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EFFECTS OF pH ON LOCOMOTER ACTIVITY AND DRIFT OF STREAM INSECTS

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**EFFECTS OF pH ON LOCOMOTOR ACTIVITY
AND
DRIFT OF STREAM INSECTS**

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ABSTRACT

Effects of acidification on the drift-related light response of the mayfly Stenonema modestum were investigated in a laboratory stream. The use of interference of the photoresponse of Stenonema as a bioassay for sublethal effects of acidification was evaluated. Acidification to pH 5 caused significant changes in the phototactic response of Stenonema, interfering with the ability of the mayfly nymphs to respond synchronously to changes in light intensity. The effect of acidification varied with season. Also, at pH 5 locomotor activity of Stenonema was depressed during midday, increased in the evening and was little affected in the early morning. At more extreme pH depressions to pH 3.45 changes were observed in the photokinetic response of Stenonema, evidenced by progressive delays in the initiation of evening activity under the rocks. During the spring (May) a short term alteration was also seen in the midday activity rates. There was no effect of lowering the pH to 5 on the response of Stenonema to introductions of the stonefly predator Amphinemura nigratta. Stenonema nymphs had lowered molting success at pH 5.

INTRODUCTION

The deleterious effect of acid precipitation on lakes and streams is well established (see review by Haines, 1981). Acidification results in changes in water chemistry that generally reduce the value of aquatic resources. Pollutants such as heavy metals, transported directly in acid rain or released by acidified soils, interfere with direct use of water for human consumption. Effects of acidification on aquatic plants and animals include a loss of many species and a reduction in the efficiency and productivity of food webs. This results in serious reduction in sporting and recreation value.

Extreme cases of acidification are easily recognized. However, experimental research on Canadian lakes indicated that important changes in the food web may occur at pH values not generally considered to be detrimental (Schindler, et al., 1985). Since research on stream acidification is not so well documented, the point at which lowered pH may be considered critical in a stream has not been clearly defined.

The major objective of this research was to develop a laboratory bioassay to determine the sublethal effects of acidification, using an infra-red video tracking system to examine the behavioral responses of mayfly nymphs in artificial laboratory streams. Since drift behavior is strongly regulated by natural changes in light, changes in the "normal" light response were used as a measure of pH-induced behavioral responses. Also, the interaction of pH and other natural variables such as time of year and the presence of predators were examined.

METHODS

Mayfly nymphs of the genus Stenonema were collected early in the morning of the first day of each experiment in riffles below the dam in the Bellamy River in Madbury, NH. The insects were collected by two methods: kicking over large stones and capturing any dislodged nymphs in nets, and picking up stones containing nymphs and gently shaking them into a bucket of water taken from the river. Both season and water level in the river determined which method was used. During February and May the temperature of the water was near freezing and the level was very high (due to spring flooding) and the kick method was used, whereas in October and June, the water was warm and the level low, so the second method was used. Once collected, the insects were kept in river water and brought to the field research laboratory, AFAIR (Anadromous Fish and Aquatic Invertebrate Research) at the University of New Hampshire in Durham, NH. Stoneflies of the genus Amphinemura were also collected by one of the above methods from the Oyster River in Durham, NH.

In the laboratory two troughs of an experimental plexiglass model stream (Figures 1 and 2) were filled with filtered water from the Oyster River that had been pumped into the laboratory. Oyster River water was used because it was convenient, has an ambient pH near neutrality, and because both the Stenonema mayflies and Amphinemura stoneflies used in the experiments are abundant there. Mayfly nymphs were not collected from the Oyster River because their riffle habitat is much smaller than that in the Bellamy River, and it was desired not to eliminate the population in the Oyster River, whereas only a small number of stonefly nymphs were taken. Each trough emptied into a small reservoir and the water was recirculated to the top by an electrical pump at a rate of 24 liters

per minute. Each trough measures 0.15 m wide x 0.25 m high x 2.4 m long, with a center depth of 3 - 5 cm. The water was continuously aerated by flowing over a plexiglass wall at the top of the trough. The entire model stream rests on a 1-meter high frame. Black plastic covers the underside of the bottom of the stream and surrounds the frame so as to create a viewing area underneath for the video system (Figure 2). Four small unglazed ceramic tiles (5 cm x 5 cm) were placed over windows cut into the plastic in the bottom of the troughs; this placement allowed the insects to be videotaped from below (Figure 3). Nymphs were sorted by size and situated on the tiles by placing them in a small beaker of water, pouring them through a plastic tube and allowing them to attach to the tiles before the tube was lifted. The tube prevented nymphs from swimming downstream before they had attached to the tiles. Stenonema modestum was chosen because previous work showed that individuals would remain on the tiles for long periods of time (Cook and Haney, 1984). Amphinemura nigratta was chosen as a predator because previous videotapings showed them eating Stenonema (unpubl.) Equal numbers of nymphs were placed on a total of eight tiles and an attempt was made to put comparable numbers of each size mayfly on each tile. Small detritus covered pebbles obtained from the Oyster River were placed on top of all tiles as a source of food for these grazing insects. Nymphs were placed in the stream by noon of the day they were collected to allow them to acclimate to the model stream before videotaping began. At the end of each experiment nymphs were collected and counted from each trough. Dead nymphs and nymphs that died attempting to molt were also counted. Manipulations of pH were begun after the insects had been in the troughs for 24 hours. Each experiment included one treatment and one control trough. To avoid possible position effects, the troughs used as the control and treatment were switched for each run.

The experimental system contained both the model stream and several remote recording devices. The videotaping system consisted of a Daage Video Camera (Model 65) with a phototube sensitive into the far infrared range that was placed in the viewing area underneath the model stream and a Gyr Time-lapse Video Recorder for VHS video cassettes located in the recording laboratory (Figure 1). Continuous illumination of the viewing windows was done with an IR cutoff filter (750 nm) placed over a high intensity lamp source which was also located in the viewing area. The pH was monitored twice daily with an Orion Ionalyzer and a Beckman combination pH electrode. Acidic conditions were created by adding concentrated sulfuric acid dropwise to the appropriate trough until the target pH was reached, generally within one-half hour. Ambient light conditions were continuously monitored with a Licor underwater quantum sensor with 2-pi collector. The light sensor was placed facing upwards and adjacent to the tiles in trough 4 (Figure 1). Temperature was monitored continuously with a thermistor placed in trough 2. All continuous data were recorded by a Licor-1000 eight-channel data-logger as mean values over ten-minute intervals.

In October, a preliminary experiment was performed to test the usability of the four tiles. Previously, several small stones or one large rock had been used as the substrate in the troughs, but quantifying the activity was difficult due to the irregularities in size and coloring of natural stones. The preliminary experiment was also used to test the effect of the number of insects per rock on their daily activity rates. In this experiment, small nymphs were placed in one of the troughs and large nymphs in the other. Different numbers of nymphs (1, 4, 8 or 12) were placed on each tile and the activity videotaped for 48 hours. Densities were re-established each morning.

Once the preliminary work was satisfactorily completed, two identical experiments were performed: one in February, the other in May. These experiments were designed to detect changes in activity of nymphs under acidic conditions, nymphs subjected to the presence of stonefly predators and nymphs subjected to both treatments. The experiments were run for six or eight days and nights. The schedule of treatments was as follows: the first 24 hours, no treatment; the second 24 hours, reduced pH in the treatment trough (pH was reduced from the ambient pH 7.0 to a low of pH 5); the third through fifth 24 hours, two stoneflies of the genus Amphinemura were placed in both troughs; the last 24 hours, the stoneflies were removed. Water temperature in the model stream during the February and June experiments was kept at 10°C (+1°C) using an immersion cooler. During the May run, the water temperature varied between 17-19°C.

