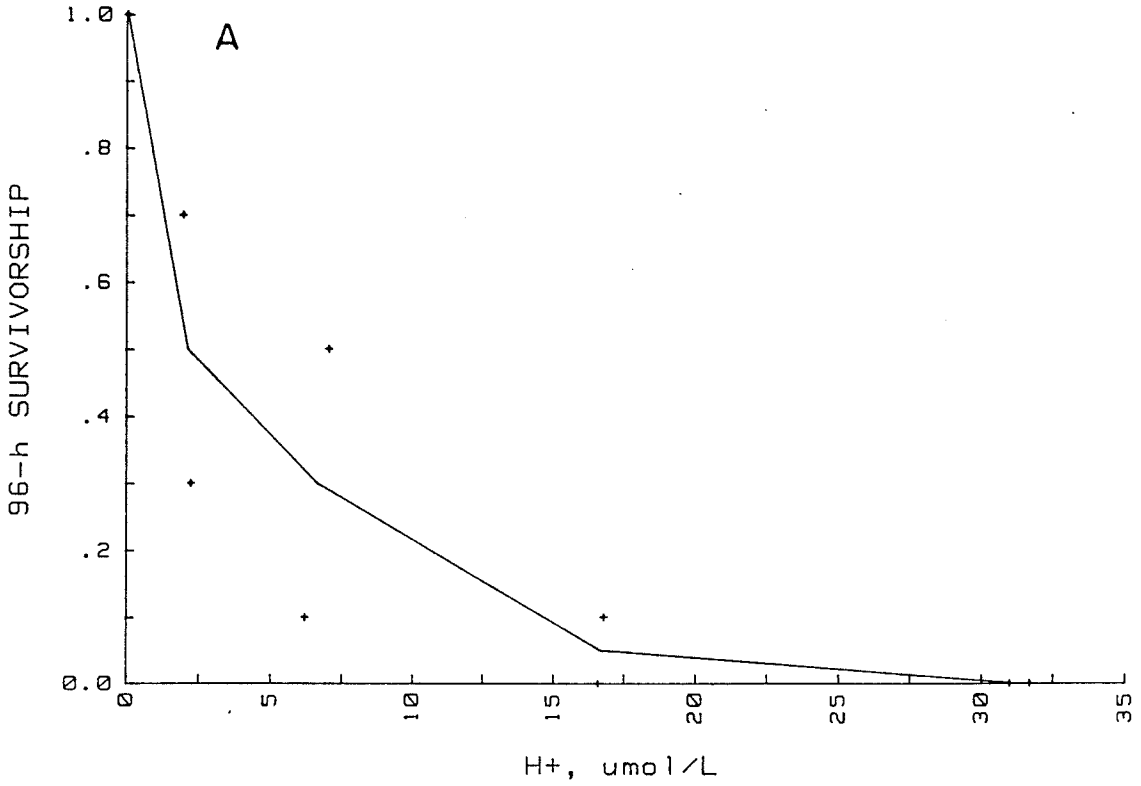
Table 7. Acute toxicity test A: ANOVA and multiple comparison of means for 96-h survivorship of Hyalella azteca at various pH's. Means underscored by the same line are not significantly different.

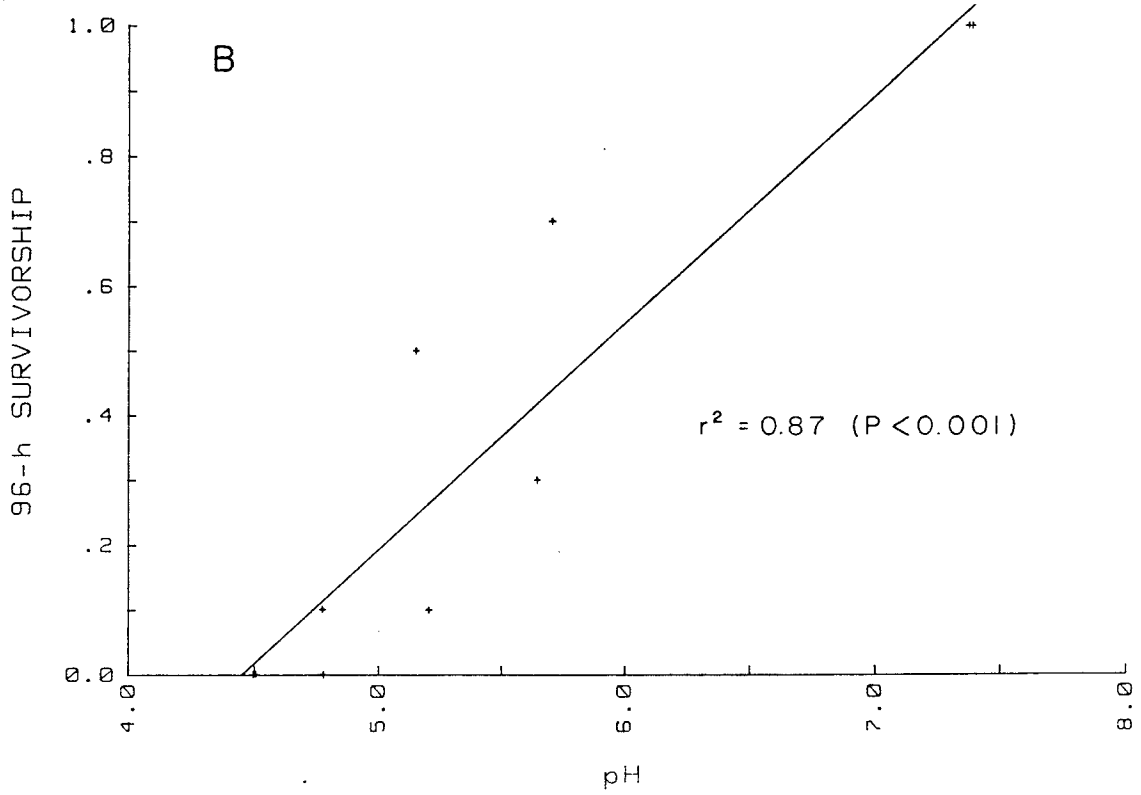
Source of Variation	df	SS	MS	F	P
pH	4	1.316	0.329	9.97	<0.025
Error	5	0.165	0.033		
Total	9	1.481			

Comparison of Means (least significant difference at alpha = 0.05)

pH of treatment	7.38	5.67	5.18	4.78	4.50
survivorship at 96 h	1	0.5	0.3	0.05	0

Figure 25. Acute toxicity test A: relationship between 96-h survivorship of H. azteca and hydrogen ion concentration. A: linear plot; B: logarithmic plot.


A
96-h SURVIVORSHIP
1.0
.8
.6
.4
.2
0.0
0
5
10
15
20
25
30
35
H+, umol/L


B
96-h SURVIVORSHIP
1.0
.8
.6
.4
.2
0.0
4.0
5.0
6.0
7.0
8.0
pH
r² = 0.87 (P<0.001)

of logit P vs time regressions) increased in a curvilinear manner as concentration of H^+ increased (Fig. 26). Estimated time to 50% mortality decreased exponentially as H^+ concentration increased (Fig. 27), and the estimated pH at which mortality reached 50% in four days (96-h LC50) was 5.48.

Size and age were only weakly related to survival time in acidified lake water. The slopes of both regressions of relative survival time against head length (Fig. 28A) and number of antennal segments (Fig. 28B) were both significantly ($P<0.05$) greater than zero. However, the r^2's were only 0.054 and 0.067 for the regressions involving head lengths and antennal segments, respectively.

Acute Toxicity B

Mean pH's of aquaria were 7.32, 7.34, 7.35, 5.65, 5.65, 5.67, 5.14, 5.15 and 5.16, with 95% confidence intervals averaging $\pm$ 0.04 pH units (Fig. 29).

Survivorship of H. azteca differed significantly ($P<0.005$) between ELA and LaSalle River populations in the unacidified treatment (Fig. 30A). After five days, mean survivorship of LaSalle River amphipods was 87%, whereas only 40% of ELA amphipods were still alive. This unexpected difference complicates the analysis of effects due to pH, and is discussed below. At pH 5.65 and 5.15, survivorship varied more among treatment replicates than between populations (Fig. 30B,C). Although mean survivorship of LaSalle River amphipods was higher than ELA amphipods in both treatments, the differences were not significant ($P>0.05$).

Figure 26. Acute toxicity test A: relationship between mortality rates of H. azteca in aquaria and mean hydrogen ion concentration. Mortality rates are represented by the slopes of logit P vs. time regressions (see text for details).

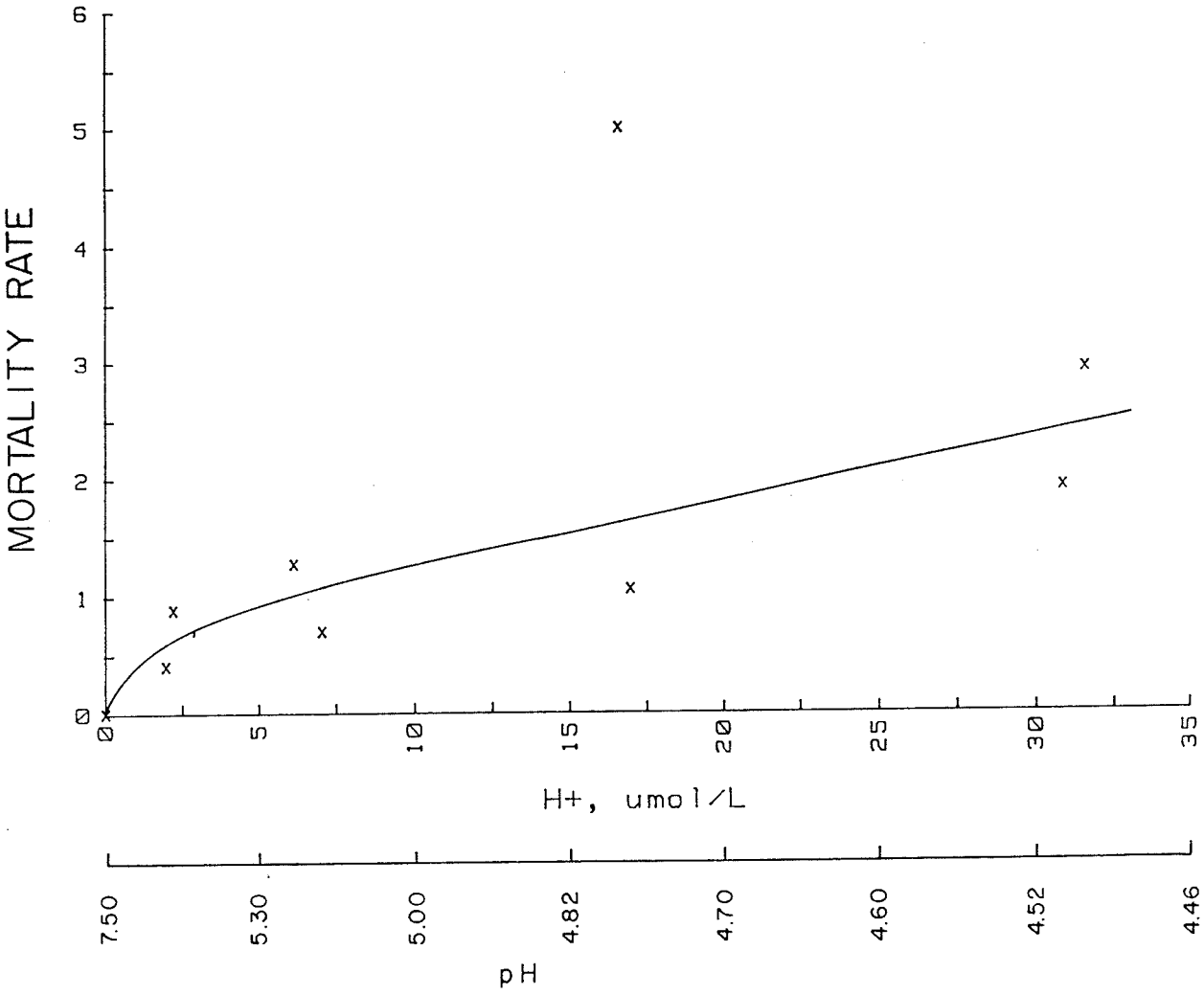

MORTALITY RATE
0
1
2
3
4
5
6
0
5
10
15
20
25
30
35
H+, umol/L
7.50
5.30
5.00
4.82
4.70
4.60
4.52
4.46
pH

Figure 27. Acute toxicity test A: relationship between estimated time to 50% mortality of H. azteca in aquaria and mean hydrogen ion concentration.

