Final Report

# AN INTEGRATED WATERSHED STUDY: AN INVESTIGATION OF THE BIOTA IN THE EMERALD LAKE SYSTEM AND STREAM CHANNEL EXPERIMENTS

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## *Abstract*

Changes in populations of stream algae and invertebrates have been associated with acid deposition in Europe, Canada, and the northeastern United States. Alpine regions in the western United States have very limited capacity to neutralize acids, and acidification of surface waters has been reported from montane lakes and streams. It is not known, however, if these pulsed acidifications are having any effects on the aquatic biota of mountainous areas in western North America. In the summer of 1986 we performed a series of stream channel experiments to determine the effects of different levels of acid input on common invertebrates and algae found in High Sierra streams. Twelve small channels, each 2.4 m X 20 cm X 20 cm, were constructed next to the Marble Fork of the Kaweah River in Sequoia National Park. Water from the Marble Fork was diverted through these channels and channels were stocked with natural substrates, algae, and invertebrates. During acid pulses sufficient acid was added to one set of four channels to reduce pH to 4.6, another set of four channels received sufficient acid to reduce pH to 5.2, and a third set of four channels was unmanipulated, acting as a control at pH 6.5. Every two weeks acid was added for eight hours in late morning and afternoon to simulate the magnitude and timing of acidic summer rain storms. Benthic densities of invertebrates and algae in each channel were measured before and after acid additions, and invertebrate drift was measured before, during, and after acidification. Four experiments were run through the summer and early autumn.

The response of the stream periphyton community was measured during one of the experiments. Declines in total diatom abundance were noted in acidified channels relative to controls one week after acid additions. Periphyton responses to acidification were species-specific. Achnanthes minutissima, Taxon 99396-3SN (Cymbella failaisencis or Gomphonema sp.), Gomphonema subclavatum, Achnanthes levanderi, and Fragilaria vaucheri were deleteriously affected by acid additions, whereas Eunotia spp. tended to be more abundant in acidified than control channels.

Baetis was the most sensitive of the invertebrate taxa, drifting at significantly higher rates in treatment than control channels. The percentage of dead Baetis in the drift increased 2.5 to 8x during acidification. Similar responses were noted in other Ephemeroptera. Drift rates of Epeorus and

Paraleptophlebia increased significantly in response to acid pulses in two experiments, particularly when pH was reduced to 4.6. Drift rates of the stonefly Zapada increased in pH 4.6 channels relative to 5.2 and control channels during the acid pulse in one experiment. Other taxa, such as chironomid larvae, exhibited less consistent responses to acidification, although drift of chironomid larvae increased in pH 4.6 channels in one experiment. Benthic densities of Baetis declined to 10 to 16% of controls 2 days after acidification. Paraleptophlebia also declined in response to acid pulses in one experiment. Other taxa either exhibited no statistically significant responses or were present in too low densities for statistical analyses.

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## *Disclaimer*

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## *Summary and Conclusions*

Many lakes and streams in the High Sierra are very dilute and weakly buffered; consequently they are quite sensitive to increased inputs of acid. Episodes of surface-water acidification have been recorded for some of these High Sierra waters but effects on the aquatic biota are largely unknown. Changes in invertebrate and algae populations have accompanied acidification of stream waters in other parts of the world. Because some stream invertebrates and algae show substantial responses to acid inputs, stream invertebrate and algae populations can act as sensitive indicators of environmental stress. Because the effects of cultural acidification depend on the chemistry of receiving waters, the biogeochemistry of affected watersheds, the species composition of aquatic plant and animal communities, and the genetic composition of plant and animal populations, it is necessary to examine the responses of the aquatic biota to acid inputs for each region separately. Unfortunately, there is little detailed data on the effects of acidification on aquatic plants and animals in the western U.S. For that matter, data on the acid sensitivity of stream algae and invertebrates in the western U.S. is practically nonexistent. Such data are needed, however, to assess the current status of High Sierra stream systems, to act as a baseline for comparison to future changes, and to make predictions regarding the probable effects of acidification.

In the summer of 1986 we performed a series of stream channel experiments to determine the effects of different levels of acid input on common invertebrates and algae found in High Sierra streams. Twelve small channels, each 2.4 m X 20 cm. X 20 cm., were constructed next to the Marble Fork of the Kaweah River in Sequoia National Park. Water from the Marble Fork was diverted through these channels and channels were stocked with natural substrates, algae, and invertebrates. During acid pulses sufficient acid was added to one set of four channels to reduce pH to 4.6, another set of four channels received sufficient acid to reduce pH to 5.2, and a third set of four channels was unmanipulated, acting as a control (pH ca. 6.5). Every two weeks acid was added for eight hours in late morning and afternoon to simulate the magnitude and timing of acidic summer rain storms. Benthic densities of invertebrates and algae in each channel were measured before and after acid additions, and invertebrate drift was measured before, during, and

after acidification. Four experiments were run through the summer and early autumn.

Additions of acid resulted in declines in diatom abundance and shifts in the relative abundances of diatom species. Achnanthes minutissima, Taxon 99396-SN, Fragilaria vaucheri and, perhaps, Gomphonema subclavatum and Achnanthes levanderi were reduced by acid inputs, whereas Eunotia spp. tended to be more abundant in acidified than control channels. There were no differences in diatom live to dead ratios among treatments during or after acid pulses. The only common alga that was not a diatom, Zygnema sp., showed no response to acid inputs. Among the invertebrates, Baetis nymphs showed the most sensitive responses to declines in pH, showing increases in drift density and declines in benthic density with decreasing pH. Other mayfly species, such as Epeorus sp. and Paraleptophlebia sp., showed increases in drift activity with increased inputs of acid, particularly when pH was reduce to 4.6. In an experiment spanning nearly a month, benthic densities of Paraleptophlebia declined with decreasing pH. A caddisfly (Amiocentrus) and a stonefly larva (Zapada) also showed increased drift during pulses of strong acid (channel pH = 4.6). Chironomids and most other taxa showed few consistent responses to acidification, although chironomid drift was enhanced in strongly-acidified channels (pH = 4.6) in one experiment. The large proportion of dead individuals in the drift of channels showing significant acidification effects emphasized the importance of acid toxicity in enhancing drift rates for sensitive taxa (Baetis, Epeorus).

These data allow us to determine the effects of acid inputs on common stream invertebrates and algae found in the High Sierra. These data pinpoint sensitive taxa which can act as indicators of environmental stress, and also allow us to predict changes in community composition resulting from the early phases of cultural acidification.

## *Recommendations*

Stream invertebrates and algae show a variety of responses to acidification. In our studies we have measured the responses of common stream algae and invertebrates to pulsed inputs of acid. These data allow us to calibrate our monitoring programs so that we can pinpoint sensitive taxa which can act as indicators of environmental change owing to acidification. This information can help us evaluate the current status of High Sierra streams, and allow us to make predictions regarding the effects of increased acidic inputs on High Sierra systems. Based on our past investigations we recommend that the CARB do the following:

1. Continue qualitative monitoring of aquatic invertebrate populations in streams in the Marble Fork basin. Our investigations indicate that the disappearance of Baetis, in particular, may act as a potent early-warning indicator of acid stress.

2. Fund laboratory and field studies to experimentally examine the pH tolerances of different diatom species. Experimental data on the pH tolerances of diatoms are virtually nonexistent, despite the importance of diatom microfossils in reconstructing the pH histories of lakes.

3. Experimentally examine the effects of acidification on trophic interactions. The effects of acidification on stream algae and invertebrates may have important repercussions for their predators and prey. For example, reductions in stream invertebrates may cause algal blooms, thereby degrading water quality, or may result in significant alterations in the food base for fish. Potential experiments could examine the effects of acidification on stream invertebrates, and, ultimately, the fish which prey upon them.

4. Experimentally examine relationships between flooding and acidification. Because most inputs of acid are associated with potential flooding events (snow-melt, heavy rains) it is important to distinguish the effects of acidification from the effects of natural disturbances associated with flooding.

5. Integrate data on the effects of acid inputs on stream invertebrates and algae with data from other projects in the Integrated Watershed Study (IWS) to (a) provide predictions of changes in biological populations with changes in surface water chemistry and (b) assist in setting deposition standards which would prevent damage to aquatic resources in the High Sierra.

Our field experiments have shown the responses of aquatic invertebrates to increased acidic inputs. CARB-sponsored modeling efforts, which integrate hydrological, meteorological, and biogeochemical data, are examining the responses of surface-water chemistry to different acid deposition scenarios. The models' output will allow investigators to predict the effects of changes in the loading of acidic species in wet and dry fall on the chemical composition of lake and stream water. These changes in surface-water quality in lakes and streams can be used in conjunction with the dose-response relationships developed for algae and invertebrate populations to predict biological responses to changes in water quality and, ultimately, acid loading. Model outputs can be used in empirical equations that relate surface-water pH and alkalinity to biological response.

The lakes and streams of the High Sierra are the most sensitive receptors for acidic wet and dry fall in the State of California. This means that they will be the first ecosystems to change with increases in acid deposition. Changes in surface-water chemistry will result in changes in biological populations. The CARB should consider the implementation of deposition standards for acidic pollutants in sensitive areas. Such standards would prevent the deterioration of surface-water quality that would lead to changes in fish and invertebrate populations. The modeling efforts mentioned above would allow scientists to determine the effects of different acid-loading levels on surface-water chemistry and, in turn, on biological populations. Deposition standards could be defined by "critical loadings" of acids, nitrates and sulfates that would result in deleterious changes in water quality. The regulatory strategy to achieve those standards would then have to be formulated and implemented by the CARB to achieve this "level of protection".

## *Introduction*

### *Background*

Many lakes and streams in the High Sierra are sensitive to acidic deposition, and acid precipitation is known to occur in these mountains. The lakes and streams in the Emerald Lake watershed (Sequoia National Park) are representative of subalpine and alpine Sierran waters, and changes in the chemistry and biology of these waters provide excellent early-warning signals of acid deposition's effects. Changes in stream algae and invertebrate communities have accompanied cultural acidification in the northeastern United States, Canada, and Scandinavia. These community effects include declines in species diversity, changes in species composition, declines in invertebrate abundance, increases in algal standing stocks, and the local extinction of some species. Stream invertebrates and algae show some of the first responses to acidification, because acid pulses in streams often precede chronic acidification in bodies of freshwater. Stream invertebrate and algae populations, then, are sensitive indicators of environmental stress owing to acidic inputs. The work on stream algae and invertebrates described below is an integral part of the Integrated Watershed Study (IWS) supported by the California Air Resources Board. This research has allowed us to characterize the responses of stream algae and invertebrates to pulsed acidic inputs. This research calibrates our monitoring data set by allowing us to determine which taxa are particularly sensitive to acid inputs, i.e. indicating the taxa to focus our attention on in monitoring efforts. In addition, these studies allow us to predict the effects of increased acidification on some characteristics of High Sierra streams, which should ultimately allow the formulation of deposition standards for protecting sensitive waters. Data collected during these experimental studies will be used in concert with data from other IWS projects to assess the current status of, and stresses to, High Sierra waters.

### *Literature review and justification for approach*

Reduction of pH in stream waters, caused by acidic deposition, has many effects on benthic invertebrate and algal communities (Hall et al. 1980, Raddum 1980, Burton et al. 1982, 1985, Hall and Ide 1987). Increased acidity has been shown to decrease the diversity of benthic invertebrate and algal communities; to decrease the abundance of benthic invertebrates; to increase

the abundance of periphyton; and to alter invertebrate life histories (Friberg et al. 1980, Roff and Kwiatkowski 1977, Almer et al. 1978, Okland and Okland 1980, Mackay and Kersey 1985, Simpson et al. 1985, NRC Canada 1981, Hall and Likens 1979, Howells et al. 1983, Bell 1971, Fiance 1978, Zischke et al. 1983, Burton et al. 1985, Lacroix 1985, Hendrey 1976, Kimmel et al. 1985, Hornbach and Childers 1987). Increased acidity can change community composition as sensitive organisms are replaced by more tolerant ones (Leivestad et al. 1976, Henriksen et al. 1980, Eriksson et al. 1980, Burton et al. 1982, Zischke et al. 1983, Hunter et al. 1985, Hall et al. 1980, Hall and Ide 1987). For example, many mayfly nymphs are particularly sensitive to acidic inputs, whereas alderfly and some chironomid larvae are relatively tolerant of acidic conditions (Sutcliffe and Carrick 1973, Grahn et al. 1974, Bell 1971, Friberg et al. 1980, Singer 1981, Hagen and Langeland 1973). Similarly, many species of diatoms will disappear as pH decreases, whereas other diatoms (e.g. Tabellaria, Eunotia) or filamentous algae (particularly Mougeotia) increase with declining pH (Hendrey 1976, Hall et al. 1980, Muller 1980, Turner et al. 1987). In addition, acidic inputs can affect organisms through their influence on other organisms in the trophic web. For example, fish can be eliminated by acid deposition with important repercussions for the invertebrates on which they prey (Henrikson and Oscarson 1978, Eriksson et al. 1980). Similarly, the elimination of sensitive invertebrate grazers by acid inputs may result in algal blooms (Griffiths 1987, Hendrey 1976).

Mobilization of toxic metals, especially aluminum, occur in low pH waters, and these metals have several deleterious effects on algae, invertebrate, and fish populations (Hall et al. 1980, Howells et al. 1983, Hall et al. 1987, Starodub et al. 1987, Burton and Allan 1986, see review in Cooper et al. 1988). For fish and invertebrates some of these changes may be mediated through effects on ion-exchange phenomena, blood chemistry, and respiratory structures (Burton et al. 1985, McDonald 1983, LaCroix 1985). Particular life history stages of stream organisms can be especially sensitive to acid stress (Menendez 1976, Daye and Garside 1977, Burton et al. 1985, France and Stokes 1987).

Increased drift of benthic invertebrates often follows acidification of streams (Hall and Likens 1979, Hall et al. 1980, Overrein et al. 1980, Singer 1982, Zischke et al. 1983, Hopkins et al. 1988, Ormerod et al. 1987). Mayflies (family Ephemeroptera) are especially sensitive to low pH waters (Bell 1971,

Sutcliffe and Carrick 1973, Fiance 1978, Howells et al. 1983, MacKay and Kersey 1985) and often show a higher drift rate than other benthic invertebrates (Overrein et al. 1980). Since many stream fish, such as trout, feed primarily on drifting invertebrates (Jenkins et al. 1970), the changes in stream acidity can greatly affect their food resources.

Acidic precipitation (pH 4-5) sometimes falls on the Sierra Nevada of California during the summer and autumn (Melack et al. 1982). During periods of heavy rainfall, watersheds may not be able to neutralize acidic inputs and short-term pH depressions of lake and stream waters may occur (Dillon et al. 1984, Melack et al. 1987). These episodic depressions may have a substantial impact on the aquatic biota well before long-term declines in pH are detectable (Servos and Mackie 1986).

A variety of approaches have been used to assess the effects of increased acidity on freshwater organisms. For example, surveys or laboratory bioassays have often been used to determine the effects of acidification on benthic invertebrates (Sutcliffe and Carrick 1973, Singer 1981, Raddum 1980, Okland 1980, Okland and Okland 1980, Roff and Kwiatkowski 1977, Bell 1970). The survey approach involves comparisons of the distribution and abundance of aquatic organisms in relation to the pH of the waters in which they live. However, causation cannot be inferred from correlation, for relationships can be confounded or obscured by the effects of other, perhaps unmeasured, variables. In the same geographical area, streams and lakes not affected by acidification may be different in many ways from those experiencing decreases in pH, thus obviating the usefulness of comparisons. Laboratory bioassays, in which pH tolerances of various organisms are determined (Bell 1971, Hollet et al. 1986), cannot adequately represent the complex biological and chemical interactions that occur in an organism's natural habitat. It is difficult to extrapolate the results obtained in the laboratory to organisms in their natural environments. Laboratory microcosms and mesocosms, which mimic natural assemblages, sediments, and water (Tonnessen 1983) have been used as compromises between bioassay-type and field experiments. Although certainly an improvement, in some cases microcosms quickly diverge from larger natural systems as some processes, such as mixing, are hindered or modified by the small size of the container. Also, other natural conditions, such as weather changes, are difficult to simulate.

Stream ecologists have increasingly used cages to analyze species interactions (Peckarsky and Dodson 1980). It is becoming increasingly apparent, however, that cages alter abiotic conditions, such as current and sediment conditions, and provide increased surfaces for insect and algae attachment, thereby confounding the interpretation of experimental results (Walde and Davies 1984). Furthermore, chemicals experimentally added to in situ cages are quickly washed to other parts of the stream. Results from studies using artificial substrates cannot always be extrapolated to natural substrate conditions owing to differences in the responses of organisms to artificial vs. natural substrates (Rosenberg and Resh 1982).

As alternatives, whole system acidification experiments have been performed (Hall and Likens 1979, Hall et al. 1980, Schindler 1980a, b, Schindler et al. 1985, Ormerod et al. 1987), but it is difficult to find replicate systems both for examining inter-system variability and for concurrent controls. Using separate streams or lakes as controls does not provide good replication because of stream-to-stream or lake-to-lake variability. It is often difficult to determine densities of the biota of whole lakes and streams because the organisms in these systems are highly aggregated (Allan 1984, Hurlbert 1984, Morin 1985). A number of studies have indicated that large numbers of samples are needed to calculate density estimates for many freshwater, particularly benthic, organisms (Resh 1979, Allan 1984). Whole stream experiments often use a downstream-manipulative versus upstream-control experimental design. The problem with this design is that it does not allow for the replication of treatments (Allan 1984, Hurlbert 1984). Furthermore, upstream and downstream areas are not independent, as upstream processes affect downstream results. Finally, whole-system manipulations are logistically difficult, requiring large amounts of manpower, money and time. They can also have effects that are biologically and socially unacceptable, potentially altering whole systems irreversibly.

Experimental stream channels and large lake enclosures, then, seem to constitute good compromises between the naturalness of whole system manipulations and the control and replication of laboratory experiments; they approximate natural conditions while providing the replication needed for statistical analyses. Artificial stream channels have been successfully used in past studies to examine the effects of increased acidity on stream systems (Zischke et al. 1983, Burton et al. 1985, Servos and Mackie 1986, Griffiths

1987). Based on our analysis of the literature and our experience with stream experiments we felt that the stream channels used in these studies provided the best compromise in terms of replicability, ease of manipulation and size, and duplication of nearly natural conditions. Simulations of acidic rain events (containing sulfuric and nitric acids), of similar acidity to those known to occur during summer storms (Melack et al. 1982), were performed in stream channels operated near Emerald Lake. These acidic rains, together with acidic snow-melt, comprise the primary acid inputs into Sierra Nevada aquatic systems. Except during these events, the pH of freshwater habitats in the western Sierra Nevada is near a pH of 6.2-6.5. Most previous stream or stream channel experiments have added acid continuously to stream sections or channels for periods of time (Hall et al. 1980, Burton et al. 1985). Although these experiments may indicate biotic responses to severe, continuous acid exposure, they provide us with little information regarding the effects of episodic acid deposition on streams.

We measured benthic (bottom) densities of invertebrates and algae, as well as invertebrate drift rates, in response to decreased pH, because benthic densities and drift are indicators of acid stress (Fiance 1978, Hall and Likens 1980, Hall et al. 1980, Howells et al. 1983, Hall et al. 1987, Hendrey 1976). The replicated stream channels used in this study allowed more precise evaluation of the effects of acid deposition on the stream biota and allowed us to predict the effects of acidic inputs on this system.

Information from these stream channel experiments revealed which invertebrates in Sierra Nevada aquatic habitats were particularly vulnerable to acid stress. These indicator organisms are being closely observed, together with changes in stream chemistry, in order to monitor the effects of acidic inputs on a representative Sierra Nevada running-water system, i.e. the streams flowing into and out of Emerald Lake. In this report we describe the results of stream channel experiments examining the effects of acidification on stream benthos and drift.

*Objectives*

The overall objective of this research was to determine the responses of invertebrates and algae commonly found in High Sierra streams to inputs of acid under natural conditions. We achieved this objective by conducting field experiments in streamside channels stocked with natural substrates,

invertebrates, and algae. In these experiments we exposed replicate channels to differing levels of acidity, and monitored the response of stream chemistry, invertebrate and periphyton (algae) densities and drift (organisms carried downstream in the current) to acidic inputs. These data allow us to make specific predictions as to the effects of increased acidic inputs on the biota of High Sierra streams. By using these data, in conjunction with monitoring data on stream invertebrates and algae from a representative, sensitive basin (Emerald Lake basin), we will determine the current status of, and stresses to, aquatic populations in High Sierra streams.

*History of project*

The stream channel project is an extension of on-going limnological investigations being conducted at Emerald Lake with support from the California Air Resources Board (CARB). We began CARB-funded limnological investigations at Emerald Lake in the summer of 1984. The Emerald Lake watershed was chosen by the California Air Resources Board as the site for its Integrated Watershed Study because it is sensitive to acid deposition, representative of subalpine and alpine waters of the Sierra Nevada, and accessible at all times of the year. The overall purpose of the Integrated Watershed Study is to assess the current status of a representative Sierran system and to determine current and potential responses to acidic inputs. The ongoing ARB limnological investigations at Emerald Lake include: monitoring water chemistry, planktonic and benthic populations, as well as performing experiments to predict the effects of increased acidification on stream and lake organisms (Melack, Cooper, and Holmes 1987; Cooper, Jenkins, and Soiseth 1988).

We have studied stream invertebrate and algae populations as part of the Melack, Cooper, Holmes project since the summer of 1984. We collected invertebrate samples from Emerald Lake's inlet and outlet streams during the summers of 1984 - 1987, and we collected additional samples from the outlet stream during other seasons. Stream algae samples were taken from these streams during the summers of 1984 - 1986. This basic measurement program will allow us to use the aquatic biota as indicators of the current status of Emerald Lake's inlet and outlet streams, and will allow us to examine correlations between biotic changes and changes in stream chemistry. In the summer of 1985 we also performed a pilot experiment using streamside channels

to assess the sensitivity of High Sierra streams to acidic conditions, and to predict the effects of acid deposition on these streams (Melack et al. 1987, Hopkins et al. 1988). This experiment was conducted along the Marble Fork of the Kaweah River instead of in the Emerald Lake streams for the following reasons: (1) There was not sufficient water in the Emerald Lake inlet or outlet streams to provide the water necessary to feed experimental stream channels. (2) Addition of effluent water from experimental channels to the Emerald Lake streams could affect their chemistry and biota. In contrast, the Marble Fork is usually much larger and would be, therefore, less affected by chemical inputs. In our preliminary experiment effluent from stream channels had a negligible effect on the pH of the Marble Fork. (3) Operation of the stream channels would interfere with our monitoring program on the Emerald Lake streams. (4) All of the common taxa present in the Emerald Lake streams are also present in the Marble Fork. The Marble Fork has a variety of additional taxa, some of which are reportedly sensitive to acid stress. The results of the Marble Fork experiments are not only applicable to the Emerald Lake system, because of the similarity in their flora and fauna, but are more generally applicable to streams in the High Sierra, given the high diversity of organisms found in the Marble Fork. (5) The stream channels are much less visible to the public at the Marble Fork site than at the Emerald Lake site. This reduced the aesthetic and visual impacts of the channels, and also reduced the probability of vandalism.

Unfortunately, because of a drought during the summer of 1985, flows in the Marble Fork became very low and there was only sufficient flow to feed four of the 12 experimental stream channels. Acid was added to two of the channels in sufficient quantities to reduce pH to 5.0 for 6 hours, and the other two channels functioned as controls (pH ~ 6.2). Benthic samples were taken from channels the day before and two days after acid additions, and drift samples were taken at periodic intervals before, during, and after acid additions. Because the Marble Fork dried up completely shortly after we added acid to half of our channels, we had to discontinue this experiment. The results of this experiment were best regarded as preliminary because of the small number of replicates for each treatment, the short duration of the experiment, and complications introduced by the Marble Fork drying. This experiment showed, however, that we could set up replicated stream channels with similar biota, and that we could successfully manipulate levels of acidity

in stream channels. Furthermore, the preliminary results from this experiment showed that some taxa showed sensitive responses to acid additions. For example, Baetis sp. drift in acidified channels was 7 times higher than in control channels during acid additions, and the percentage of drifting baetids that were dead was higher in acidified than in control channels.

Building on the experience gained during the 1985 field season we acquired CARB funding to conduct a series of well-replicated, rigorous stream channel experiments in the summer of 1986. These experiments allowed us to determine and predict the effects of increased acid inputs on the biota of High Sierra streams. A large advantage of channel experiments is that they allow the precise control of experimental parameters (e.g. acid levels) while exposing the stream community to natural conditions, thereby combining the control of a laboratory assay with the biological reality of whole system manipulations. These experiments were conducted next to the Marble Fork of the Kaweah River for the reasons cited above. Because of the similarities between the Marble Fork's biota and the biota of the Emerald Lake streams, we can use these data to determine the sensitivity of organisms from Emerald Lake streams to acid inputs.

The stream channel project of the Integrated Watershed Study was funded by the CARB for one and one-half years (June 1986 to December 1987). A series of four, short-term (2 weeks) acidification experiments were performed during the summer of 1986. Because of problems in maintaining precise pH levels in the second experiment, we report the results of the first, third, and fourth experiments here. In these experiments we introduced acid to some experimental channels in pulsed additions to simulate low-pH rain events. Most previous stream or stream channel experiments have added acid continuously to stream sections or channels for periods of time. Although these experiments may indicate biotic responses to severe, continuous acid exposure, they provide us with little information regarding the effects of episodic acid deposition on streams. Currently, most pH depressions in Sierra Nevada lakes and streams are temporary, and result from episodic inputs of acid, during rain events or snow-melt. Because these episodic events can have large effects on the aquatic biota, the biota can be used as early-warning signals of environmental stress, even though stream or lake chemistry will show few responses at most times. In this study we added acid, in 8-hour pulses, to stream channels and examined the responses of the stream biota to these pulsed acidic inputs. The

results of such experiments are especially relevant in areas like California where acid deposition is just beginning to be recognized as a problem. In California, changes in the stream biota can be used as early-warning signals of cultural acidification. We summarize the results of our investigations below.

## *Materials and Methods*

Twelve experimental stream channels were set up next to the Marble Fork of the Kaweah River, in Sequoia National Park on the western side of the Sierra Nevada, California, U.S.A. (elevation 2720 m, latitude 36°35'N, longitude 118°40'W).

Channel design and sampling protocol were refined based on the experiment of the summer of 1985. Equipment was lifted by helicopter to the research camp on 24-25 June, 1986. On-site channel construction began 26 June and was completed on 8 July, although high discharge of the Marble Fork precluded final water diversion into the channels until 21 July. Flow was sufficient to run all 12 channels through 16 September and nine channels until the end of the last experiment on 1 October 1986.

Channels were similar in design to those of 1985. The bases consisted of two 1.2 x 2.4 meter plywood sheets treated with three coats of Varathane (nontoxic). These were each divided into six, 20 cm x 2.4 meter, channels with 20-cm high Plexiglas sides acting as partitions between channels (Fig. 1). A 6-cm high Plexiglas plate was affixed to the downstream end of each channel to insure adequate water depth throughout the channel. Immediately in front of each downstream plate were small retainers on the sides of the Plexiglas walls to hold drift nets (mesh size 250 um). The gradient in each set of stream channels was adjusted to provide a drop of 5 cm from the upstream to the downstream end of each channel.

Water was diverted from a pool in the Marble Fork approximately 40 m upstream and elevated 2 m above the channels. Two flexible plastic pipes transported the unfiltered water to a 0.5 cubic meter central reservior. Three 12.7-cm diameter, 5-meter PVC pipes carried water from the bottom of the front panel of the reservoir to the channels. Four PVC "T's" were placed at approximate 0.6-m intervals along each delivery pipe, and flexible plastic pipes (internal diameter 12 cm) carried water from each T to the stream channels. Flow into individual channels was regulated by 12.7-cm diameter butterfly valves that connected the delivery pipes to each of the pipes delivering water from the T's to experimental channels. During acidifications 2 30-liter plastic containers discharged acid solution through Tygon tubing to the appropriate delivery pipe. Precise control of acid flow was accomplished with the use of

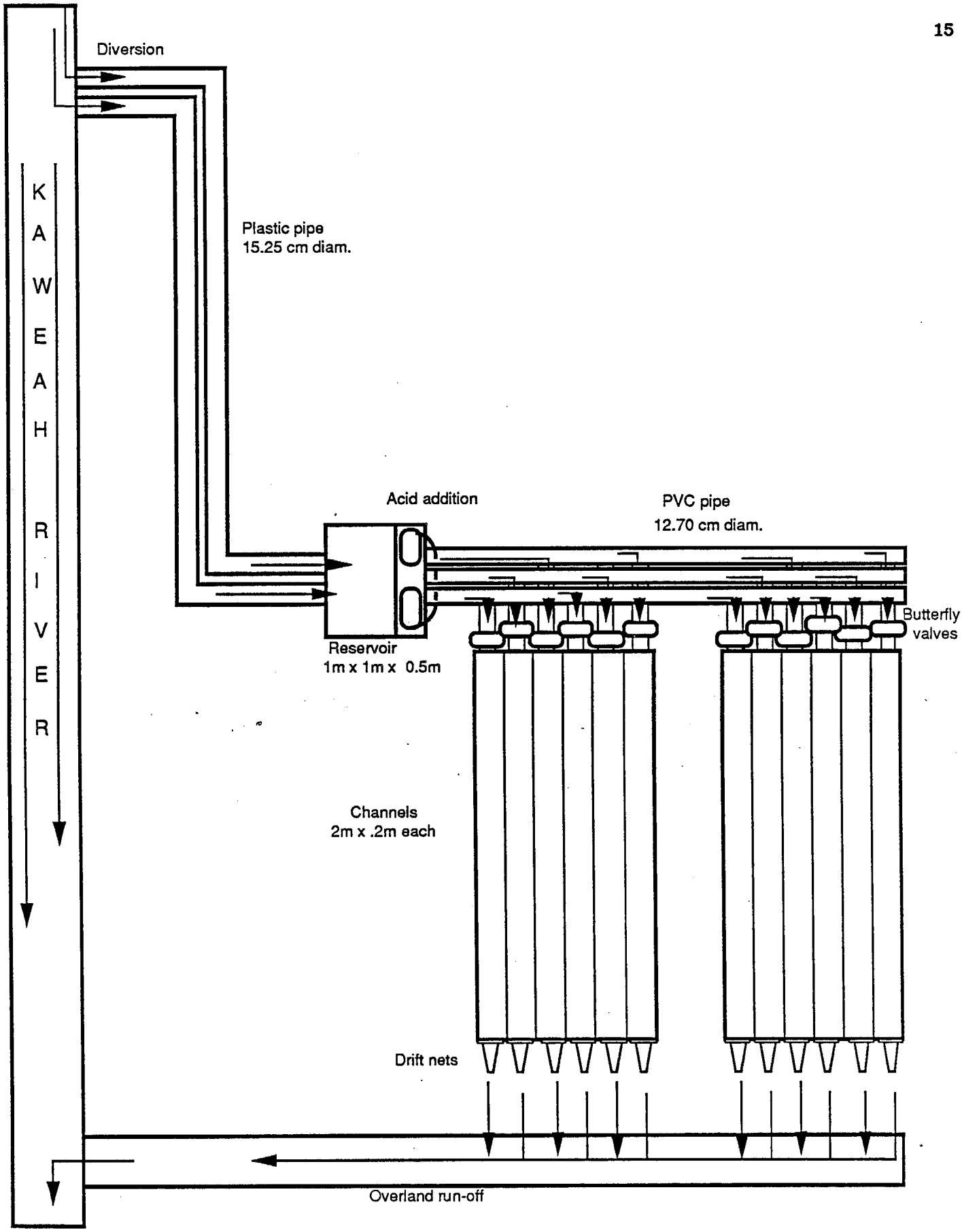


Figure 1. Design of replicate experimental stream channels set up along the Marble Fork of the Kaweah River. July-October 1986. Arrows indicate direction of water flow.

stopcocks mounted in the Tygon tubing. Discharge waters from the channels flowed through open plastic pipes back into the Marble Fork.