The final experiment was performed in June, and was designed to determine a threshold pH at which a large change in normal daily activity occurred. The pH was lowered daily in the control trough, until a final low pH of 2.0 was reached and the animals died.

Nymph activity was quantified by the following method. A ten-minute period from each hour during the daytime period and the nighttime period was viewed on the videotape. The ten-minute interval was chosen so that activity, and thus the insects' light response, could be correlated to the light data which, as noted above, was recorded as the mean light intensity during 10-minute intervals. The total number of nymphs under each tile and the number of those that moved during the first five-minute interval were recorded (each nymph was only counted once even if it moved several times). This procedure was repeated for the second five-minute interval. The percentage of nymphs moving during the ten minute period was calculated as an average percentage of nymphs active per 5 minutes.

$$\% \text{ active} = \frac{(\# \text{ moved, 1st five minutes}) + (\# \text{ moved, 2nd five minutes})}{2} \div (\text{5 min})^{-1} \times \frac{(\# \text{ under, 1st five minutes}) + (\# \text{ under, 2nd five minutes})}{2} * 100$$

After the activity was quantified, the values were plotted against time at midpoint in the 10-minute observation interval. The 24-hour cycle was divided into three periods that correspond to the normal change in activity of the nymphs. Activity is low during the daytime, increases at sunset, is maintained at a high level throughout the night, then decreases during the sunrise period to the daily low. These periods were then used to calculate the average number of nymphs under the tiles during the daytime period as:

$$\text{Average \# under tile (daytime period)} = \frac{(\# \text{ under 1st 5 min}) + (\# \text{ under 2nd 5 min})}{\# \text{ observations in daytime period}}$$

The total amount of activity for each period was found by integration of the data using planimetry.

Drift could not be assessed because too few animals drifted per night to be statistically significant and the addition of enough insects to the system to be able to study drift would have adversely affected the activity data by overcrowding the tiles.

Relative light change (S) was calculated according to Ringelberg (1964) for continuous light change:

$$S = \frac{\ln I - \ln I}{d t \text{ (sec)}}$$

where $\ln I$ and $\ln I$ are the natural log of light intensities at time zero and a subsequent time respectively, and $d t$ is the time interval in seconds.

The light response model utilized in this study is based on Elliot (1968) and Haney et al. (1983). According to the model, the normal sequence of activities of mayflies that result in evening drift include (1) the activation of

an overall increase in movement that is undirected (photokinetic) under the control of an endogenous rhythm entrained by the relative change in light and (2) the subsequent movement of animals to the upper side of the rocks at the time when the light intensity falls below a critical threshold (phototactic response). Thus, when undisturbed, the timing of the photokinetic response is independent of the light intensity, but begins at a critical threshold of relative light change. In contrast, the phototactic response is inversely related to the absolute light intensity, i.e., when the light intensity is high, the mayflies move onto the upper rock surface later in the evening.

Haney et al. (1983) proposed that the timing of the photokinetic activation is closely associated with the relative light change threshold (0.0017/sec) determined by Ringelberg (1964). In the present study this threshold value (RS) is used as a convenient time-marker for calculating temporal deviations of the photokinetic and phototactic responses. It is assumed that the actual threshold value for Stenonema is probably not precisely 0.0017/sec.

The times of initiation of photokinetic activation (IEAT) and initiation of leaving the underside of the rock (ILUT) were estimated by determining the first sequence of three activity values above (IEAT) or below (ILUT) the daytime average levels. The actual time was calculated as the midpoint between the first of these three points and the previous data point. Temporal deviations were expressed as the advance (-) or delay (+) relative to the time at which the Ringelberg threshold value was exceeded: IEAT - RST, or ILUT - RST.

Statistical comparisons were made using Analysis of Variance for tests of differences between means or development of simple linear regressions. Unless otherwise stated, statistical significance was considered at $p < 0.05$.

RESULTS

1. Density experiments

A. Effect of density on daily activity

Two channels were run with unacidified water. Each channel contained either large or small Stenonema modestum nymphs at initial densities on the four rocks of 1, 4, 8 and 12 animals per rock. Although there was some movement of mayflies between rocks, the pattern of density differences was maintained by daily replacement during the two-day experiment. Analysis of variance was used to determine whether the number of Stenonema influenced either the overall activity (total activity between 1700 and 1200 h) or the degree of evening activity (the percent of the total activity that occurred between 1700 and 1900 h). Densities of 1-12 animals per rock were not correlated with either total activity or the partitioning of activity into the twilight period. There was no effect of density for either large or small Stenonema.

B. Effect of density on light model parameters

There was no effect of Stenonema density on any of the model parameters examined, such as the timing of the evening activity and movement of mayflies to the upper side of the rock. These results indicated that further studies with Stenonema in the experimental streams should employ 8 - 10 mayflies per rock as this would provide an ideal number of individuals for counts of activity and movement and would still be below a density at which disturbance effects are seen.

2. Effect of acidification and stonefly predators on the daily activity pattern of Stenonema

February and May experiments were designed to determine the activity response of Stenonema with and without a predator present. Work of Peckarsky (1980) indicated mayfly behavior was modified by the presence of predatory stoneflies and that the mayfly-stonefly interaction probably involved olfactory cues. Recent work by Malmgren and Watson (1987) demonstrated that acidification alters the olfactory-mediated behavior of salmon in freshwater. The following experiments were intended to examine possible interactive effects of acidification and the presence of stonefly predators on the activity pattern of Stenonema.

Daily activity was divided into three periods: sunrise (midnight to one hour after sunrise), midday (one hour after sunrise to two hours before sunset) and sunset (two hours before sunset to midnight). Activity rates (% active per 5 min) were integrated for each period using a computer tablet.

During February, there was no significant effect of either pH 5 or the presence of the predatory stonefly Amphinemura nigratta on the activity rates in any of the three time periods. In May, midday activity rates immediately after addition of acid to pH 5 were roughly one-half the control channel ($p < 0.07$). No significant effect of acidification or predator was seen in the sunrise or sunset periods (Duncan's multiple range test, $p < 0.05$).

Stenonema collected in February were conspicuously different from those collected in May (Figures 4 - 7). In February, nymphs in the field were generally deep in the sediments. They were also much "quieter" when placed in the laboratory stream, as evidenced in the lower midday activity rates

(Table 1). It appears that there are seasonal differences in the responsiveness of Stenonema to acid and predators, for during the winter Stenonema showed no significant activity response to either presence of Amphinemura or acidification.

Midday activity averaged for the entire experiment was lower at pH 5 in February and May. Sunset activities were higher or variable at pH 5 and the least effect of pH was seen in sunrise period (Tables 1 and 2). This suggests that acidification to pH 5 depresses Stenonema activity during the daytime, but elevates their activity when they are most active in the evening. The result is exaggerated differences between daytime and nighttime activities.

3. Effect of acidification on the light response of Stenonema

Density experiments with large and small Stenonema run at pH 7 in October were also used to examine light responses without acidification. Experiments comparing control (pH=7) and acidified (pH=5) channels were conducted in February and May. The results were first analyzed by month (within-month effects) and by comparison of months (seasonal changes).

A. Within-month effects

As predicted by the light response model, timing of the photokinetic activation was not related to the light intensity in any of the experiments. Also, as seen in Tables 3 and 4, the phototactic timing correlated with the light intensity in October ($p < 0.07$) and February ($p < 0.07$). However, at pH 5 there was no significant correlation ($p < 0.10$) between light intensity and ILUT, indicating an interfering effect of pH. During the May

experiment, ILUT was not correlated with light intensity in either the control or acidified channel.