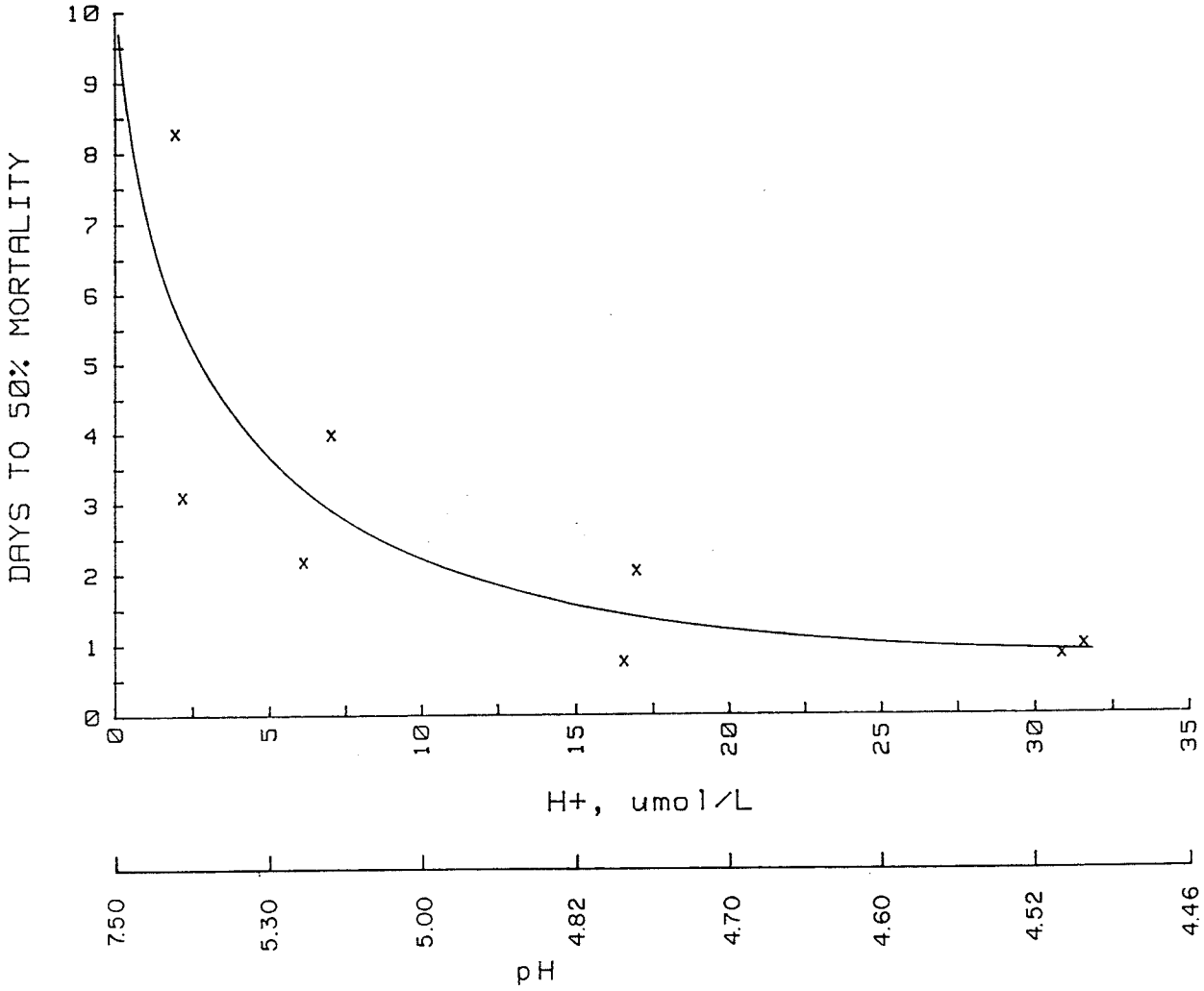
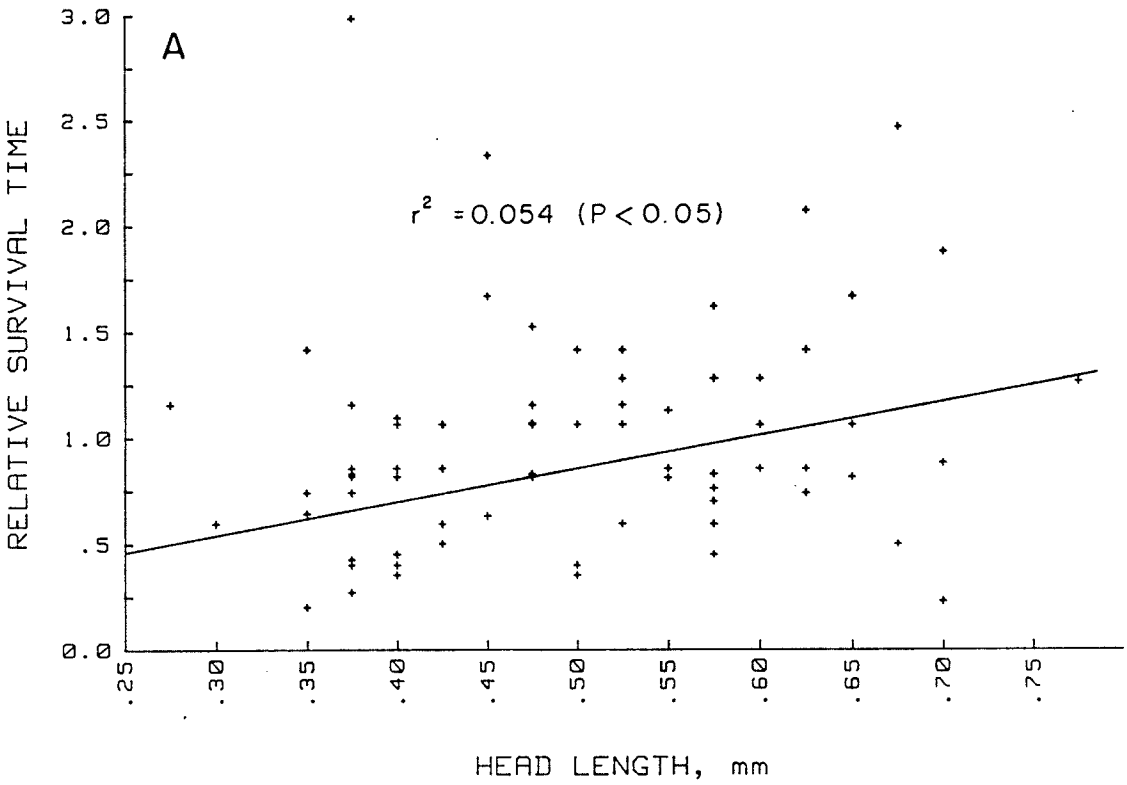

DAYS TO 50% MORTALITY
10
9
8
7
6
5
4
3
2
1
0
0
5
10
15
20
25
30
35
H+, umol/L
7.50
5.30
5.00
4.82
4.70
4.60
4.52
4.46
pH

Figure 28. Acute toxicity test A: linear regressions of relative survival time of individual H. azteca vs. head length (A), and number of antennal segments (B). Survival times of amphipods from acidified treatments (pH 4.50 - 5.70) were pooled after dividing by the mean survival time for the aquarium in which the amphipod was placed.


A
r^2 = 0.054 (P < 0.05)
RELATIVE SURVIVAL TIME
3.0
2.5
2.0
1.5
1.0
.5
0.0
.25
.30
.35
.40
.45
.50
.55
.60
.65
.70
.75
HEAD LENGTH, mm

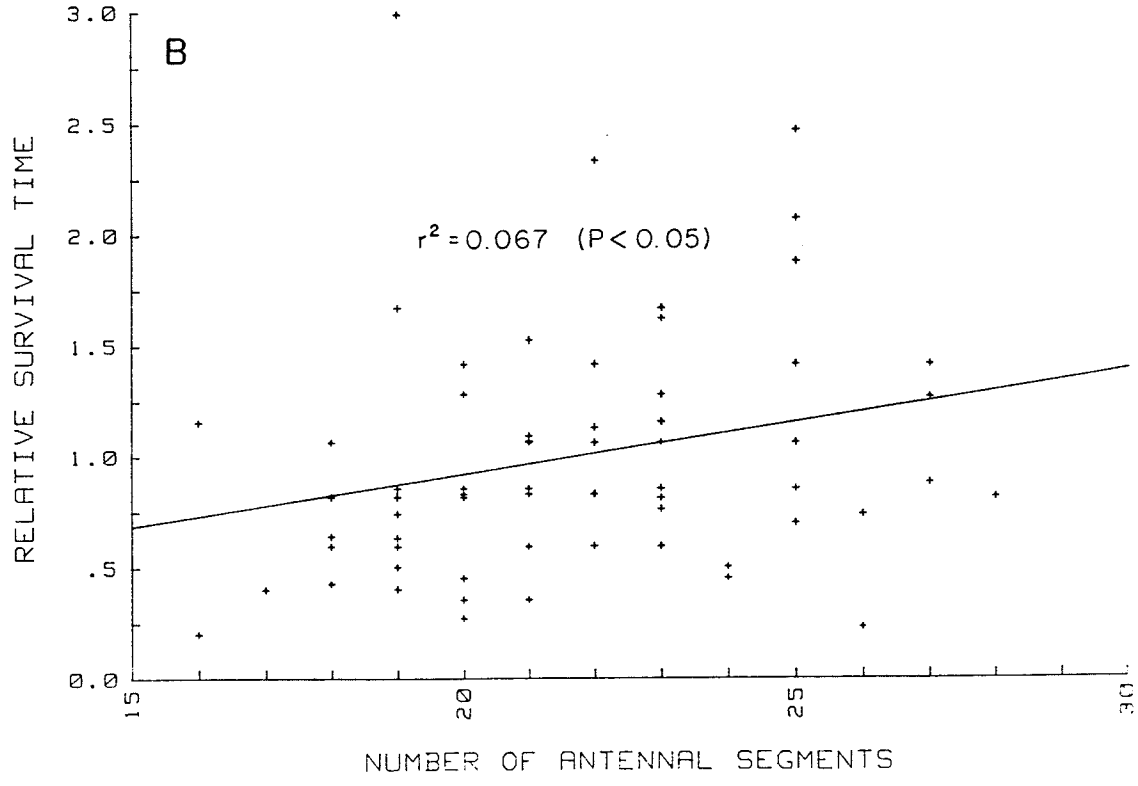

B
r^2 = 0.067 (P < 0.05)
RELATIVE SURVIVAL TIME
3.0
2.5
2.0
1.5
1.0
.5
0.0
15
20
25
30
NUMBER OF ANTENNAL SEGMENTS

Figure 29. Acute toxicity test B: pH of aquaria. Intended treatments were: unacidified = aquaria 1,4,8; pH 5.60 = aquaria 3,5,9; pH 5.12 = 2,6,7.