Acidification experiments were conducted on 4-8 August (Experiment 1), 19-22 August (Experiment 2), 3-6 September (Experiment 3), and 16-19 September 1986 (Experiment 4). Each experiment consisted of a single 8-hour pulse of three treatment levels of acid: pH 6.5 (control, no acid), pH 5.2, and pH 4.6. Experimental channels were acidified with solutions of nitric and sulphuric acids (1:1 by equivalents). Acid additions were conducted in late morning and afternoon (1000 - 1800 hrs), to simulate acid precipitation events which primarily occur in the afternoon in the summer months (Fig. 2). In two of the treatment channels (one each at pHs 4.6 and 5.2), pH was monitored continuously, with pH in remaining channels being measured every 60 minutes. Continuous pH measurements were made using portable pH meters and gel-filled electrodes. We measured the pH of water samples from other channels in the field with a Ross combination electrode (8104) and an Orion SH250 pH meter. pH was also frequently measured in effluent waters from channels, at the confluence of discharge from channels and the Marble Fork, and in downstream stretches of the Marble Fork to insure that acidified effluent from channels was not depressing Marble Fork pH levels.

Water samples were collected for chemical analyses in the laboratory before, during, and after acidification. Laboratory measurements of pH, which we used to verify the accuracy of the field measurements, were made on unfiltered samples with a combination electrode suitable for use in dilute waters (Sargent-Welch S-30072-15) and a Fisher Acumet 805 pH meter. Acid neutralizing capacity (ANC) was determined by incremental titration of the same sample with 0.1 N HCL. Filtered samples (Gelman AE glass-fiber filters) were analyzed by ion chromatography for nitrate and sulfate. Unfiltered subsamples which had been acidified in the field were analyzed for total iron and total aluminum by atomic absorption with the graphite furnace technique.

The stream channels were leached with stream water for 2 weeks and stocked with substrate from the Marble Fork. Six large rocks (approximately 9 cm diam.) and 2 cm of gravel (0.5-2.0 cm) and sand were placed in each channel. Invertebrates were seeded in, and also allowed to naturally colonize, all channels for a two-week period prior to the first, second, and third acid events. Invertebrates were collected by gentle kick sampling into drift nets (mesh size 250 um), placed into buckets of water, and divided equally among

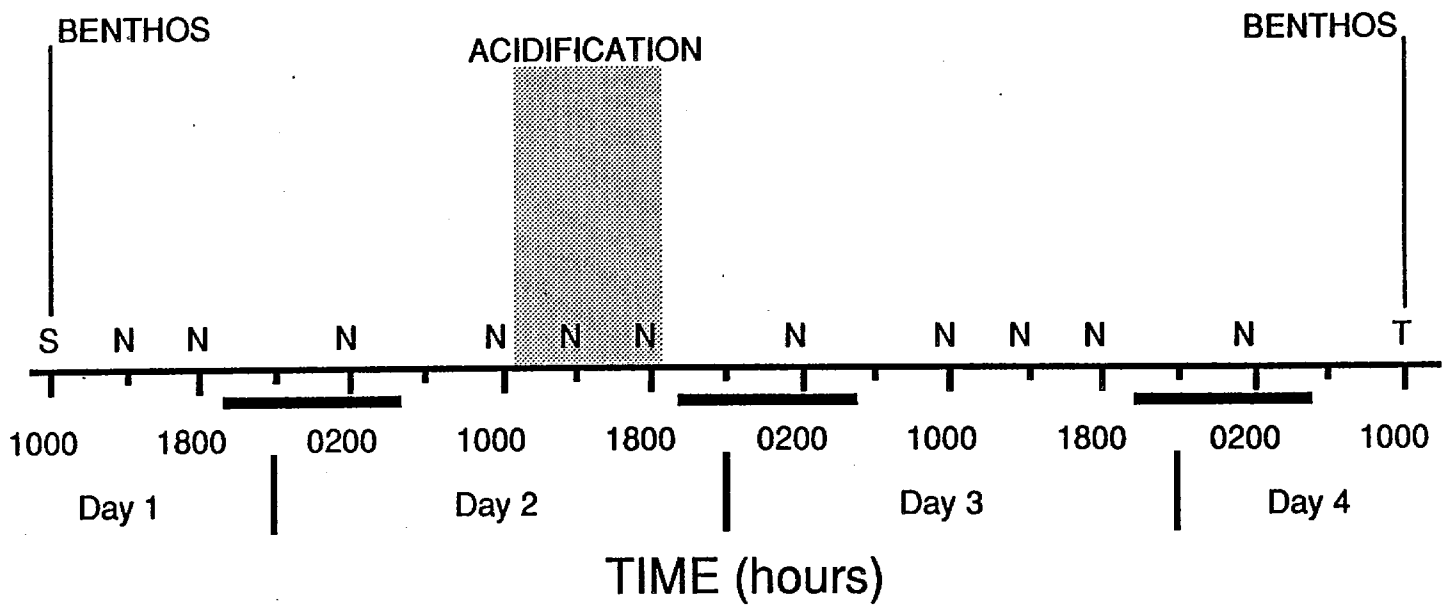


Figure 2. Design of experiments. The shaded area indicates the time of acid additions, the dark horizontal bars under the time line represent the periods of darkness (night), vertical lines between days indicate 2400 hrs (midnight). Benthic samples were taken 24 hours before (S) and 40 hours after (T) acid additions. Drift nets were first set in place at time S, emptied and replaced at each of the "N's", and finally removed at time T.

the channels. Colonization via drift was prevented between the third and fourth acid events to determine long-term stream community responses to repeated acid exposure. Colonization was also excluded for 24 hours before and 40 hours after each acid pulse to prevent "swamping" effects of natural invertebrate drift from the Marble Fork of the Kaweah (Hopkins et al. 1988). Invertebrate exclusion was effected by directing diverted water through a 250-um mesh net (1-m diameter X 1.5-m length) located in the reservoir. This net was monitored and changed daily and emptied only during the day, when natural drift was minimal. Between Experiments 3 and 4 a small tear formed in the block net; however, the net was repaired within 24 hours. The passage of invertebrate drift through this small tear may have accounted for some of the increase in densities of some taxa between the third and fourth acidifications. In Experiment 2 it was difficult to regulate pH levels because of rains and subsequent high discharge, so the results of that experiment will not be discussed here.

Invertebrate drift was monitored for 24 hours prior to, eight hours during, and 40 hours after each acid pulse (Fig. 2). Nets (250um mesh) fixed to the end of each channel were emptied every four hours during the time corresponding to acid addition (approx. 1000-1800 hours), and every eight hours for the remainder of each 24-hour period. Discharge was measured in each channel before and after each drift sample was taken, by collecting the discharge in a calibrated bucket set at the end of each channel. Using these data, flow into channels was regulated to insure equal discharge among channels. Drift rates were calculated as numbers per unit volume ($m^3$) to correct for flow differences among channels. Live vs. dead counts for common taxa in the drift were obtained by immediately washing the contents of the drift nets into buckets and counting the numbers of live and dead individuals of selected taxa. An individual was considered dead if it did not move when prodded. Benthic invertebrate densities were determined 24 hours prior to, and 40 hours and 2 weeks after each acid event (Fig. 2). Stream benthos was sampled by cleaning all substrates in a 10 X 20 cm area on the bottom of each channel into a drift net (250 um) placed just downstream. Two such samples were collected from each channel at each sampling time and combined for analyses. Invertebrate samples were preserved in 70% ethanol and were later counted to family or genus under a dissecting microscope at 12X.

Periphyton community composition was monitored during Experiment 3. Ceramic tiles (1 cm x 1 cm), with the uppermost surface ground smooth, were incubated for 3 weeks in the Marble Fork of the Kaweah River, and then placed in the experimental channels. After two additional weeks three tiles were removed from each channel immediately prior to acid addition to eight of the channels. This sampling time is designated T-0 hours. After the eight-hour acid pulse (T-8 hrs) three more tiles were removed from each of the channels. Another set of 3 tiles was removed from each channel 24 hours after T-0 (T-24) and again one week (T-168 hrs) after experiment initiation. Each collected tile was placed in a vertical position in a plastic vial and immediately preserved with 8% gluteraldehyde. In the laboratory each tile was photographed to permit subsequent estimation of macroalgal abundance. The organisms attached to the tile were then removed by using the acetate-peel method, cleaned, and mounted (Melack et al. 1987). Diatom enumeration and identification followed our previous protocols (Melack et al. 1987). Most diatom identifications were verified with the PIRLA (Paleolimnological Investigations of Recent Lake Acidification) diatom iconograph or with collections at the Academy of Natural Sciences in Philadelphia.

For invertebrate benthic densities or drift, analyses of variance were performed on appropriately transformed data (Allan 1984, Allan and Russek 1985) to determine if there were significant treatment effects on drift rates, benthic densities, and percentage dead in the drift. If significant treatment effects were indicated, these analyses were followed by multiple range tests to determine which treatments were different. For algal data, the abundance of each taxon in a channel was obtained by calculating its average abundance for the three replicate tiles collected from each channel at each sampling time. These mean abundances were $\log_{10}$ transformed, a mean and confidence limit for each treatment were then calculated for the log-transformed values for the four replicate channels, and means and upper and lower confidence limits were back-transformed to the arithmetic scale by taking anti-logs. We performed one-way ANOVAs on log-transformed densities of the 14 most common taxa, and total algal abundance, to see if there were significant effects of treatment (pH levels) on algal abundance. We followed ANOVAs showing significant treatment effects with Duncan's multiple range test to see which treatments (pH 6.5 vs. 5.2 vs. 4.6) were significantly different. The log-transformed mean

numbers of algal cells per channel at each time were used as replicates in all statistical analyses.

## Results

*Physical and chemical factors*

The mean discharge of experimental stream channels declined from 3.2 l/sec during Experiment 1 to 0.7 l/sec in Experiment 4 (Fig. 3). In Experiment 1 discharge remained relatively constant over the experiment, whereas discharge declined over the three days Experiment 3 lasted. There were no significant differences in discharge among treatments in Experiments 1 and 3. Owing to low discharge at the outset of Experiment 4, three channels were closed (1 from each treatment) to insure adequate discharge for the remaining channels. Before acid addition, discharge among treatments in Experiment 4 tended to follow the pattern control > 4.6 > 5.2. There were no significant differences in discharge among treatments, however, during or after acidification. After acid additions runoff from an early winter storm caused a significant increase in water flow in the remaining nine channels.

Mean channel water depth showed the same patterns as discharge. Mean depths over the substrate (± 1 SD) were 10.7 (± 0.6 cm), 7.0 (± 0.5 cm), and 6.0 (± 0.32 cm), during experiments 1, 3,and 4, respectively. Water temperatures in the experimental channels were similar to those in the Marble Fork. Temperatures ranged from 10 to 19° C in August and 5 to 16° C in September.

Control channel pHs were relatively constant for the duration of each experiment (Fig. 4; Tables 1-3). During experimental acidifications, the pHs of channels to which acid was added were within 0.2 pH units of target pHs (5.2 and 4.6), except in the case of the pH 4.6 treatment of Experiment 1 (Table 2). Concentrations of acid neutralizing capacity (ANC), nitrate, and sulfate directly reflect the additions of nitric and sulfuric acids (Tables 1-3). Concentrations of total iron and total aluminum were low and variable, and did not show consistent increases in the acidified channels (Table 4).

*Algae*

Diatoms were counted and identified on 144 tiles, i.e. 3 replicate tiles from each of 12 channels collected four times (T-0,T-8,T-24, and T-168 hrs) during Experiment 3. Approximately 55 diatom taxa were observed. On average only 13 of these taxa occurred on 18 or more of the 36 tiles collected each time. Twenty species in the controls, 22 species at pH 5.2, and 25 species at pH 4.6 were found on one tile only. Of the taxa occurring on a single tile

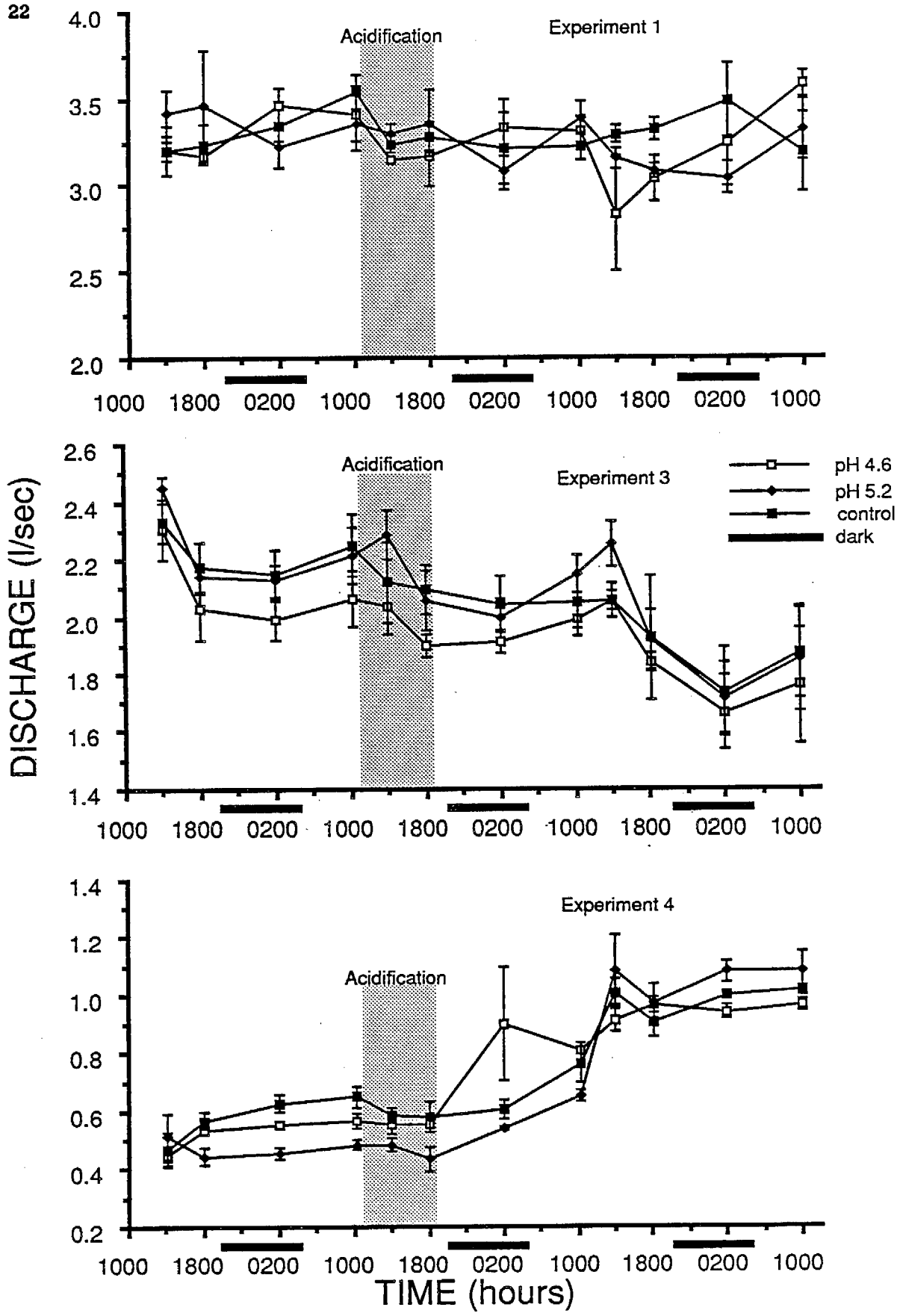


Figure 3. Mean discharge (liters/sec) from channels in each treatment, taken at drift sets throughout the three 72 hour experiments. Experiment 1: 5-8 August; Experiment 3: 3-6 September; Experiment 4: 17-19 September 1986. The shaded areas indicate the time of acid additions, the dark horizontal bars under the time line represent the periods of darkness (night). Vertical lines represent ±1 standard error (S.E.)

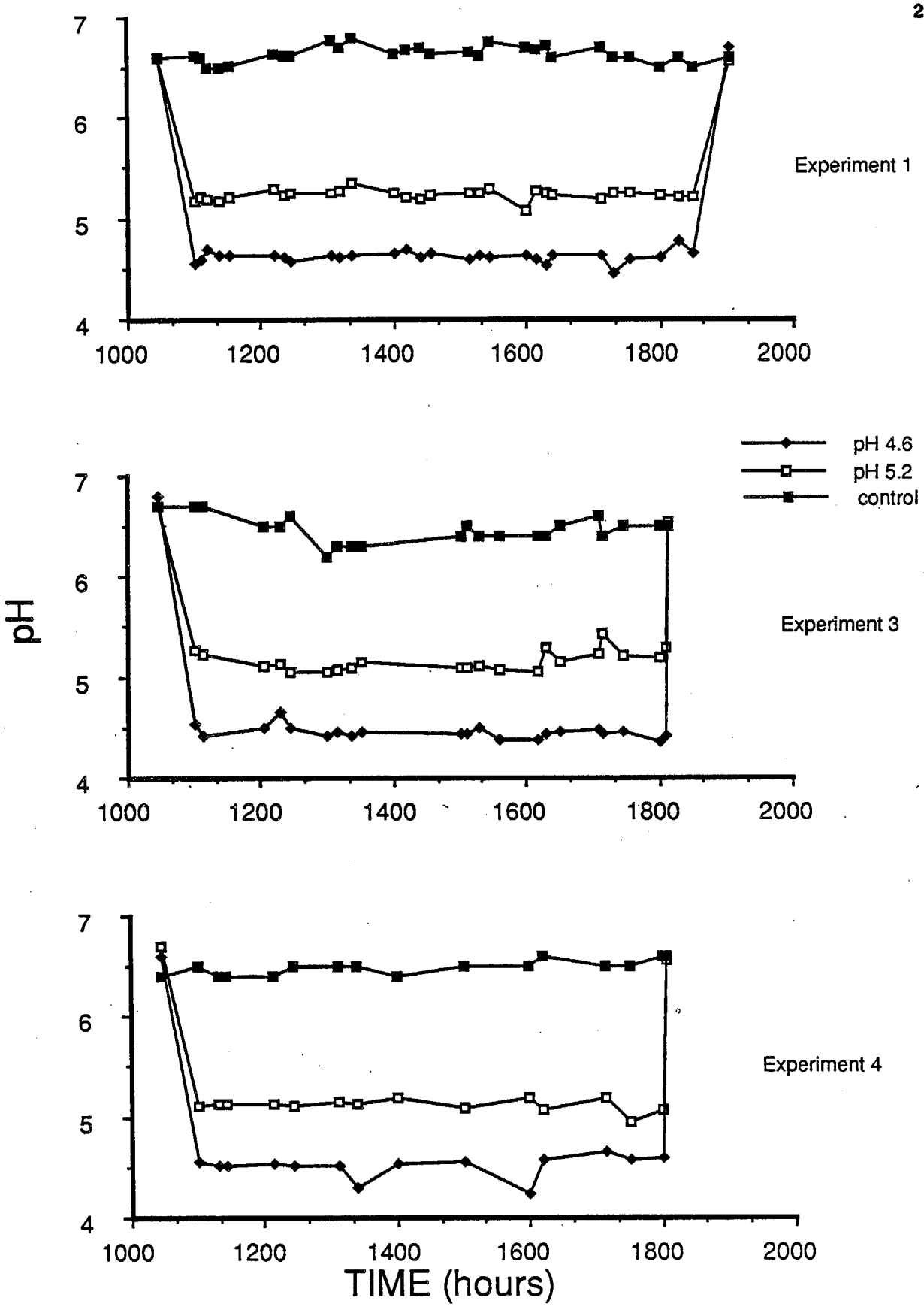


Figure 4. Treatment pH's from the channels for each of the three reported experiments. Values are from one replicate within each treatment level, taken at approximate 15 minute intervals over the 8 hour acidification.

Table 1. Chemical conditions in the experimental channels during Experiment 3 (2-4 September 1986). Times are as follows: TBF - 1700 h, 2 September (day before acidification); T1 - 1115 h, 3 September (60 min after acidification); T2 - 1730 h, 3 September (45 min before end of acidification); TAF - 0830 h, 4 September (day after acidification). Data are means (standard deviations in parentheses) for all four channels.

| | | treatment | | |
|---|---|---|---|---|
| variable | time | control | pH 5.2 | pH 4.6 |
| $H^+$ ($\mu eq\ L^{-1}$) | TBF | 0.21 (0.06) | 0.17 (0.03) | 0.20 (0.05) |
| | T1 | 0.48 (0.17) | 6.72 (0.70) | 26.9 (14.70) |
| | T2 | 0.20 (0.02) | 5.11 (1.43) | 23.9 (4.70) |
| | TAF | 0.24 (0.01) | 0.23 (0.04) | 0.23 (0.03) |
| pH | TBF | 6.67 | 6.76 | 6.71 |
| | T1 | 6.32 | 5.17 | 4.57 |
| | T2 | 6.69 | 5.29 | 4.62 |
| | TAF | 6.62 | 6.64 | 6.63 |
| ANC ($\mu eq\ L^{-1}$) | TBF | 53.0 (2.2) | 49.8 (1.8) | 50.9 (1.7) |
| | T1 | 44.6 (3.4) | NA | NA |
| | T2 | 49.1 (1.6) | NA | NA |
| | TAF | 47.2 (1.1) | 45.1 (1.5) | 47.6 (1.6) |
| $NO_3^-$ ($\mu eq\ L^{-1}$) | TBF | 0.0 (0.0) | 0.2 (0.2) | 0.1 (0.1) |
| | T1 | 0.0 (0.0) | 22.9 (0.4) | 53.9 (1.4) |
| | T2 | 0.1 (0.1) | 23.1 (4.2) | 32.9 (11.0) |
| | TAF | 0.2 (0.3) | 0.1 (0.1) | 0.1 (0.1) |
| $SO_4^{2-}$ ($\mu eq\ L^{-1}$) | TBF | 4.6 (0.1) | 4.8 (0.4) | 4.6 (0.3) |
| | T1 | 5.0 (0.2) | 29.0 (0.6) | 58.8 (1.7) |
| | T2 | 5.3 (0.1) | 30.5 (3.1) | 39.2 (10.3) |
| | TAF | 4.2 (0.3) | 4.3 (0.3) | 4.1 (0.4) |

Table 2. Chemical conditions in the experimental channels during Experiment 1 (5-7 August 1986). Times are as follows: TBF - 1600 h, 5 August (day before acidification); T1 - 1115 h, 6 August (20 min after acidification); T2 - 1745 h, 6 August (20 min before end of acidification); TAF - 1400 h, 7 August (day after acidification). Data for $H^+$, pH and ANC are means (standard deviations in parentheses) for all four channels; $NO_3^-$ and $SO_4^{2-}$ were measured in one channel from each treatment.

| | | treatment | | |
|---|---|---|---|---|
| variable | time | control | pH 5.2 | pH 4.6 |
| $H^+$ ($\mu eq\ L^{-1}$) | TBF | 0.33 (0.02) | 0.33 (0.02) | 0.35 (0.04) |
| | T1 | 0.39 (0.03) | 4.45 (0.25) | 13.0 (0.90) |
| | T2 | 0.31 (0.03) | 7.02 (0.53) | 5.62 (1.50) |
| | TAF | 0.27 (0.03) | 0.32 (0.02) | 0.28 (0.06) |
| pH | TBF | 6.48 | 6.48 | 6.45 |
| | T1 | 6.41 | 5.35 | 4.89 |
| | T2 | 6.51 | 5.15 | 5.25 |
| | TAF | 6.56 | 6.50 | 6.55 |
| ANC ($\mu eq\ L^{-1}$) | TBF | 23.8 (1.0) | 25.1 (1.8) | 24.4 (3.0) |
| | T1 | 21.1 (1.2) | 0.5 (0.9) | NA |
| | T2 | 25.9 (1.0) | -1.7 (1.5) | NA |
| | TAF | 24.3 (0.1) | 22.6 (2.1) | 26.6 (1.2) |
| $NO_3^-$ ($\mu eq\ L^{-1}$) | TBF | 0.1 | 0.0 | 0.3 |
| | T1 | 0.6 | 13.3 | 26.4 |
| | T2 | 0.1 | 12.3 | 11.6 |
| | TAF | 0.1 | 0.1 | 0.4 |
| $SO_4^{2-}$ ($\mu eq\ L^{-1}$) | TBF | 3.1 | 3.5 | 3.8 |
| | T1 | 1.9 | 16.1 | 22.5 |
| | T2 | 3.5 | 18.2 | 19.6 |
| | TAF | 3.3 | 3.4 | 3.2 |

Table 3. Chemical conditions in the experimental channels during Experiment 4 (16-18 September 1986). Times are as follows: TBF - 1400 h, 16 September (day before acidification); T1 - 1100 h, 17 September (60 min after acidification); T2 - 1745 h, 17 September (15 min before end of acidification); TAF - 0845 h, 18 September (day after acidification). Data for $H^+$, pH and ANC are means (standard deviations in parentheses) for all four channels; $NO_3^-$ and $SO_4^{2-}$ were measured in one channel from each treatment.

| | | treatment | | |
|---|---|---|---|---|
| variable | time | control | pH 5.2 | pH 4.6 |
| $H^+$ ($\mu eq\ L^{-1}$) | TBF | 0.17 (0.03) | 0.21 (0.05) | 0.22 (0.02) |
| | T1 | 0.17 (0.01) | 5.60 (1.04) | 21.00 (1.80) |
| | T2 | 0.34 (0.06) | 4.83 (0.61) | 20.50 (1.30) |
| | TAF | 0.43 (0.13) | 0.31 (0.02) | 0.31 (0.01) |
| pH | TBF | 6.77 | 6.68 | 6.66 |
| | T1 | 6.78 | 5.25 | 4.68 |
| | T2 | 6.52 | 5.32 | 4.69 |
| | TAF | 6.37 | 6.50 | 6.51 |
| ANC ($\mu eq\ L^{-1}$) | TBF | 49.7 (1.3) | 47.4 (1.1) | 46.6 (0.2) |
| | T1 | 52.6 (0.5) | NA | NA |
| | T2 | 51.4 (1.7) | NA | NA |
| | TAF | 50.8 (1.3) | 50.9 (0.8) | 52.6 (1.5) |
| $NO_3^-$ ($\mu eq\ L^{-1}$) | TBF | 0.0 | 0.0 | 0.1 |
| | T1 | 0.1 | 19.7 | 34.1 |
| | T2 | 0.0 | 18.1 | 28.7 |
| | TAF | 5.5 | 8.3 | 8.3 |
| $SO_4^{2-}$ ($\mu eq\ L^{-1}$) | TBF | 7.1 | 4.3 | 4.3 |
| | T1 | 4.3 | 27.8 | 41.5 |
| | T2 | 4.1 | 26.0 | 36.7 |
| | TAF | 7.2 | 6.9 | 8.2 |

Table 4. Concentrations of iron and aluminum in the experimental channels. Data are means (standard deviations in parentheses) for 2 channels, except for the control in Experiment 3 (3 channels). TBF - day before acidification; T2 - 20-60 min before the end of acidification.

| | | Fe (µM) | | Al (µM) | |
|---|---|---|---|---|---|
| experiment | treatment | TBF | T2 | TBF | T2 |
| 1 (5-6 Aug) | control | 0.37 (0.26) | 0.50 (0.07) | 0.26 (0.01) | 0.79[a] |
| | pH 4.6 | 0.17 (0.06) | 0.08 (0.00) | 0.28[a] | 0.39 (0.02) |
| 3 (2-3 Sep) | control | 0.32 (0.05) | 0.25 (0.05) | 0.35 (0.03) | 0.48 (0.03) |
| | pH 4.6 | 0.33 (0.05) | 0.86 (0.28) | 0.33 (0.03) | 0.87 (0.40) |
| 4 (16-17 Sep) | control | 0.31 (0.09) | 0.19 (0.06) | 0.43 (0.02) | 0.52 (0.02) |
| | pH 4.6 | 0.26 (0.03) | 0.18 (0.07) | 0.31 (0.02) | 0.50 (0.20) |

[a] data available for only one of the replicates

approximately one half were alive at the time of collection. The only alga that was not a diatom that was commonly observed on tiles was Zygnema sp.

Total numbers of diatoms declined during acidification, and diatom density 24 hours after experiment initiation tended to be lower at pHs 4.6 and 5.2 than in the controls ($P = 0.066$, ANOVA, Figure 5). Diatom abundance increased in all treatments during the ensuing week and there was a statistically significant effect of treatment on diatom density after 168 hours (7 days, $P < 0.05$, ANOVA). After 7 days, diatom densities in control channels were significantly higher than those in pH 4.6 channels ($P < 0.05$); however, densities in pH 5.2 channels were not significantly different from those in control or pH 4.6 channels ($P > 0.05$, Duncan's multiple range test).

Achnanthes minutissima was the dominant taxon, with mean abundances ranging between 15,000 and 28,000 valves per $cm^2$. There was a tendency for this diatom's abundance to be lower in acidified than in control treatments after acid addition, and this pattern was statistically significant after one week ($P < 0.05$, ANOVA, Fig. 6). At T-168 hrs the valve concentration in the controls was significantly greater than at pH 4.6 ($P < 0.05$); however, densities at pH 5.2 were not significantly different from those at the other two pH levels.

A number of other taxa showed similar responses to acid inputs. The entity we have tentatively designated 99396-SN (either Cymbella failaisencis or Gomphonema 35N) decreased in abundance following the addition of acid and by T-24 hrs the abundance of this diatom was significantly lower in both acid treatments than in the control, which increased in abundance over this same time interval ($P < 0.05$, Duncan's multiple range test, Figure 7). The abundance of Gomphonema subclavatum also tended to be lower in the acid than the control channels at T-24 ($P = 0.06$, ANOVA, Fig. 9). Although densities were highly variable, Fragilaria vaucheri was significantly more abundant in control than in acidified channels at the end of acidification (T-8, $P < 0.05$, Duncan's multiple range test, Fig. 10).

Species of Eunotia tended to be more abundant in acidified than control channels at some point after acid addition. Eunotia tenella tended to be more abundant at pH 5.2 than at pHs 6.5 or 4.6 at T-24 hours ($P = 0.13$, ANOVA, Fig. 8). Another species of Eunotia, Eunotia tenella "long form", also appeared to thrive in acid channels, tending to be more abundant in acid than control

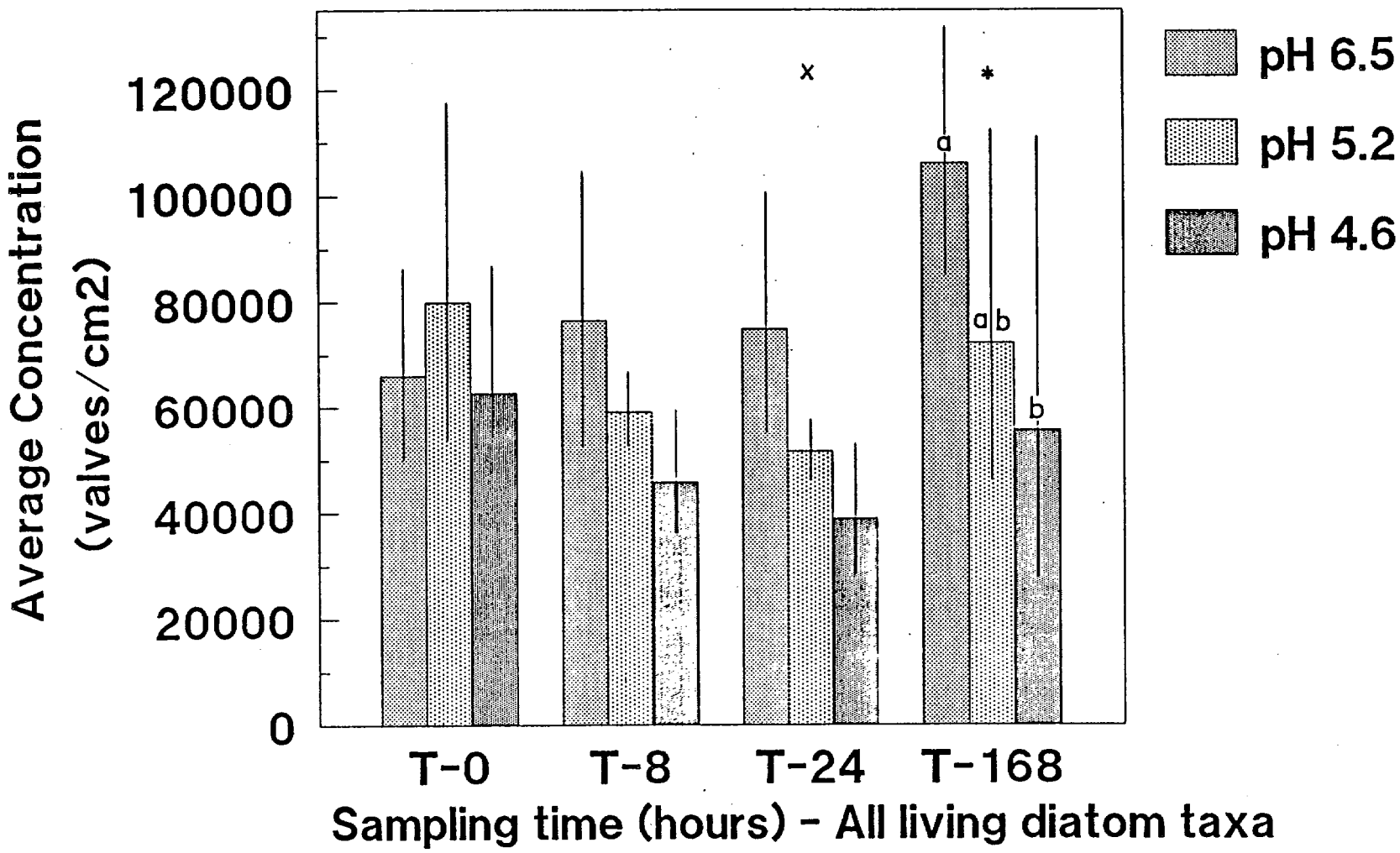


Figure 5. Average abundance (live valves/$cm^2$ $\pm$ 2 standard errors (SEs)) of the living diatom taxa on artificial substrates collected at T-0, T-8, T-24, and T-168 hours. Statistically significant effects of treatment are indicated by an * ($P < 0.05$), marginally significant effects by an x ($0.05 < P < 0.10$, ANOVA). For significant ANOVAs, treatments with the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test).