Acidification to pH 5 interfered with the ability of Stenonema to move to the upper surface of the rocks in response to light intensity. These directed movements were broadly spread out through time. There was, however, no measurable effect of low pH on the photokinetic response of Stenonema. The relationship between ILUT and the light intensity was significant at $p < 0.10$, but not at $p < 0.05$. This weak relationship is probably due to the very limited range of light conditions within each experimental period of 3-7 days.

B. Seasonal changes

Using the combined data from February and May the relationship between light intensity and ILUT became highly significant ($p < 0.0006$) at pH 7 (Table 5). The data at pH 5 were much more variable (Table 6), but showed a significant effect of light intensity on ILUT ($p < 0.04$). Thus, the pH effect was essentially the same as seen in the individual months, i.e., greater variability in response with the acidification. Surprisingly, with the combined months, there was also a significant effect of light intensity on the time of photokinetic activation (pH 7, $p < 0.0001$; pH 5, $p < 0.0003$) (Tables 7 and 8).

The preceding results indicate pH 5 altered the phototactic response of Stenonema as evidenced by the correlation between the ILUT and the light intensity at the time of the relative light threshold. Also, seasonal differences indicate Stenonema may be less susceptible to acid stress in February than in May.

4. Effect of extreme acid stress

The final experiment was conducted to examine the response of Stenonema to a period of continued lowering of pH that would be similar to animals in the field exposed to a brief pH "shock" as, for example, may occur during spring snow melt. During June, the pH in the control channel was held at pH 7, while in the experimental channel the pH was reduced from 4.5 on day 1 to pH 3.8, and 3.45 on subsequent days. This approximates the lowest pH depression expected if the stream were to contain only rainwater from a severe acid rain event.

The response of leaving the underside of the rocks in relation to light intensity was very similar to the results from our previous experiments, i.e., pH disrupted the phototactic response.

Decreasing pH did not cause a progressive change in the time of leaving the rock underside relative to the control channel. The dominant effect of low pH on the phototactic response was an increase in the variability.

An unexpected effect of the high acidity was a shift in the time of the photokinetic response relative to the control channel. As pH was decreased below 4.5, there was a continual advance in the photokinetic activation time from approximately one-half hour later than the control channel at pH 4.5 to more than one-half hour earlier than the control channel at pH 3.45 (Table 9). Thus, during the extreme acidification of an acid rain event one might expect alteration of the mayfly behavior due to disruption of both the phototactic and the photokinetic responses.

5. Light manipulations to test the light response model

The strong correlation between light intensity and the time of

initiation of photokinetic activity is inconsistent with the light response model. This suggests three different possibilities: (1) the relative light threshold response is dependent on the light intensity and that the model is incorrect; or (2) the relative light threshold changes with the age of the mayfly nymphs; or (3) the relative light change threshold changes with the different environmental conditions associated with season (i.e., temperature and photoperiod).

To test the first possibility that the photokinetic activation is influenced by the light intensity, one of the two channels was covered with varying amounts of window screen or black plastic to reduce the light intensity from 2-4 orders of magnitude. At the same time, by exposing both channels to the same natural light cycle, the relative light change was allowed to be the same in both channels. Model predictions would be that the time of initiation of activity under the rock should be the same for both channels, but the time of moving to the upper side of the rock should be later on the brighter channel.

Stenonema clearly showed no difference in the time of photokinetic activation in the channels, despite the considerable difference in light intensity. Estimated times of activation differed less than 5 min at light differences of 10^2 and 10^4 times. This experiment supports the photokinetic assumption of the model.

To address the question of whether the Stenonema have a different threshold for the photokinetic response at different ages, a comparison was made of the times of activation of large and small Stenonema in the October experiment. Large nymphs were roughly the maximum size attained by

Stenonema, whereas the small animals were about one-third the body length of the large animals. The times of activation on day one were 19:10 (10 min s.d.) and 19:00 (10 min s.d.) for large and small animals respectively. On day two of the experiment the respective photoactivation times were 18:54 (21 min s.d.) and 18:50 (26 min s.d.). Thus, there is no significant difference in the photokinetic activation times of Stenonema of contrasting size and presumably contrasting age.

It can be concluded that the dependence of photokinetic activation revealed in the comparison of data from different months is probably due to seasonal shifts in stimulus thresholds for photokinetic activation. Such shifts in thresholds are probably related to seasonal changes in environmental conditions such as photoperiod and temperature or physiological condition of the nymphs, rather than simply the age or size of the animals.

6. Observations on the effect on molting and response to extreme pH depression

During the February, May and June experiments up to about 50% of the late instar Stenonema nymphs successfully molted in the control channels. At pH 5 far fewer animals attempted to molt and most attempts were unsuccessful. This indicates that reduction of the stream water to pH 5 was sufficient to cause physiological stress in Stenonema.

At the end of the June experiment pH was lowered to from 3.45 to pH 2 over a period of a few hours during the midday period and the response of the mayflies was monitored. Surprisingly, Stenonema nymphs did not leave the rocks, but rather became increasingly inactive. After a few hours at pH 2 all animals died, with some remaining attached to the rocks. It appears that Stenonema does not attempt to escape from pH-stressful conditions by leaving the rocks during the day.

DISCUSSION

The results presented support the concept that photoresponse of mayfly nymphs could be a useful bioassay of the sublethal levels of acidification. At pH 5, a level of acidification that caused deleterious effects on molting, Stenonema showed significant changes in its phototactic behavior. Quite likely, the observed differences in photoresponse would result in asynchronous drift behavior and consequent elevated mortality in nature. As the pH drops below 4.5 acidification alters the photokinetic as well as phototactic behavior. Interference of pH with the photokinetic response could result from a reduction in the sensitivity of nymphs to the light stimulus, thereby causing increasing delays in response as the acidity increases. This delay in response may also reflect diminishing physiological vigor at very low pH. It is unlikely that even severe pH depressions would cause catastrophic drift of Stenonema as seen by Hall et al. (1980) with the artificial acidification of a small stream.

A useful application of the technique described would be to determine the pH threshold at which an alteration in the photoresponse is observed. It could be anticipated that such pH thresholds may vary with the test species chosen. Mayfly nymphs are especially useful, since they are often among the first organisms to disappear with acidification and thus are probably sensitive bioindicators. It is also likely that for a given species the pH threshold may vary depending on the water chemistry. Variations in the concentrations of heavy metals such as aluminum and copper would probably alter the pH threshold as well as the response curve to the entire range of pH changes, as the solubility of aluminum is not a simple linear function of pH. In the stream water used in these experiments, aluminum is present only in concentrations well below those toxic to mayfly nymphs (Cook and Haney, 1984).

Nymphs of Stenonema have well-defined diel activity patterns that are easily studied in a laboratory stream. An infra-red sensitive video monitoring system provides a means of directly observing behavioral responses of the animals with little disturbance. Using time-lapse recordings played back at speeds that compressed real time 60 times (one minute real time equals one sec) analysis of the activity rates and density changes under eight rocks for a 24-hour period was accomplished in 2-3 hours. A major advantage of the method described is that it employs a natural photoresponse as a bioassay tool. Mayfly nymphs appear to remain on natural activity cycles in the laboratory streams for at least one to two weeks. The effects measured under these conditions should have direct application to altered behavior in natural streams. The use of an outside window for photoperiod has the advantage of providing animals with the changes of a natural light environment as well as the advantage of technical simplicity. However, a simulated daylight system in which light intensity and rate of light change could be carefully regulated would offer the possibility of controlling light conditions over the entire experiment. By controlling both light intensity and the rate of light change, interference effects of pH should be more easily and clearly defined. The expense, however, of such a solar simulator may be prohibitive with current technology.