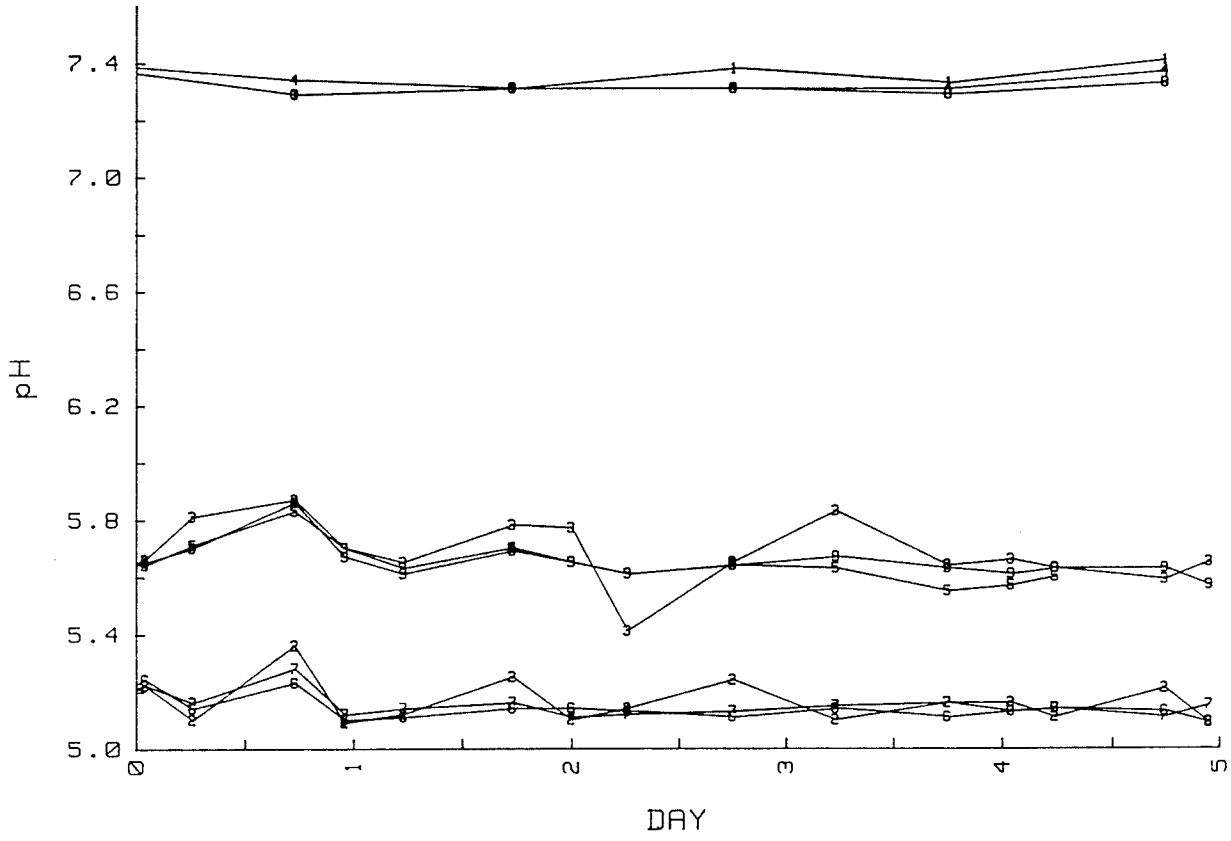
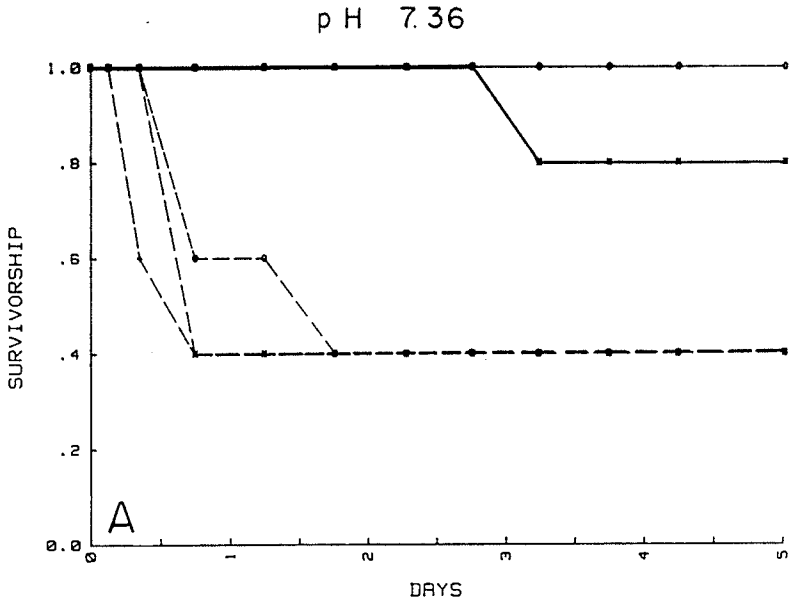
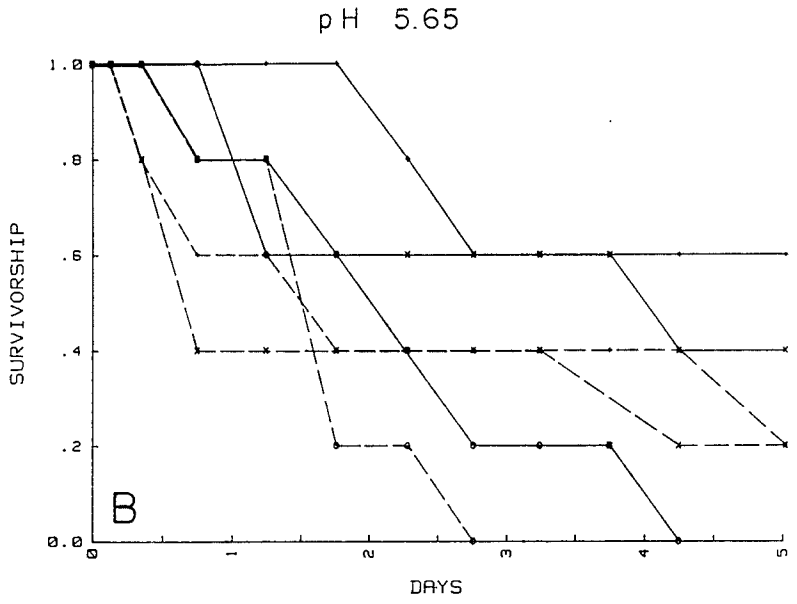
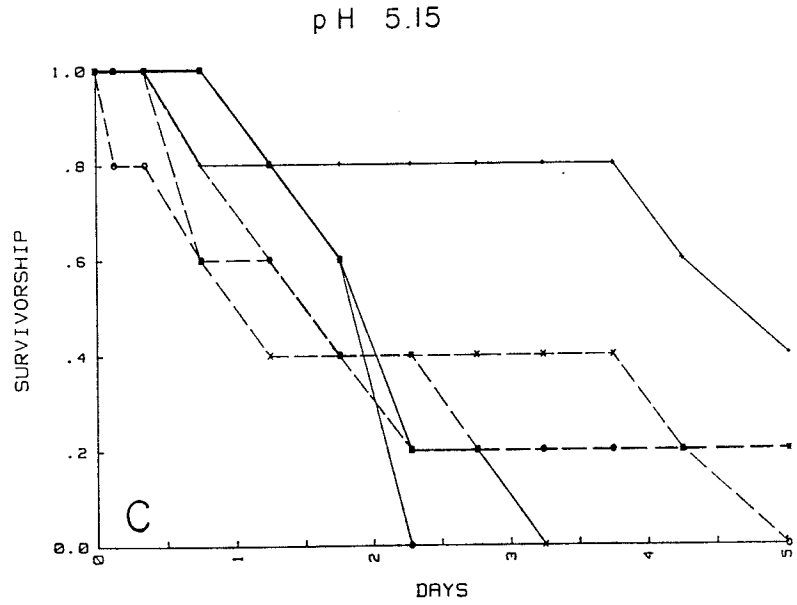

7.4
7.0
6.6
6.2
5.8
5.4
5.0
pH
0
1
2
3
4
5
DAY

Figure 30. Acute toxicity test B: differences in survivorship of H. azteca from the LaSalle River and from ELA. Solid line = LaSalle River; dashed line = ELA. +,o,x = replicate aquaria.


pH 7.36
SURVIVORSHIP
1.0
.8
.6
.4
.2
0.0
A
0
1
2
3
4
5
DAYS


pH 5.65
SURVIVORSHIP
1.0
.8
.6
.4
.2
0.0
B
0
1
2
3
4
5
DAYS

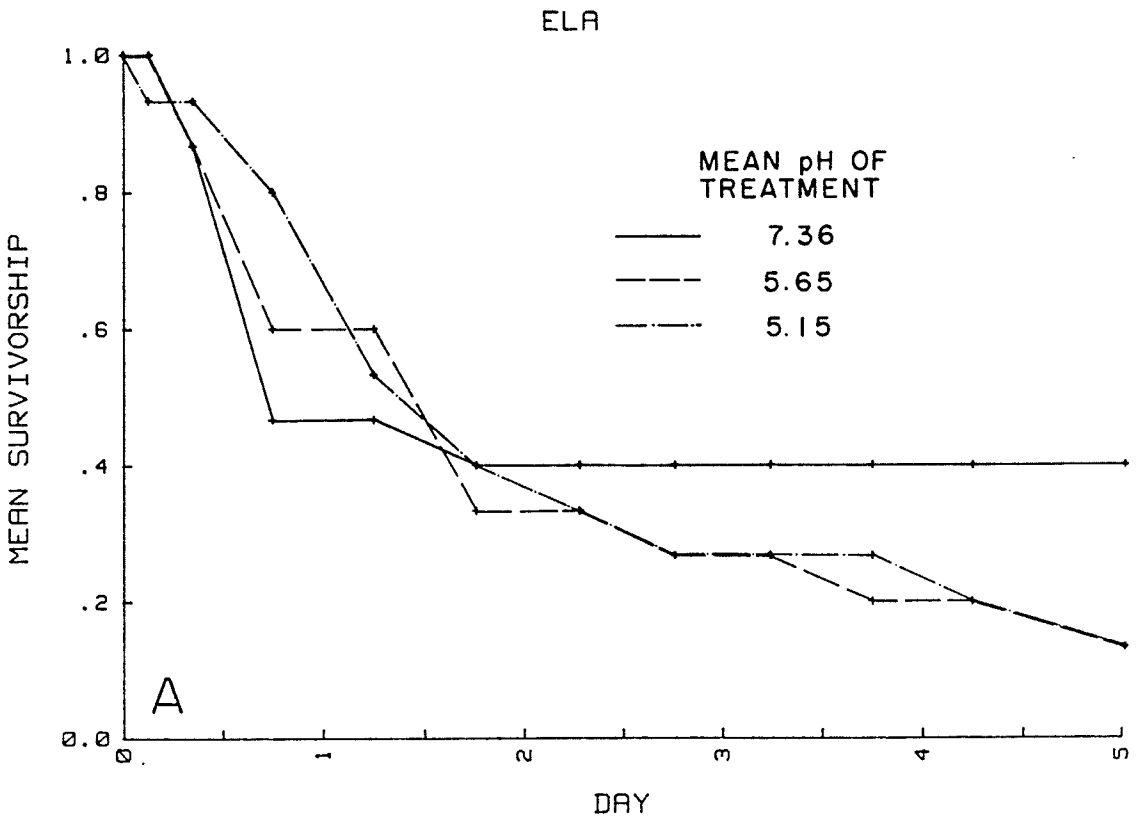

pH 5.15
SURVIVORSHIP
1.0
.8
.6
.4
.2
0.0
C
0
1
2
3
4
5
DAYS

Acidification affected survivorship of LaSalle River amphipods more than ELA amphipods. The latter showed no significant differences in survivorship among the three treatments (Fig. 31A). LaSalle River amphipod survivorship however, was much lower ($P<0.005$) at pH 5.65 and 5.15 than at pH 7.35 (Fig. 31B). However, the difference in survivorship between pH 5.65 and 5.15 was not significant.