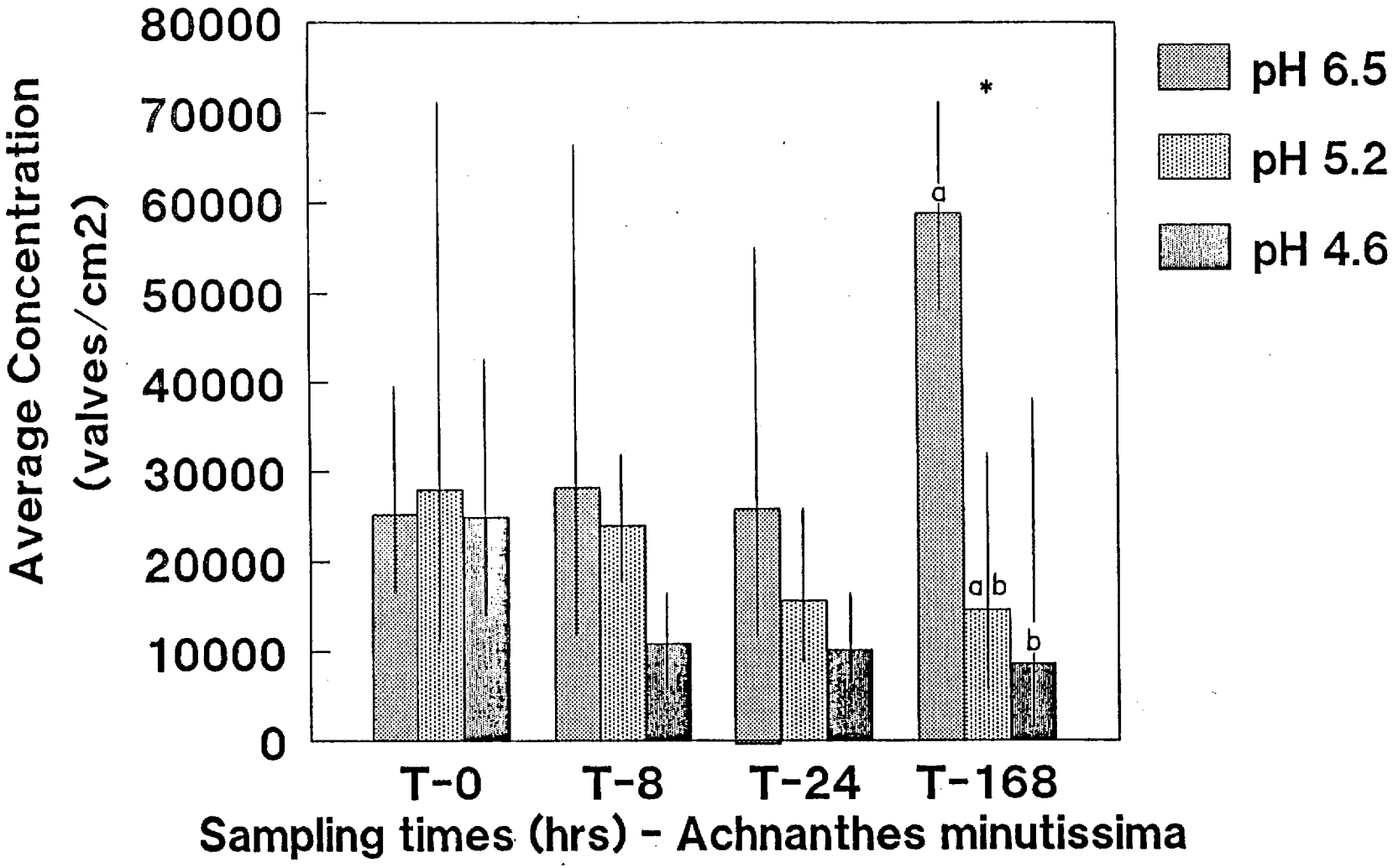


Figure 6. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of Achnanthes minutissima at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

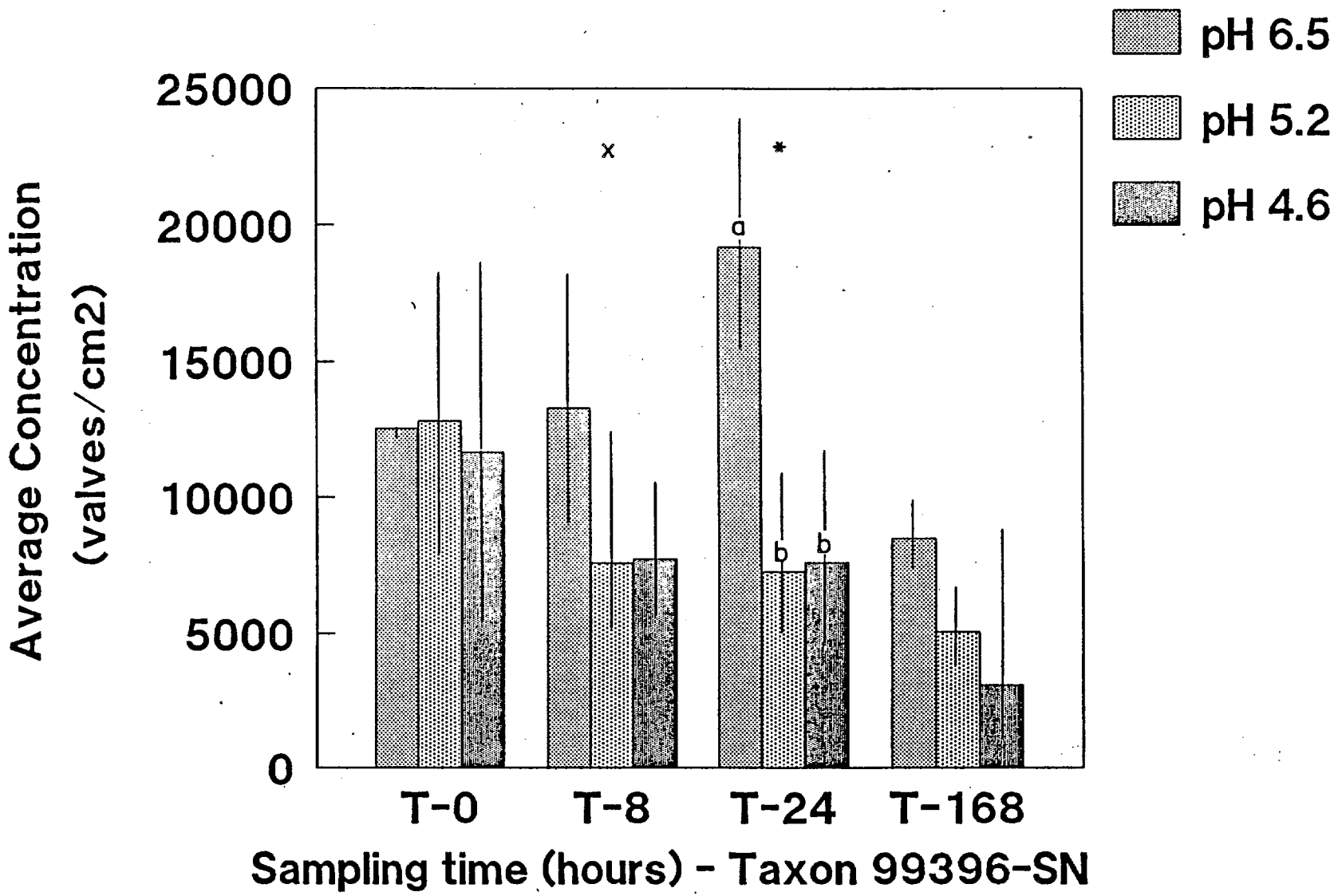


Figure 7. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of taxon 99396-SN at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

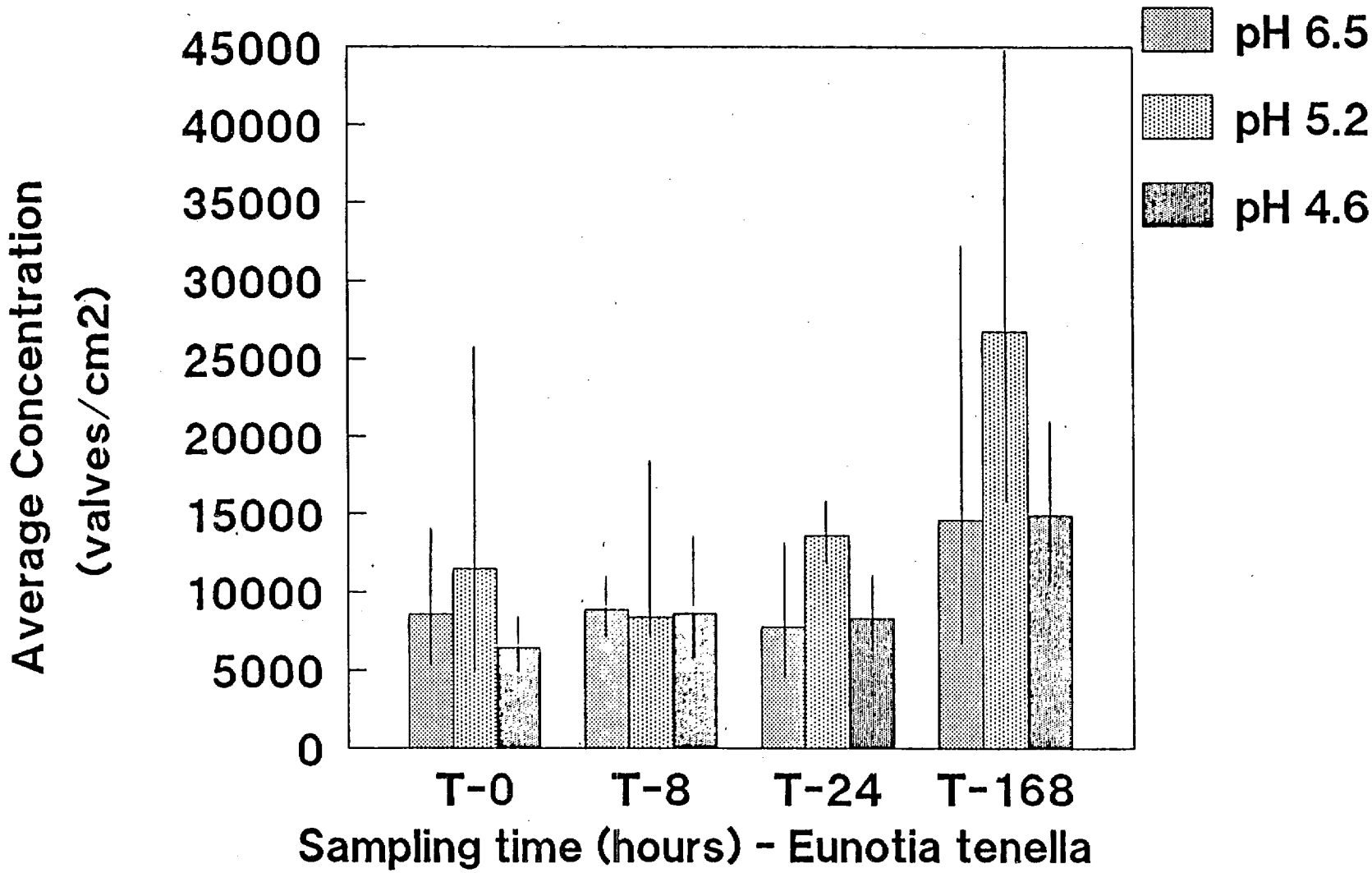


Figure 8. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of Eunotia tenella at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

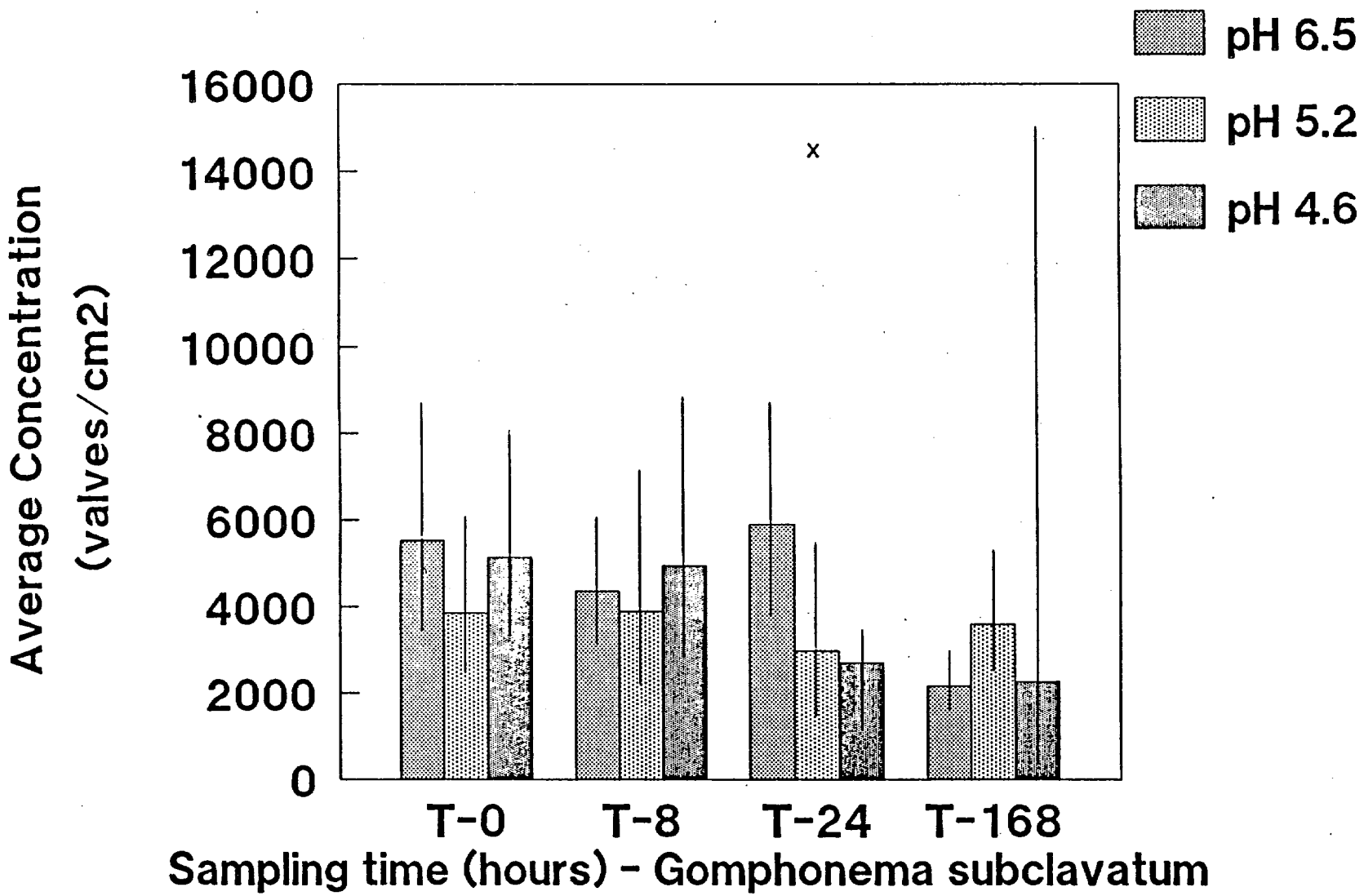


Figure 9. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of Gomphonema subclavatum at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

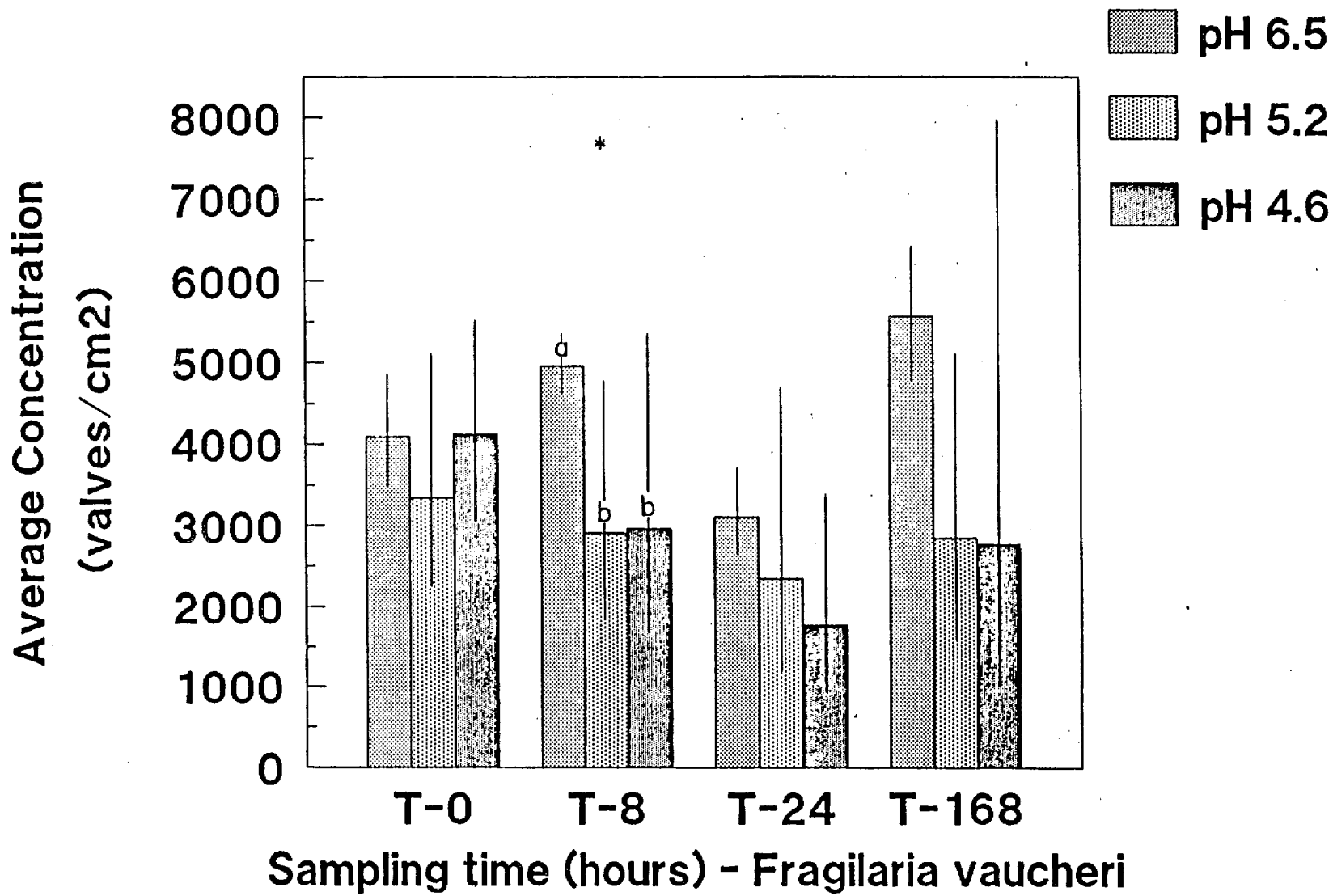


Figure 10. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of Fragilaria vaucheri at T-0, T-8, T-24, an T-168 hours. Other designations as in Figure 5.

channels after one week (Fig. 12). High variability in the control channels, however, precluded statistically significant results.

Most other taxa either showed few responses to acid manipulations or occurred at such low, variable densities that it was difficult to discern response patterns. There was a suggestion of declines in the abundance of Achnanthes levanderi in acid channels during acid additions, but few consistent trends were apparent (at T-8, P = 0.09, ANOVA, Fig. 11). Similar to Eunotia tenella "long form", Tabellaria flocullosa appeared to be more abundant in the acid than control channels at T-168 hrs, but these trends were not significant (Fig. 13). It is difficult to conclude anything about the response patterns of Synedra sp., Cymbella minuta, Achnanthes marginulata, and A. austriaca v. helvitica to acid inputs owing to low, variable densities (Figs. 14 - 17). The only common alga that was not a diatom, Zygnema sp., showed no response to acid inputs (Fig. 18).

In summary, the algal assemblage colonizing tiles in experimental stream channels showed a variety of responses to pulsed acidification. Total numbers of diatoms were lower in acid than control channels after acidification and there were both long (one week) and short-term (one day) shifts in species composition in response to the 8-hour acid pulse. Abundances of Achnanthes minutissima, Taxon 99396-SN, and Fragilaria vaucheri were significantly depressed, relative to controls, in at least one acid treatment after acid additions, and Gomphonema subclavatum and Achnanthes levanderi tended to show similar responses. Eunotia tenella tended to be more abundant at pH 5.2 than in the controls after acid addition. Trends in the mean abundances of the other common taxa suggested effects of acid inputs; however, most of these responses were not significant, usually owing to low, variable densities. Changes in the abundances of diatoms over the experiment were mediated by changes in immigration or emigration or by differential growth. There was little indication of differential survivorship among treatments as the ratio of live to dead cells remained relatively constant across treatments.

*Invertebrates*

Stream invertebrates found in the benthos and drift of the experimental stream channels are listed in Table 1. The species found in channels are the same as those found in the Marble Fork of the Kaweah River.

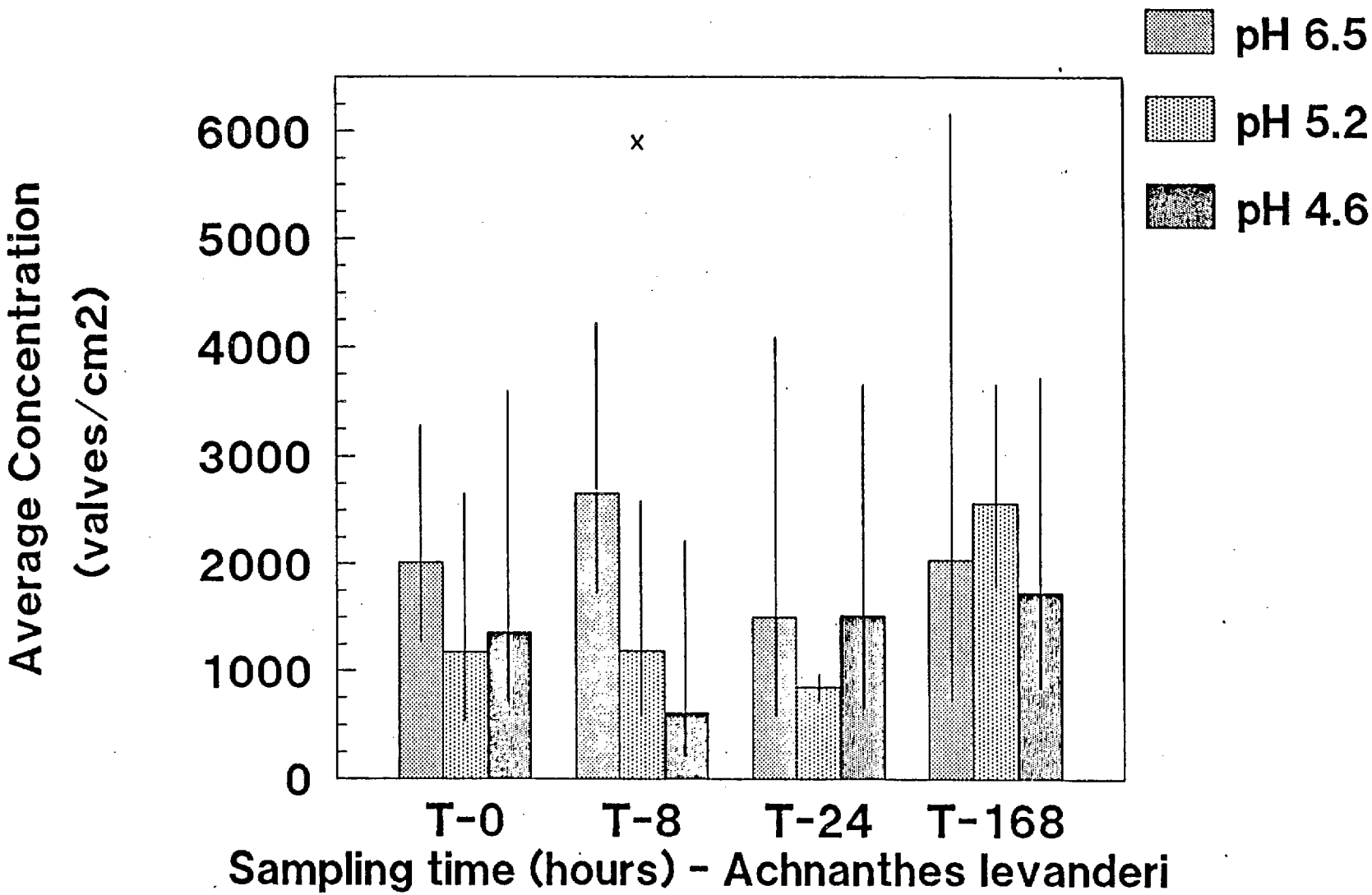


Figure 11. Average abundance (live valves/$cm^2$ ± 2 SEs) of Achnanthes levanderi at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

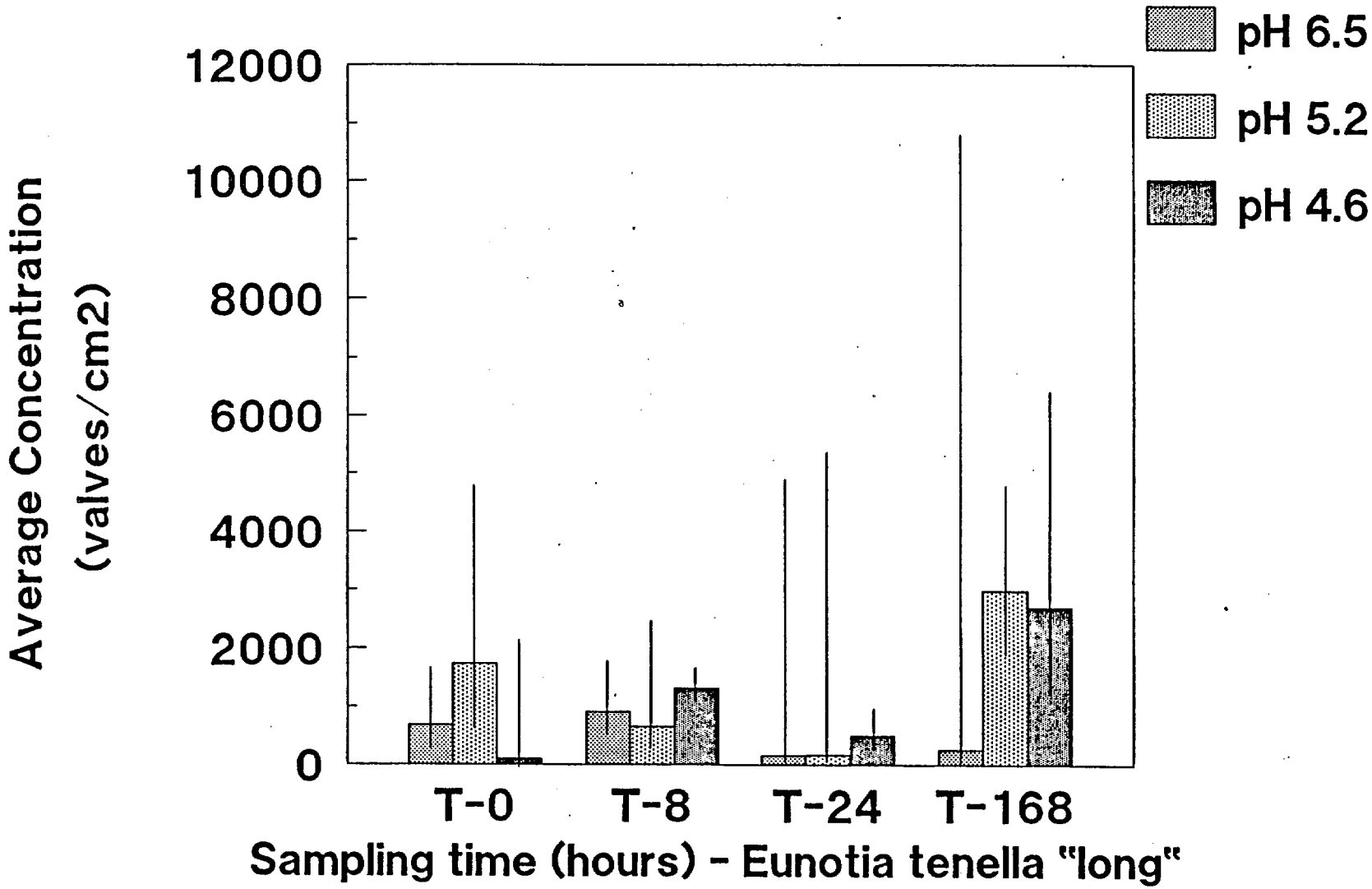


Figure 12. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of Eunotia tenella "long" at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