SUMMARY AND CONCLUSIONS

An infra-red video monitoring system is described that permits observation of the behavioral responses of mayfly nymphs under quasi-natural and undisturbed conditions. The sensitivity of the method was adequate to resolve disturbances in the photoresponse of Stenonema. The technique has considerable potential for use as a bioassay tool to evaluate critical levels at which environmental perturbations such as acidification effect significant alterations in the natural behavior of stream mayfly nymphs. With minor modifications, its use could be adapted to other species of stream invertebrates.

Results of this study indicate acidification can interfere with drift-related behavior by altering the reaction of Stenonema to light changes. This interference appears to occur at two different mechanisms, depending on the degree of acidification. At relatively small pH depression (pH 5) Stenonema fail to move to the upper side of the rock at the appropriate time (phototactic interference). Day-night difference in Stenonema activity are greater at pH 5 due to both a depression of daytime and stimulation of nighttime activities. One might expect higher evening drift rates in response to moderate acidification. With more extreme acidification, there is a gradual advance in the time of the beginning of evening activity (photokinetic interference).

Seasonal differences in the effect of acid on the photoresponse suggest the effect of a pH depression on these mayflies may differ depending on whether it occurs in winter or in the spring. Possibly, animals in a reduced metabolic state in winter are less sensitive to the effects of an acid rain event.

The findings of this study also contribute to a better understanding of the mechanism underlying the stream drift phenomenon. Observations on the relationships of light to the autonomy of the photokinetic and phototactic responses of Stenonema provide direct support for assumptions of the light-response model.

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REFERENCES

- Cook, W. and J. F. Haney. 1984. The acute effects of aluminum and acidity upon nine stream insects. Technical Completion Report No. 46. Water Resource Research Center, Durham, NH.
- Elliott, J. M. 1968. The daily activity patterns of mayfly nymphs (Ephemeroptera). J. Zool. Lond. 155:201-221.
- Haines, T. 1981. Acid precipitation and its consequences for aquatic systems. Trans. Am. Fish. Soc. 110: 669-707.
- Haney, J. F., T. Beaulieu, R. Berry, D. Mason, C. Miner, E. McLean, K. Price, M. Trout, R. Vinton and S. Weiss. 1983. Light intensity and relative light change as factors regulating stream drift. Arch. Hydrobiol. 97: 73-88.
- Hall, R. J., G. E. Likens, S. B. Fiance and G. R. Hendry. 1980. Experimental acidification of a stream in the Hubbard Brook Experimental Forest, New Hampshire. Ecology 61: 976-989.
- Malmgren, C. and W. Watson. 1987. Modification of olfactory-related behavior in juvenile Atlantic salmon by changes in pH. J. Chem. Ecol. 13 (3): 533-546.
- Pecarsky, B. 1980. Predator-prey interactions between stoneflies and mayflies: behavioral observations. Ecology 61: 932-943.
- Ringelberg, J. 1964. The positively phototactic reaction of Daphnia magna Straus. Neth. J. of Sea Res. 2: 319-406.
- Schindler, D., K. Mills, D. Malley, D. Findlay, J. Shearer, I. Davies, M. Turner, G. Linsey and D. Cruikshank. 1985. Long-term ecosystem stress: The effects of years of acidification on a small lake. Science 228: 1395-1400.

FIGURE LEGENDS

- FIGURE 1** Diagram of the AFAIR laboratory experimental stream and the remote sensing instruments located in an adjacent room. The experimental stream is illuminated with natural lighting from a south-facing window.
- FIGURE 2** Side view of the experimental stream showing the position of the video camera and infra-red light source, the recirculating water system and the Nitex basket between the stream outlet and the reservoir.
- FIGURE 3** A sample hard copy of a video-taped experiment, showing the eight rocks (unglazed tiles) and the Stenonema nymphs on the underside of the rocks.
- FIGURE 4** The diel pattern of activity and abundance on the underside of the rocks averaged for the four rocks in the channel.
- FIGURE 5** See description in Figure 4.
- FIGURE 6** See description in Figure 4.
- FIGURE 7** See description in Figure 4.