Mortality rates of ELA and LaSalle River populations of H. azteca also showed different responses to acidification. The slopes of logit P vs time regressions increased from <0.2 at ~0.046 umol H^+/L (pH 7.35) to >1.0 at ~7.0 umol/L (pH 5.15) for LaSalle River H. azteca, but increased only slightly (~0.5 to 0.8) over this pH range for ELA amphipods (Fig. 32). Note that at 2.2 umol H^+/L (pH 5.65) for ELA amphipods, and at 2.2 and 7.0 umol/L for LaSalle River amphipods, the slope of one of the treatment replicates is substantially different that the other two (Fig. 32). Thus it is difficult to determine the significance of the differences in mortality rates among pH treatments. The estimated time when mortality reached 50% for the LaSalle amphipods decreased exponentially as H^+ concentration increased (Fig. 33), and the 4-day LC50 appeared to be pH ~5.6 (~2.3 umol/L). Estimated time to 50% mortality for ELA amphipods showed little change with increase in H^+ concentration, and high mortality in the control treatment prevented estimation of 96-h LC50.

Linear regressions of relative survival time against head lengths of amphipods, with both ELA and LaSalle River populations pooled and separated, showed no effects of head length on relative survival time.

Figure 31. Acute toxicity test B: differences in mean survivorship between pH treatments. (A) ELA H. azteca, (B) LaSalle River H. azteca.


ELA
MEAN SURVIVORSHIP
1.0
.8
.6
.4
.2
0.0
MEAN pH OF TREATMENT
7.36
5.65
5.15
A
0
1
2
3
4
5
DAY

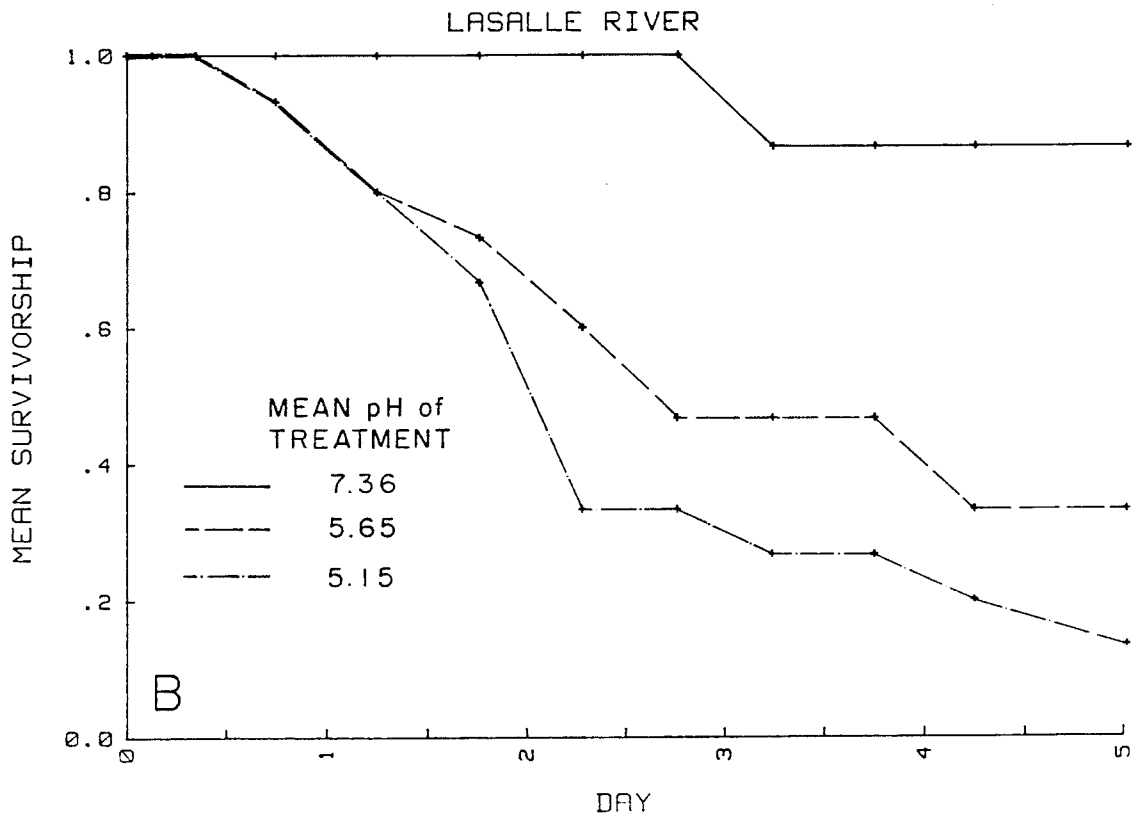

LASALLE RIVER
MEAN SURVIVORSHIP
1.0
.8
.6
.4
.2
0.0
MEAN pH of TREATMENT
7.36
5.65
5.15
B
0
1
2
3
4
5
DAY

Figure 32. Acute toxicity test B: relationship between mortality rates and mean hydrogen ion concentration for H. azteca from different populations. Mortality rates are represented by slopes of logit P vs. time regressions (see text for details).

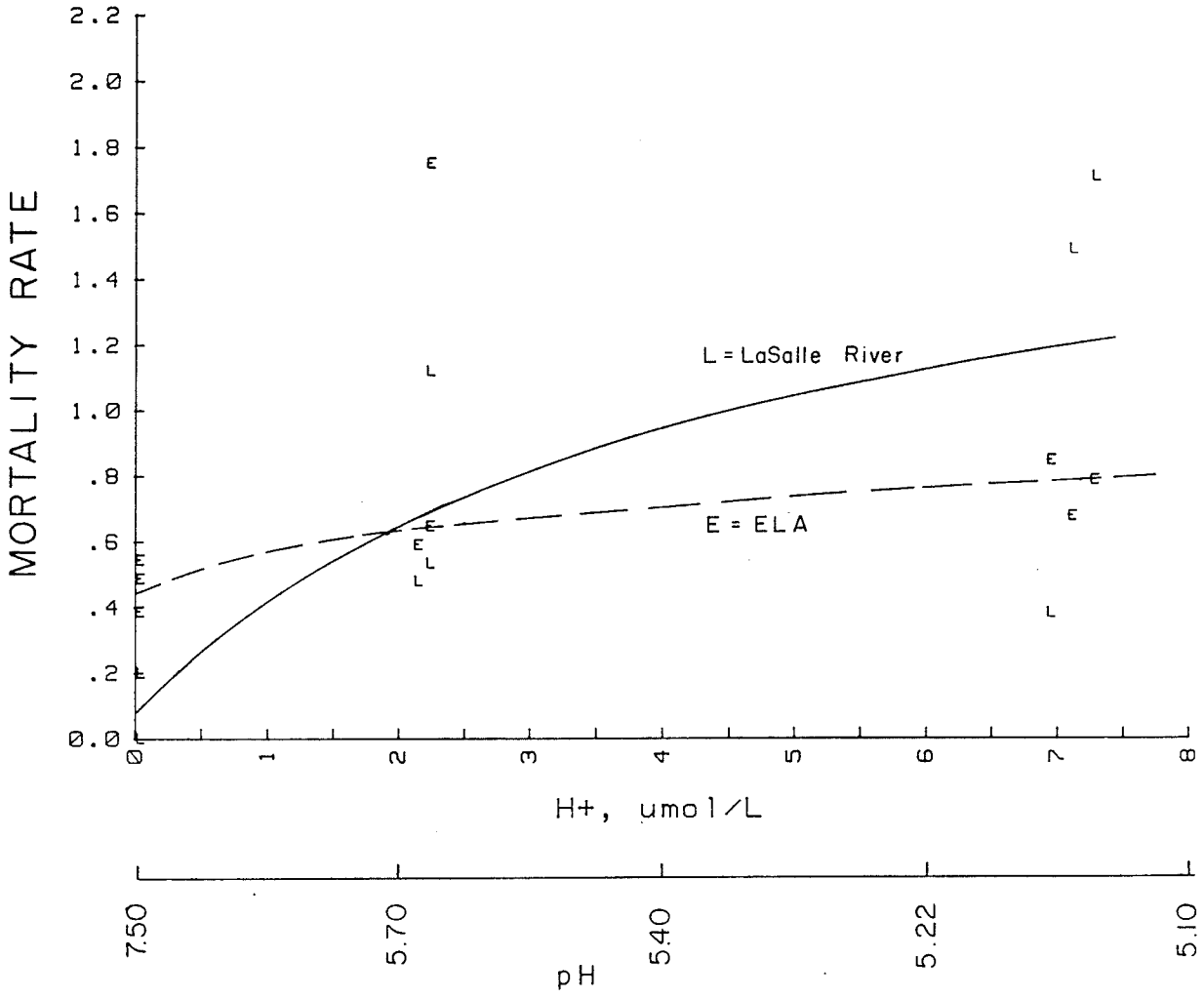
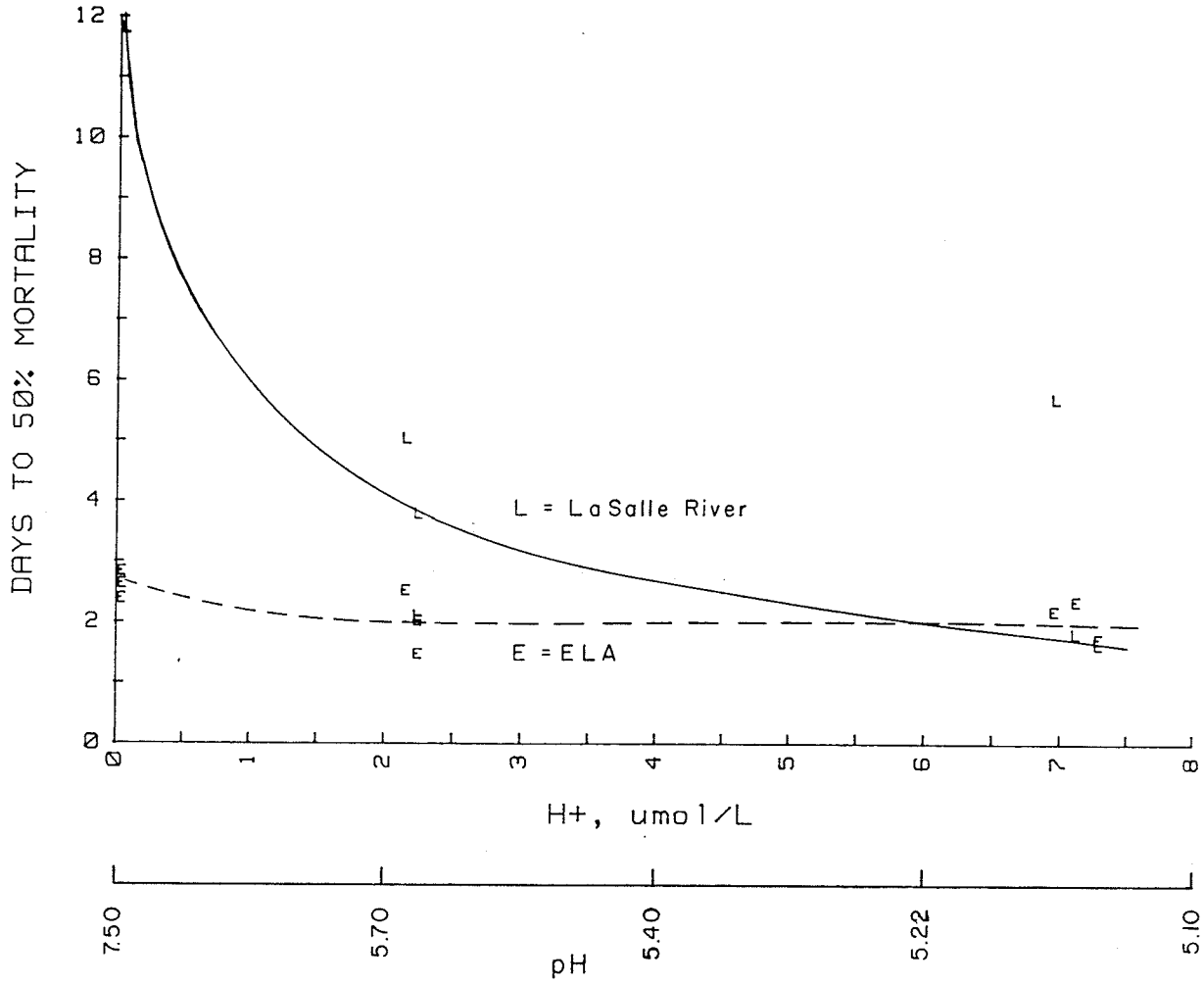

MORTALITY RATE
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0.0
L = LaSalle River
E = ELA
0
1
2
3
4
5
6
7
8
H+, µmol/L
7.50
5.70
5.40
5.22
5.10
pH

Figure 33. Acute toxicity test B: relationship between estimated time to 50% mortality and hydrogen ion concentration for H. azteca from different populations.