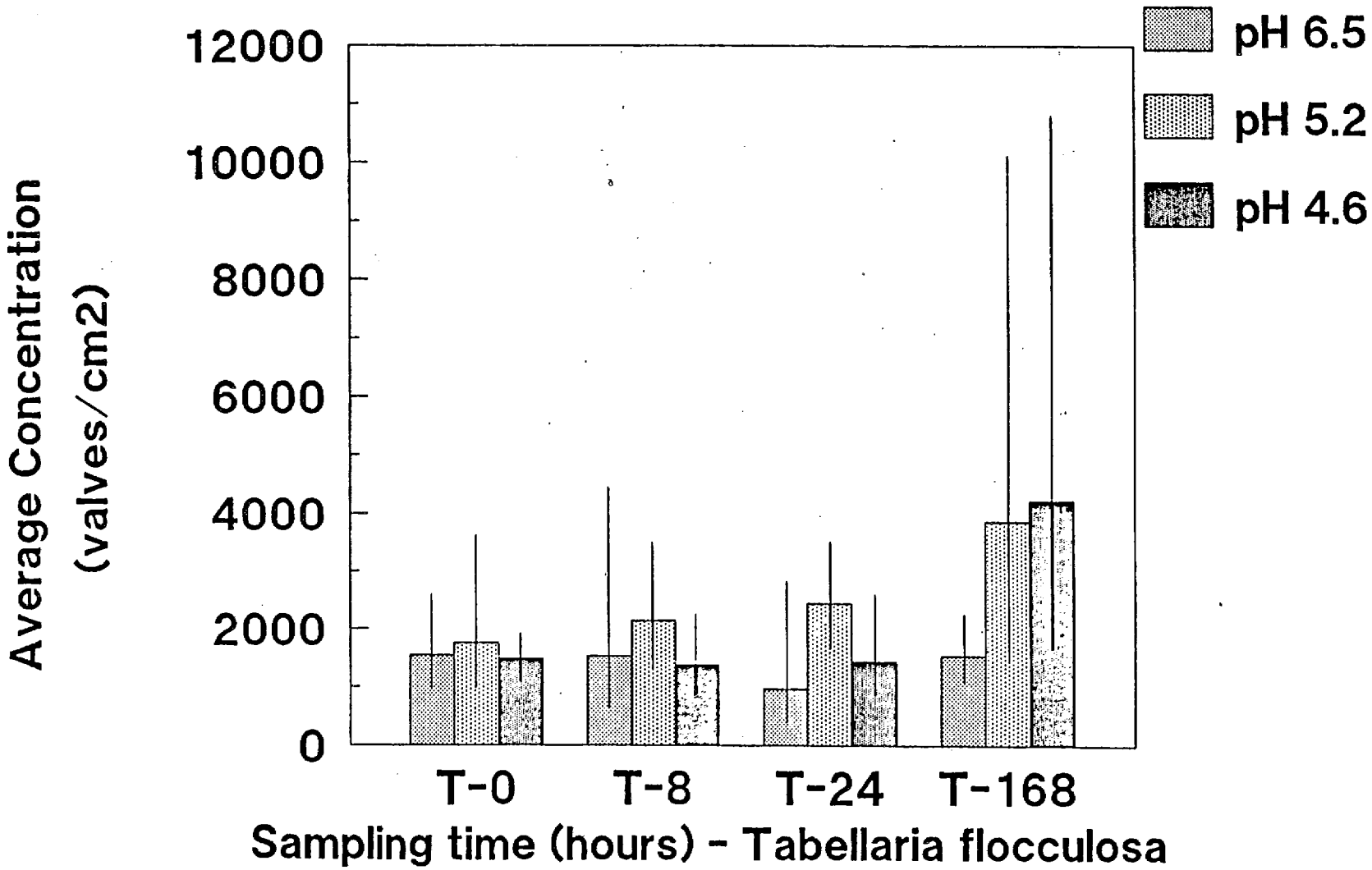


Figure 13. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of Tabellaria flocullosa at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

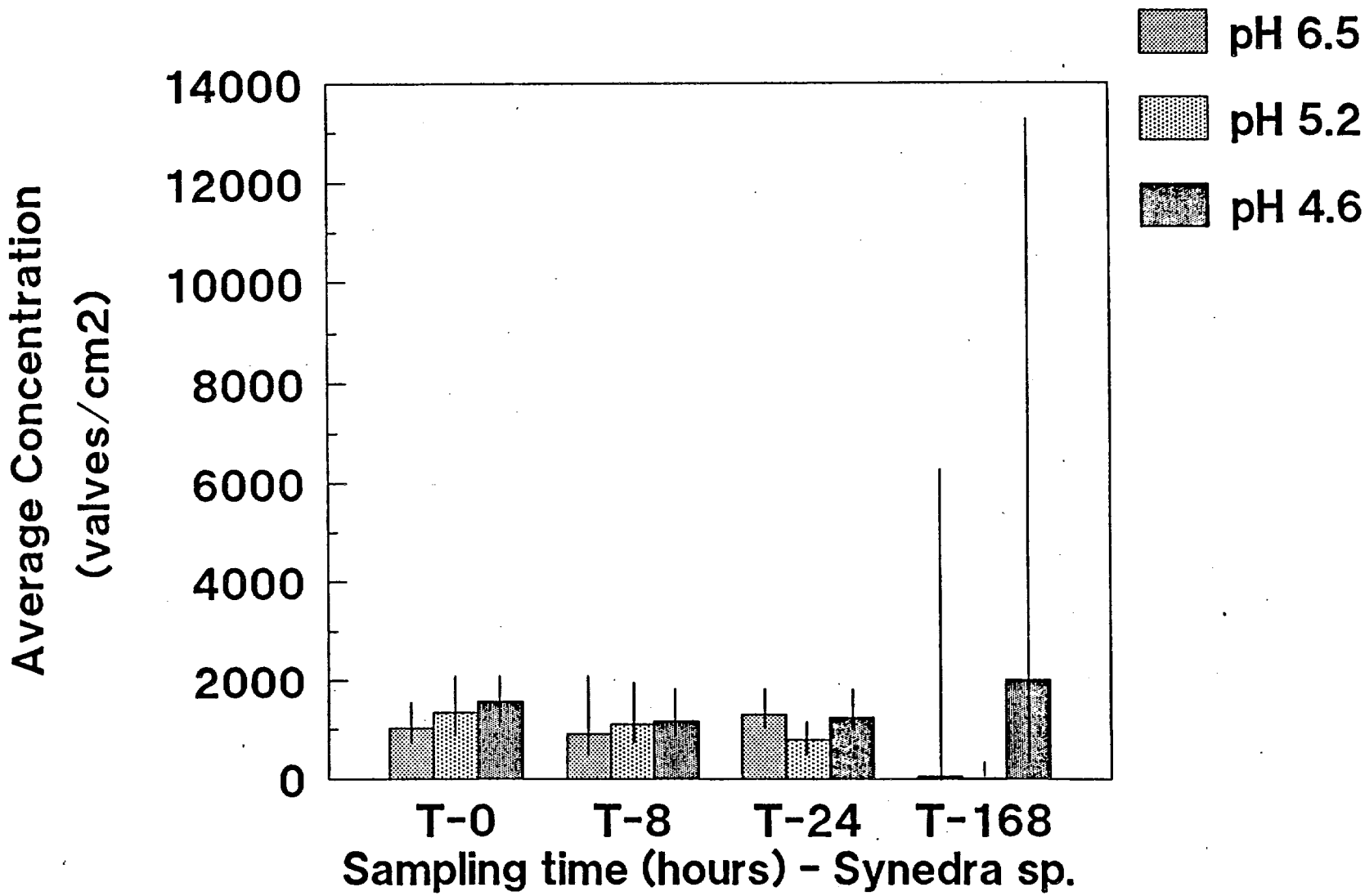


Figure 14. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of Synedra sp. at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

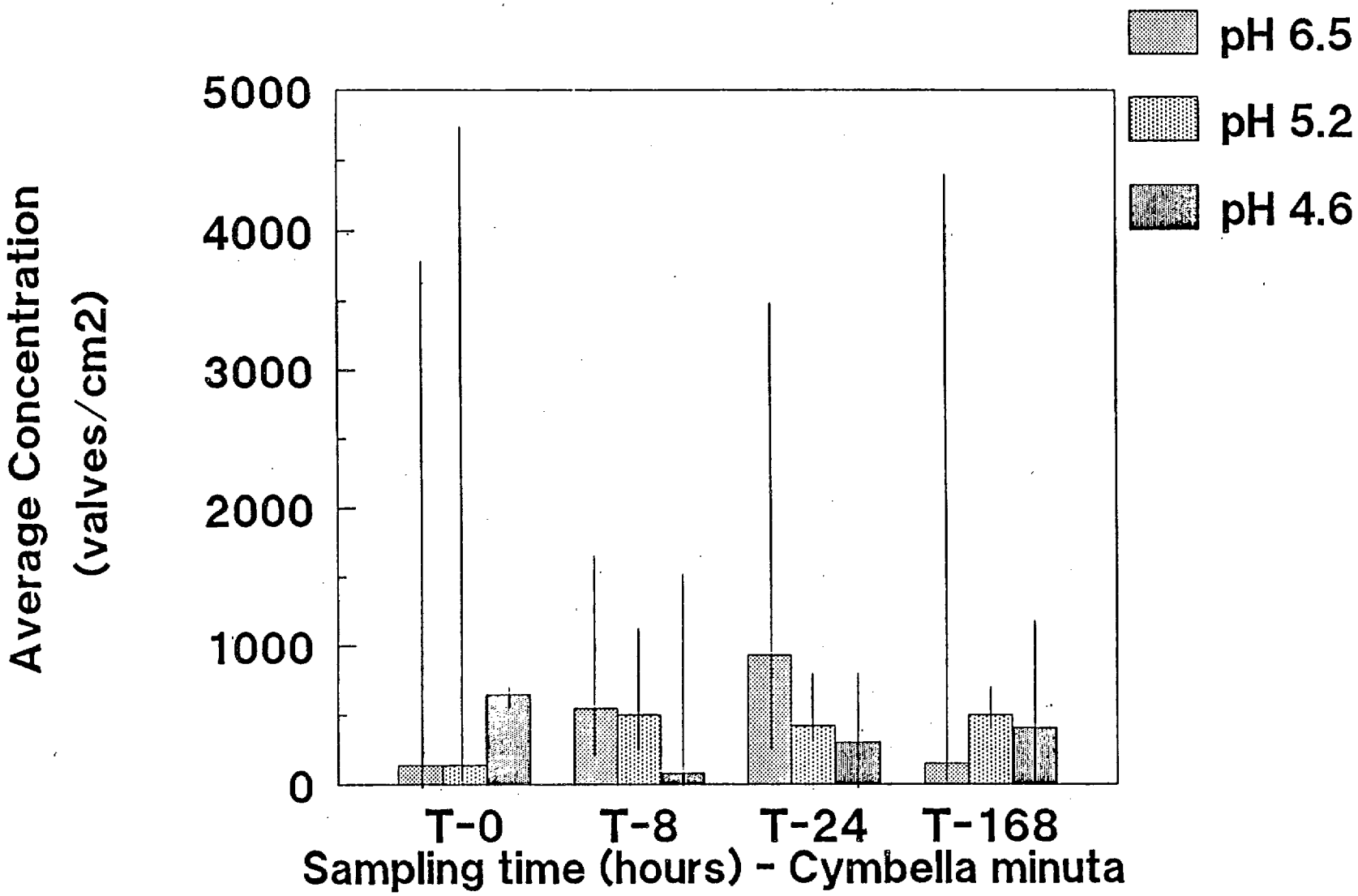


Figure 15. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of Cymbella minuta at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

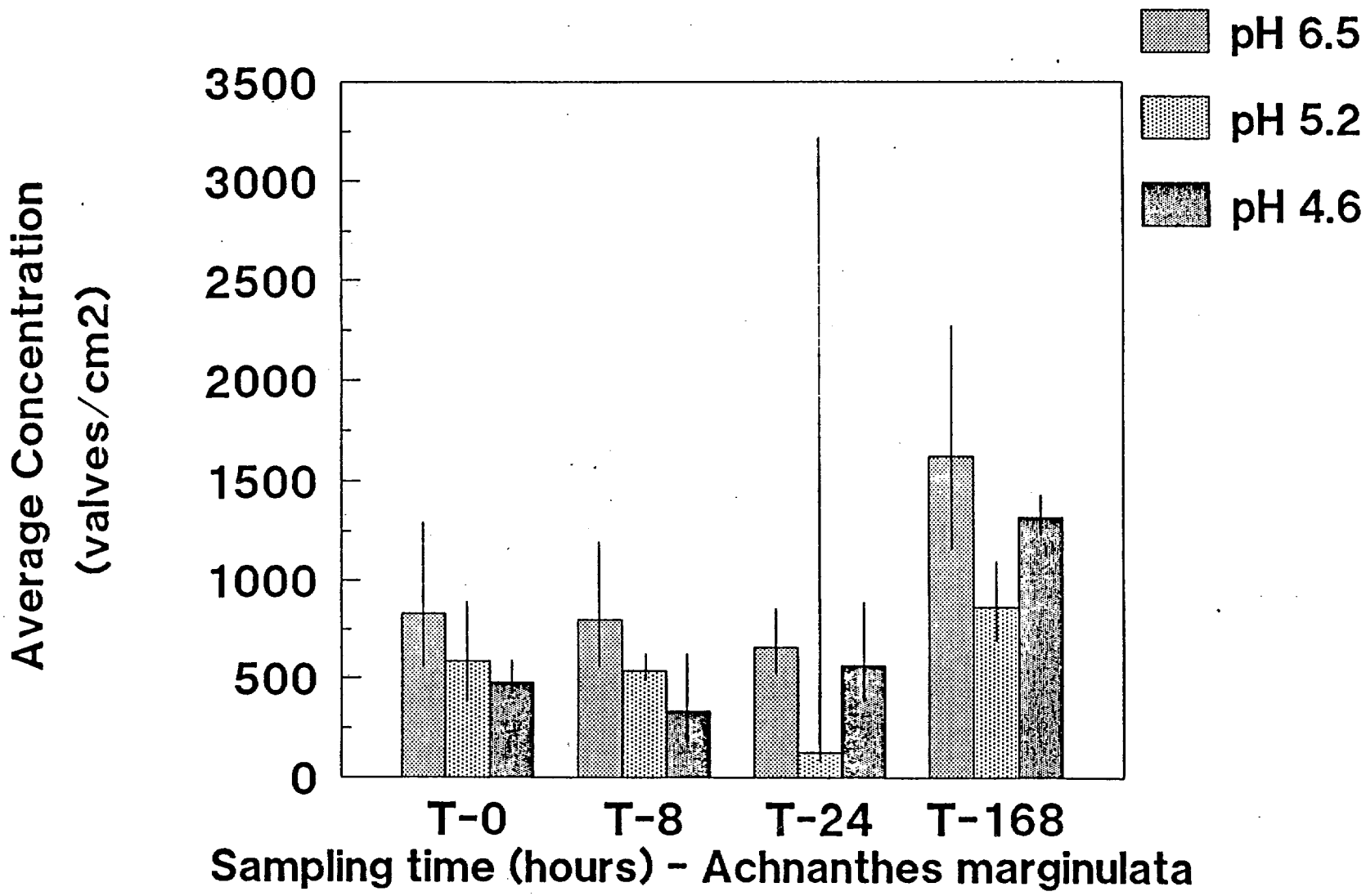


Figure 16. Average abundance (live valves/$cm^2$ $\pm$ 2 SEs) of Achnanthes marginulata at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

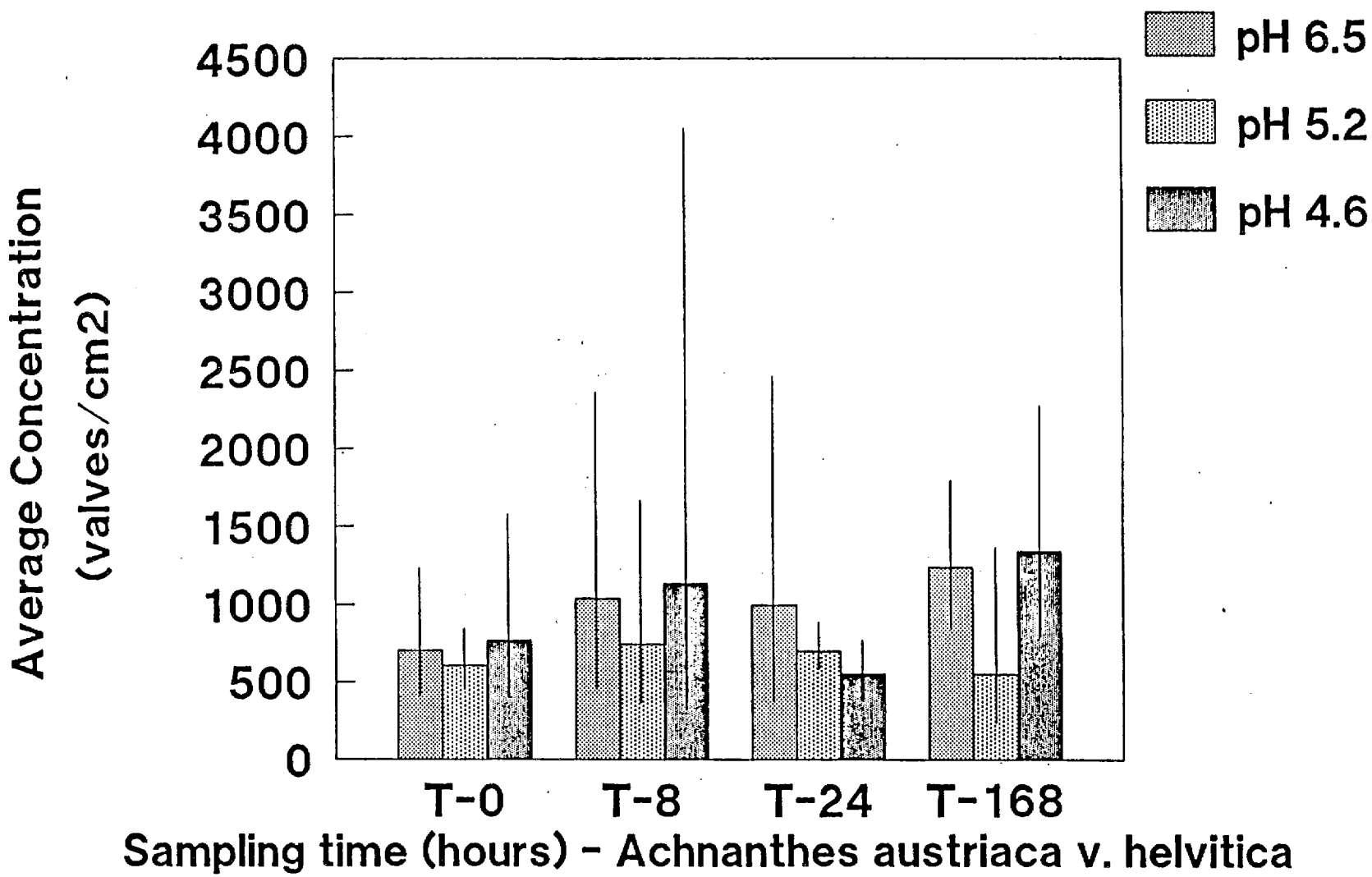


Figure 17. Average abundance (live valves/$cm^2$ ± 2 SEs) of Achnanthes austriaca v. helvitica at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

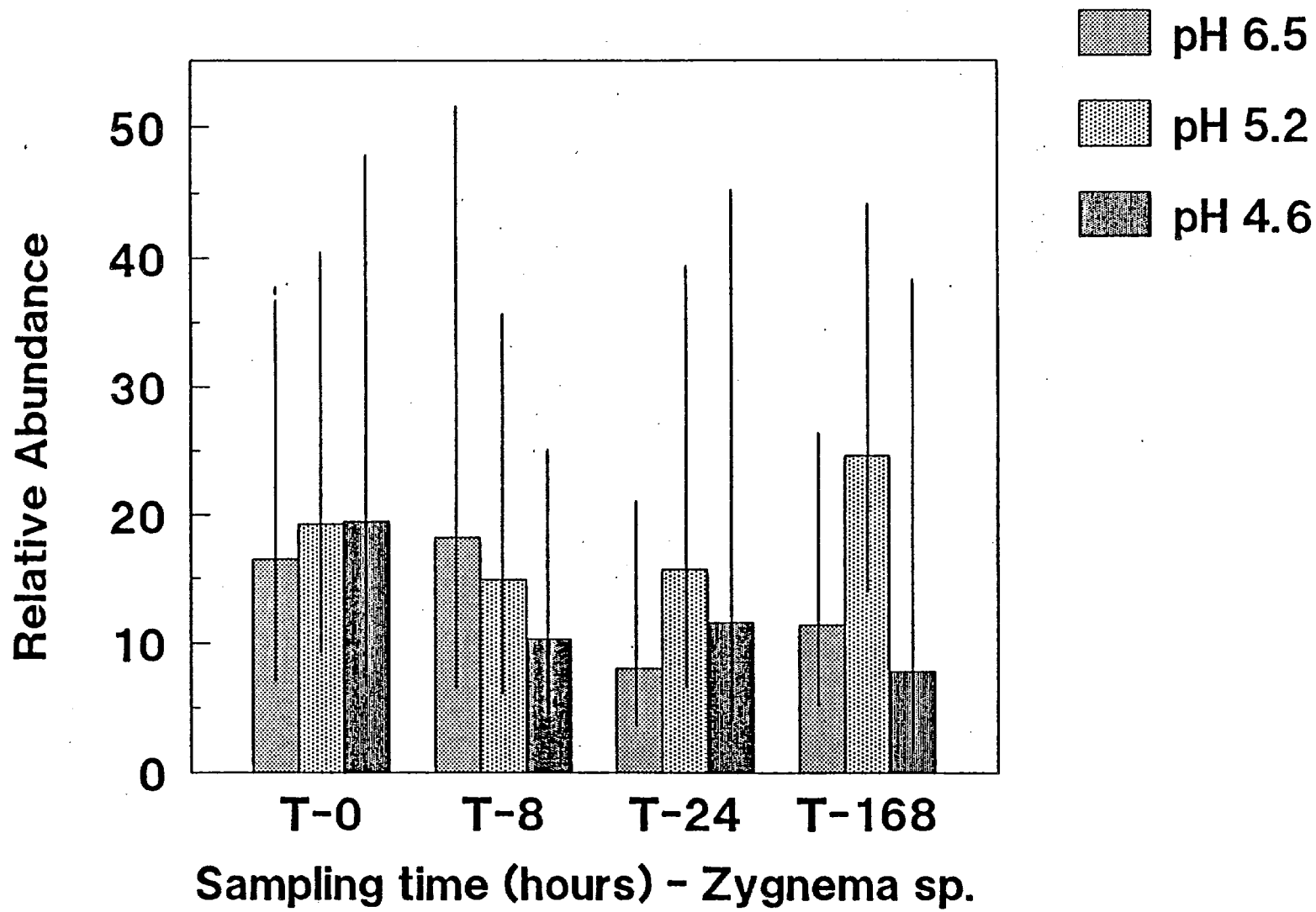


Figure 18. Mean relative abundance (± 2 SEs) of Zygnema sp. at T-0, T-8, T-24, and T-168 hours. Other designations as in Figure 5.

Table 5. Invertebrate taxa collected from drift and benthic samples collected from the Marble Fork experimental stream channels, July - October, 1986.

**Phylum Arthropoda**
**Class Insecta**

**Order Collembola**

**Order Ephemeroptera**
Siphlonuridae
Ameletus
Baetidae
Baetis
Heptageniidae
Cinygma
Cinygmula
Epeorus
Heptagenia
Ephemerellidae
Attenella
Caudatella
Drunella
Ephemerella
Serratella
Leptophlebiidae
Paraleptophlebia

**Order Plecoptera**
Leuctridae
Perlomyia
Nemouridae
Amphinemura
Nemoura
Malenka
Zapada
Leuctridae
Perlomyia
Perlodidae
Megarcys
Isoperla
Skwala
Chloroperlidae
Alloperla
Sweltsa

**Order Megaloptera**
Sialidae
Sialis

**Order Trichoptera**
Brachycentridae
Amiocentrus
Micrasemis
Hydropsychidae
Hydropsyche
Hydroptilidae
Ochrotrichia
Limnephilidae
Ecclisomyia
Dicosmoecus
Neophylax
Desmona
Polycentopidae
Polycentropus
Rhyacophilidae
Rhyacophila

**Order Coleoptera**
Dytiscidae
Oreodytes
Uvarus
Agabus
Coptotomus
Hydrovatus
Hydroporus
Rhantus
Hydrophilidae
Tropisternus
Elmidae
Zaitzevia
Narpus

**Order Diptera**
Deuterophlebiidae
Deuterophlebia
Tipulidae
Dicranota
Pedicia
Simuliidae
Prosimulium
Simulium
Twinnia

Chironomidae

Dixidae
Dixa
Meringodixa
Empididae
Clinocera
Muscidae
Limnophora

**Class Arachnida**
Hydracarina

**Phylum Nematoda**

**Phylum Annelida**
**Class Oligochaeta**

*Drift-* Drift rates for the mayflies Baetis and Epeorus, the blackfly Simulium, midges (chironomids), and most other larvae, as well as adult beetles (Hydroporus) and water mites, were similar in control and treatment channels prior to acidification in all experiments. Baetis showed the most sensitive drift responses to acid inputs (Figs. 19 and 20). There was significantly higher drift in acidified than control channels during both the first and last four hours of the acidification period in Experiments 1 and 3 (Fs= 8.5 - 38.2, P < 0.01 to 0.0001). Baetis drift from the experimental channels declined during the acidification period, but was still significantly higher than from controls. Drift rates in acid treatments were 11 to 26 times greater than those in controls during the first four hours of acidification and 4 to 7 X higher than controls during the last four hours of acidification. Following acidification Baetis drift was often significantly greater in control channels than acid treatment channels in Experiments 1 and 3 (Fs = 5.9 to 61.6; P< .05). Baetis drift behavior during acidification in Expt. 4 was similar to that in the previous experiments. Although not significant, acid channel drift rates were 2.5 - 5.5 times higher than controls (Fig. 21). After acidification Baetis drift in control channels was higher than that in channels to which acid was added.

Prior to acid addition approximately 20% of Baetis in the drift were dead (mean of all channels combined) in Expt. 1, 10% in Expt. 3, and 20% in Expt. 4 (Figs. 22 - 24). Throughout acid addition the percent of drifting Baetis that were dead in the pH 4.6 channels increased to 80%, 82%, and 58%, respectively, in these experiments. In Expt. 1 the proportion of dead Baetis increased with decreasing pH; all treatments were significantly different during acidification (F=9.0 - 47.4 p<.05, arcsine square root transformed data, ANOVA). The percentage of dead drift declined following the end of acidification. Approximately 71 % of the increased drift in pH 5.2 channels and 76 % of the increased drift in pH 4.6 channels during the acid pulse could be attributed to the drift of baetids killed by acid ("toxic" drift). In Expts. 3 and 4 the proportion of dead Baetis in the drift also showed significant responses to acid inputs (Figs. 23 and 24). In Expt. 3 the proportion of drifting baetids that were dead were higher at pH 4.6 than 5.2 and higher at 5.2 than in the controls. Seventy-three % of the drift at pH 5.2 and all of the baetid drift at pH 4.6 during acidification could be attributed to toxic drift (drift of dead individuals). In Experiment 4 the only significant effect of treatment on

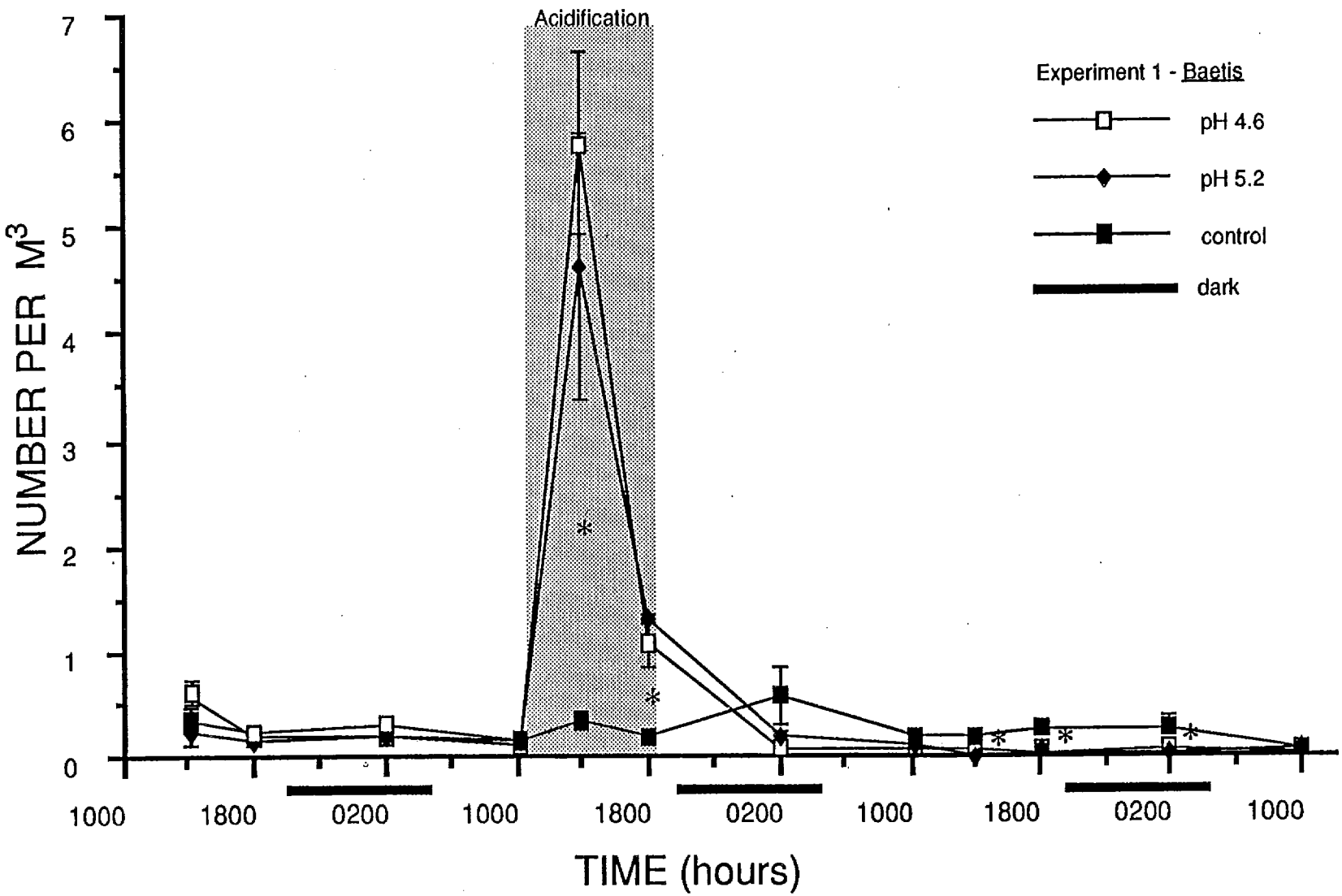


Fig. 19. Baetis drift rates (number / $m^3 \pm 1$ S.E.) for each treatment throughout the course of Experiment 1. Shaded area indicates the time of acid addition. Lower dark bars indicate periods of darkness. The treatment(s) above an asterisk were significantly different from the treatment(s) below an asterisk (*$P < 0.05$, Duncan's multiple range test). If an asterisk was located above the treatment with the highest mean value, this indicates that treatments with the highest and lowest means were significantly different ($P < 0.05$), but neither was different from the treatment with the intermediate value.