FIGURE 1

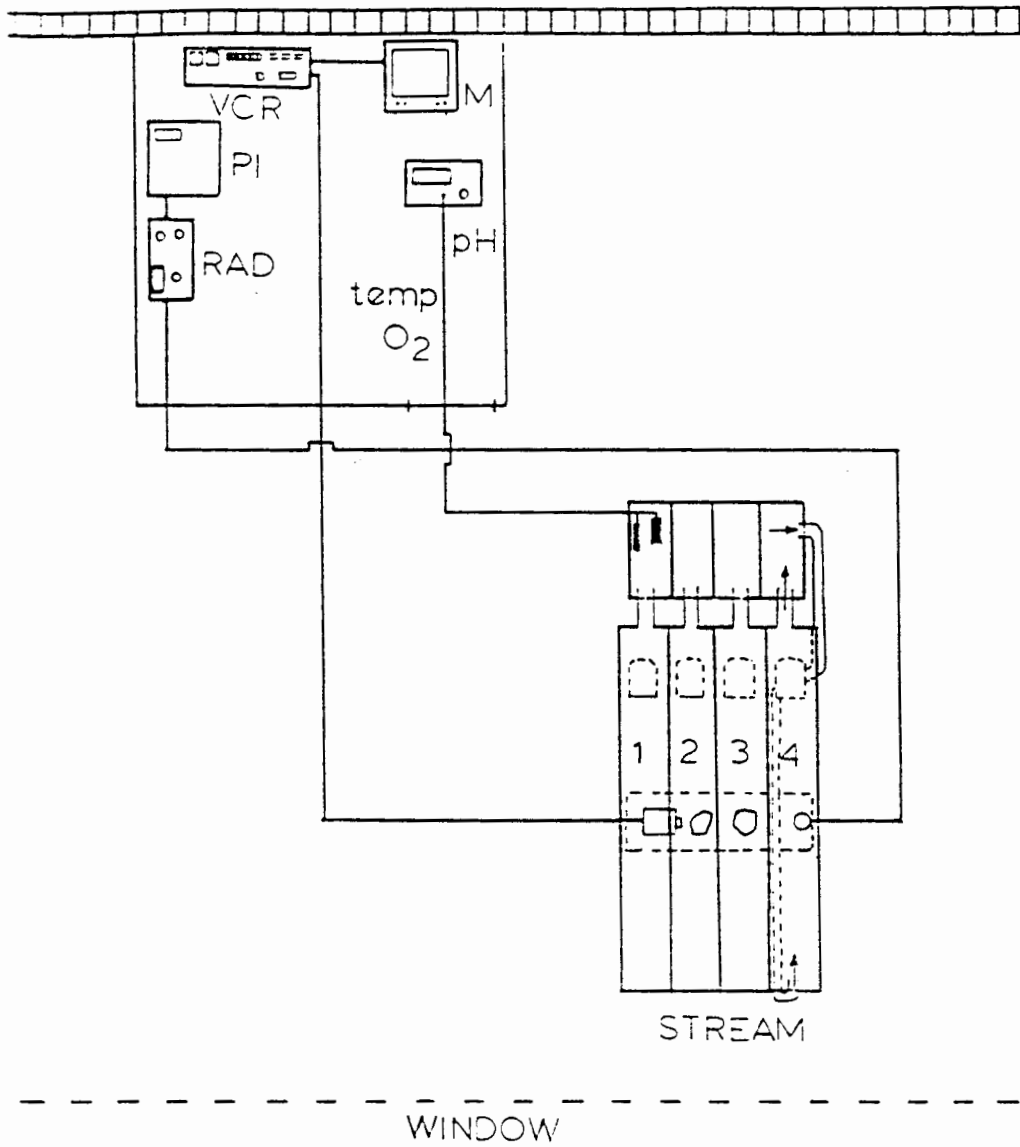


FIGURE 2

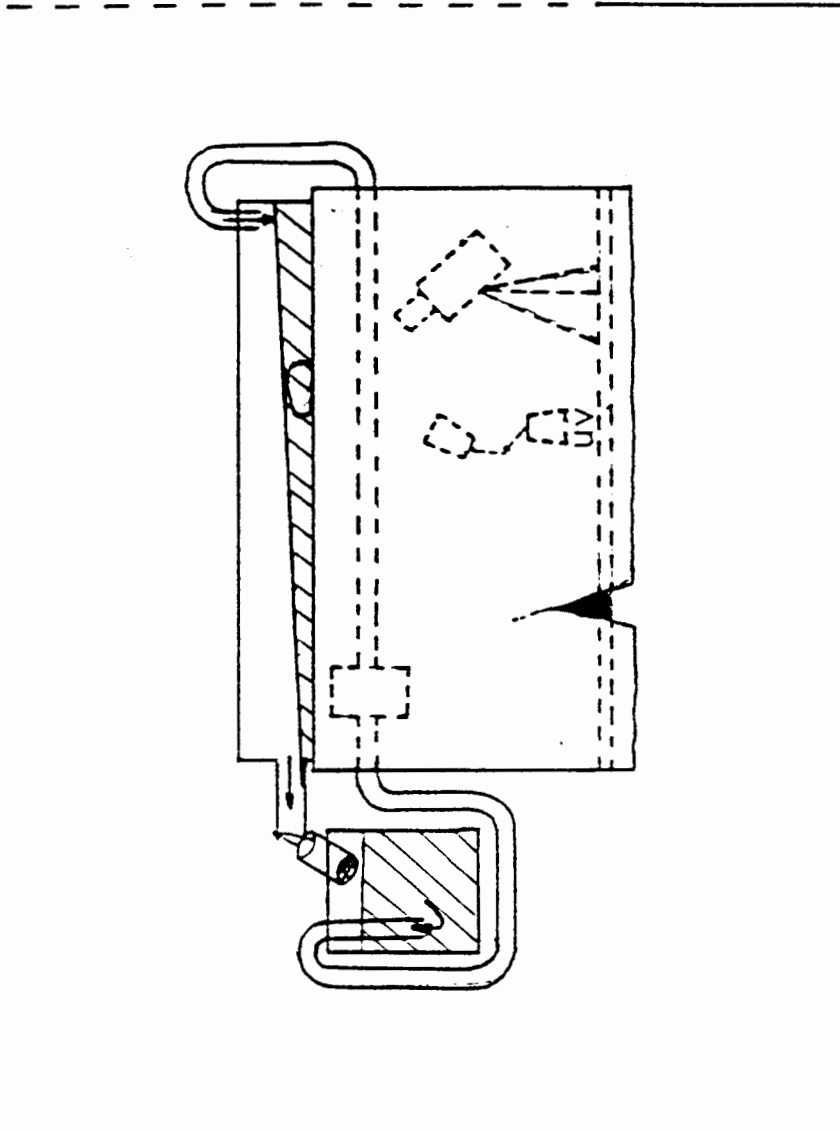
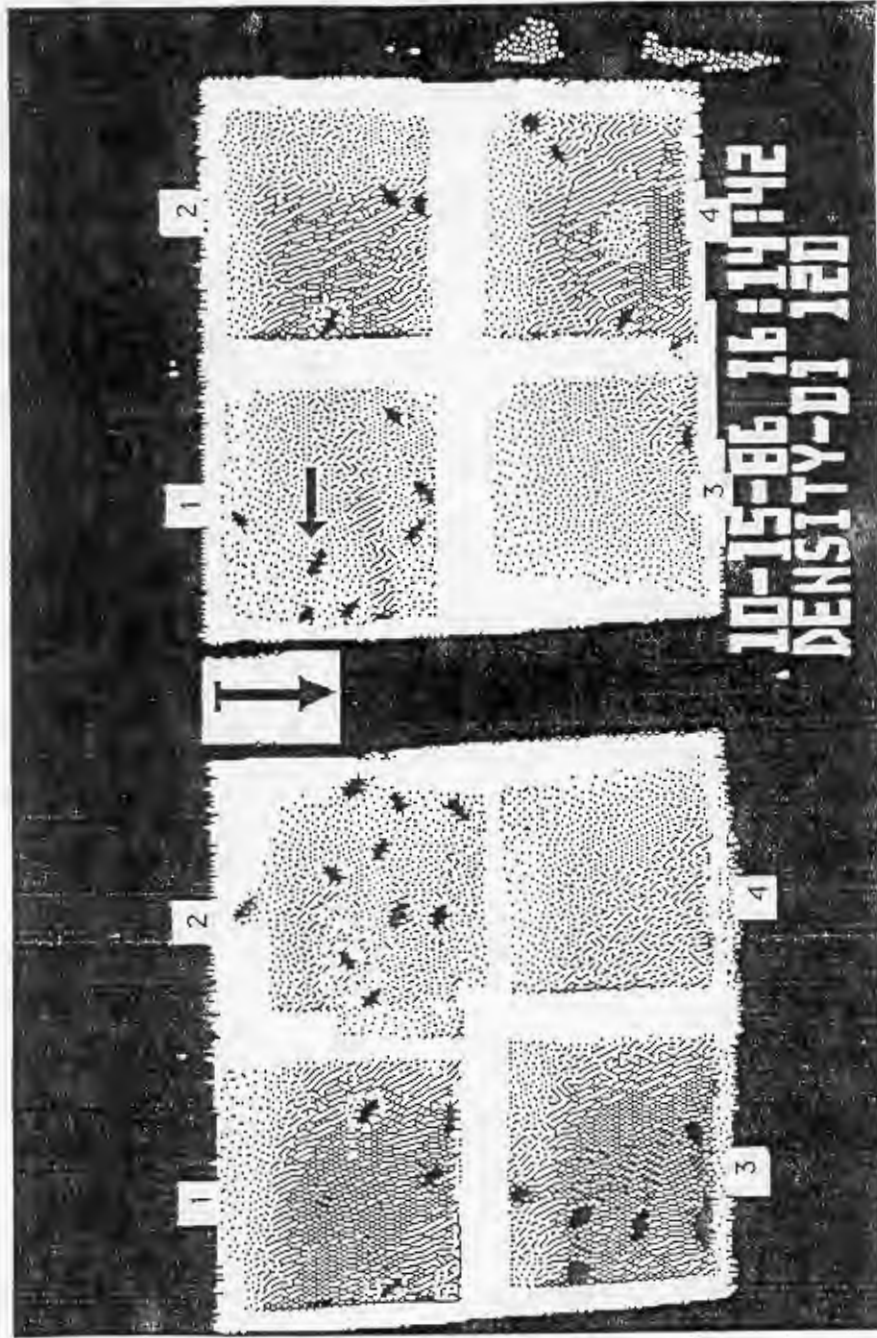
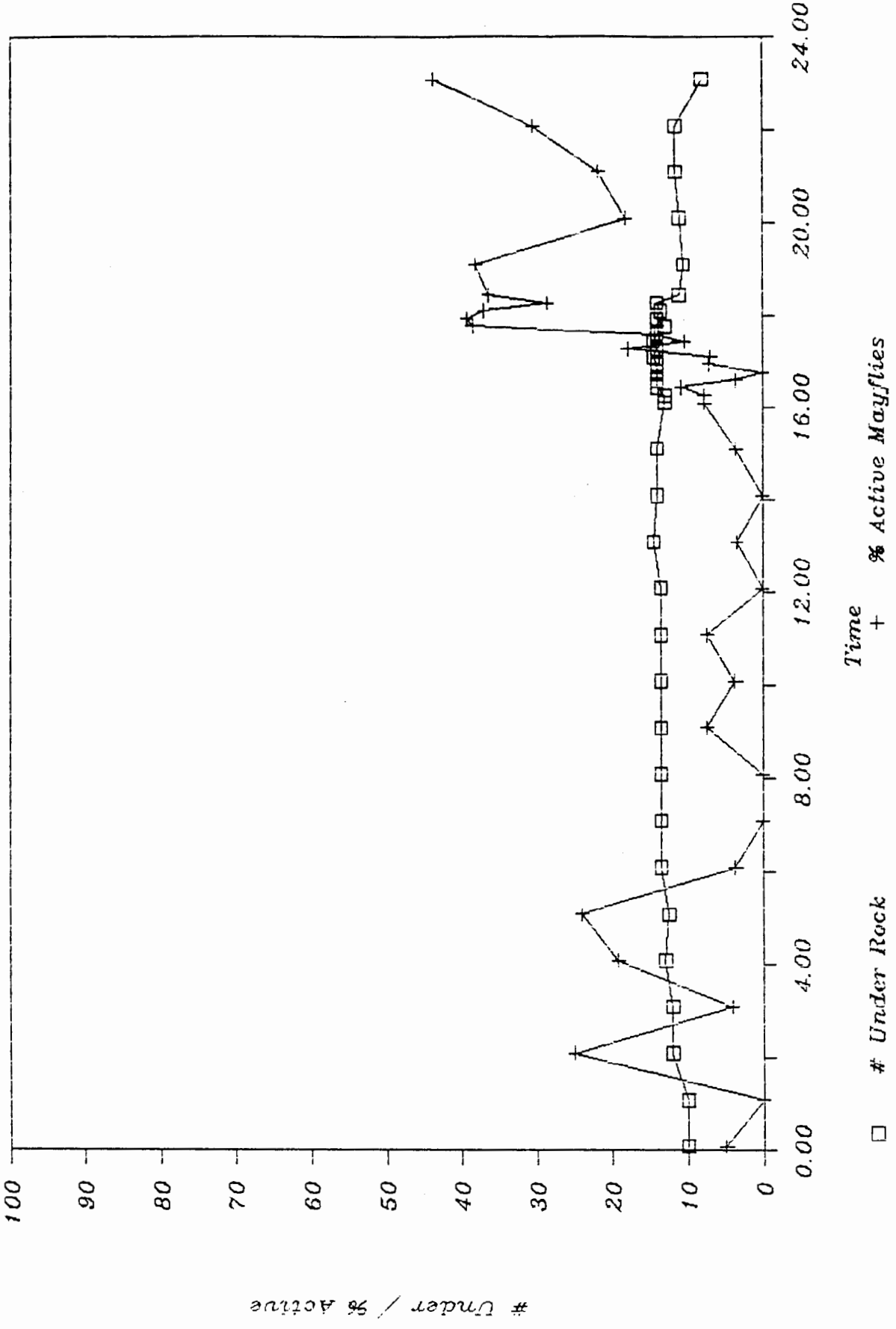