DAYS TO 50% MORTALITY
12
10
8
6
4
2
0
L = LaSalle River
E = ELA
0
1
2
3
4
5
6
7
8
H+, umol/L
7.50
5.70
5.40
5.22
5.10
pH

Chronic Toxicity

Mean pH's of treatment replicates were all within 0.03 pH units of the target pH's of 5.52, 5.70 and 6.00. In control aquaria, mean pH's were 7.42 to 7.44. Overall the average 95% confidence interval of the variation in pH within aquaria was ± 0.025 (Fig. 34). The concentration of calcium in aquaria after 30 days ranged from 2.72 - 3.31 mg/L, which indicates that concentrations did not become limiting to *H. azteca* or change substantially during the experiment.

Survivorship of newborn amphipods was poor in all acidified aquaria. In 10 out of 12 acidified treatment replications, there were no survivors after 16 days (Fig. 35A-D). Survivorship also was poor in some control aquaria, indicating that stress from sources other than reduced pH caused mortality. Analysis of survival times by Kruskal-Wallis H-tests (Table 8) showed that survivorship was significantly ($P<0.005$) different among treatments in all time replicates. The distributions of individual survival times (time replicates pooled), shown as percent relative frequencies of the number of days survived, are different among treatments (Fig. 36A-D). Although amphipods tended to survive longer in time replicates 3 and 4 than in 1 and 2, the same relationship is evident in all time replicates (Fig. 35). As pH decreased, both the mean and the standard deviation of the survival times decreased, from 13.7 ± 7.9 days at pH 7.44 to 5.08 ± 1.9 days at pH 5.51 (Fig. 36). Control survival times were significantly greater than those in acidified treatments within all time replicates. However, among pH 5.99, 5.70 and 5.51, differences were not significant (Table 8).

Figure 34. Chronic toxicity test: pH of aquaria. Intended treatments were: unacidified = aquaria 0; pH 6.00 = aquaria 1; pH 5.70 = aquaria 2; pH 5.52 = aquaria 3.

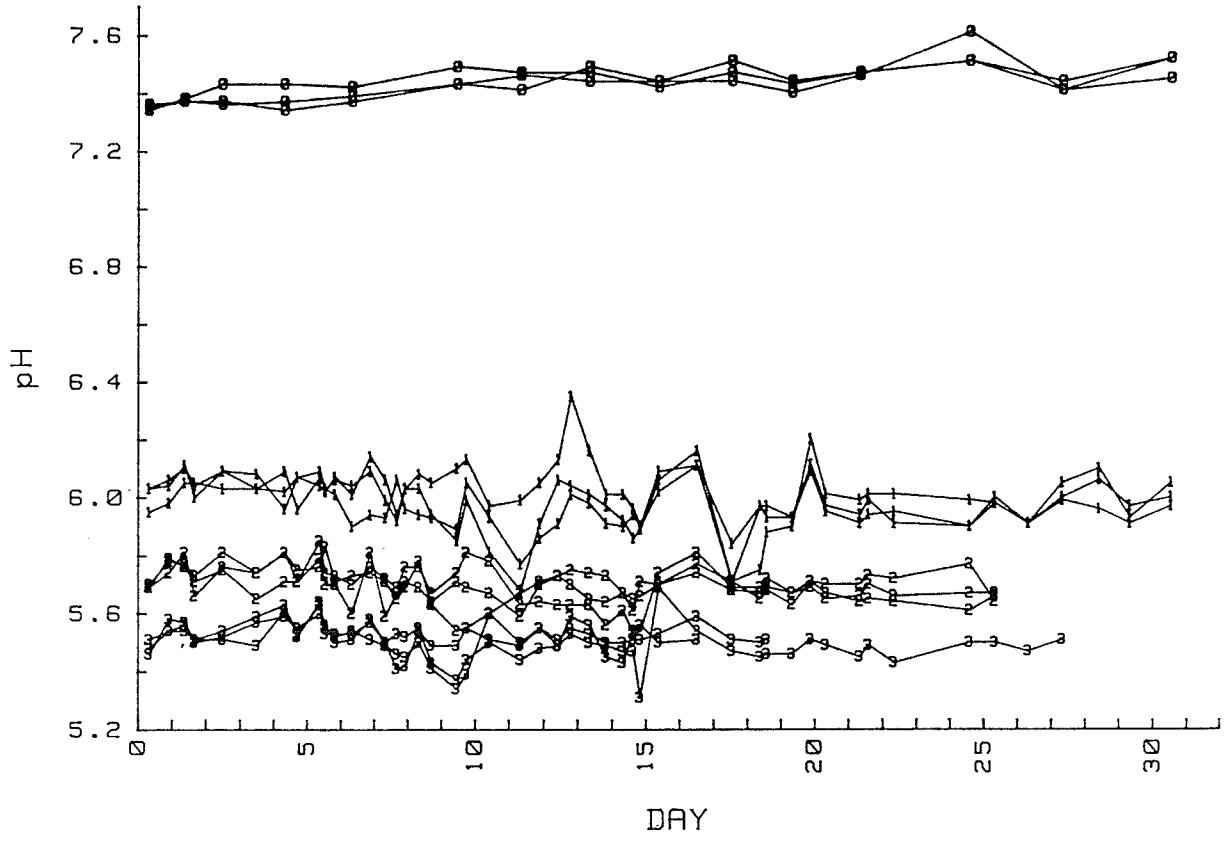
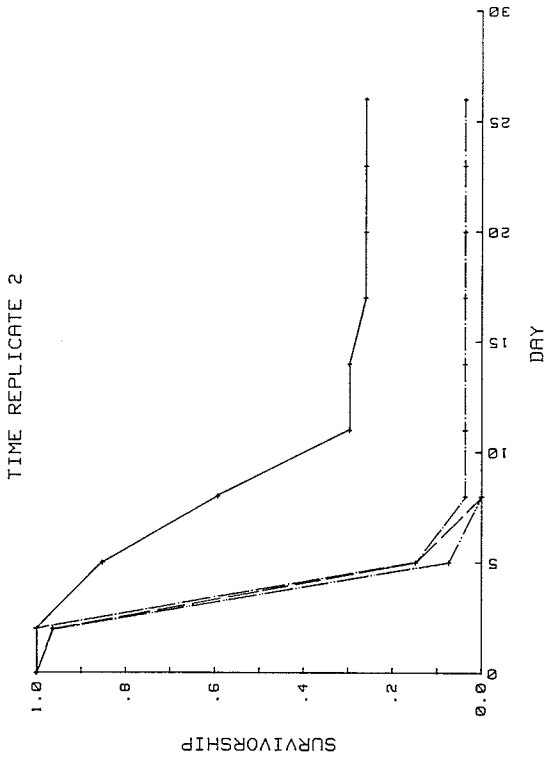
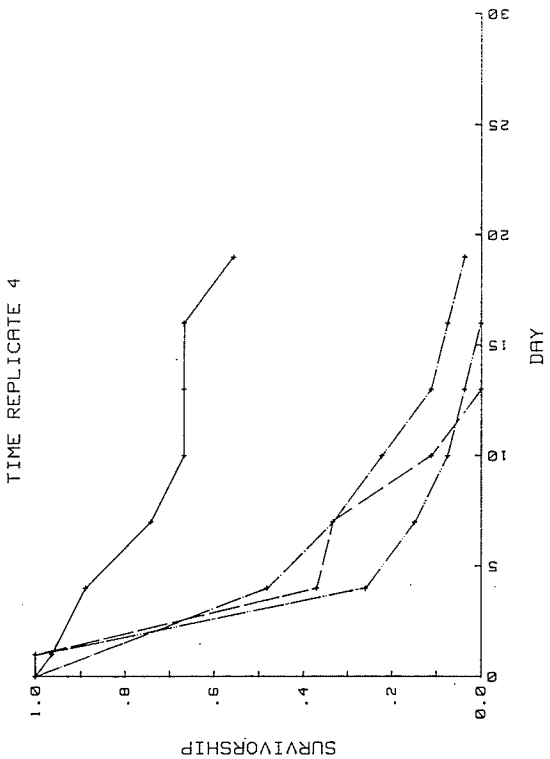

7.6
7.2
6.8
6.4
6.0
5.6
5.2
pH
0
5
10
15
20
25
30
DAY

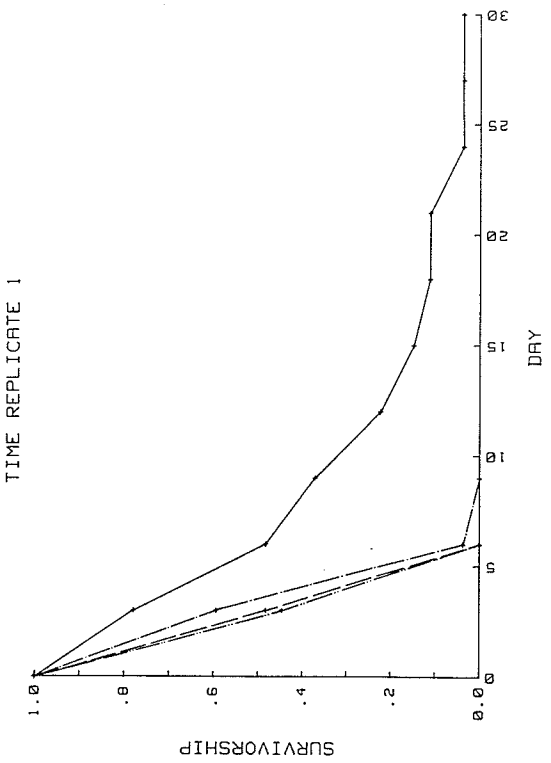
Table 8. Chronic toxicity test: Analyses of differences in survivorship of Hyalella azteca between pH treatments within time replicates, using K-sample Kruskal-Wallis H-tests.

Treatments Compared	Time Replicate	Significance Level
1. All treatments	1	$P < 0.005$
2. All treatments	2	$P < 0.005$
3. All treatments	3	$P < 0.005$
4. All treatments	4	$P < 0.005$
5. pH 5.51, 5.70, 5.99	1	n.s.
6. pH 5.51, 5.70, 5.99	2	n.s.
7. pH 5.51, 5.70, 5.99	3	n.s.
8. pH 5.51, 5.70, 5.99	4	n.s.

Figure 35. Chronic toxicity test: mean survivorship of newborn H. azteca in pH treatments within time replicates. Each curve is based on (3 amphipods per vial) X (3 vials per aquarium) X (3 aquaria per treatment) = 27 amphipods. ——— = pH 7.44; —·—·—·— = pH 5.99; — — — — = pH 5.70; —··—··— = pH 5.51.


