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THE ACUTE EFFECTS OF ALUMINUM AND ACIDITY UPON NINE STREAM INSECTS

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**THE ACUTE EFFECTS OF ALUMINUM
AND ACIDITY UPON NINE STREAM INSECTS**

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TECHNICAL COMPLETION REPORT

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ABSTRACT

The effects of increased aluminum concentrations and decreased pH upon the immature stages of five caddisflies, two mayflies, a stonefly, and a beetle were tested. To do this, individuals were removed from two riffle habitats in southern New Hampshire, placed into artificial streams and subjected to additions of aluminum salts and sulfuric acid for a three day period. The acute mortality and the drifting behavior of the insects over this three day period were then analysed using multiple linear regression. Aluminum additions caused increased mortality in the stonefly Nemoura nigratia and the caddisfly Macronema spp.; aluminum additions also increased the drift of the caddisfly Potamyia flava, but the response was small and likely due to the increased salinities in aluminum treatments. Aluminum, hydrogen ions, and their interaction affected the drifting behavior of the water penny beetle, Psephenus herricki. While concentrations of aluminum and hydrogen ions did not affect the drift of the mayfly Stenonema spp., high variability of aluminum concentrations caused increased drift for this genus; Macronema spp. also showed increased drift in response to large variations in aluminum concentrations. The effect of aluminum upon molt success was minor, but the effect of high acidity upon molt success was pronounced.

INTRODUCTION

The alterations of precipitation chemistry caused by industrial pollutants have been acknowledged for many years (Likens and Bormann, 1974). Recently the influence of "acid precipitation" upon the leaching of cations from the soil into streams has been investigated (Johnson et al.,

1981; Webb, 1982). During short periods of warm weather during the spring the melting of acidified snow may cause short term increases in cations (especially potassium and aluminum) leaching into streams (Cronan and Schofield, 1979); this effect is most pronounced where a thick snow cover reduces ice formation within the soil (Johnson et al., 1981). The objective of this study was to investigate the effects of these short term alterations in water chemistry upon drift and mortality of stream insects.

Other investigators have presented studies on the effects of acidity and heavy metals upon stream insects. Some of these studies involved alterations of water chemistry under laboratory conditions in order to compute an LD₅₀ (e.g. Bell and Nebeker, 1964; Bell, 1971); others involved investigating aquatic environments with altered chemical conditions (Koryak et al., 1972; Fiance, 1978; Winner et al., 1980; Hall et al., 1980; Peckarsky and Cook, 1981; Havas and Hutchinson, 1982; 1983). Few of these studies specifically investigated the effects of chemical alterations upon insect behaviors such as drift.

In our study, insects collected from a variety of riffle habitats were placed into artificial streams and subjected to additions of aluminum potassium sulfate, sulfuric acid, and potassium hydroxide. Two effects of these alterations were then examined: drift, the behavior by which live insects leave a habitat by entering the water column; mortality, the acute physiological reaction to the chemicals, including molt failure. Drift and mortality under varying chemical conditions were computed for: the caddisflies Macronema spp. (Hydropsychidae), Hydropsyche betteni, H. elissoma (Hydropsychidae), Potamyia flava (Hydropsychidae), and Chimarra obscurra (Philopotamiidae); the water penny beetle, Psephenus herricki (Psephenidae); the stonefly Nemoura nigratta (Nemouridae); the mayflies

Ephemerella catawba (Ephemerellidae) and Stenonema spp. (Heptageniidae). Multiple linear regression was then used to determine if the concentrations of aluminum and hydrogen ions significantly affected drift or mortality.

METHODS

The insects used in this study were collected in riffles of two southern New Hampshire rivers: the North River in Nottingham NH supplied Nemoura nigratta, Psephenus herricki, Ephemerella catawba, and Stenonema spp.; the Oyster River in Lee NH supplied Stenonema spp., and further downstream below a dam in Durham NH the Oyster River supplied the Trichoptera. At the site of collection both the North and Oyster Rivers are third order, but the North River is larger, more rapid and clearer than the Oyster River below the dam in Durham. Thus the insects studied came from two communities, one dominated by filter feeding Trichoptera (the Oyster River) the other dominated by grazing stoneflies, beetles and mayflies, as well as predatory stoneflies (the North River). The insects were collected by raking and brushing the substrate and capturing the dislodged animals with a hand net. Fresh individuals were collected for each weekly experimental run. Therefore individuals from different age classes were used at the end of the experiment (in May) than at its commencement (in March). Captured individuals were placed in cooled water and returned to the laboratory.