FIGURE 3



Channel C: February 18, 1987
(pH 7)

FIGURE 4



Channel B: February 18, 1987
(pH 5)

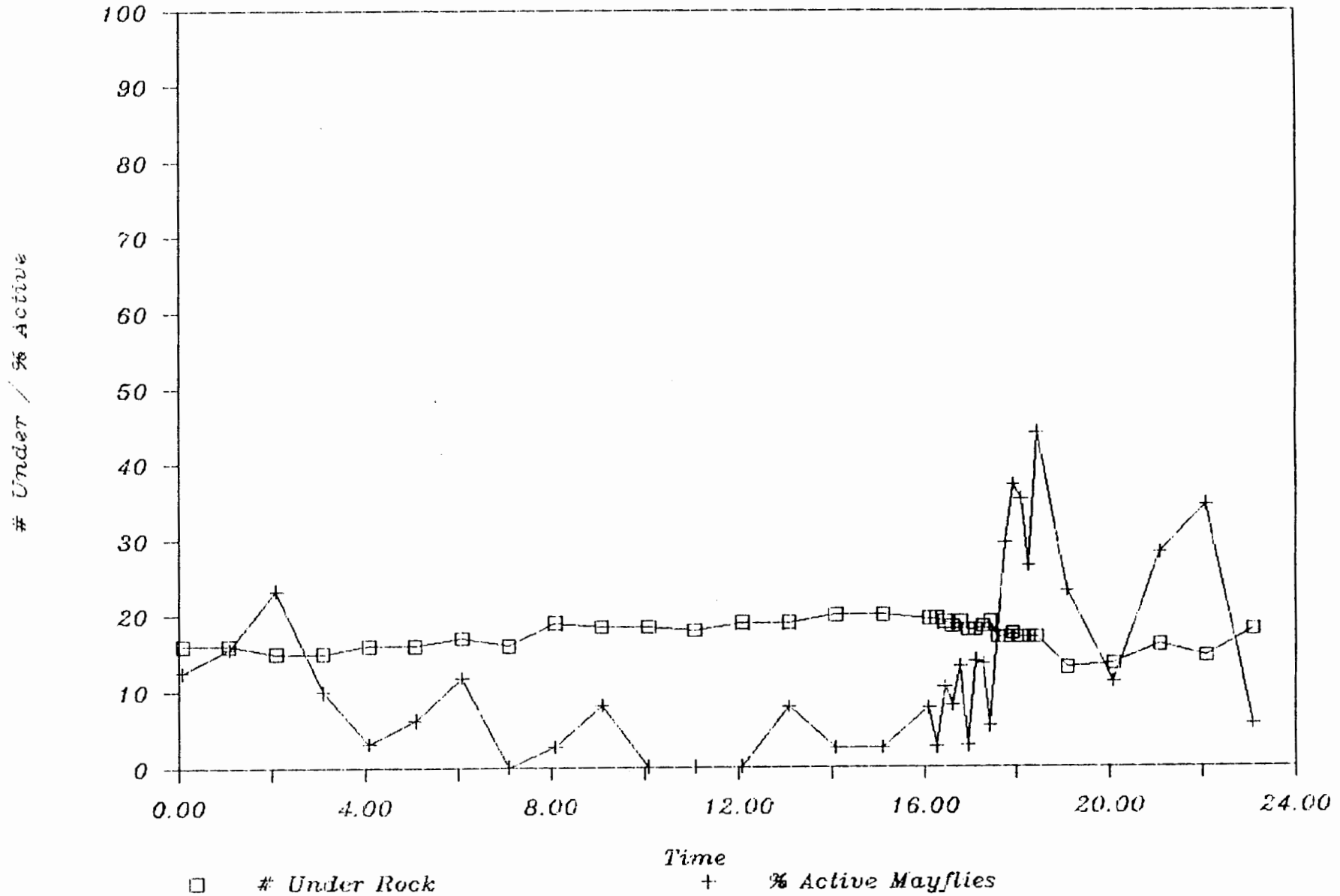


FIGURE 5

Channel B: May 7, 1987
(pH 7)