TIME REPLICATE 2
SURVIVORSHIP
DAY
1.0
.8
.6
.4
.2
0.0
0
5
10
15
20
25
30


TIME REPLICATE 4
SURVIVORSHIP
DAY
1.0
.8
.6
.4
.2
0.0
0
5
10
15
20
25
30


TIME REPLICATE 1
SURVIVORSHIP
DAY
1.0
.8
.6
.4
.2
0.0
0
5
10
15
20
25
30

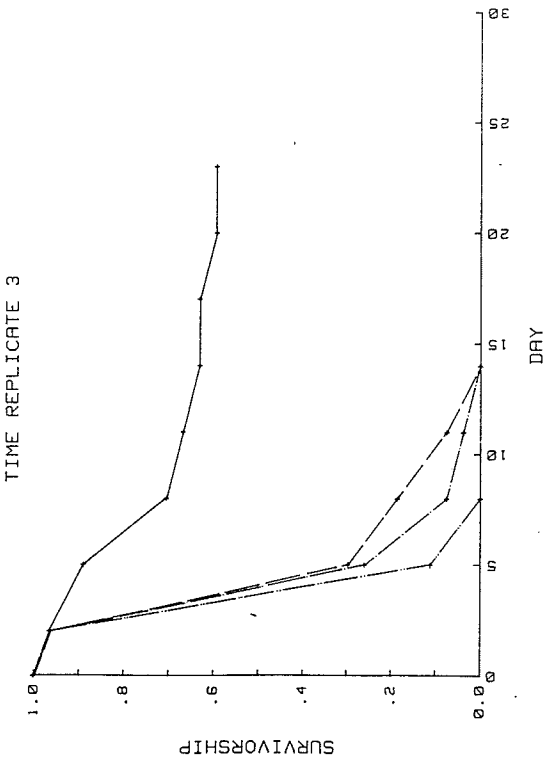
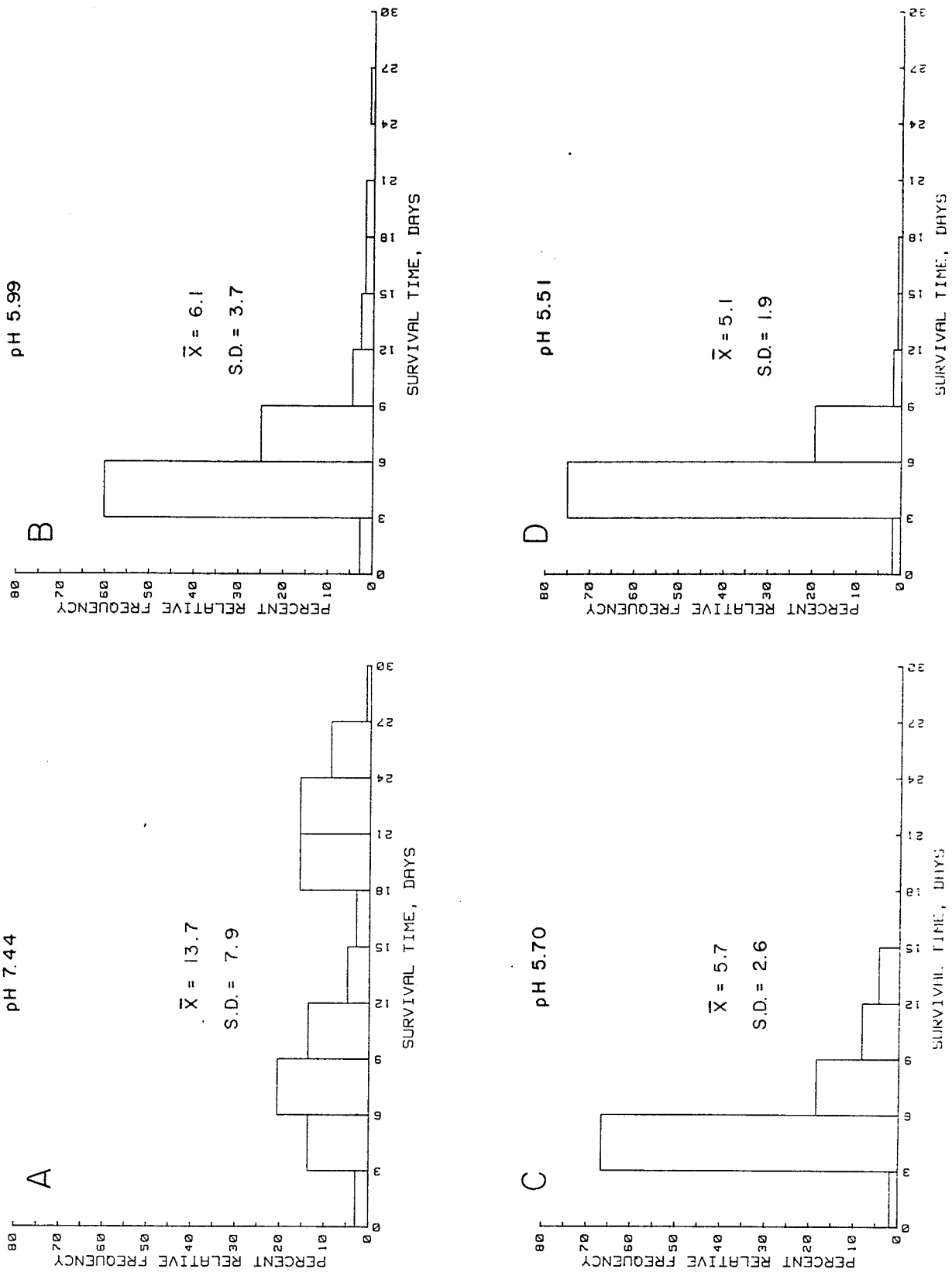

TIME REPLICATE 3
SURVIVORSHIP
DAY
1.0
.8
.6
.4
.2
0.0
0
5
10
15
20
25
30

Figure 36. Chronic toxicity test: distributions of survival times of H. azteca under various pH's. n = 108 for all treatments except pH 7.44, for which n = 102.


A
pH 7.44
$\bar{X}$ = 13.7
S.D. = 7.9
B
pH 5.99
$\bar{X}$ = 6.1
S.D. = 3.7
C
pH 5.70
$\bar{X}$ = 5.7
S.D. = 2.6
D
pH 5.51
$\bar{X}$ = 5.1
S.D. = 1.9
PERCENT RELATIVE FREQUENCY
SURVIVAL TIME, DAYS

DISCUSSION

Hydrogen Ion Concentration and Survivorship

The concentration of hydrogen ions strongly affects survivorship of populations of H. azteca when exposed to acidified lake water. In general, survivorship in acidified treatments does not become significantly lower than that in control treatments until pH < 6.1. At pH 6.32 (transplantation experiment) and pH 6.12 (bag acidification), no effect of acidification on survivorship was detected. At pH below 6.1 and above 5.5, survivorship of amphipods at a specific pH and defined period of exposure varied among experiments, possibly due to stress from handling and containment, differences in temperature, or differences in populations (with respect to acclimatization to dilute waters).

Survivorship of newborn amphipods at pH 6.0 was assessed in the chronic toxicity experiment, and ranged from 15 to 43% at 5 days, and 0 to 22% at 10 days (mean survivorship within time replicates). These values are much lower than results for other amphipods of all ages at lower pH and longer exposures (e.g. 75% survivorship after 20 days at pH 5.80 in acidified bags, and 50% survivorship after 5 days at pH 5.67 in acute toxicity test A). Because survivorship was also poor

under control conditions (Fig. 35), factors other than H^+ ions probably contributed to mortality in the acidified treatments. Very young amphipods have higher metabolic rates, molt more frequently and have relatively greater demands for essential ions and nutrients than older amphipods, all of which increase sensitivity to environmental stresses compared to more mature individuals (Buikema and Benfield 1979). Increased sensitivity to low pH also would be expected; however, in the bag acidification experiment, survivorship of newborn individuals was >60% after 10 days at pH as low as 5.62 (Fig. 22). Although temperatures were lower in bags, this still suggests that H^+ ions alone do not account for such poor survival in the chronic laboratory toxicity test.

In transplanted enclosures of amphipods, the least artificial conditions of the experiments, survivorship was approximately 40% at pH 5.66 after 20 days, whereas in acidified bags of lake water survivorship at pH 5.62 after 20 days was 70%. The main differences in the conditions of these two experiments were in the water temperatures and chemicals added. Temperatures were 20-27^{o}C for the first 20 days of the transplantation experiment versus 14-20^{o} for the bag acidification experiment. Lakes 302S and 223 received H_2SO_4, whereas the bags of lake water received HCl and NaOH. Lower temperatures can reduce osmoregulatory stress caused by acidified waters, because turnover rates for water and ions decrease with temperature (Sutcliffe 1978). Mortality also can be reduced at high concentrations of Na^+, which can lessen the loss of Na^+ and thus partially alleviate stress of low pH (see Part I). Meinel et al. (1985) found that Na^+ appeared to moderate H^+ stress. Survival rate

of Gammarus fossarum was increased, but only at very low pH (4.0,4.5), with concentrations of Na^+ 1.7-17 times background level. In comparison, the maximum addition of Na^+ to the acidified bags was only on the order of 30% of background concentration - much lower than the amount that produced an effect on amphipods in the study of Meinel et al. (1985). Thus, differences in temperature rather than Na^+ concentration may be more important in explaining differences in survivorship of amphipods in the transplantion and bag acidification experiments.

Survivorship in laboratory experiments was generally lower than in the field experiments. At pH ~5.7 after 5 days, survivorship ranged from 30-50% in the acute and chronic toxicity tests, whereas in the acidified lakes and bags, survivorship was 85-95% after 5 days at pH 5.6-5.8. Amphipods in the laboratory experiments were held in 4-L vessels of lake water in a light and temperature controlled environmental room set to conditions similar to midsummer littoral zones of ELA lakes. Although concentrations of Ca^{2+} were not depleted after 30 days, it is possible that other chemical conditions may have changed, such as the accumulation of a contaminant or the depletion of a required substance. This could cause additional stress, and would have a greater effect in 4 L (laboratory tests) than in 500 L (bag acidification experiment) of water. Handling of amphipods for assessment of survivorship was also greater in the acute toxicity tests than in the other experiments - 12 times over 5 days compared to $\leq$ 3 times per 5 days. While care was taken to minimize disturbance due to handling, survivorship still could have been affected, especially that of newborn amphipods.

At pH $\leq$ 5.5 survivorship of H. azteca was poor. After 4 days, mortality was 50% in the acute laboratory experiments at pH 5.5. In populations transplanted into L223 (pH 5.45), an average of only 11% of the original amphipods were alive after 20 days. All individuals died after 3 days at pH 4.50 and 4.78 in acute toxicity test A, and almost all amphipods died after 15 days in the chronic toxicity test at pH $\leq$ 6.0, although this latter mortality is probably not solely due to H^+ stress (see above).

Relationships between concentration of H^+ and ability to survive were observed using several statistics - survivorship (i.e. number or proportion of individuals alive or estimated to be alive at a given time), rate of mortality (slope of a linear regression of logit transformed survivorship against time), estimated time at which mortality equals 50% (calculated from mortality rates), and distributions of survival times of amphipods in treatments. Survivorship decreased as concentration of H^+ increased in a linear manner up to at least 4 umol/L (Fig. 18,21). Over a range of H^+ concentrations from 0.04 - 32 umol/L, the response was curvilinear on the arithmetic scale (Fig. 25A), and linear on a pH scale (Fig. 25B). Mortality rates and estimated time to 50% mortality also increased and decreased, respectively, non-linearly as concentration of H^+ increased from 0.04 - 32 umol/L (Fig. 26,27,32,33), although this trend was somewhat unclear in acute toxicity test B due to greater variation in survivorship among treatment replicates. These relationships suggest that the range of 0.04 - 4 umol H^+/L (pH$\geq$ 5.4), over which the rate of change in response was greatest, is the most critical to survivorship. In fact, it is in this range that survivorship became significantly

lower than that at neutral conditions, and that long-term survival of populations became doubtful.

Survival times of amphipods in the chronic toxicity test were distributed differently among treatments, although these differences were not significant among pH 5.51, 5.70 and 5.99. As pH decreased, lifetimes of amphipods tended to be shorter. Variation in survival times also decreased, presumably because H^+ concentration became the major cause of mortality and overrode other factors affecting survival time.