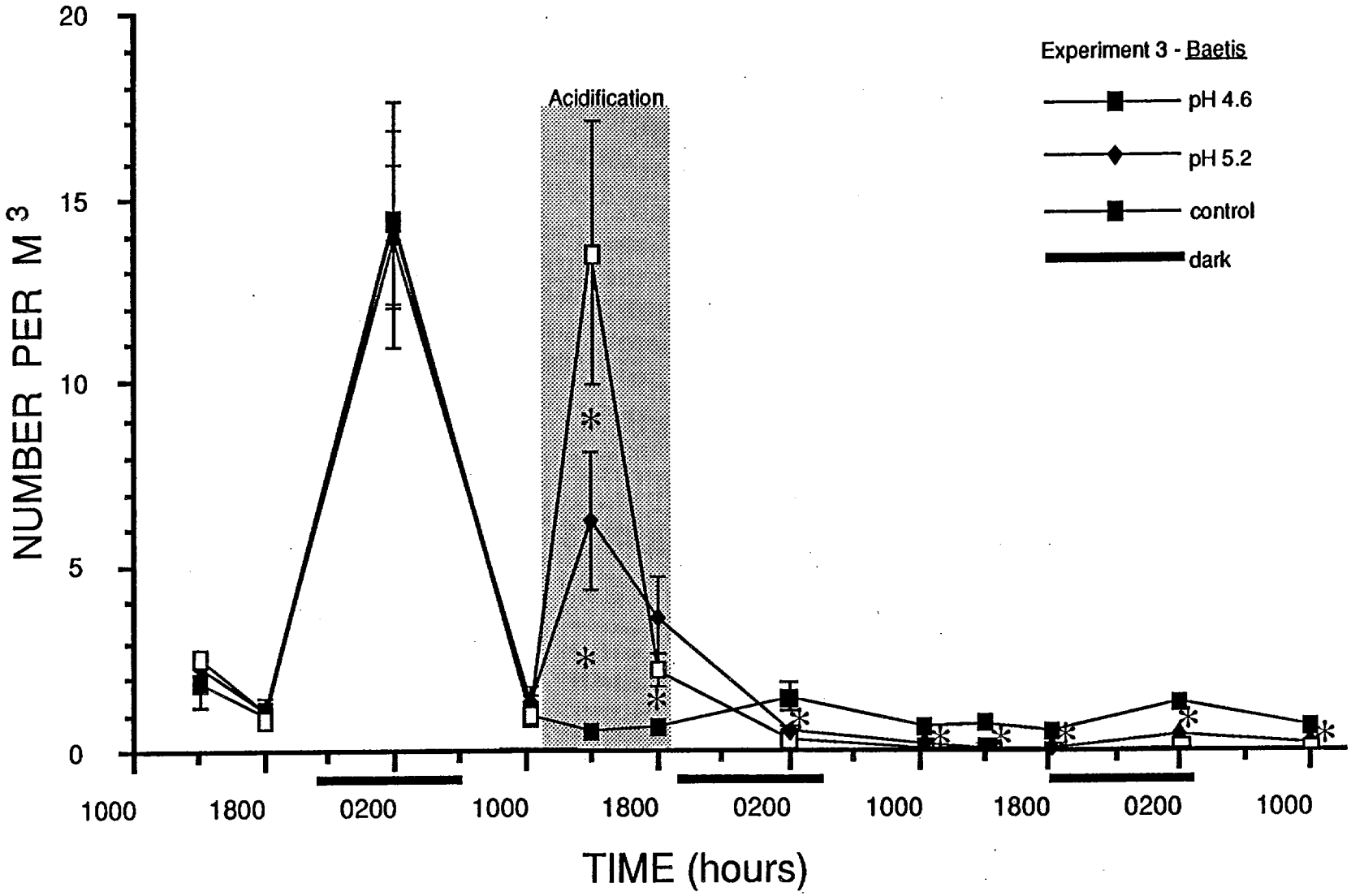


Figure 20. Baetis drift rates (number / $m^3$ ± 1 S.E.) for each treatment throughout the course of Experiment 3. Other designations as in Figure 19.

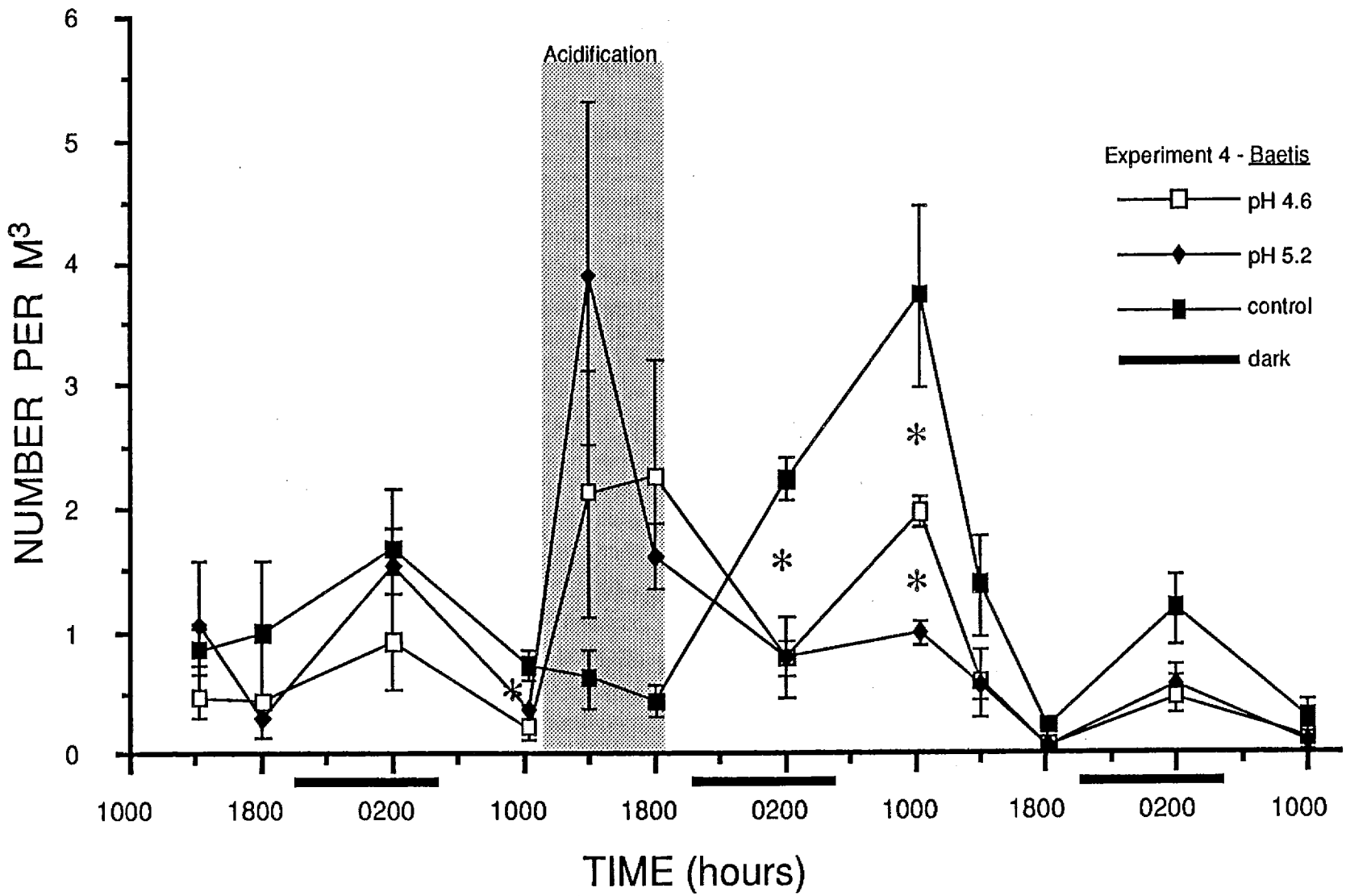


Figure 21. Baetis drift rates (number / $m^3 \pm 1$ S.E.) for each treatment throughout the course of Experiment 4. Other designations as in Figure 19.

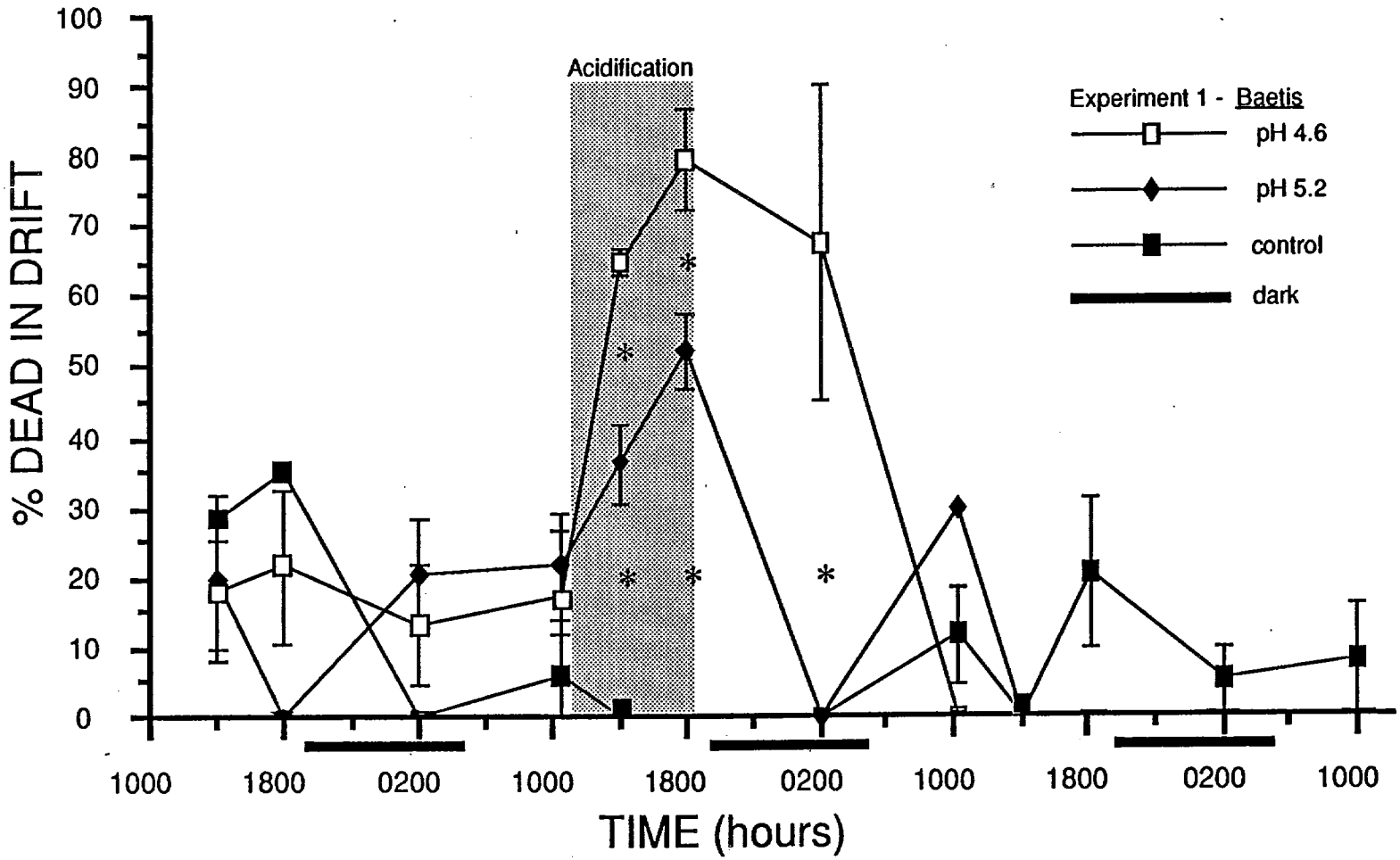


Fig. 22. The percentage of Baetis that were dead in each drift set over the course of Experiment 1 (± 1 S.E.). Other designations as in Figure 19.

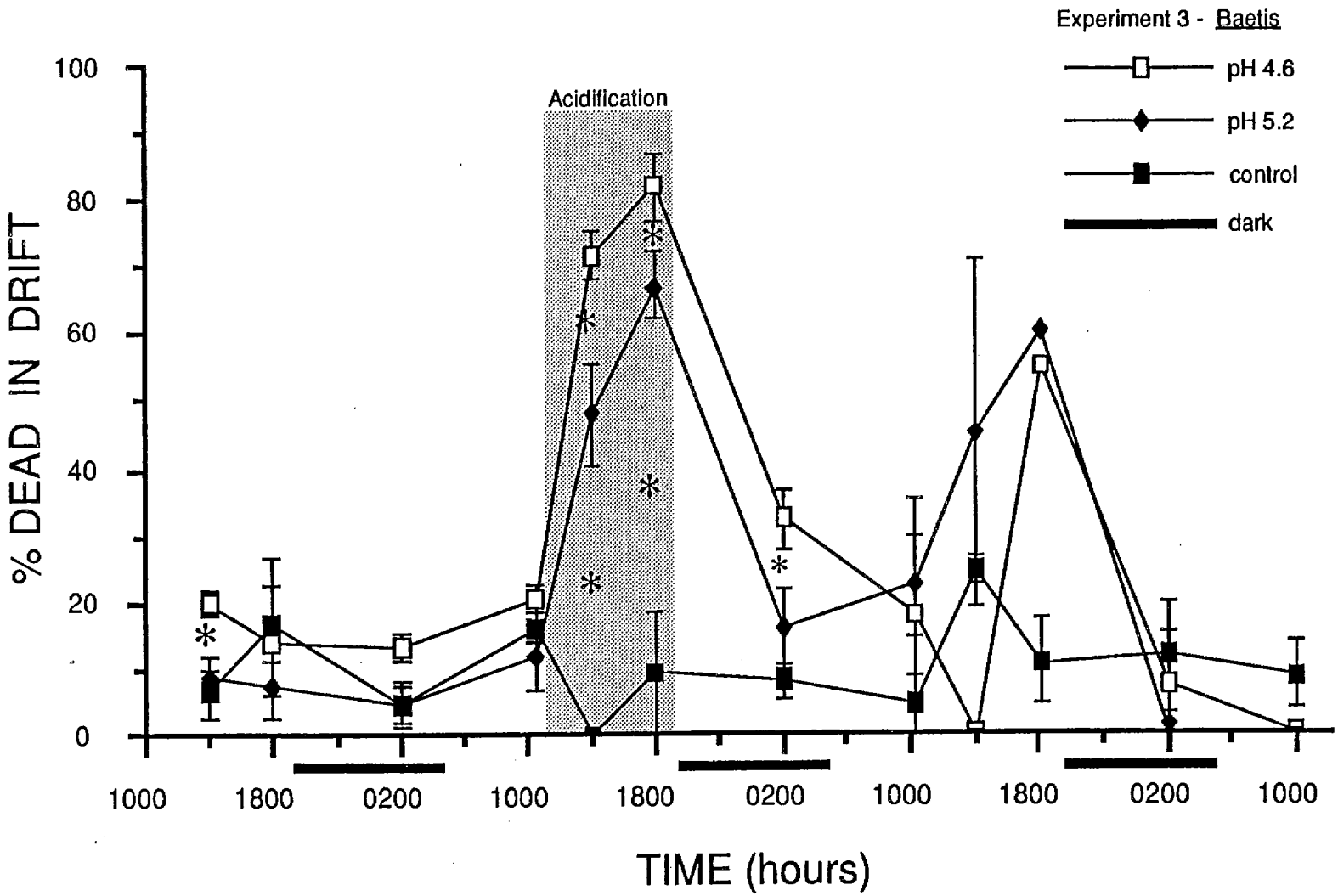


Figure 23. The percentage of Baetis that were dead in each drift set over the course of Experiment 3 ( ± 1 S.E.). Other designations as in Figure 19.

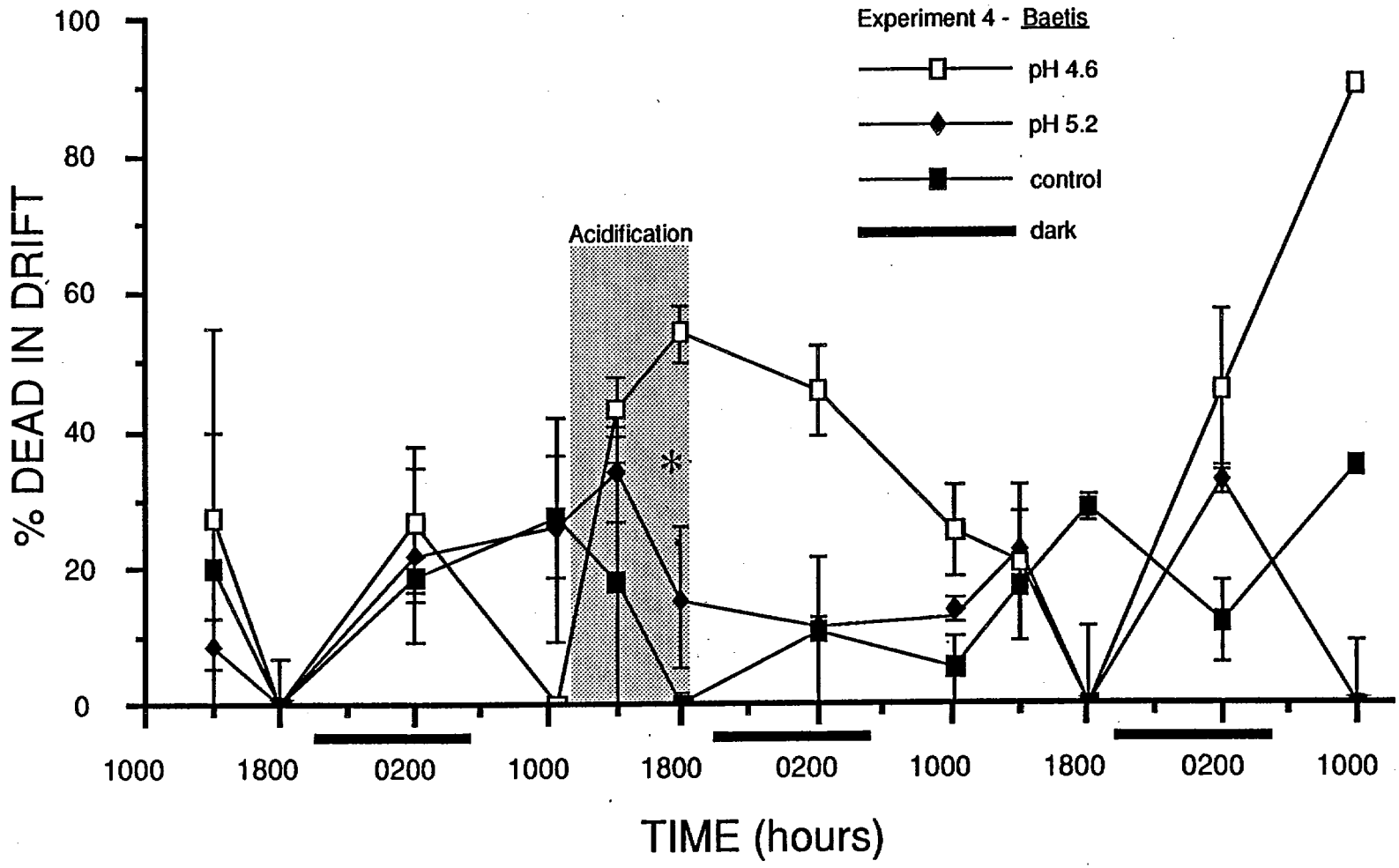


Figure 24. The percentage of Baetis that were dead in each drift set over the course of Experiment 4 ( ± 1 S.E.). Other designations as in Figure 19.

the proportion of drifting baetids that were dead occurred in the second half of the acid pulse when pH 4.6 channels were greater than pH 5.2 channels and controls. In Experiment 4 during the acid pulse, 31 % of the increased drift at pH 5.2 and 82 % of the increased drift at pH 4.6 could be attributed to toxic drift.

In Experiment 3 a pronounced nocturnal peak in baetid drift disappeared, even in control channels, after acid was added (Fig. 20). In contrast, there was no nocturnal drift peak before experimental manipulations in Experiments 1 and 4 (Figs. 19, 21). We attributed this variability in the presence of nocturnal peaks in drift to variation in benthic density among experiments. Our results suggest that baetid drift rates are density-dependent, being higher when baetid benthic densities are higher. Baetid benthic densities were over twice as high at the outset of Experiment 3 than at the outset of Experiments 1 and 4, or at the end of Experiment 3 (Figs. 41, 42). We postulate that when baetid benthic densities are high, baetid drift rates are high as baetids try to avoid intense competition for food or space. Below some threshold density, however, resources are sufficient to support resident baetids and baetids show little tendency to disperse. It should be remembered that a block net precluded incoming drift for the entire time that nets monitored drift leaving channels. We hypothesize that, after a large number of baetids left channels at night at the beginning of Experiment 3, benthic densities were reduced to the point that competition, and dispersal rates, were drastically reduced (Fig. 20). Similarly, benthic densities at the beginning of Experiments 1 and 4 were already low enough that baetid drift rates were low (Figs. 19, 21). The observation that benthic densities of Baetis were similar in control channels at the beginning and end of Experiments 1 and 4, and at the end of Experiment 3, lends some support to this idea (Figs. 41, 42). Density-dependent drift responses may have accounted for variability in nocturnal drift peaks for other taxa, such as Epeorus and chironomids (Figs. 25, 26, 30). Although density-dependent drift responses can account for increased drift when benthic densities are high, they do not explain why increased drift occurs at night. Although it is commonly observed that invertebrate drift is highest at night, the explanation for this diel periodicity remains controversial and may be related to diel changes in risk of predation (Allan 1978).

Nymphs of the mayflies Epeorus and Paraleptophlebia also exhibited significant increases in drift rates in acidified channels during acidification

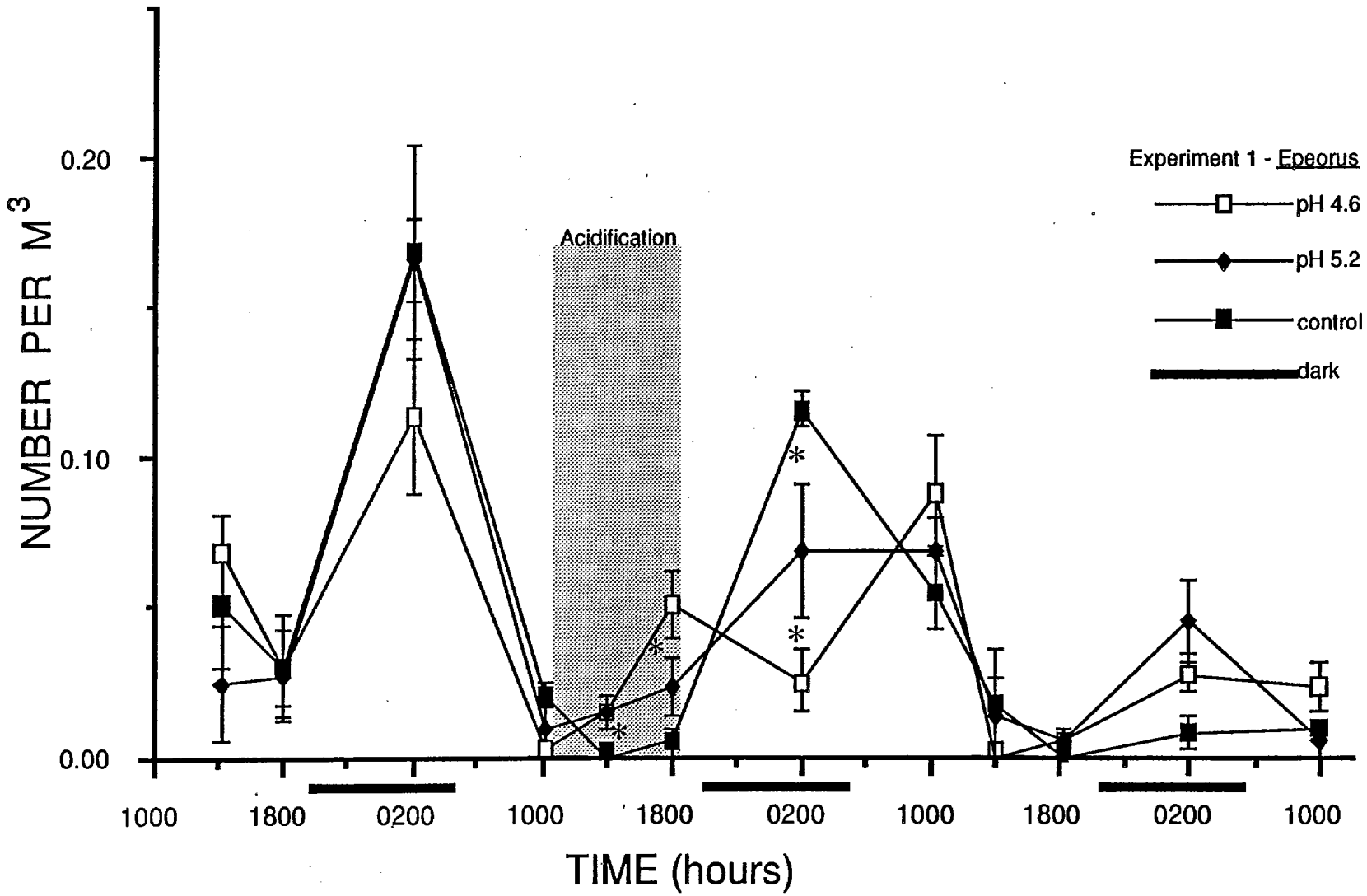


Fig. 25. Epeorus drift rates (number / $m^3$ ± 1 S.E. ) for each treatment throughout the course of Experiment 1. Other designations as in Figure 19.

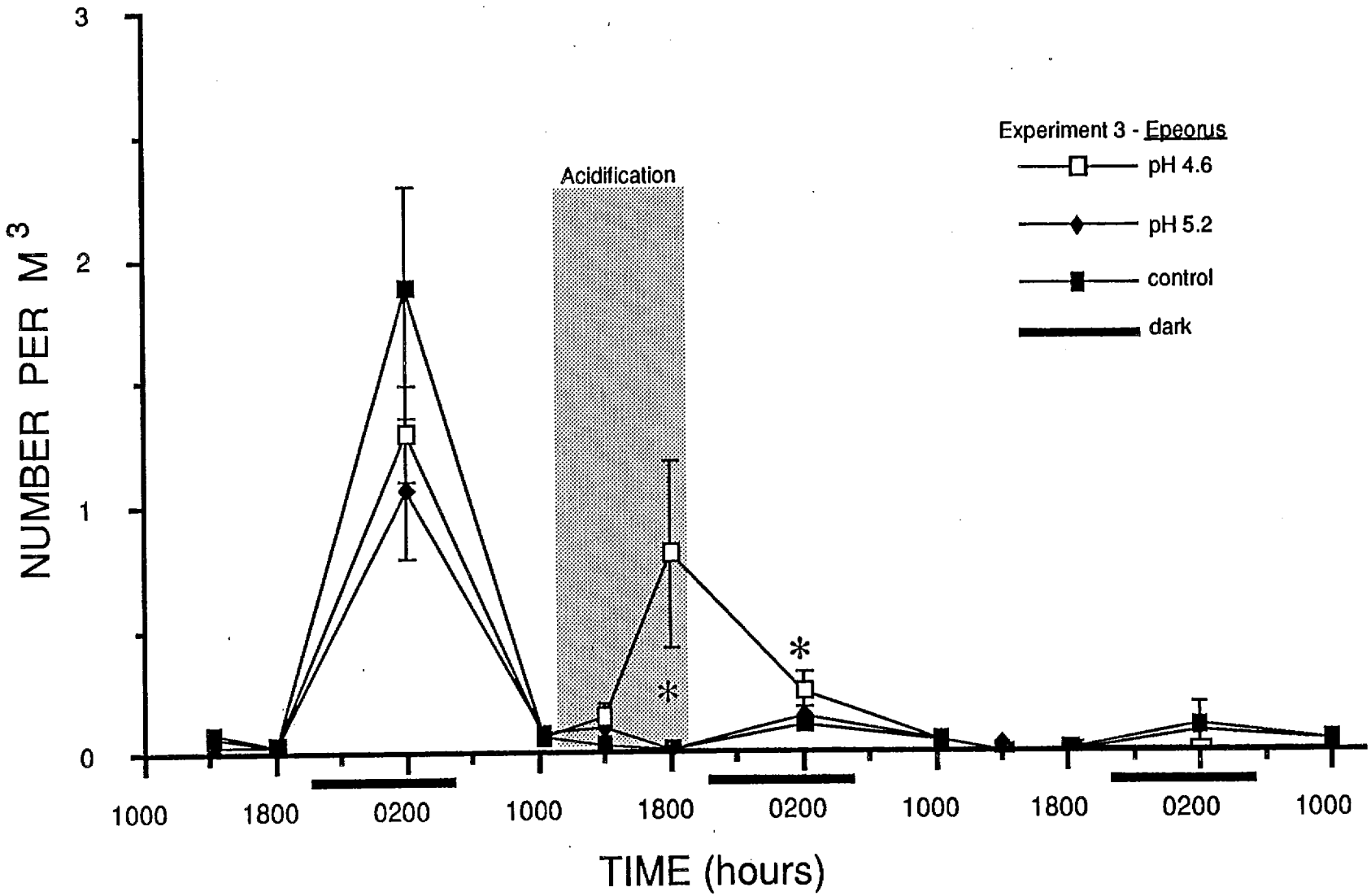


Figure 26. Epeorus drift rates (number / $m^3$ ± 1 S.E.) for each treatment throughout the course of Experiment 3. Other designations as in Figure 19.

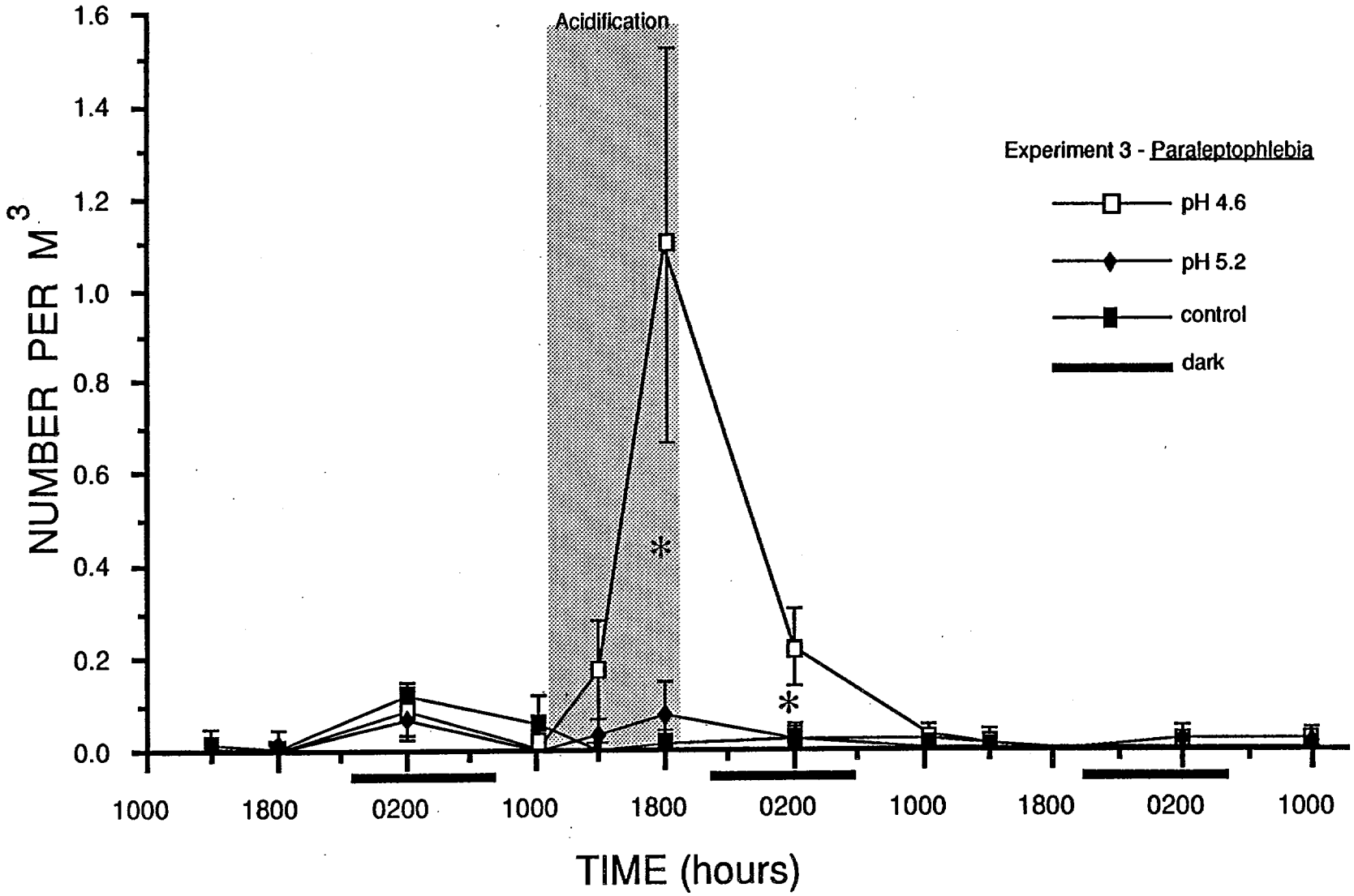


Figure 27. Paraleptophlebia drift rates (number / $m^3$ ± 1 S.E.) for each treatment throughout the course of Experiment 3. Other designations as in Figure 19.