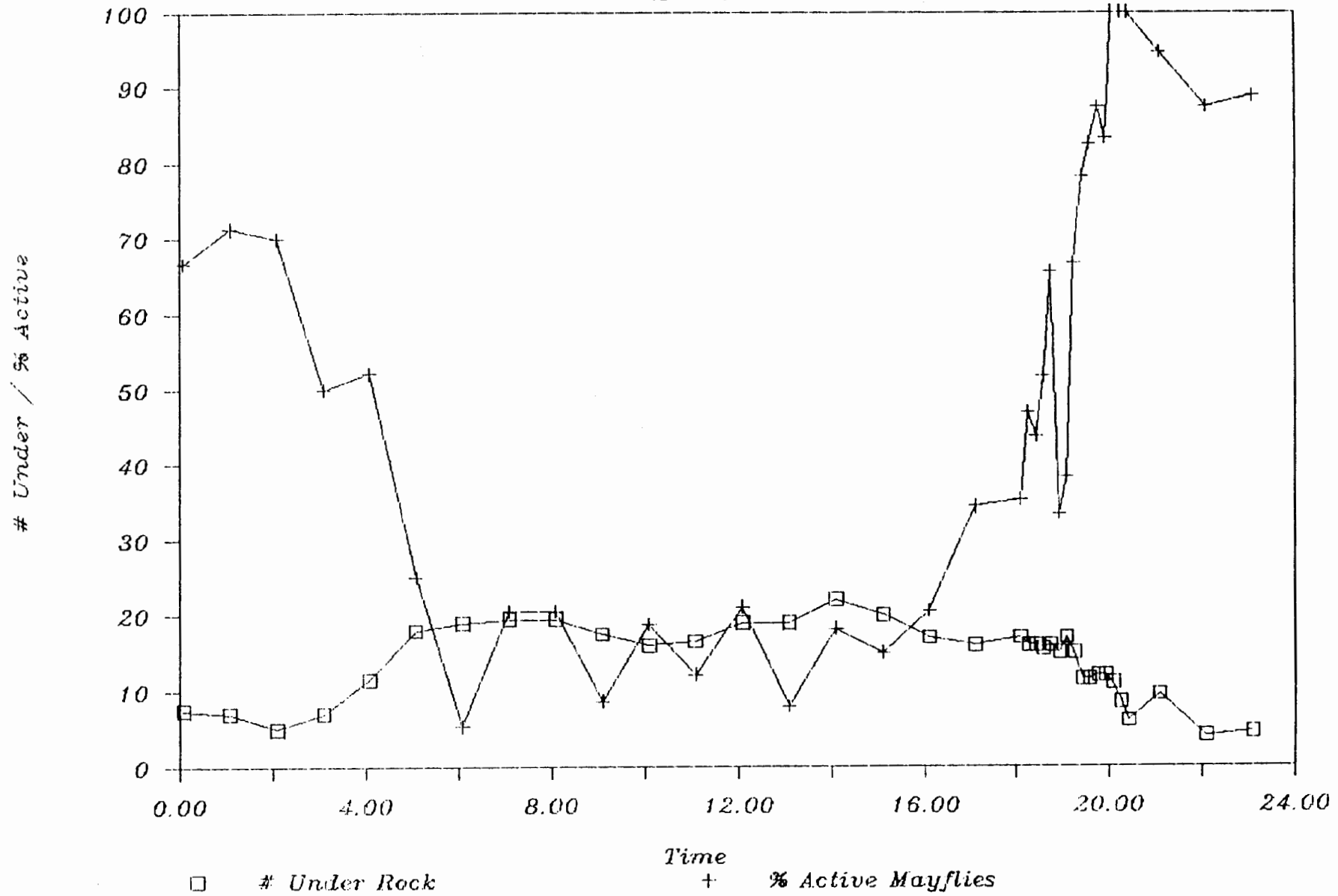


FIGURE 6

Channel C: May 7, 1987 (pH 5)

FIGURE 7

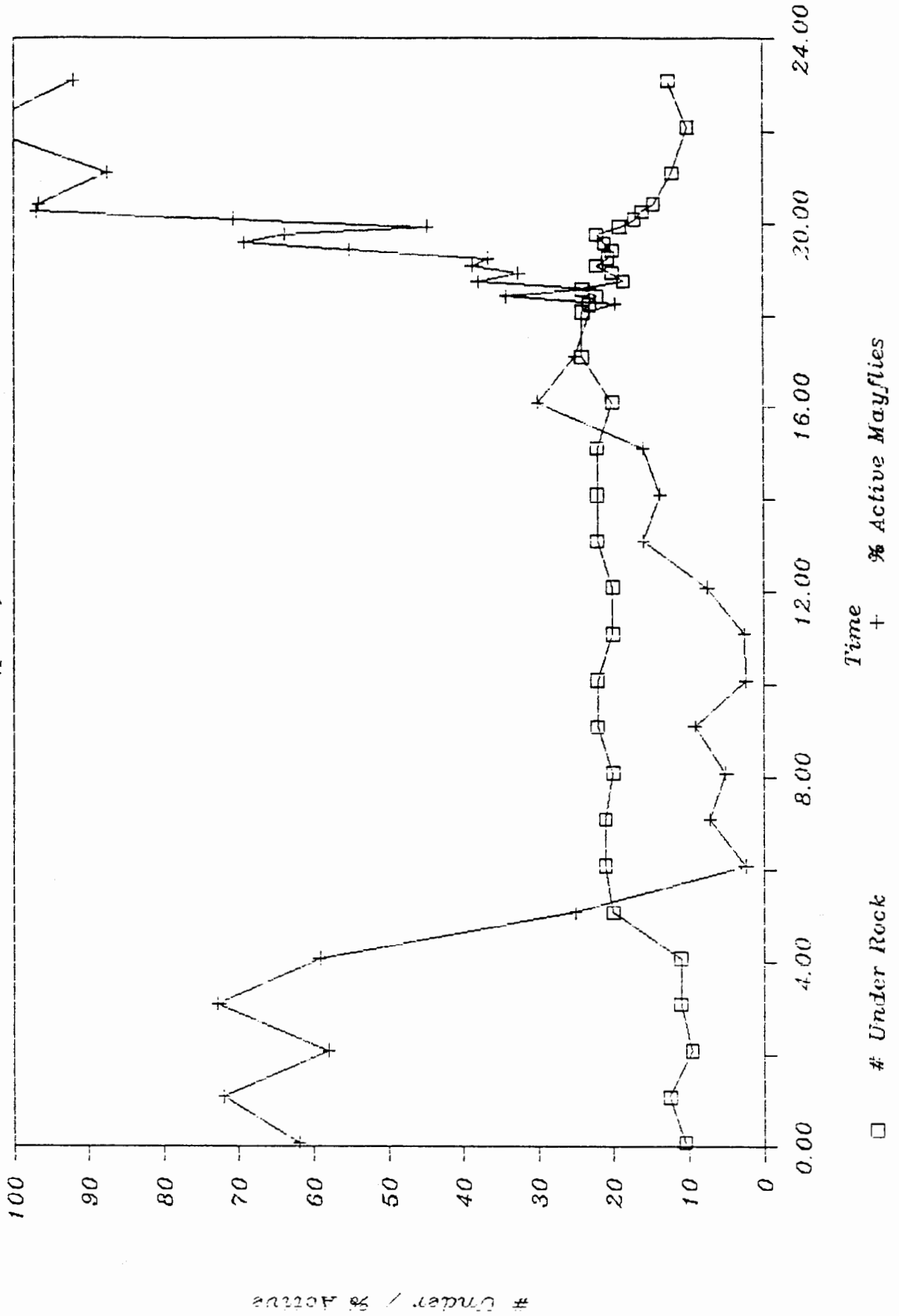


TABLE 1

Total activities of Stenonema integrated from daily activity curves for each of the three activity periods. Values are averages of 3 - 4 rocks with standard deviation of mean in parentheses. Activity rates were expressed as % active per 5 minutes.