Population Type and Tolerance to Acidic Conditions

Hyalella azteca used in these experiments came from two different sources: (1) L239 at ELA, Ontario (used in the transplantation, bag acidification, acute toxicity B and chronic toxicity experiments), and (2) the LaSalle River, Manitoba (acute toxicity A and B experiments). The responses of these two distinct populations to acidification of ELA waters were compared in the acute toxicity test B. However, caution is required in interpreting the results because ELA amphipods survived poorly under control conditions, whereas LaSalle River amphipods survived well. This suggests that a factor other than tolerance to acidic conditions affected survivorship. Most ELA amphipods were collected from L239 one day before the experiment, in contrast to LaSalle River amphipods, which were taken from a laboratory culture established several months before the experiment. In such cultures, selection tends to favor individuals that are more tolerant of laboratory conditions. Therefore, it is not surprising

that ELA amphipods, perhaps stressed from collection and transport to the laboratory, suffered higher mortality than LaSalle River amphipods. If this mortality factor was great compared to the effect of low pH, the response to low pH may be obscured.

Notwithstanding, LaSalle River amphipods survived more poorly at pH 5.15 and 5.65 than at pH 7.36, whereas ELA amphipods showed no differences in survivorship between control and acidified treatments (Fig. 31). (Factors other than pH should have affected amphipods in all treatments equally.) These results support the hypothesis that ELA amphipods, which come from waters with much lower concentrations of major ions than the LaSalle River, are better adapted to taking up and maintaining required ions, such as Na^+ and Ca^{2+}, and thus more tolerant of the osmoregulatory problems associated with reduced pH. The conclusion that should be drawn is that different populations may not respond in the same way to acidification, and that the chemical environment to which these populations may be acclimatized must be considered. This is true when comparing responses of LaSalle River amphipods in the acute toxicity tests with those of the ELA amphipods in the other experiments. If LaSalle River amphipods are used to estimate responses of amphipods from soft waters, such as in ELA and other Precambrian Shield lakes, the reduction in survivorship due to acidification is likely to be overestimated rather than underestimated.

Size, Age and Tolerance to Acidic Conditions

Smaller and younger amphipods should be more sensitive to acidic conditions because of their larger surface area to volume ratios,

higher metabolic rates, and greater frequency of molting (Buikema and Benfield 1979), all of which require the regulation of internal concentrations of ions (Sutcliffe 1978). In the bag acidification experiment and acute toxicity test A, the effects of size or age of an individual on survival time at low pH were significant, but minor. Amphipods <3 days old placed into acidified bags survived more poorly than adults, at pH 5.62, 5.80 and 6.12 (Fig. 22), although in one of the bags at pH 6.12 initial mortality was more likely due to handling stress than to H^+. Juvenile amphipods (~3 mm in body length) did not show higher mortality than adults (≥ 5 mm body length) at any pH except 5.62, and the difference at pH 5.62 was not significant. This suggests that the size difference between adults and juveniles does not affect survivorship, at least between pH 5.8 and 6.1. At pH 4.5-5.6, size (based on head lengths) and age (based on the number of antennal segments) were only slightly correlated with relative survival time of adults and juveniles in acute toxicity test A (Fig. 28), but the relationship was not evident in acute toxicity test B. Once the newborn stage is passed, size and age are apparently minor factors affecting the ability of H. azteca to survive acidic conditions.

Comparison with Other Studies

The survivorship of H. azteca in the experiments of this study can be compared to the results of two other examinations of the responses of H. azteca to experimental acidification. Zischke et al. (1983) acidified outdoor artificial channels to pH 5 and 6 with sulfuric

acid. After approximately 1 mo, abundance of H. azteca, initially one of the dominant members of the naturally colonized macroinvertebrate community, decreased sharply in the pH 5 channel. Size frequencies in the two acidified channels and the pH 8 reference channel were similar, however, indicating a lack of difference in tolerance among size classes. The fact that H. azteca of any size were observed at pH 5 after three months disagrees with the results of the present study. However, conditions in the acidified channels could have decreased sensitivity and vulnerability to low pH. Concentration of Ca^{2+} was 30-43 mg/L, compared to 2-3 mg/L for ELA water. Elevated Ca^{2+} concentration has been correlated to greater tolerance of acidic water by crustaceans in field studies (Okland and Okland 1986), so higher Ca^{2+} content of the channel water may have reduced mortality resulting from failure of Ca^{2+} maintenance at low pH. Amphipods in the channels also were not confined to enclosures. If pH near the sediments was greater than pH higher in the water column, as in some lakes of Ontario (Oliver and Kelso 1983, Kelly et al. 1984, Dermott et al. 1986), amphipods may not have experienced as severe an exposure to acidic conditions as if held in the water column. Therefore, higher Ca^{2+} content and sediment buffering of pH may account for the survival for 3 mo of some H. azteca at pH 5 in an artificial channel.

Stephenson and Mackie (1986) estimated the 96-h LC50 to be pH 4.4 for H. azteca in Precambrian Shield lake water at $4^{o}C$. This compares to a 96-h LC50 of pH 5.5 obtained for H. azteca in ELA water at $23^{o}C$ in the present study. Temperature is known to affect toxicity of a wide range of chemicals; survivorship generally increases with a

decrease in temperature (Mayer and Ellersieck 1986). Survival of fish under acid stress is better at lower temperatures (Kwain 1975, Spry et al. 1981), and amphipods in Norwegian lakes tolerate low pH in cool waters better than in warm waters (Okland and Okland 1985). At low temperatures metabolic rates are slower. Turnover rates for water and ions in amphipods at 20°C are approximately double the rates at 10°C (Sutcliffe 1978). At 4°C, amphipods are not very active and therefore much less susceptible to the osmoregulatory stress of low pH compared to more active amphipods at 23°C. Mayer and Ellersieck (1986) noted that in most studies addressing the effects of temperature on the toxicity of chemicals, there was a 2X to 4X increase or decrease (depending on the specific toxicant) in LC50 per 10°C change, which equals a 4X to 16X increase or decrease over 20°C. The difference in the 96-h LC50 of H^+ concentration for H. azteca estimated at 23°C (this study) and at 4°C (Stephenson and Mackie 1986) was 12.6X in terms of umol H^+/L (LC50 of pH 5.5 to 4.4). Thus the difference in 96-h LC50's could be due to difference in temperature.

The tolerance of several gammarid amphipods to acidification has been assessed under laboratory conditions in other studies. Gammarus lacustris tolerated pH <5.5 poorly over exposure periods of 45-96 h (Borgstrom and Hendrey 1976), which is similar to the responses of H. azteca in the present study. After 10 days at pH 5.0, survivorship of G. fossarum was 60%, whereas at pH 6.0 it was ~90%. At pH 4.5, all animals were dead after 5 days (Meinel et al. 1985). This suggests that G. fossarum is more tolerant of acidification than H. azteca, which had only 10-50% survivorship at pH 5.2 after 5 days, and 0%

survivorship at pH 4.5 after 3 days in acidified aquaria. Brehm and Meijering (1982) showed that for a 12-h exposure to acidified brook water, survivorship was markedly reduced at pH 4.6 for G. fossarum, and pH 4.3 for G. pulex. It is difficult to compare these 12-h survivorship data with survivorship of H. azteca in the present study because of differences in the ranges of pH treatments (pH 3-5 for Brehm and Meijering vs. pH 4.5-5.6 for this study). However, because G. pulex had a lower critical pH than G. fossarum, and G. fossarum appeared more tolerant of acidification than H. azteca (above), G. pulex may be more tolerant of low pH than H. azteca, based on laboratory results. This conclusion is supported by data on the natural distributions of amphipods across pH gradients (Fig.6, Part II). Therefore, H. azteca is as or more sensitive to low pH than other species of amphipods.

Temperatures in the gammarid bioassays cited above were ~6-15°C, whereas for the experiments done here using comparable pH's temperatures were 15-20°, or a constant 23°C. Although slower responses (that is, lower mortality rates) would be expected at lower temperatures (see above), temperatures of all the experiments appear to be representative of the natural conditions of the species, so comparisons of tolerances to low pH for the purpose of making inferences about natural populations is valid.

PART IV. GENERAL DISCUSSION

The overall objectives of this study were to determine whether the ability of populations of H. azteca to survive is affected by acidification, and to estimate the minimum pH allowing long-term survival. An examination of the combined results of the natural distribution of Hyalella azteca at ELA (Part II), the five experiments done (Part III), and L302S historical observations (Part II) best addresses the consequences of environmental acidification to H. azteca. It is clear from the results of this study that acidification over the range of pH 7.4 - 4.5 strongly reduces survivorship of H. azteca. The relative abundance of natural populations in ELA lakes tends to decrease as pH decreases. In the experiments, which involved placing groups of amphipods into acidified lakes, limnocorrals or laboratory aquaria, the effects of pH on survivorship were highly significant, indicating a causal relationship between acidification and ability to survive. However, estimating the critical pH for this relationship is not straightforward because of variation in both the conditions of and responses to acidification among the approaches used to measure the effects of low pH.

One way to deal with information from the seven approaches used in this study is to determine for each one the highest pH at which survivorship is significantly lower than that at neutral pH, and then use these determinations to derive a common or composite estimate of critical pH. This estimate represents the point at which stress from

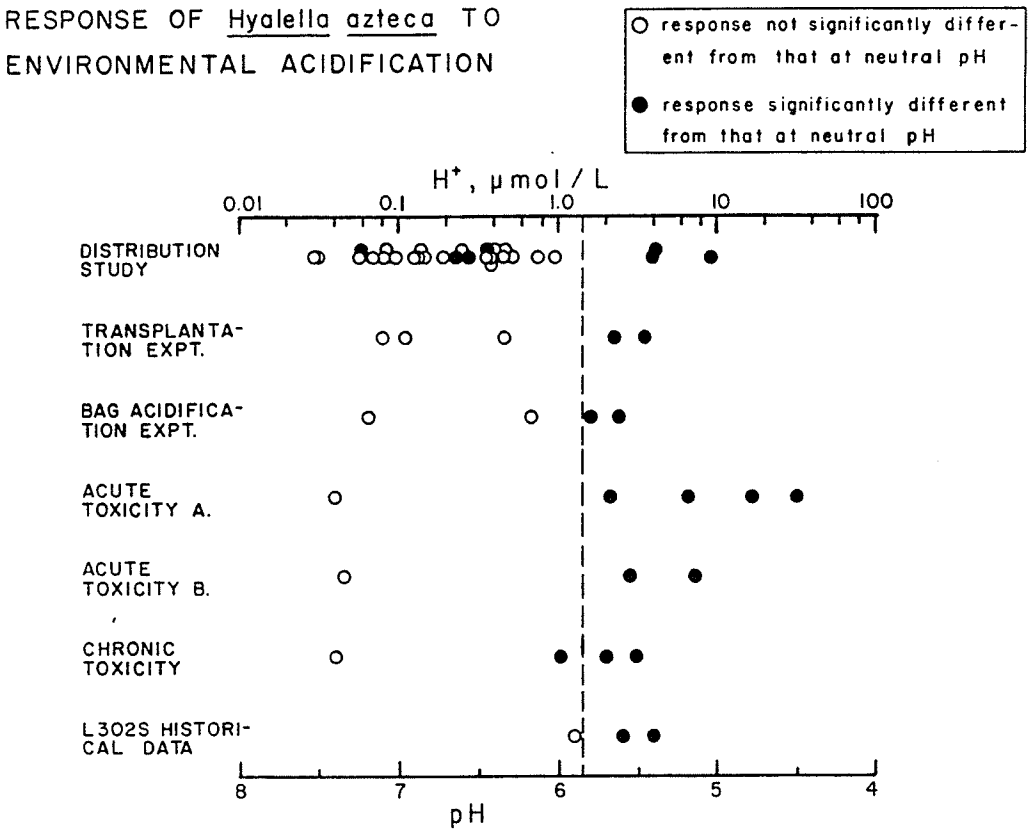
the toxicant is first detected in a population. The concentration of H^+ at which survivorship starts to decrease does not necessarily mean that long-term survival is unlikely, but it is still probably higher than the concentration (in umol/L) at which functions such as growth, reproductive output, behavior and various physiological activities are affected. Impairment of these functions can, in the long run, result in a reduction in survivorship of a population.