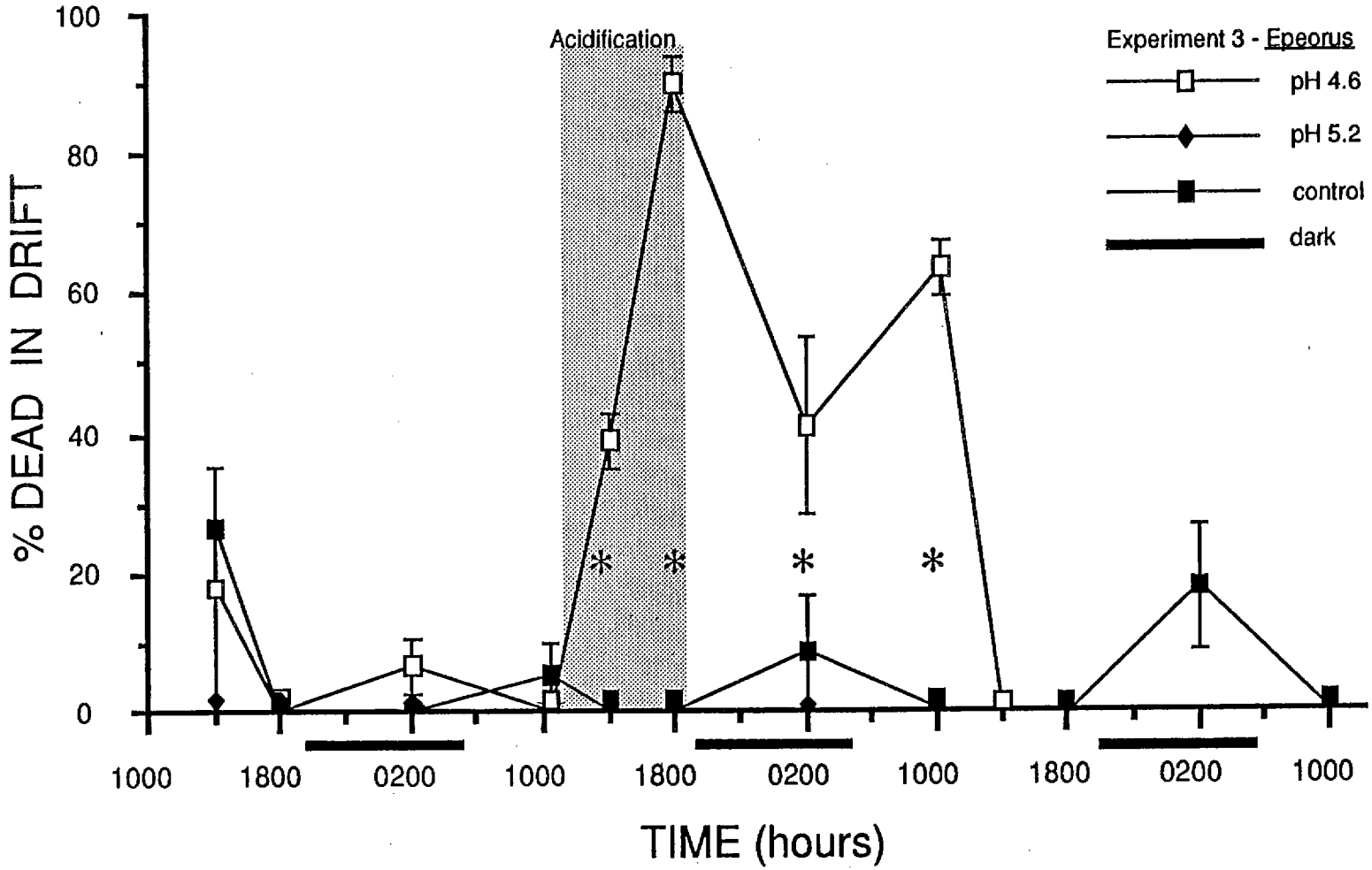


Figure 28. The percentage of Epeorus that were dead in each drift set over the course of Experiment 3 ( ± 1 S.E.). Other designations as in Figure 19.

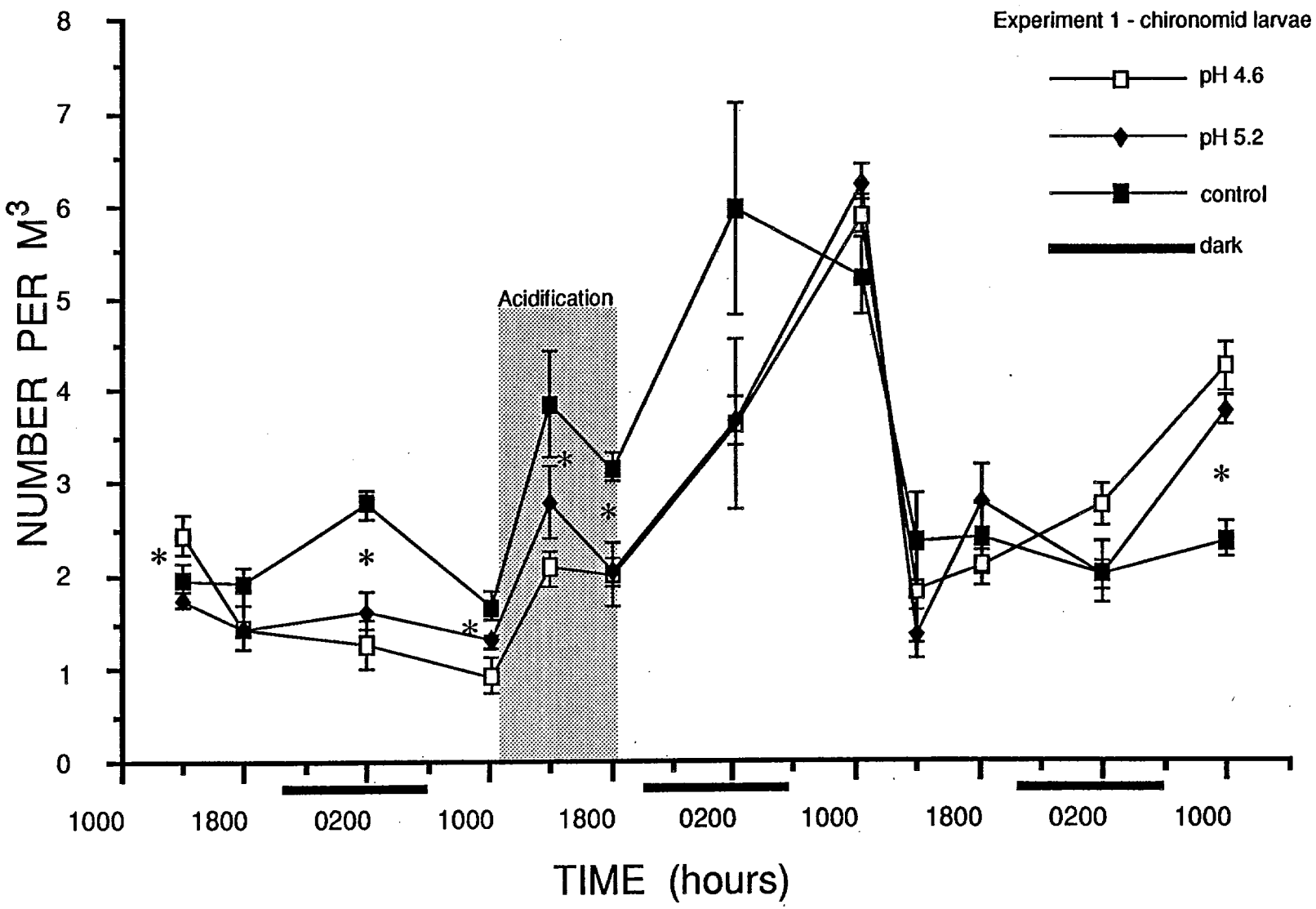


Fig. 29. Chironomid larvae drift rates (number / $m^3$ ± 1 S.E. ) for each treatment throughout the course of Experiment 1. Other designations as in Figure 19.

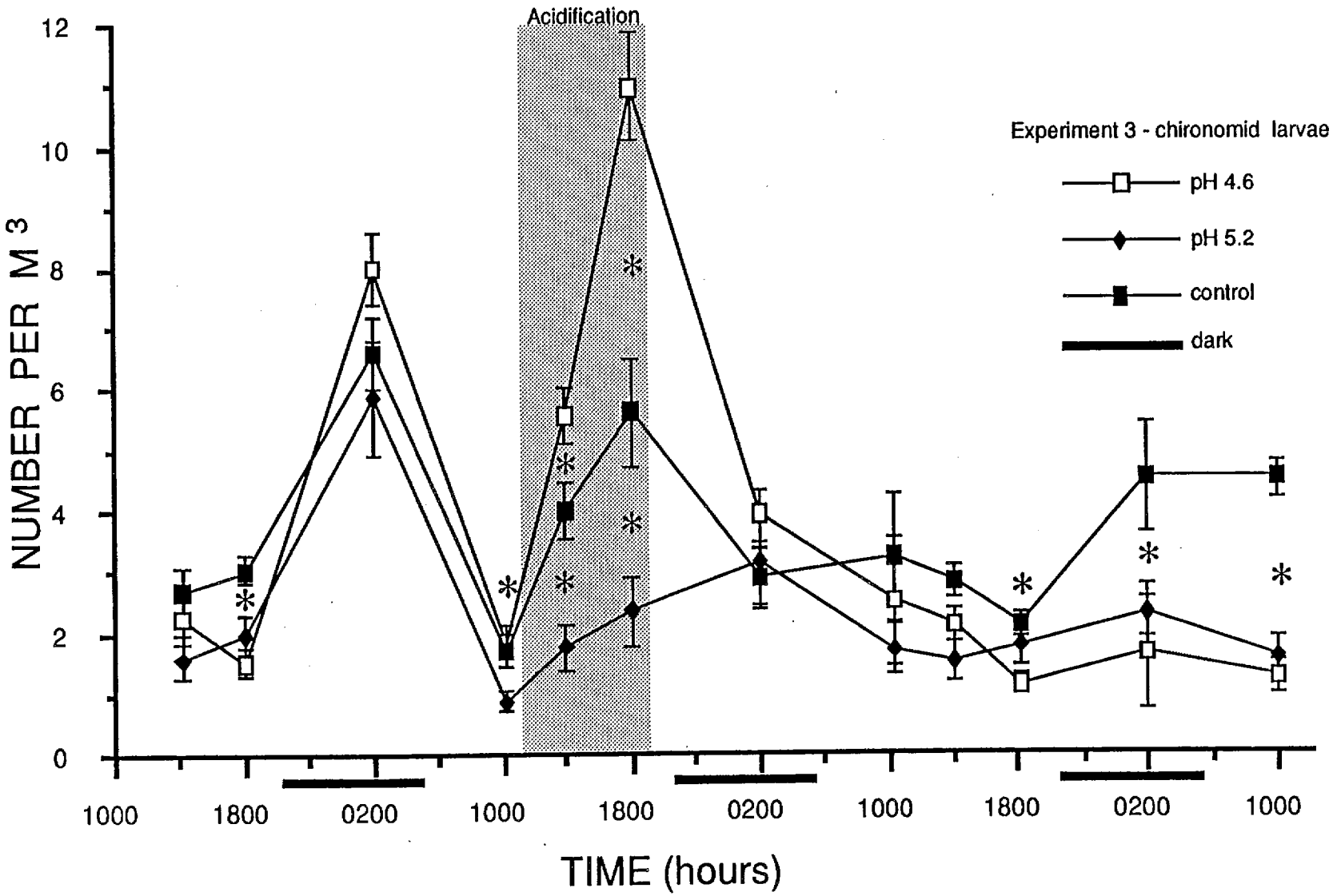


Figure 30. Chironomid larvae drift rates (number / $m^3$ ± 1 S.E.) for each treatment throughout the course of Experiment 3. Other designations as in Figure 19.

(Figs. 25 - 27). Epeorus drift abundance was low in Expt. 1 but was higher in acidified channels than controls during the first half of the acid pulse, and higher in the pH 4.6 than the pH 5.2 and control channels in the second half of the pulse ($F=6.8$, $p<.018$; $F=5.8$ $p<.024$). Conversely, nocturnal drift following acidification was 2x higher in control relative to acidified channels ($F=10.8$, $p<.004$). Live to dead ratios of drifting Epeorus were not monitored in Experiment 1. For the remainder of the sampling period drift rates in control and acidified channels were similar. In Expt 3 significantly greater drift was recorded for the 4.6 channels relative to other treatments during the second half of and immediately after acidification ($F=8.2$ $p<.014$, $F = 5.5$, $p<0.02$). The percentage of drifting Epeorus that was dead was significantly higher in the pH 4.6 channels than in the pH 5.2 or control channels, and became as high as 90% (Fig. 28). Approximately 45% of the increased drift in the pH 4.6 channels could be attributed to toxic drift. Paraleptophlebia was almost absent from drift throughout the experiments, although significantly higher drift was recorded from pH 4.6 than pH 5.2 or control channels during and immediately after the acid pulse in Expt. 3 (Fig. 27) ($F=7.3$ & $5.7$, $p<.05$).

Drift responses of chironomids were temporally variable. Larval drift was significantly more abundant in control than treatment channels prior to and during acidification in Experiment 1, but significantly higher in acid channels than controls over a day and a half after acid additions stopped ($F=4.6$ - $11.1$ $p<.05$) (Fig. 29). Chironomid drift during acidification in Experiment 3 showed the pattern pH 4.6 > control > pH 5.2 but control drift was also higher than the acid treatments at one point before acidification (Fig 30). Higher drift occurred in control channels than in acidified channels in the last three net sets ($F=4.8$ - $29.4$ $p<.05$). There were no consistent patterns in chironomid larval drift rates among treatments in Experiment 4 (Fig 31). Drift rates of chironomid pupae were occasionally higher in control than in acidified channels both before (Expt. 1) and after (Expts. 1 and 3) acid pulses (Figs 32 and 33).

Acidification enhanced the numbers of dead drifting chironomids in Expt. 3 (Fig. 34). The percentage of drifting chironomid larvae that were dead were 2.5 to 3.3 times higher in pH 4.6 than control channels, and 1.6 to 2.9 times higher in pH 5.2 than control channels. During the first four hours of the acid pulse the percentage of dead drifting chironomids were significantly higher in acid than control channels, whereas during the last four hours of

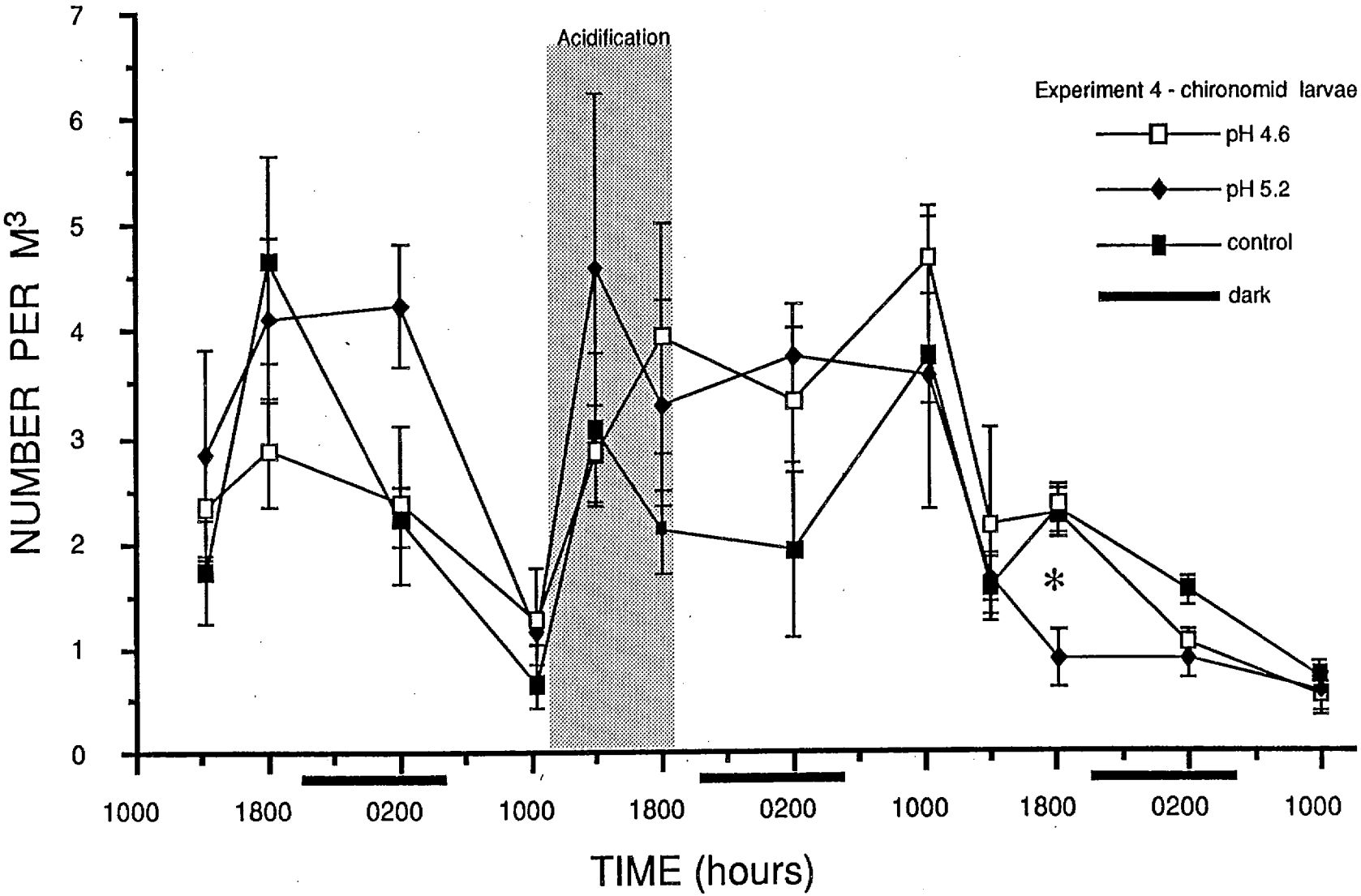


Figure 31. Chironomid larvae drift rates (number / $m^3 \pm 1$ S.E.) for each treatment throughout the course of Experiment 4. Other designations as in Figure 19.

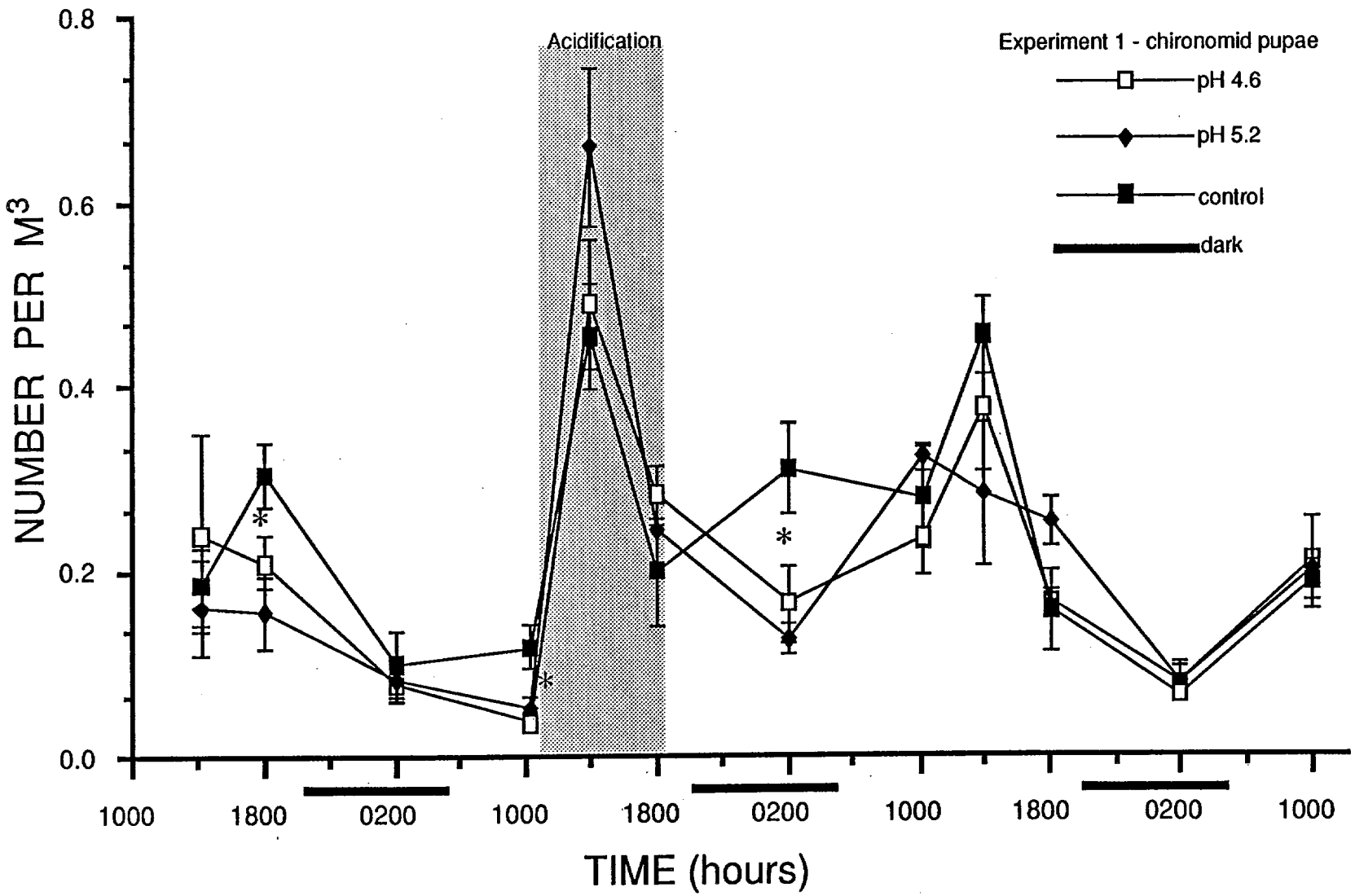


Fig. 32. Chironomid pupae drift rates (number / $m^3$ ± 1 S.E. ) for each treatment throughout the course of Experiment 1. Other designations as in Figure 19.

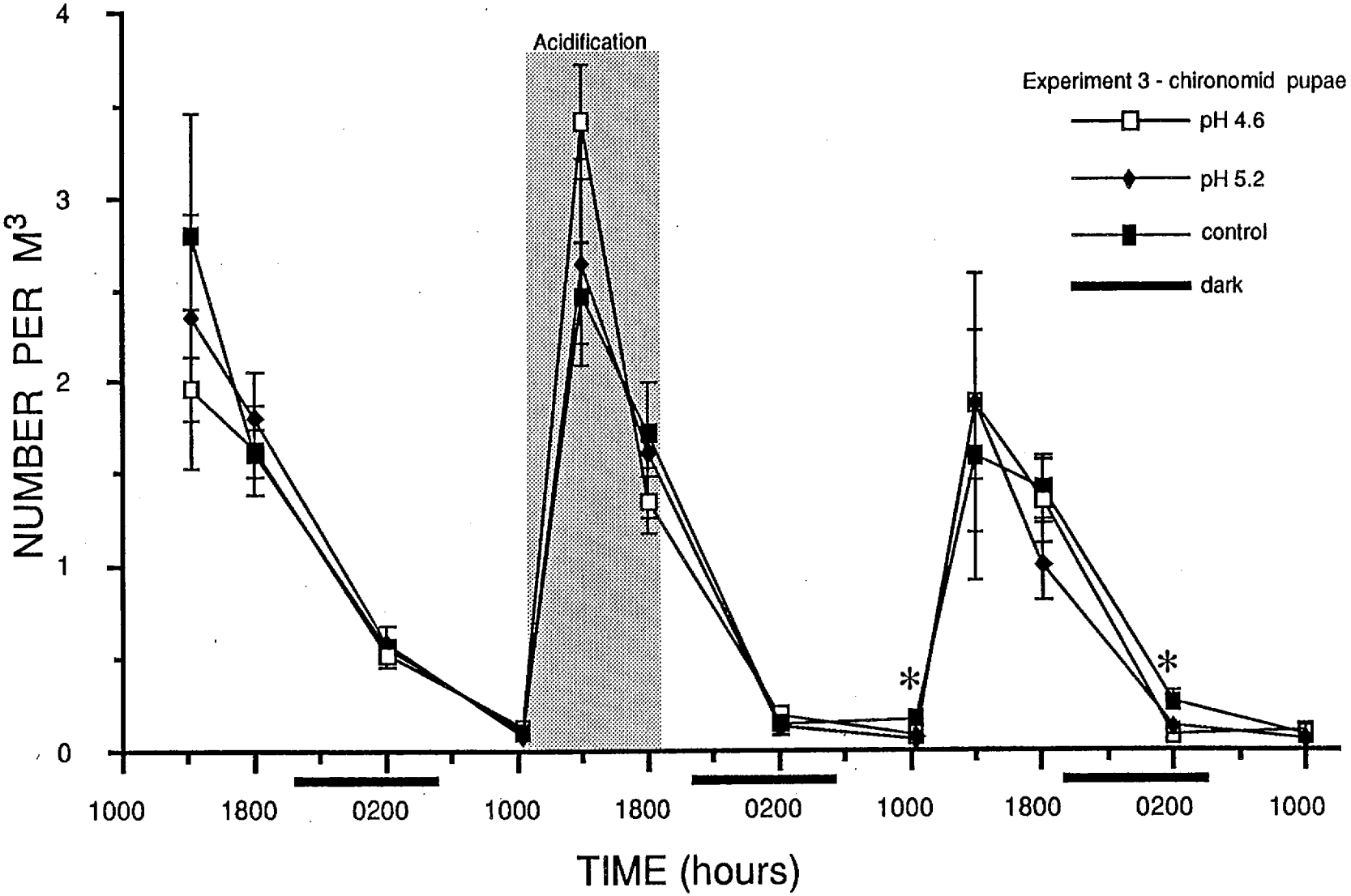


Figure 33. Chironomid pupae drift rates (number / $m^3$ ± 1 S.E.) for each treatment throughout the course of Experiment 3. Other designations as in Figure 19.

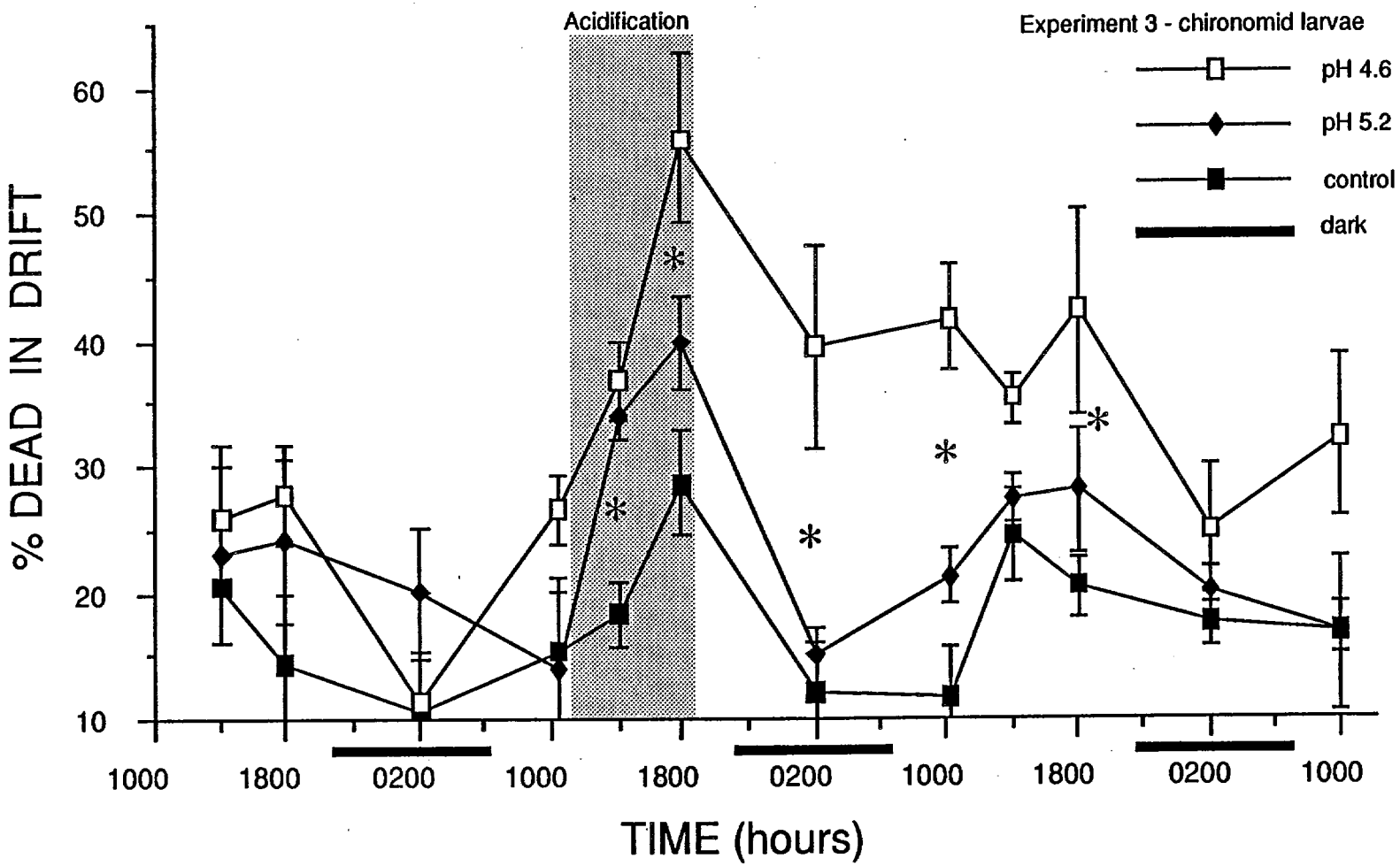


Figure 34. The percentage of chironomid larvae that were dead in each drift set over the course of Experiment 3 ( ± 1 S.E.). Other designations as in Figure 19.

the pulse pH 4.6 channels had higher proportions of dead chironomids than pH 5.2 or control channels. All increased drift in the acidified channels during acidification was owing to the drift of dead individuals. A higher percentage of dead chironomids was observed in the drift of pH 4.6 channels as opposed to pH 5.2 or control channels for some time after acid additions stopped. No consistent patterns in the proportion of drifting chironomids that were dead were noted in other experiments.

Drift rates of a number of rarer taxa were affected by acidification. Mites in Expt. 1 showed higher drift in control than in acidified channels during the second half of the acid pulse, although drift appeared to be higher in pH 5.2 than pH 4.6 and control channels in the preceding period (Fig. 35). On the other hand, larvae of the stonefly Zapada in Expt. 3 showed increased drift in 4.6 channels relative to controls during the second half of the acidification ($F=7.3$, $p<.02$, Fig. 36). Although rare, drift rates of larvae of the caddisfly Amiocentrus tended to increase in acid channels relative to controls in Experiment 3 ($p<.054$) (Fig. 37). Adult winged insects tended to have higher drift rates in control channels relative to acidified channels after acidification (Figs. 38, 39). Because this group was composed primarily of chironomid adults it is not surprising that they show the same response patterns as chironomid pupae. Other taxa showed few responses to acid treatment (e.g. Fig. 40).