Date	Channel B			Channel C		
	Sunrise	Midday	Evening	Sunrise	Midday	Evening
Feb 17	NA	NA	366.0 (13.5)	NA	NA	330.8 (15.2)
Feb 18	195.0 (9.7)	49.0 (5.6) A	365.2 (11.9)	207.5 (10.7)	66.7 (5.0)	405.5 (10.4)
Feb 19	132.5 (9.5) A	63.0 (7.9) A	461.2 (10.2) A	123.0 (8.2)	85.0 (7.2)	497.5 (16.6)
Feb 20	319.5 (13.6) A	34.0 (3.9) A,P	402.0 (5.7) A,P	351.8 (15.6)	83.7 (7.7) P	412.0 (10.1) P
Feb 21	274.2 (10.2) A,P	83.2 (8.2) A,P	497.8 (10.5) A,P	437.8 (16.0) P	175.5 (8.8) P	548.0 (13.3) P
Feb 22	269.5 (12.1) A,P	82.3 (7.7) A,P	651.5 (15.4) A,P	390.5 (6.3) P	169.0 (0.0) P	544.0 (16.8) P
Feb 23	374.8 (13.7) A,P	132.8 (12.8) A	503.0 (17.4) A	278.5 (2.8) P	137.0 (2.3)	280.2 (12.3)
Feb 24	211.7 (9.2) A	143.8 (5.3) A	NA	291.0 (15.0)	164.3 (7.8)	NA
May 05	NA	NA	377.0 (13.0)	NA	NA	468.0 (8.1)
May 06	564.0 (11.8)	233.5 (11.6)	514.8 (15.4)	499.5 (11.8)	122.2 (5.5) A	401.0 (7.5) A
May 07	304.0 (13.8)	271.5 (12.1)	580.0 (12.7)	440.2 (12.3) A	125.2 (7.3) A	531.0 (13.0) A
May 08	349.5 (13.5)	485.5 (14.5) P	499.5 (14.5) P	451.8 (9.7) A	355.8 (12.8) A,P	583.8 (13.2) A,P
May 09	583.7 (16.1) P	411.3 (15.0) P	361.2 (18.0) P	498.2 (13.0) A,P	351.7 (16.3) A,P	552.7 (6.3) A,P
May 10	404.0 (17.1) P	NA	NA	400.0 (7.4) A,P	NA	NA

NA = Not Available
A = Acid (pH 5)
P = Predator (Amphinemura)

TABLE 2

Comparison of the mean activities (integrated activity units) of *Stenonema* pH 5 and pH 7, for the periods of the day and ratios of activity at pH 5 to pH 7 (5/7). Mean activities calculated for entire experiment at a particular treatment (6-8 days, n = 16-30). Means between treatments within each period and experiment were not significantly different ($p < 0.10$).

	Sunrise			Midday			Sunset		
	pH 5	pH 7	5/7	pH 5	pH 7	5/7	pH 5	pH 7	5/7
February	276.8	267.0	1.04	92.0	116.6	0.79	479.1	414.9	1.15
May	430.1	446.9	0.96	215.8	253.4	0.85	514.7	466.8	1.03

TABLE 3

Analysis of variance of the time of initiation of leaving the underside of the rock (ILUT) versus the light intensity at the time of the Ringelberg Threshold (IRST). Time is Eastern Standard Time. pH = 7.0. Experiment conducted 5 - 6 October 1986.

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
Model	1	0.1666	0.1666	5.938	0.0715
Error	4	0.1122	0.0280		
C Total	5	0.2789			
		Adj R-SQ		0.4969	

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER = 0	PROB> T
Intercep	1	0.0695	0.1048	0.663	0.5438
IRST	1	0.6404	0.2628	2.437	0.0715

TABLE 4

Analysis of variance of the time of initiation of leaving the underside of the rock (ILUT) versus the light intensity at the time of the relative light change threshold (IRST). Time is Eastern Standard Time. pH = 7.0. Experiment conducted 18 - 24 February 1987.

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
Model	1	0.5806	0.5806	4.743	0.0723
Error	6	0.7345	0.1224		
C Total	7	1.3151			
		Adj R-Sq		0.3484	

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER = 0	PROB> T
Intercep	1	-1.6920	0.9264	-1.826	0.1176
IRST	1	10.1391	4.6557	2.178	0.0723

TABLE 5

Analysis of variance of the deviation of the time of leaving the underside of the rock (ILUT) from the time of the relative light change threshold (RST) versus the light intensity. Time deviation in minutes. Light intensity in $\mu\text{mol sec}^{-1}$. February and May data combined. pH = 7.

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
Model	1	6.3043	6.3043	19.041	0.0006
Error	15	4.9663	0.3311		
C Total	16	11.2706			

Adj R-Sq

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER = 0	PROB> T
Intercep	1	-0.8145	0.2641	-3.084	0.0076
IRST	1	6.4996	1.4895	4.364	0.0006

TABLE 6

Analysis of variance of the deviation of the time of leaving the underside of the rock (ILUT) from the time of the relative light change threshold (RST) versus the light intensity. Time deviation in minutes. Light intensity in $\mu\text{mol sec}^{-1}$. February and May data combined. pH = 5.

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
Model	1	1.4041	1.4041	5.007	0.0374
Error	19	5.3281	0.2804		
C Total	20	6.7322			
			Adj R-Sq	0.1669	

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER = 0	PROB> T
Intercep	1	-0.4375	0.2260	-1.936	0.0679
IRST	1	2.8490	1.2732	2.238	0.0374

TABLE 7

Analysis of variance of the deviation of the time of evening activity initiation (IEAT) from the time of the relative light change threshold (RST) versus the light intensity at the time of RST (IRST). Time deviation in minutes. Light intensity in $\mu\text{mol sec}^{-1}$. February and May data combined. pH = 7.

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
Model	1	4.1505	4.1505	19.682	0.0003
Error	18	3.7958	0.2109		
C Total	19	7.9463			
			Adj R-Sq	0.4958	

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER = 0	PROB> T
Intercep	1	-0.9034	0.2105	-4.291	0.0004
IRST	1	5.0543	1.1393	4.436	0.0003

TABLE 8

Analysis of variance of the deviation of the time of evening activity initiation (IEAT) from the time of the relative light change threshold (RST) versus the light intensity at the time of RST (IRST). Time deviation in minutes. Light intensity in $\mu\text{mol sec}^{-1}$. February and May data combined. pH = 5.

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
Model	1	3.1011	3.1011	42.451	0.0001
Error	21	1.5341	0.0731		
C Total	22	4.6351			
			Adj R-Sq	0.6533	

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER = 0	PROB> T
Intercep	1	-0.8863	0.1080	-8.207	0.0001
IRST	1	3.9419	0.6050	6.515	0.0001

TABLE 9

Analysis of variance of the deviation of the initiation of evening activity (IEAT) from the time of the relative light change threshold (RST) versus pH (4.50, 4.00, 3.80, 3.45). Units of time and light as in Table 5. Experiment conducted 17 - 21 June 1987.

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
Model	1	1.1210	1.1210	16.41	0.0018
Error	11	0.7519	0.0683		
C Total	12	1.8729			
			Adj R-Sq	0.5620	

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER = 0	PROB> T
Intercep	1	2.9623	0.7337	4.037	0.0020
pH	1	-0.75	0.1872	-4.050	0.0019