Mayer et al. (1986) analysed endpoints (i.e. survival, growth, reproduction, various physiological characters, behavioral traits) for 28 chemicals and seven fish species in 34 chronic toxicity tests, and determined that the NOEC ("no observable effects concentration"), which is the highest observed concentration of a toxicant that shows no significant difference from the responses measured in a control treatment (Maki 1979, Capizzi et al. 1985), for survival was up to five times the NOEC's for all other endpoints in ~95% of the comparisons. Therefore, using a statistic based on survivorship as a criterion for estimating the lowest pH (i.e. highest H^+ concentration) allowing survival over an indefinite time period is conservative.

Figure 37 summarizes observations of the response of H. azteca to concentrations of H^+ for each source of information used in this study. The response is in terms of survivorship, or presence in a lake (which is a gross measure of survivorship), and is indicated to be either different or not different from the level of response shown under control or neutral pH conditions. For the distribution study, the response level for neutral pH is presence in the lake, and the significantly different response, though not in a statistical sense, is absence. The L302S historical data also involve presence/absence

Figure 37. Compilation of responses to acidification observed for Hyalella azteca under field and laboratory conditions. Responses are in terms of either presence or absence in a lake (Distribution study, L302S historical data) or survivorship (acidification experiments). Dashed line indicates the pH below which responses appear to become significantly different from those at neutral or control pH in the same set of observations.

RESPONSE OF *Hyalella azteca* TO ENVIRONMENTAL ACIDIFICATION



responses. In 1983, when the mean epilimnion pH during the ice-free period was 5.9, H. azteca was present, and this is assumed applicable to previous years when the lake was unacidified. In 1984, when the pH had decéased to 5.6, H. azteca was no longer found and, this is considered a significant departure from the neutral state. For the acidification experiments, survivorship was determined as significantly different from that in control treatments by several statistical methods - ANOVA and multiple comparison of means (transplantation experiment, acute toxicity test A), comparison of 95% confidence intervals of P-L estimates of survival functions (bag acidification), and Kruskal-Wallis H-tests of survival times (acute toxicity test B, chronic toxicity test).

When all responses are shown in relation to pH (Fig. 37), overall it appears that below a pH of 5.85 (1.4 umol H^+/L) responses become significantly different from those of the neutral condition. Some exceptions should be noted. In the distribution study H. azteca was absent from four lakes having pH > 6.0. Absence from two of these lakes probably is due to low concentration of Ca (<1.24 mg/L). The high concentration of dissolved organic carbon or periodic decrease in pH, as suggested in Part II, may explain why H. azteca does not occur in the other two lakes. In the chronic toxicity test, survivorship of newborn amphipods at pH 6.0 was significantly lower than that in control treatments, likely due to handling and container stress. Thus, after allowing for certain biases and other factors that affect the responses to acidification, the results shown in Fig. 37 suggest that H. azteca cannot survive indefinitely in environments when pH remains <5.8 (the highest pH in all approaches, except the chronic

toxicity test, showing a significantly different response from that at neutral pH).

Inferring a pH tolerance limit for H. azteca is constrained by several conditions. The pH distribution in a lake can be heterogeneous both spatially and temporally (see Part I). In sediments, microbial reactions can increase pH 0.5 to 1.0 pH units (Kelso et al. 1982, Kelly et al. 1984, Dermott 1985), resulting in potential refugia from severely acidic conditions. Decrease of pH can also be periodic, occurring during spring runoff or individual acidic precipitation events (Gjessing et al. 1976, Nilssen 1980), and often only the upper layer of water becomes temporarily acidified. Such heterogeneity in the pH throughout a lake affects exposure to acidic conditions, and may restrict H. azteca to mean pH's above or below the pH which is limiting under chronic, unchanging conditions.

Tolerance to low pH by a species is influenced by temperature. The cooler the water, the slower the response. Thus, acidic conditions in the spring would not likely be as harmful as equivalent acidity in the summer. High concentrations of Na^+ and Ca^{2+} can also increase tolerance to low pH, although Havas (1981) suggested that Na^+ regulation is not affected until pH$\leq$ 5.0, which is well below the lowest pH H. azteca can survive in the long term. It is also likely that the range of Ca and Na concentrations of waters lying in regions susceptible to acidification (e.g. Precambrian Shield) may not be great enough to influence survivorship, except in very dilute lakes (Ca concentration $\leq$ 1.24 mg/L) and coastal lakes where concentration of Na can be more variable (Underwood et al. 1986).

Another potential factor in acidified lakes that can interact with

H^+ effects on H. azteca is metal concentrations. Metals can enter surface waters with atmospheric fallout near industrial centres, as leachates from watersheds, or from lake sediments (see Part I). Little information exists on whether metal concentrations occurring in acidified waters are toxic to H. azteca. Stephenson and Mackie (1986) pointed out that aluminum, the metal most commonly observed to increase with acidification, appears to be harmless or even beneficial to H. azteca at low pH. Cadmium has been found to impair survival and reproduction in gammarid amphipods at concentrations on the order of 1 ug/L (Lake et al. in Zauke 1982). However, Mackie (1985) reported that based on 96-h static bioassays, the lethal concentration of cadmium to H. azteca is at least two orders of magnitude higher than the levels found in Muskoka (Ontario) area lakes at pH 5.0. (The average concentration of cadmium in Muskoka area lakes was approximately 50 ng/L [M. Stephenson, pers. comm.].) In general, for other metals, concentrations that are toxic to amphipods in laboratory studies tend to exceed concentrations encountered in acidified waters, except for lakes receiving direct fallout from smelters or drainage from acid mines.

The responses to acidification shown by H. azteca from ELA confirm those observed by Stephenson and Mackie (1986) for southern Ontario. Although these authors suggested that pH 5.6 was the lower limiting pH, the present study indicates pH 5.8 as a lower limit. However, these two estimates are not necessarily in disagreement. The estimate from this study was based on (a) presence/absence data from lakes with $5.4 \geq pH \geq 6.0$ and provided no information on lakes with pH between

5.4 and 6.0; and (b) experimental data showing significantly lower survivorship at pH $\leq$ 5.8, which does not imply that extinction is imminent. Concluding that acidification below 5.8 would likely restrict long-term survival of H. azteca is therefore a conservative estimate requiring the least number of assumptions about the sensitivity of H. azteca to H^+.

The sensitivity of H. azteca to acidification below pH 5.8 is similar to that of other benthic crustaceans which, as a group, are among the earliest aquatic organisms to respond to lake acidification (see Parts I and II). Existing information on the lower limits of tolerance of pH for natural populations of benthic crustaceans (Fig. 6) indicates that acidification to below pH 5.5 would cause the disappearance of many of these species, especially groups of smaller animals such as the amphipods, notostracans and mysids. According to Jeffries et al. (1986), a considerable proportion (25%) of lakes surveyed in eastern Canada had pH $\leq$ 6.0, and about 30% of lakes had low acid neutralizing capacity (ANC) ($\leq$ 40 ueq/L). Thus, these lakes are vulnerable to acidification due to acid deposition. A substantial proportion of lakes in several regions of the eastern United States also had pH$\leq$ 6.0 or ANC $\leq$ 40 ueq/L, although the percentages were lower than in Canada (Linthurst et al. 1986). Populations of H. azteca are therefore threatened in a large number of lakes in eastern North America. They may in fact, have been eliminated from lakes in eastern Canada with pH $\leq$ 5.5, or an estimated 1-63% of all lakes, depending on subregion surveyed (Jeffries et al. 1986).

Elimination of benthic crustaceans from acidified waters would be significant because it could have important effects on the aquatic

food web. Prey species of lake trout, including the opossum shrimp (Mysis relicta), crayfish (Orconectes virilis), fathead minnow (Pimephales promelas) and pearl dace (Semotilus margarita), were severely reduced or eliminated as pH fell to 5.0 in an experimentally acidified lake (Schindler et al. 1985). Although lake trout were still numerous at pH 5.0, their condition factor declined substantially and the frequency of cannibalism increased, indicating a high level of stress (Schindler et al. 1985). The amphipod Gammarus lacustris, the tadpole shrimp Lepidurus arcticus, and several species of gastropods, the most important food organisms for trout in Norway, became rare or absent from lakes with pH<6.0 (Borgstrom and Hendrey 1976, Okland,J. 1980, Okland,K. 1980). In lakes with trout depending on these species, their loss due to acidification could cause a 10-30% reduction in trout production and a decline in quality of the more acid tolerant trout (Okland and Okland 1980). Thus, the elimination of H. azteca, a species widely consumed by fishes, could lead to similar disruptions of food webs.

Hyalella azteca may be useful as an early indicator of ecological effects of acidification. Because the species is found in almost any standing body of water (except naturally acidic or very low calcium waters), its absence or disappearance from a culturally acidified lake could be considered as unnatural. Stephenson and Mackie (1986) and Schindler and Mills (1986) suggested that H. azteca should be included in monitoring programs assessing the effects of acidification in North America. The results of the present study support this position.

Further research should address whether lake sediments insulate amphipods from the lower pH's of overlying waters. Amphipods living near such sediments would not be exposed to the same conditions of epilimnetic waters which are commonly measured in surveys. Second, the lower tolerance limit of pH for H. azteca could be estimated more precisely with in situ life table analyses of natural populations held under acidified conditions, in order to minimize mortality due to artificial factors.

PART V. SUMMARY

The effects of environmental acidification were assessed for the freshwater amphipod Hyalella azteca by (a) examining the relationship of pH to natural distributions of populations, and (b) experimentally determining the responses to acidification. The main results of these studies are :

1) pH and calcium concentration are the two primary factors affecting presence and relative abundance of H. azteca in lakes of the Experimental Lakes Area. H. azteca was not found in any of the 5 lakes that had pH 5.0 - 5.4 or concentration of Ca <1.24 mg/L, whereas in 29 out of the 31 remaining lakes with pH >6.0 and concentration of Ca >1.24 mg/L H. azteca was present.

2) Survivorship of amphipods transplanted into lakes was lower ($P<0.05$) in lakes with pH 5.66 and 5.45, than survivorship in lakes with pH 6.32, 6.92 and 7.07. The effect of H^+ concentration on survivorship after 19 days was highly significant ($P<0.001$).

3) Survivorship of amphipods held in 500-L bags of acidified lake water at pH $\leq$ 5.8 for 26 days was lower ($P<0.05$) than survivorship in unacidified (pH 7.18) bags. At pH 5.62, 5.80 and 6.12 survivorship of newborn amphipods was lower ($P<0.05$) than that of adults and juveniles.

4) Effect of H^+ concentration on 96-h survivorship of H. azteca in 4-L aquaria of acidified lake water was significant ($P<0.025$), and survivorship in all acidified treatments (pH $\leq$ 5.6) was lower ($P<0.05$) than that in control (pH 7.38) treatments. The 96-h LC50 was estimated as pH 5.5. There was a weak correlation between increasing size, as measured by head length, and survival time of adult and juvenile amphipods in acidified treatments in one experiment, and no correlation in another.

5) Hyalella azteca collected from the LaSalle River, having water of greater ionic strength than ELA water (60 vs 3 mg Ca/L), showed lower ($P<0.005$) survivorship in acidified (pH 5.15,5.65) than in unacidified (pH 7.36) 4-L aquaria, whereas amphipods from ELA did not. However, poor survivorship of ELA amphipods in the unacidified treatment indicated that low pH alone did not account for all mortality.

6) Amphipods that were $\leq$ 3 days old and placed into acidified (pH 5.51, 5.70 and 5.99) 4-L aquaria survived more poorly ($P<0.005$) than those in control (pH 7.44) aquaria, although mortality due to handling and containment again was evident.

These results indicate that pH's <5.8 strongly affect the ability of H. azteca to survive, and that populations will decline in habitats where the pH remains below this level. Natural populations in a considerable proportion of lakes in eastern North America may, therefore, be affected by acidification.

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