*Benthic densities* - There were no significant differences (ANOVA) in pretreatment benthic densities in any of the experiments for any of the common taxa (chironomid larvae, chironomid pupae, Baetis, Paraleptophlebia, Epeorus, Isoperla, Rhyacophila, Hydroporus, mites, oligochaetes). The most abundant taxa in these experiments were chironomid larvae, chironomid pupae, and Baetis nymphs. Baetis benthic densities were significantly less in acid channels than in controls in Experiments 1 and 3 ($F= 31.05$, $P<.0001$ and $F=11.044$, $p<.004$, respectively, Duncan's Multiple Runs test) In Experiment 1 Baetis densities in pH 4.6 and 5.2 channels were, respectively, 10 and 16 % of control levels two days after acidification (Fig. 41). In Experiment 3 Baetis densities in pH 4.6 channels were lower than those in pH 5.2 channels which were lower than those in controls (5 and 27 % of controls, respectively, Fig. 42). Baetis densities in control channels remained constant during Experiment

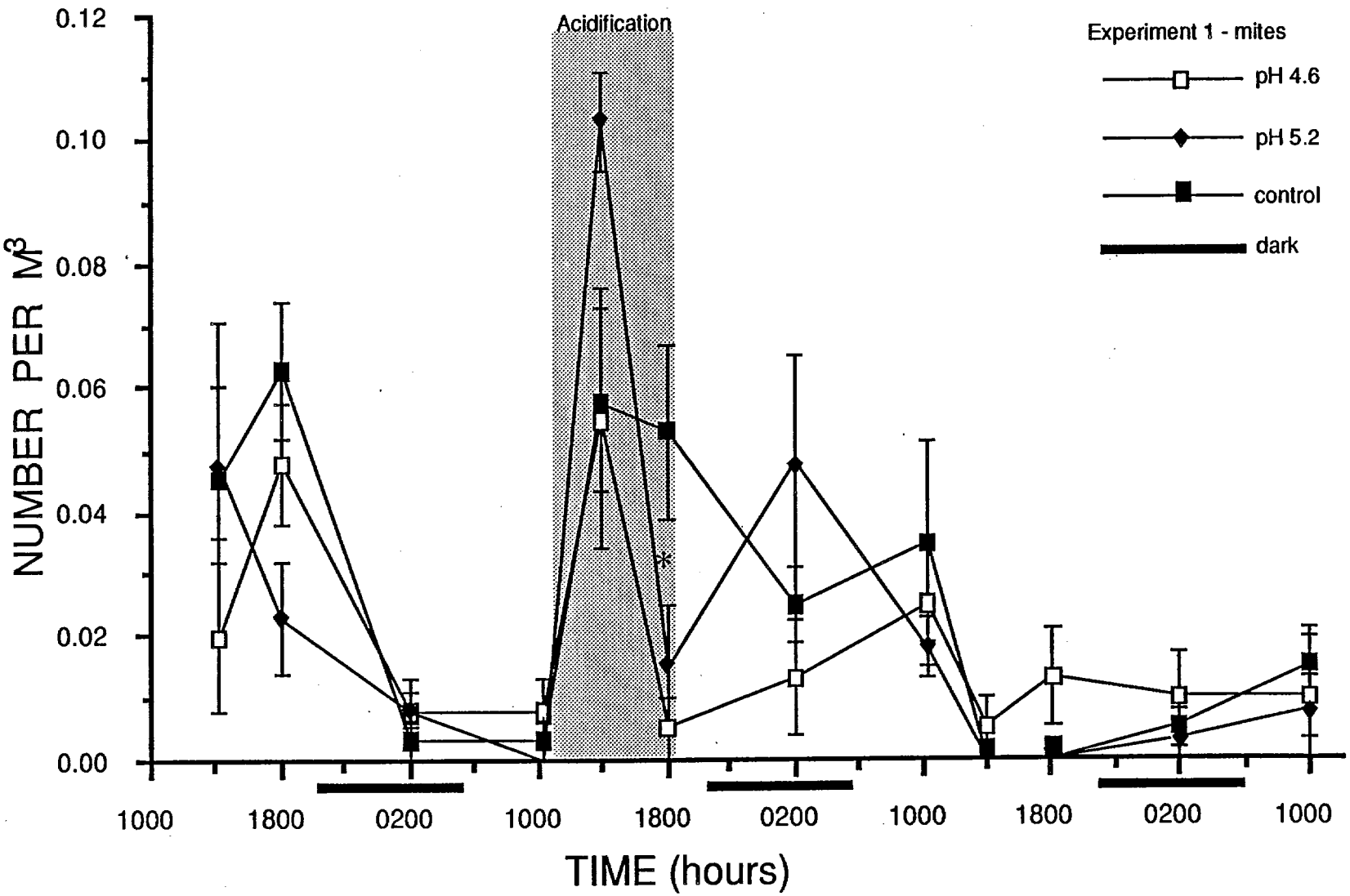


Fig. 35. Aquatic mite drift rates (number / $m^3$ ± 1 S.E. ) for each treatment throughout the course ofExperiment 1. Other designations as in Figure 19.

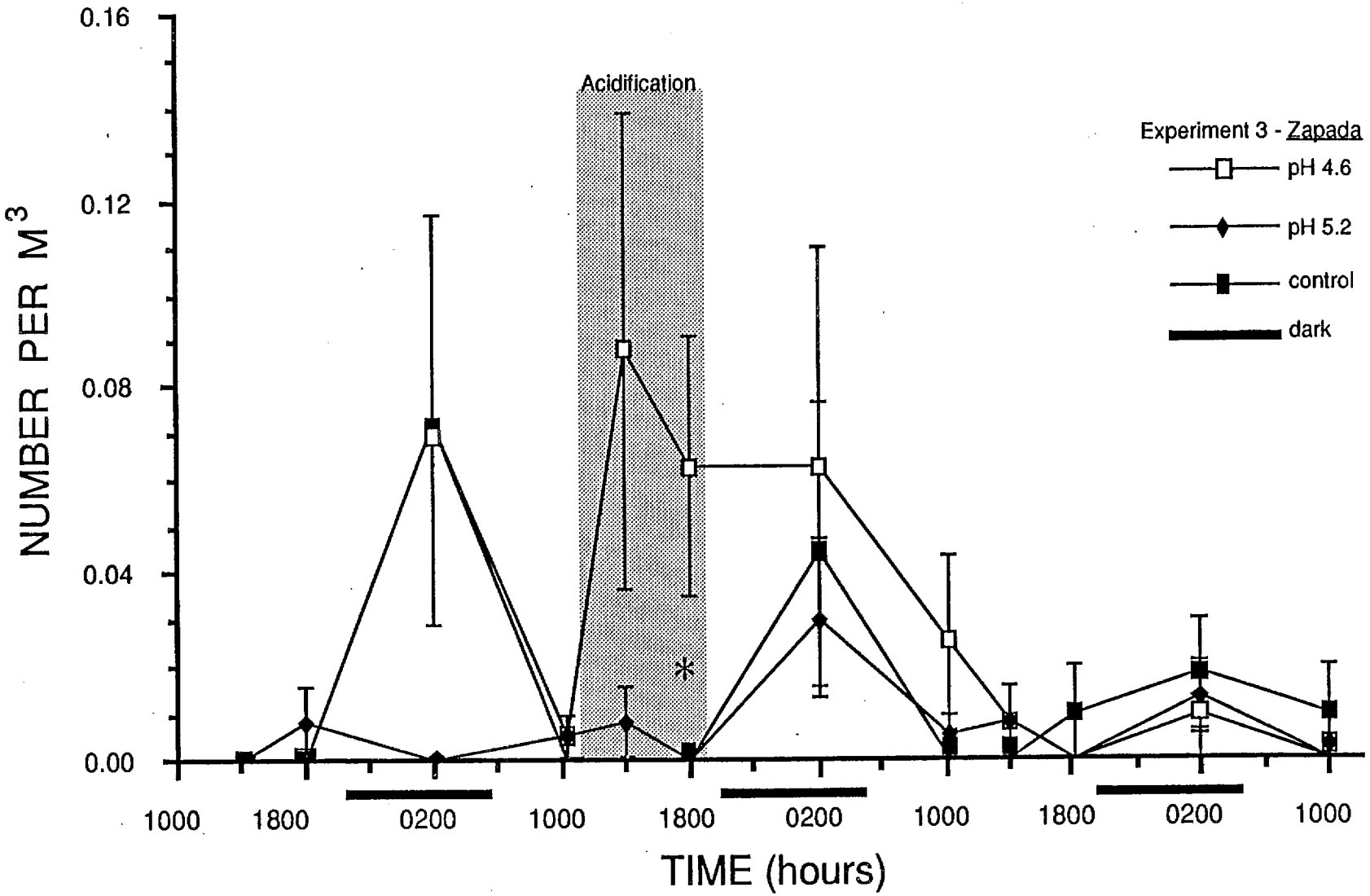


Figure 36. Zapada drift rates (number / $m^3$ ± 1 S.E.) for each treatment throughout the course of Experiment 3. Other designations as in Figure 19.

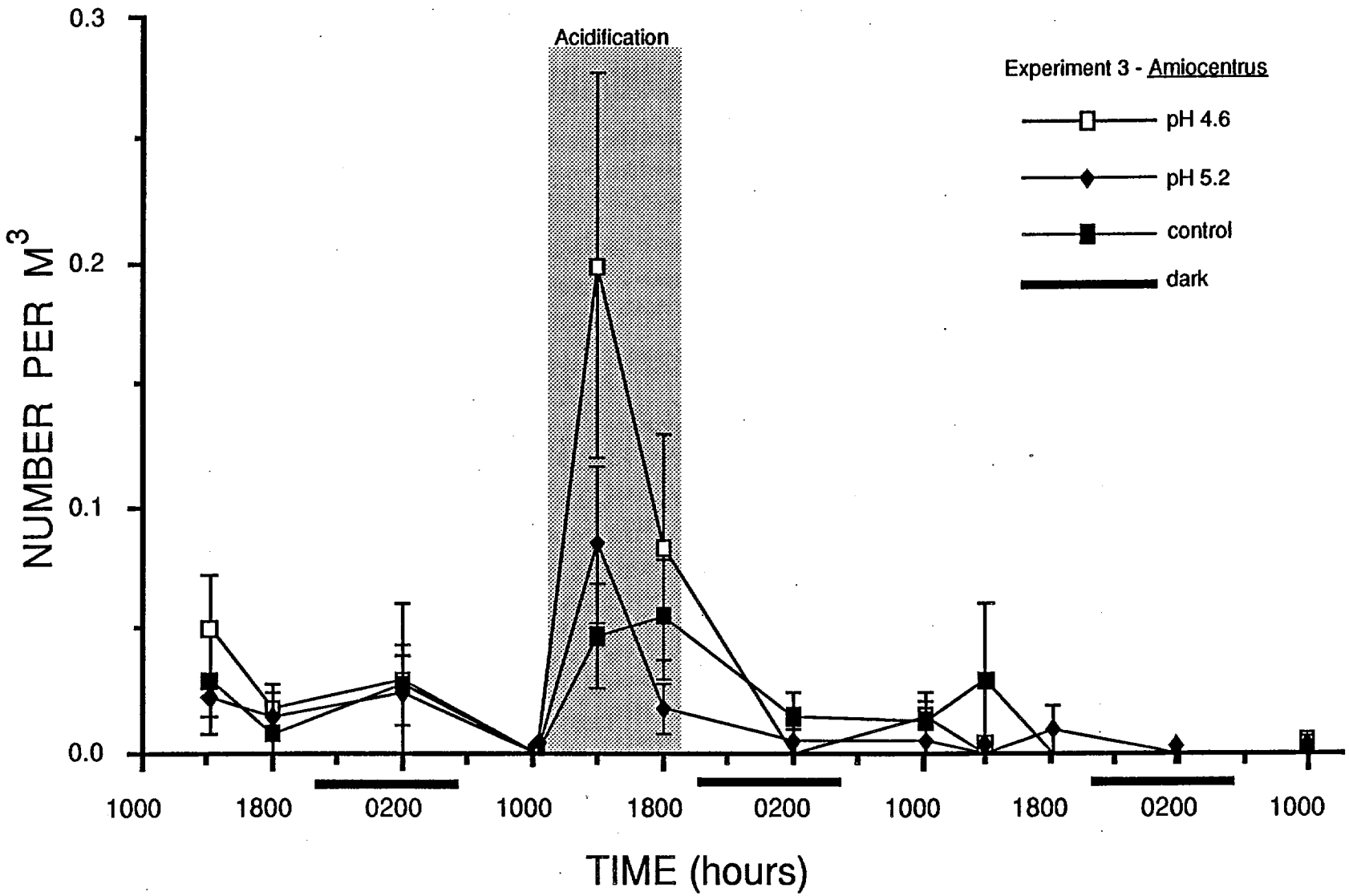


Figure 37. Amiocentrus drift rates (number / $m^3$ ± 1 S.E.) for each treatment throughout the course of Experiment 3. Other designations as in Figure 19.

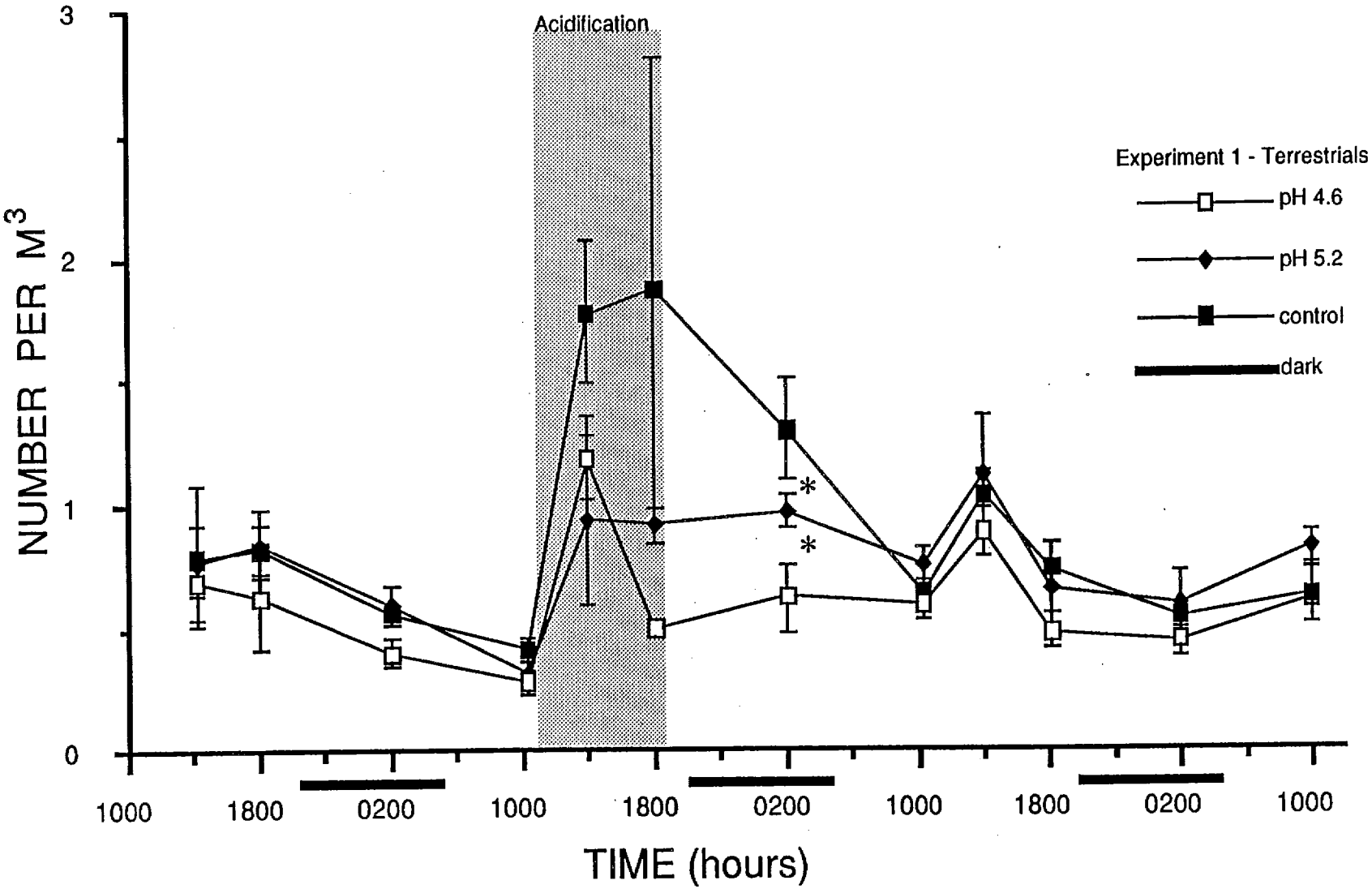


Fig. 38. Terrestrial invertebrate drift rates (number / $m^3$ ± 1 S.E. ) for each treatment throughout the course of Experiment 1. Other designations as in Figure 19.

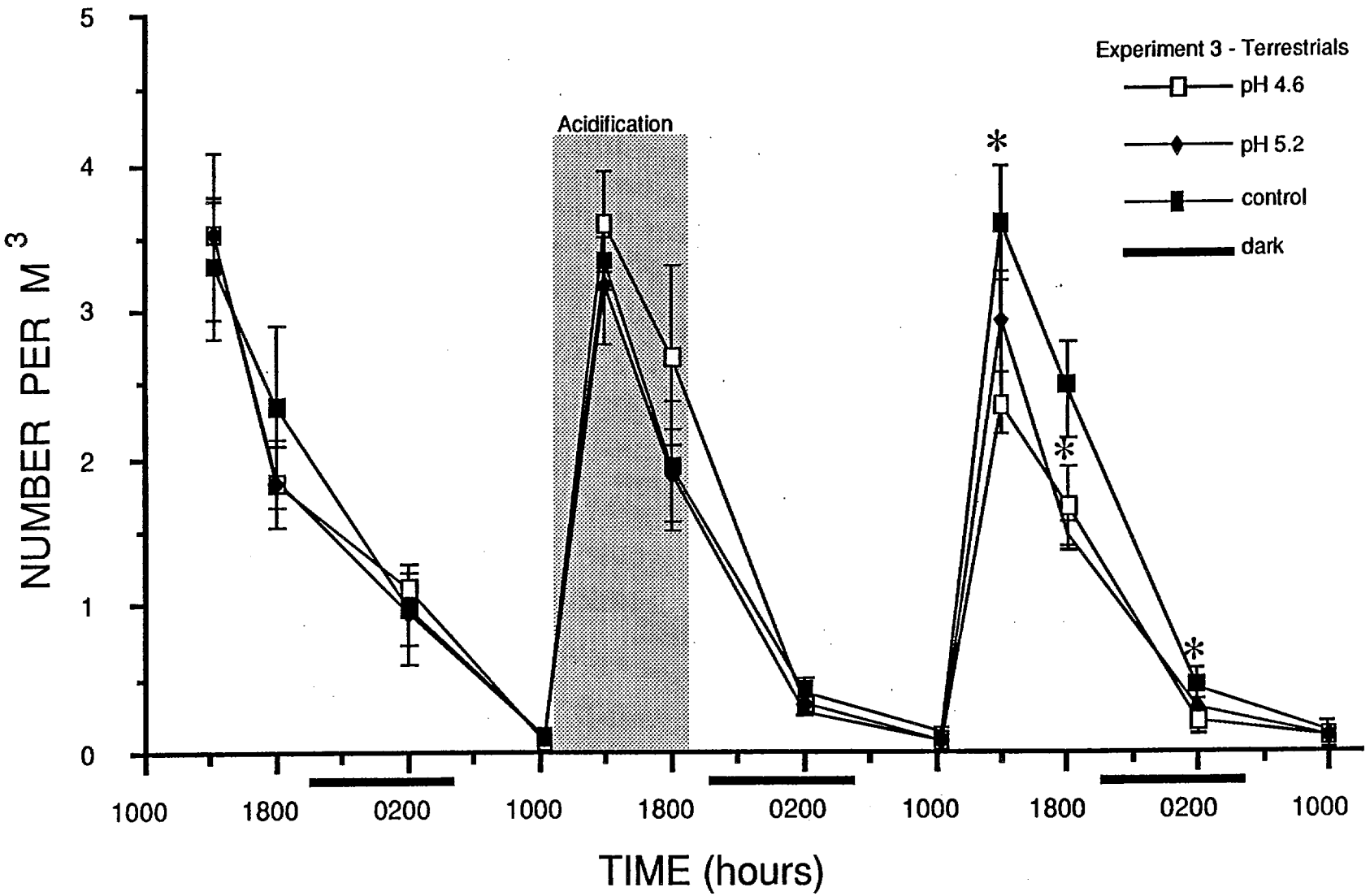


Figure 39. Terrestrial invertebrate drift rates (number / $m^3$ ± 1 S.E.) for each treatment throughout the course of Experiment 3. Other designations as in Figure 19.

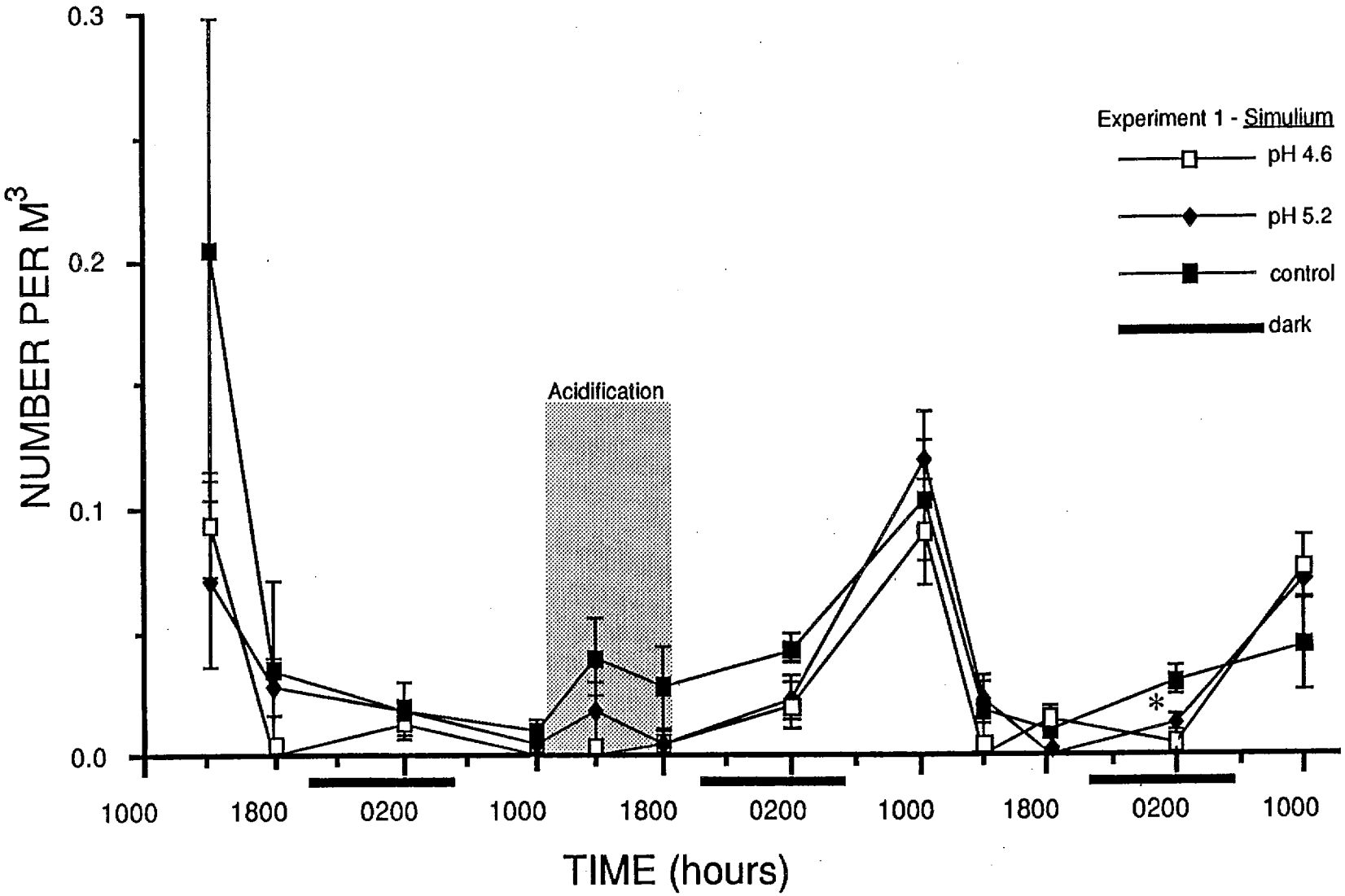


Fig. 40. Simulium larvae drift rates (number / $m^3$ ± 1 S.E. ) for each treatment throughout the course of Experiment 1. Other designations as in Figure 19.

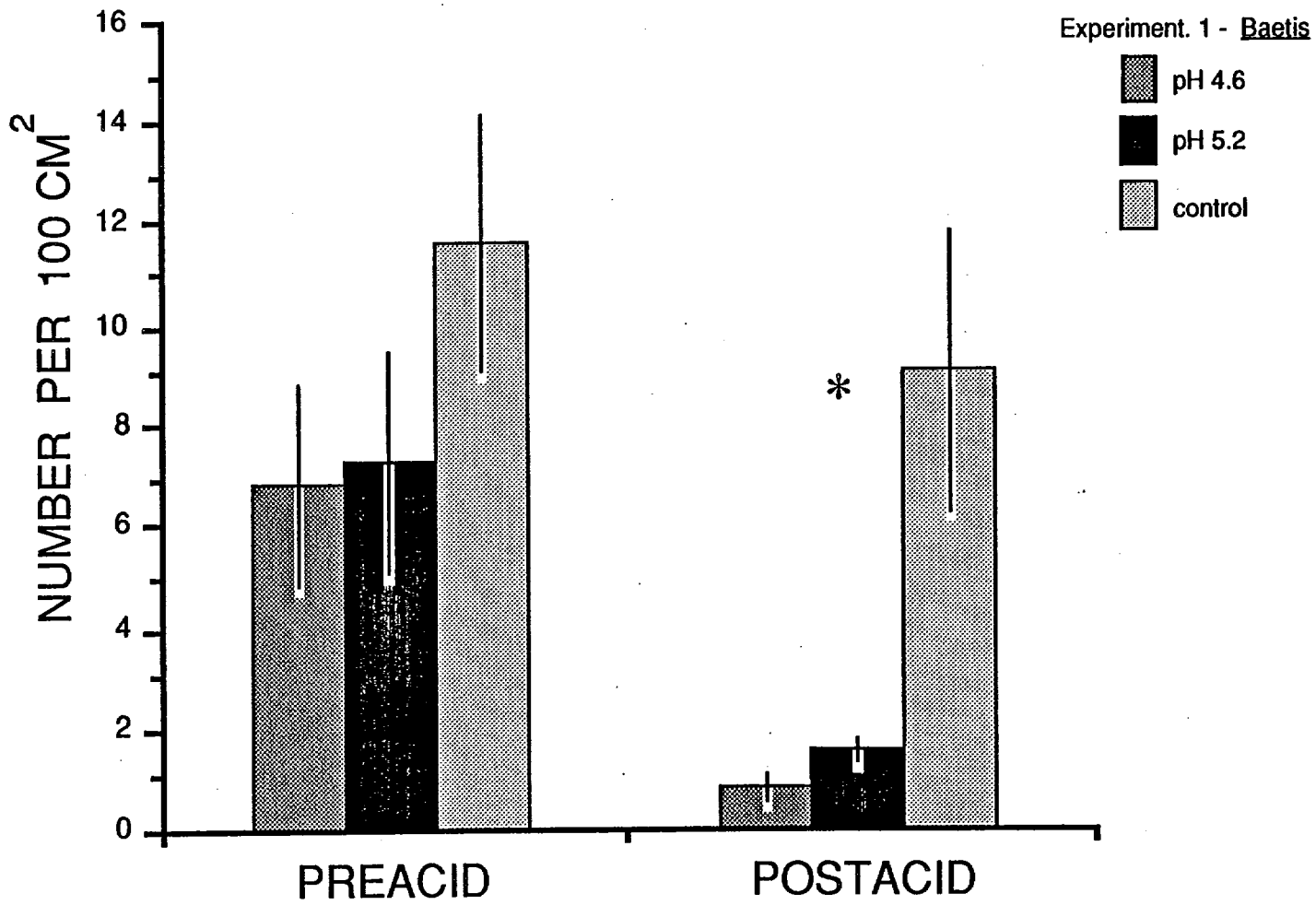


Figure 41. Benthic densities (number per 100 $cm^2$ ± 1 S.E.) of Baetis in treatment channels in Experiment 1. Preacid samples were taken 24 hours prior to acid addition, postacid were taken 40 hours after the conclusion of acid addition. An asterisk over an histogram indicates that it, and any histograms to the left, are significantly lower than the next histogram to the right ($P < 0.05$, Duncan's multiple range test)

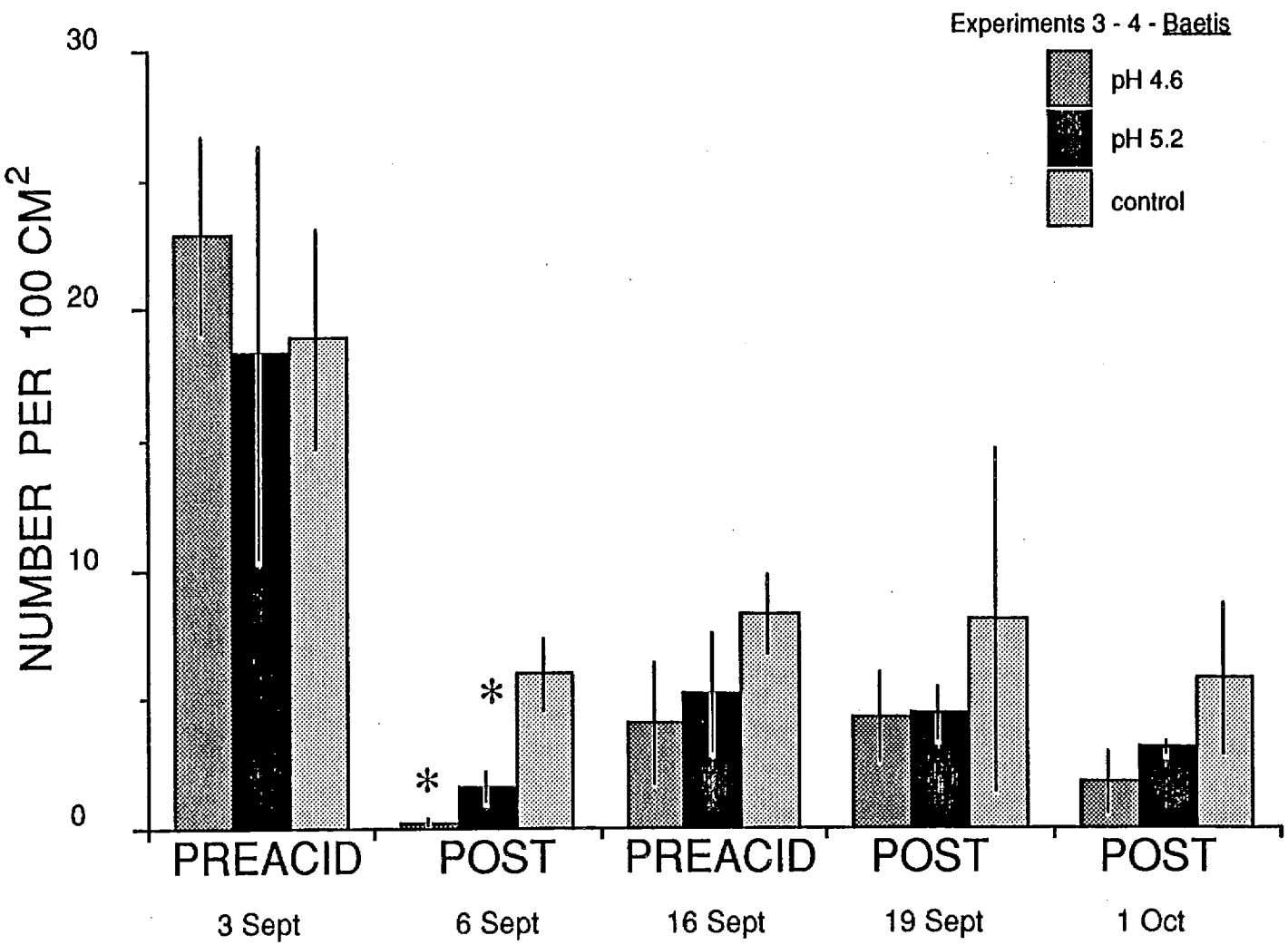


Figure 42. Benthic densities (number per 100 $cm^2$ ± 1 S.E.) of Baetis in treatment channels in Experiments 3 and 4. Preacid samples were taken 24 hours prior to an acid addition, postacid samples were taken 40 hours after the conclusion of an acid addition. An additional postacid sample was taken 2 weeks after acid addition in Experiment 4. Immigration by drifting aquatic invertebrates was excluded during this time period. A 250 µm net screened channel intake water from before benthic sample collection on 3 September until after benthic sample collection on 1 October. An asterisk over an histogram indicates that it is signifiantly lower than the next histogram to the right ($P < 0.05$, Duncan's multiple range test).

1. but declined in Experiment 3 probably in response to drift losses (see above). Because drift from the Marble Fork was prevented from entering the channels between Experiments 3 and 4, these experiments can be viewed as one long experiment with two acid pulses. Twelve days after acid was added in Experiment 3, baetid densities had recovered in pH 4.6 and 5.2 channels and there were no significant differences among treatments (Fig. 42). Baetis densities remained relatively constant during Experiment 4 and there were no significant treatment effects on baetid densities. Approximately two weeks after acid addition in Experiment 4 the mean Baetis densities in 4.6 and 5.2 channels were 32 and 56 % of those in control channels. These differences were not significant, however, primarily owing to high variation in control channels.

The densities of Paraleptophlebia appeared to be depressed by acid inputs through Experiments 3 and 4, but the only significant differences were recorded two weeks after the Experiment 4 acidification ($F = 6.8$, $P < 0.03$, Duncan's multiple range test, Fig. 43). Paraleptophlebia densities at pHs 4.6 and 5.2 were aproximately 13 and 25% of those in controls after the Experiment 3 acidification, but densities were too low and variable to indicate statistically significant effects. Densities of this mayfly increased in all channels after Experiment 3; however, treatment differences were largely maintained. Densities of Paraleptophlebia in 4.6 channels were approximately 9 to 14% of those in control channels before and after Experiment 4, whereas densities in 5.2 channels were approximately 80 % of those in controls before acidification but declined to 40 - 50 % of controls after acid inputs. Benthic densities of other taxa were not significantly affected by acid additions in Experiments 1, 3, and 4 (Figs. 44 - 47).

In summary, benthic invertebrates showed a variety of responses to acid inputs. Drift rates of Baetis increased and benthic densities declined with acidification. Lower drift rates in acidified channels than in controls after acid pulses were probably owing to the depletion of the benthic source for drift. A large part of the increased drift of Baetis in acidified channels during acid pulses was owing to the drift of individuals killed by the acid. Depending on the experiment, 31 to 73 % of the increased drift in pH 5.2 channels and 76 to 100 % of drift in pH 4.6 channels was owing to toxic drift. Although Baetis drift responded to reductions to both pHs 4.6 and 5.2, other taxa responded primarily only to the lowest pH. In the first half of

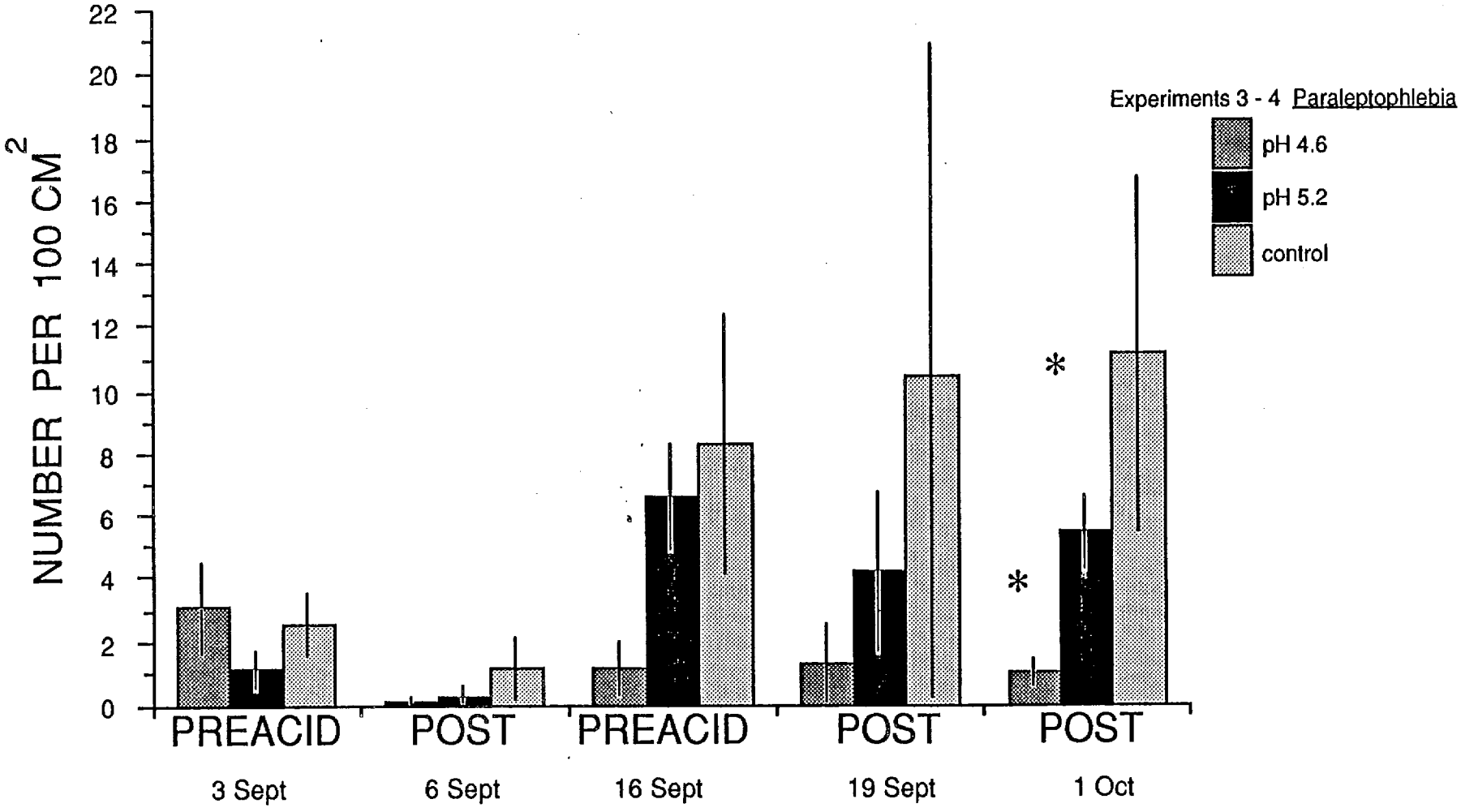


Figure 43. Benthic densities (number per 100 $cm^2$ ± 1 S.E.) of Paraleptophlebia in treatment channels in Experiments 3 and 4. Preacid samples were taken 24 hours prior to an acid addition, postacid samples were taken 48 hours after the conclusion of an acid addition. An additional postacid sample was taken 2 weeks after acid addition in Experiment 4. Migration by drifting aquatic invertebrates was excluded during this time period. A 250 µm net screened channel intake water from before benthic sample collection on 3 September until after benthic sample collection on 1 October. An asterisk over an histogram indicates that it is signifiantly lower than the next histogram to the right ($P < 0.05$, Duncan's multiple range test).

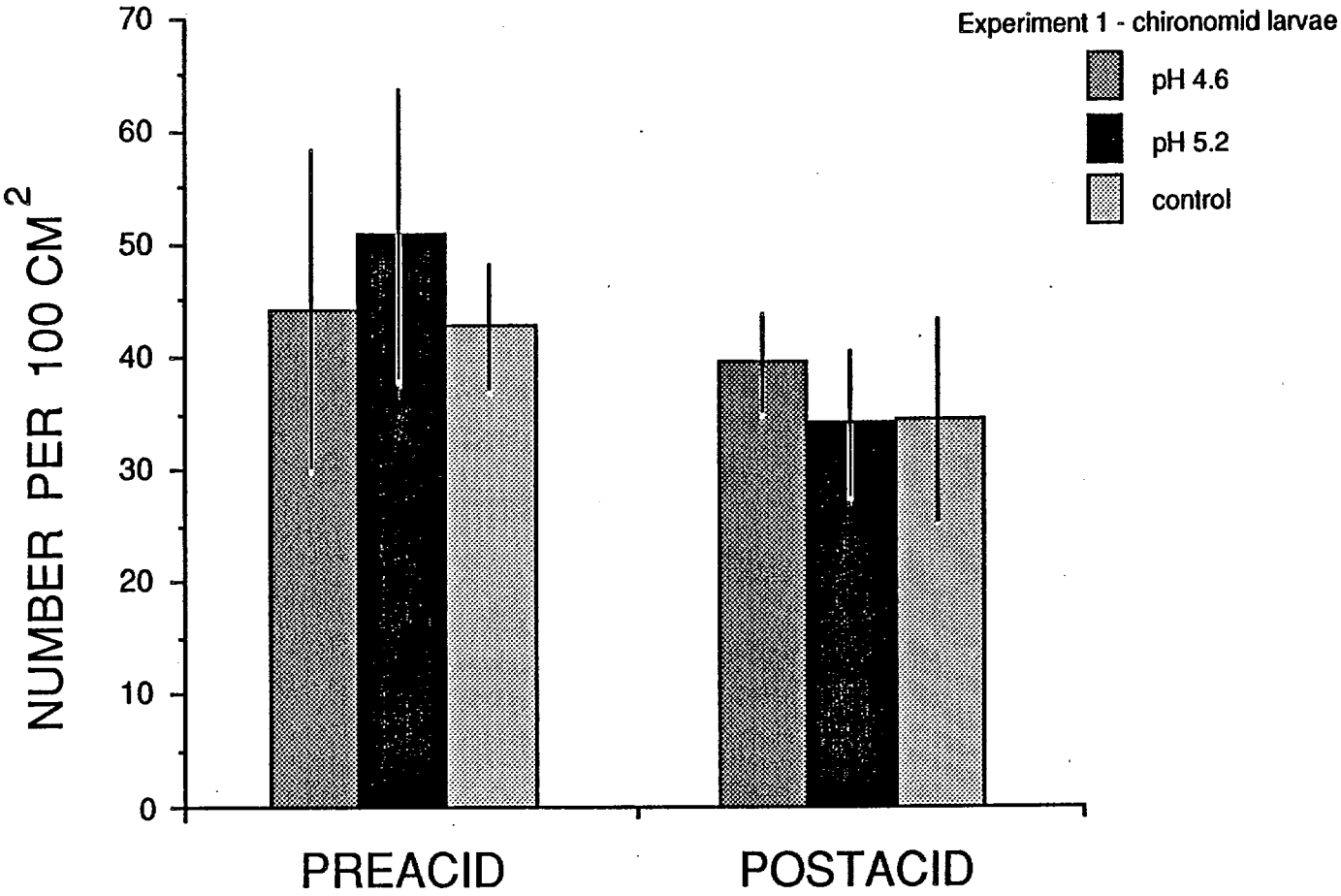


Figure 44. Benthic densities (number per 100 $cm^2 \pm 1$ S.E.) of chironomid larvae in treatment channels in Experiment 1 Preacid samples were taken 24 hours prior to acid addition, postacid were taken 40 hours after the conclusion of acid addition.

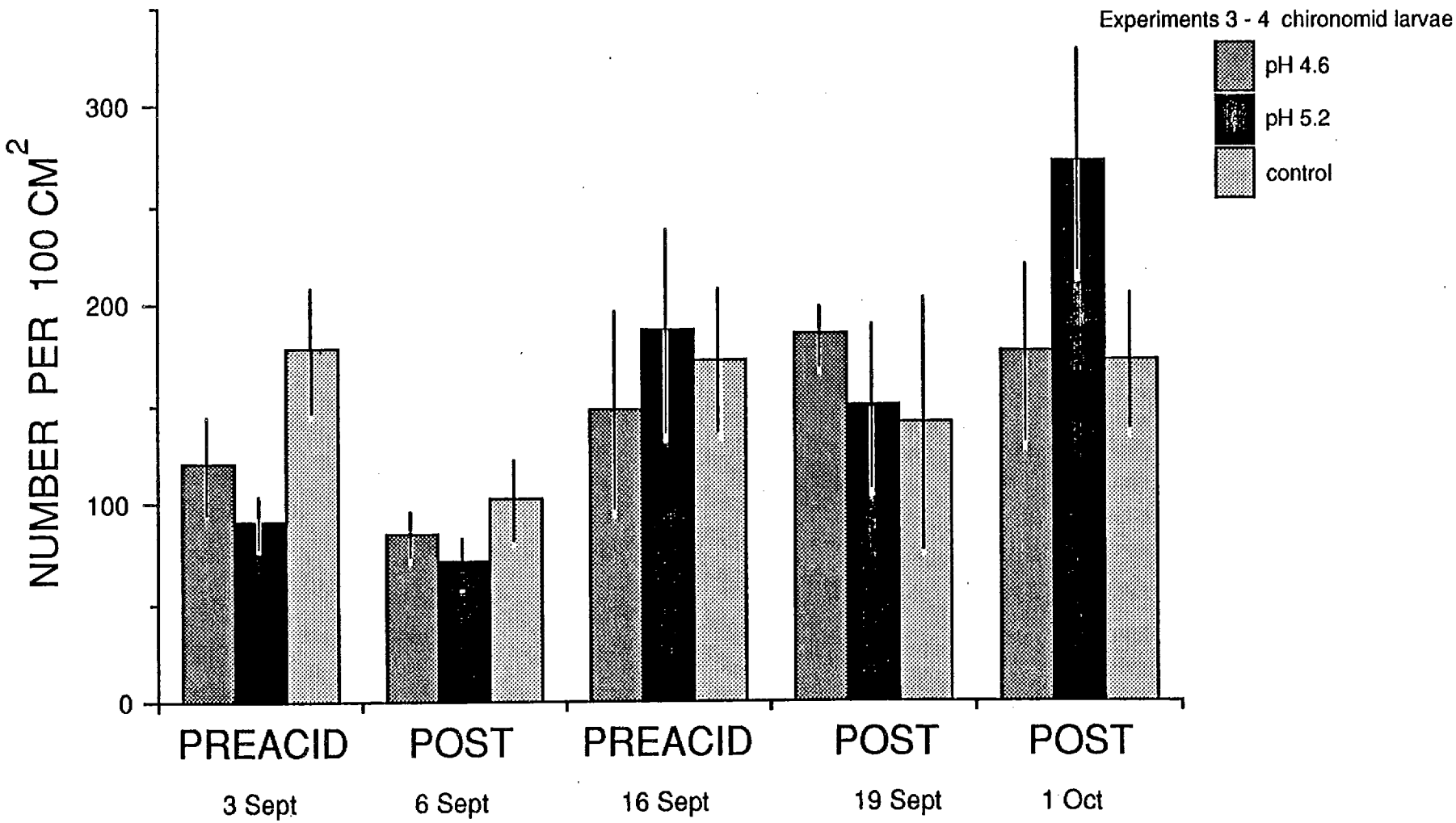


Figure 45. Benthic densities (number per 100 $cm^2 \pm 1$ S.E.) of chironomid larvae in treatment channels in Experiments 3 and 4. Preacid samples were taken 24 hours prior to an acid addition, postacid samples were taken 40 hours after the conclusion of an acid addition. An additional postacid sample was taken 2 weeks after acid addition in Experiment 4. Immigration by drifting aquatic invertebrates was excluded during this time period. A 250 µm net screened channel intake water from before benthic sample collection on 3 September until after benthic sample collection on 1 October.

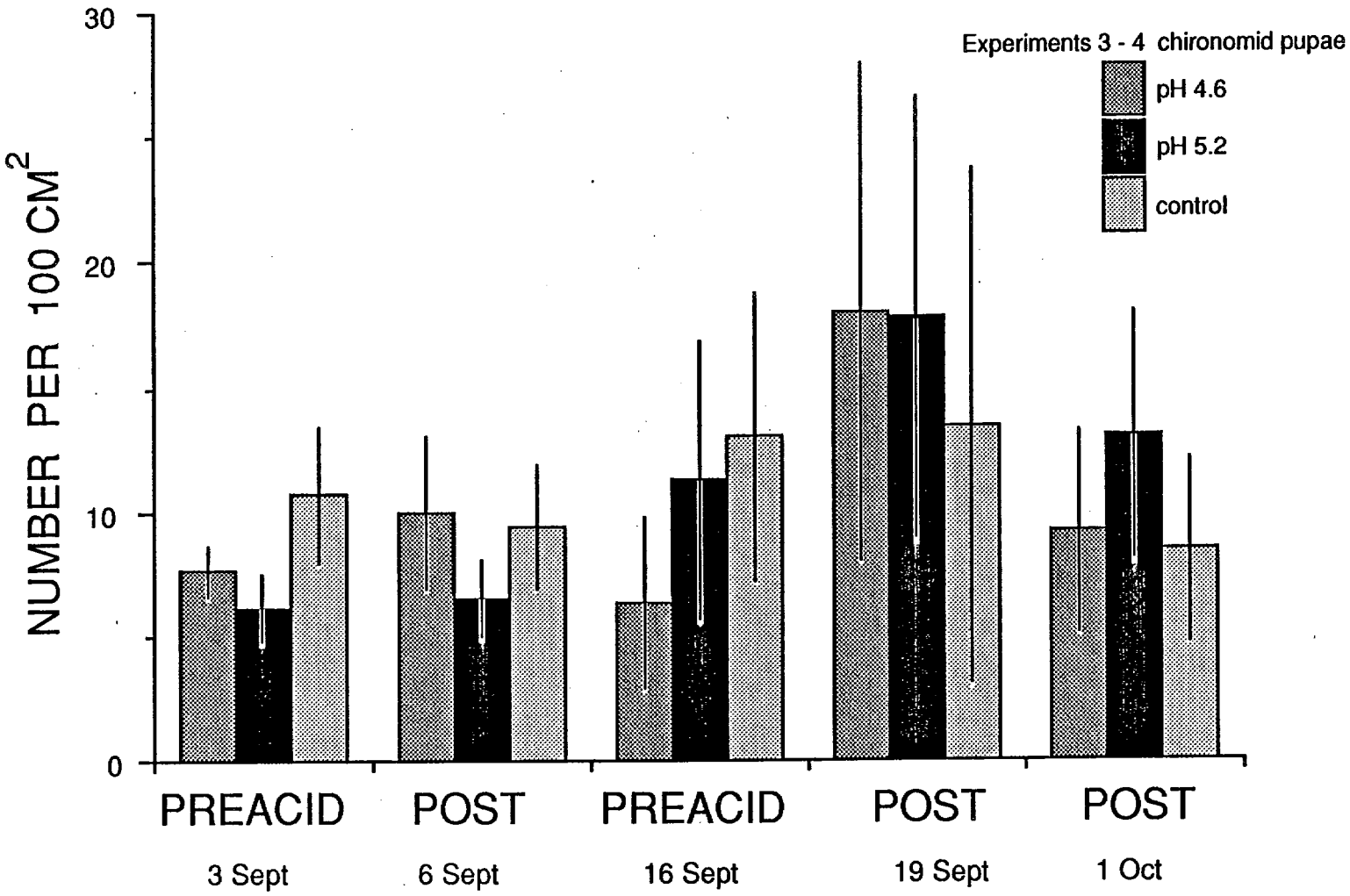


Figure 46. Benthic densities (number per 100 $cm^2 \pm 1$ S.E.) of chironomid pupae in treatment channels in Experiments 3 and 4. Preacid samples were taken 24 hours prior to an acid addition, postacid samples were taken 40 hours after the conclusion of an acid addition. An additional postacid sample was taken 2 weeks after acid addition in Experiment 4. Immigration by drifting aquatic invertebrates was excluded during this time period. A 250 µm net screened channel intake water from before benthic sample collection on 3 September until after benthic sample collection on 1 October.

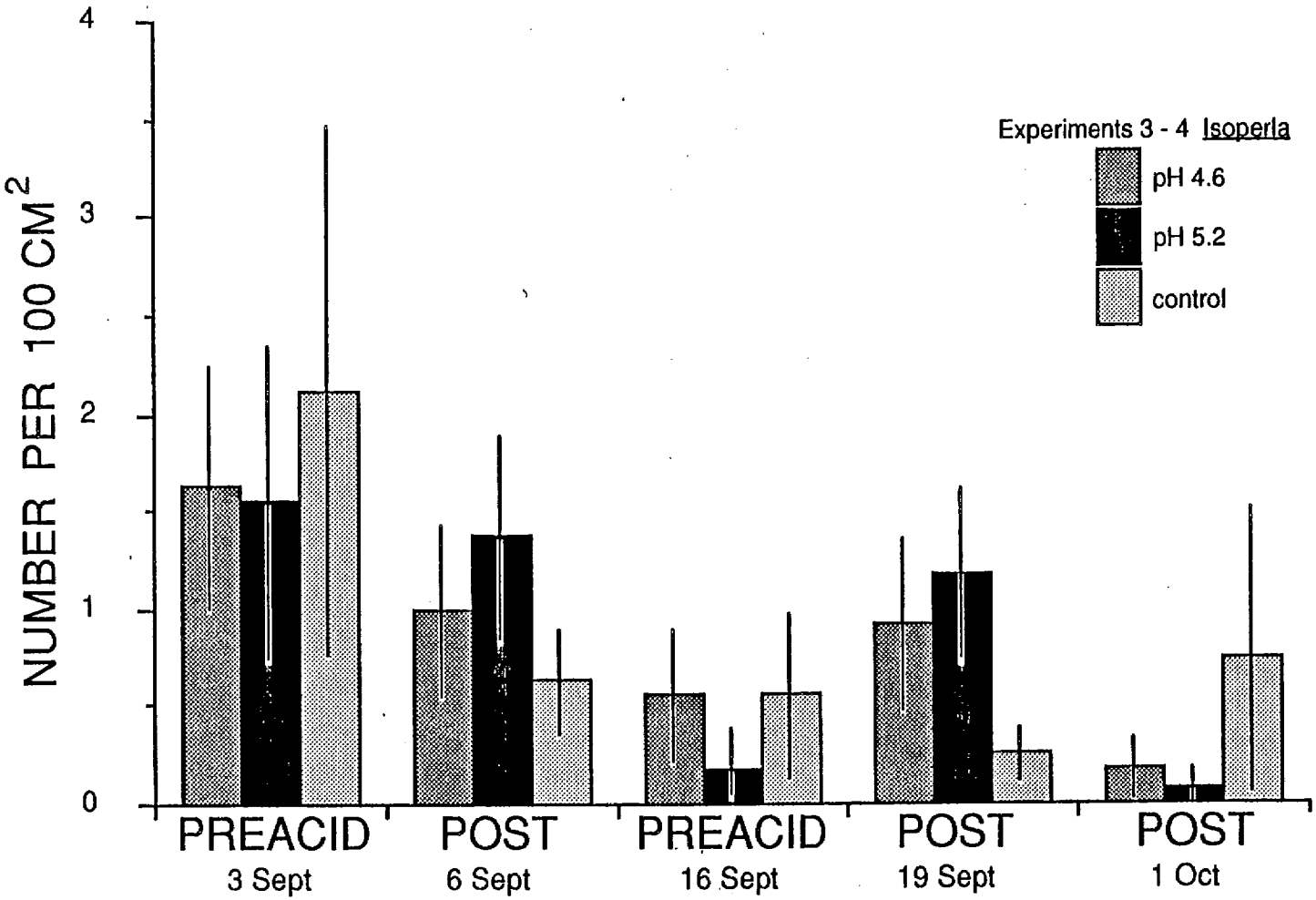


Figure 47. Benthic densities (number per 100 $cm^2$ ± 1 S.E.) of Isoperla in treatment channels in Experiments 3 and 4. Preacid samples were taken 24 hours prior to an acid addition, postacid samples were taken 48 hours after the conclusion of an acid addition. An additional postacid sample was taken 2 weeks after acid addition in Experiment 4. Immigration by drifting aquatic invertebrates was excluded during this time period. A 250 µm net screened channel intake water from before benthic sample collection on 3 September until after benthic sample collection on 1 October.

Experiment 1's acid pulse Epeorus's drift rates were significantly higher in both acidified treatments than in controls. In the second half of this experiment, and during Experiment 3, Epeorus drift was significantly higher at pH 4.6 than at pH 5.2 and 6.5. In Experiment 3, 46 % of the increased drift in pH 4.6 channels was owing to toxic drift, the remainder to the drift of live individuals. In Experiment 3 significant increases in drift relative to controls for Paraleptophlebia, Zapada, and Amiocentrus only occurred at the lowest pH (4.6). Other than Baetis, Paraleptophlebia was the only taxon that showed significant reductions in benthic density with decreasing pH. Part of the lack of statistically significant acidification effects on benthic densities is owing to the low numbers of benthic taxa collected in samples. With the exception of baetids and chironomids most taxa were too rare in samples to allow statistical testing.

Chironomid larval drift was higher in control than acidified channels both before and during the acid pulse in Experiment 1, and before and after the acid pulse in Experiment 3. During the acid pulse in Experiment 3, however, chironomid drift was higher in pH 4.6 channels than in controls and higher in controls than in pH 5.2 channels. The proportions of drifting chironomids that were dead were inversely proportional to pH in Experiment 3, and all increased drift in acidified channels during the acid pulse could be attributed to toxic drift. Control drift rates for chironomid pupae and adults were often higher than those in acidified treatments after acid pulses. The density of chironomid larvae did not appear to be affected by acid treatments in any experiment.

## *Discussion and Conclusions*

Past studies on the effects of acid deposition on aquatic communities have included surveys (Singer 1981), laboratory bioassay experiments (Bell 1971), or whole stream acidifications (Hall et al. 1980, Hall et al. 1987, Ormerod et al. 1987). Experimental stream channels provide an excellent compromise among these methods in terms of similarity to natural conditions, replicability needed for statistical analyses, ease of manipulation, and size. The experimental stream channels used in this study were representative of the natural system, containing similar substrates, and algal and invertebrate populations found in the Marble Fork of the Kaweah River. Water chemistry was identical to the Kaweah River as water was diverted directly from the natural stream into the channels.

Whole stream or stream channel designs used to test the effects of reduced pH on stream invertebrates in the past have had limited replication, and thus were not adequate for rigorous statistical analyses (Hall et al. 1980, Hall et al. 1987, Burton and Allan 1986, Zischke et al. 1983, Burton et al. 1985, Servos and Mackie 1986, Allard and Moreau 1984, 1987, Ormerod et al. 1987, Griffiths 1987). In almost all of these cases researchers misapplied statistical analyses by misidentifying the true replicates assigned to each of their experimental treatments, resulting in errors associated with "pseudoreplication" (Hurlbert 1984). In addition, in some experiments researchers did not sample stream sections prior to acid treatment, thereby failing to establish the comparability of sections assigned to different treatments.

In our experiments we found that stream invertebrates and algae showed a variety of responses to acid inputs. Despite the influx of algal drift from the Marble Fork into the experimental stream channels (drift nets only caught invertebrates) and despite the short duration of experimental acid pulses, diatoms on tiles decreased in abundance and showed shifts in the relative abundances of individual taxa with acid inputs. Most studies examining the responses of periphyton to acidification have reported increases in algal biomass, particularly in filamentous taxa like Mougeotia (Hendrey et al. 1976, Hall et al. 1980, Turner et al. 1987, Griffiths 1987). Our studies differed from previous studies in examining the effects of acid pulses on stream communities, as opposed to the effects of chronic, continuous acid additions.

Because some of the increases in filamentous algae in acidic conditions may be owing to reduced grazer density and/or concomitant decreases in microbial decomposition, it may be that our experiments were of insufficient duration or did not add acid long enough to see these effects. The only common filamentous alga on our tiles, Zygnema sp., showed no response to acid inputs. Our data do suggest that acid addition may result in short-term depressions in diatom abundance. Although there was not a pronounced shift from a diatom-dominated to a filamentous algae-dominated situation, there were changes in the abundances of individual diatom taxa. Taxa like Achnanthes minutissima, Taxon 99396-SN, Fragilaria vaucheri and, perhaps, Gomphonema subclavatum and Achnanthes levanderi declined with acidic inputs, whereas Eunotia spp. actually tended to do better under acidic conditions. In an experiment using artificial channels and algae from an acidified brook, Hendrey (1976) found that species of Eunotia and Tabellaria were among the dominant algae. Furthermore, these results agree with acid-tolerance schemes based on correlational relationships between lake pH and diatom species composition developed for paleolimnological work (Holmes 1987, Melack et al. 1987). This experimental verification of the pH preferences of some diatom species lends credence to the utility of employing diatoms as pH indicators in core pH reconstructions.

Among invertebrate taxa Baetis seems to be the most sensitive to acid inputs, showing large increases in drift and declines in benthic density with even moderate acidification. Other mayfly genera, such as Epeorus and Paraleptophlebia, also show increased drift with acid inputs, particularly when pH is reduced to 4.6. A stonefly larva (Zapada) and caddisfly larvae (Amiocentrus) also exhibited increased drift at low pH, but chironomids and most other taxa showed no consistent responses to lowered pH. Increased drift of benthic invertebrates has been reported to follow acidification of streams (Hall and Likens 1979, Hall et al. 1980, Overrein et al. 1980, Zischke et al. 1983). This acceleration in macroinvertebrate drift activity is often an initial reaction to lowered pH and levels off to pre-acid rates over time (Hall and Likens 1979, Hall et al. 1980). Ephemeroptera (mayflies) are especially sensitive to low pH waters (Bell 1971, Sutcliffe and Carrick 1973, Fiance 1978, Howells et al. 1983, Mackay and Kersey 1985, Allard and Moreau 1987, Ormerod et al. 1987, Griffiths 1987) and often have a higher drift rate in response to lowered pH than other benthic invertebrates (Overrein et al. 1980).

The increased drift of stream invertebrates during acidification may be due to a passive, "toxic" drift; i.e., an increased number of killed individuals in the drift (Griffiths and Sardella 1984). Increases in drift in response to insecticide spraying have been shown to result from an increase in the number of dead invertebrates in the drift (Eidt and Weaver 1983). The increased drift may be due to an active, behavioral response of the drifting organisms (Hall et al. 1980), or may be due to an increase in the insects' susceptibility to being accidently dislodged. The high percentage of dead Baetis, Epeorus, and, in Experiment 3, chironomids in the drift immediately following acidification of stream channels indicates that "toxic" drift may dominate observed drift responses. The mechanisms of stream drift during acid pulses is most likely species specific, as is drift behavior in unmodified waters (Otto and Sjostrom 1986), and is also dependent on exact pH levels.

The effects of acid inputs on the densities of individual benthic species seemed to depend, to some degree, on the microhabitat preferences of these species (Ormerod et al. 1987, Allard and Moreau 1987). Baetis nymphs are epibenthic and active, spending most of their time on the upper surfaces of rocks where they would be directly exposed to acidified water. Chironomids, on the other hand, often burrow into the substrate and so are buffered from contact with polluted surface water. It is perhaps not surprising, then, that benthic densities of chironomids showed no significant responses to acidification. Although pretreatment differences among treatments preclude a direct assessment of this possibility, the data suggest that chironomid drift was actually lower when acid was added. It is possible that chironomids escape deleterious conditions by burrowing. It should be remembered that our chironomid assemblage was composed of at least a dozen species representing several different functional groups (grazers, collectors, predators). Some of the effects of acidification on individual taxa may have been masked by lumping all chironomids into one taxonomic group. Finally, the lack of significant differences in benthic chironomid densities between acidified and control channels was possibly due to the influx of chironomid drift from the Marble Fork. Although our block net was sufficient to prevent the immigration of most macroinvertebrates to stream channels it did allow the passage of small chironomids.

Because stream invertebrates are an important part of the diet of many stream fish, such as trout, it is possible that the effects of acid inputs on

invertebrates may have repercussions for higher trophic levels. Prey of aquatic origin provide approximately 70 to 90% of the diets by number of trout in the outlet stream draining Emerald Lake (Cooper et al. 1988). Many of the taxa responding to acid pulses in the experiments reported here are common components of trout diets in the High Sierra (mayflies, chironomids, caddisflies, stoneflies). Baetid mayflies, which seem to be especially sensitive to acid inputs, are important components of trout diets in many mountain streams (Allan 1981). Comparisons with hatchery reared fish and fish in sparsely-populated lakes at high elevations indicate that brook trout are growing at a fraction of their potential rate in many High Sierra systems (Cooper et al. 1988). Because food is so obviously limited, we can expect that trout growth and diet would be highly sensitive to acid-induced changes in the aquatic community.

Since aquatic communities often show the first and most drastic effects of cultural acidification (Cowling and Linthurst 1981, Almer et al. 1974, Hall et al. 1987), it is important to be able to make predictions as to which organisms will be the most sensitive to acid stress. Baseline monitoring data (Hall and Ide 1986) are important in order to differentiate between natural (e.g. seasonal and annual) variation in community structure, and variations caused by anthropogenic perturbations. Experiments such as this can help us to pinpoint the early-warning signals of stream stress so that we will know which aspects or processes in Sierra Nevada streams to concentrate our attention and efforts on in future monitoring efforts. The results suggest that Baetis, in particular, may be a good "early-warning" indicator of the effects of acid deposition on Sierra Nevada streams.

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