In the laboratory the insects were sorted by eye into seven groups; Chimarra spp., Hydropsyche/Potamyia spp., Macronema spp., P. herricki, Nemoura spp., Stenonema spp., and Ephemerella spp.. Other insects (e.g. Brachyptera fasciata, Allocapnia spp., Leuctra spp., Leptophlebia spp. and Prosimulium mixtum) were occasionally also put into a trough, but

insufficient data on these species precluded analyses of their responses. An approximately equal number from each of the six groups were then placed into one of four troughs of an artificial stream. Each trough of this stream was 0.15 M wide X 0.25 M high X 2.4 M long, and was filled with a substrate of rocks 5-10 mm in diameter. The rocks were taken from a small stream, washed and autoclaved prior to the experiments; however some algae was present on the rocks and trough sides during the experiment as a food source for grazing insects. Prior to each experimental run, water was collected from below the dam on the Oyster River in Durham so that food for the Trichoptera would be near natural levels. Each trough emptied into a small reservoir where the water was recirculated to the top of the trough by an electric pump at a rate of 24 liters per minute. Each trough held 26 liters of water. Stream water was maintained at 9-12 degrees C. Lighting was provided by fluorescent lights above the troughs and was maintained at natural photoperiods. The insects were allowed to acclimate to the trough for 16-20 hours before water chemistry was altered.

The water chemistry was altered by adding aluminum potassium sulfate and sulfuric acid. Since $\text{AlK}(\text{SO}_4)$ is an acid salt potassium hydroxide was added to create high aluminum-low acidity conditions. To avoid possible position effects, each trough in each run was randomly assigned an aluminum concentration and pH to be tested. Aluminum concentrations tested were between 0.3 and 2.5 ppm Al and acidities varied between pH 4.3 and pH 6.9. Aluminum concentrations higher than 1 ppm Al are rare for acid rain events, but concentrations much higher than this have been observed in mine drainage (Peckarsky and Cook, 1981). Each weekly battery of four treatments included one control (a trough into which no chemicals were added). Total aluminum concentrations were determined colorimetrically

using the ferron-orthophenanthroline method (Rainwater and Thatcher, 1960). Iron and fluorine ions interfere with this determination. Iron concentrations were tested concurrently with aluminum and were always low, producing less than 1% error in aluminum determination. Fluorine concentrations were not routinely tested, but tests made by the Durham Water Supply indicate that concentrations are generally 0.1 ppm F, which would produce an error in aluminum determination of less than 1%. No attempt was made to determine the speciation of total aluminum. Hydrogen ion concentrations were measured with a pH probe (Orion Research models 231 and 407a). Chemical conditions were measured at least three times a day.

In the early winter (December to February) a preliminary set of experimental treatments were performed. During these runs aluminum concentrations varied dramatically around the desired mean concentration, presumably due to the precipitation of aluminum hydroxides. Aluminum concentrations were restored during this preliminary set of treatments by pouring 50-250 ml doses of $\text{AlK}(\text{SO}_4)$ solution (200 ppm Al) into the troughs; variations in pH were corrected by adding 2 N $\text{H}_2(\text{SO}_4)$ or 2 N $\text{K}(\text{OH})$. Because aluminum determinations required a period of chemical reduction, aluminum adjustments could be made only once or twice a day. Since pH measurements were made by probe, hydrogen ions could be more closely monitored and acidity could be adjusted more often than aluminum. The correction of chemistry by the addition of slugs resulted in large deviations of chemical conditions that strayed far from the natural conditions that we were simulating. Thus prior to the main (March to May) experimental set of treatments a system was developed whereby $\text{AlK}(\text{SO}_4)$, $\text{H}_2(\text{SO}_4)$ and $\text{K}(\text{OH})$ could be added continuously at low concentrations. This maintained the desired conditions without the high variation of conditions

in the preliminary set of treatments. Aluminum concentrations in the main experimental set rarely strayed beyond 25% of the mean; maximum deviation (the largest difference between chemical conditions at a given time in a trough and mean chemical concentrations in that trough, expressed as a percentage of the mean) did not differ between treatments and controls (average maximum deviation for treatments=20%, average maximum deviation in controls=17%; $t=0.59$, $df=16$, n. s.). Hydrogen ion concentrations on the other hand had high deviations, most troughs experienced a period during which hydrogen ion concentrations varied by more than 50% of the mean. However these high deviations were not confined to treatments, there was no difference between treatment maximum deviation (average=61%) and control maximum deviation (average=56%) ($t=0.51$, $df=16$, n. s.). In contrast, during the preliminary set of treatments maximum deviations of aluminum concentrations in treatments (average=47%) were significantly greater than the maximum deviations of aluminum concentrations in controls (average=5%) ($t=3.6$, $df=17$, $p<.01$). Drift and mortality from the preliminary set of data were not used in the search for significant effects due to chemical concentrations, but were used to test the effects of the variability of chemical conditions upon insect drift.

To measure insect drift out of the troughs, nets were attached to the outlet of each trough. These nets were emptied each morning and the contents were examined. Those insects that were alive in the net were identified and scored as drift for that day. Occasionally a dead molting insect (an insect that was in the process of emerging from its molting skin, or had not yet fully restored its pigmentation when it had died), a whole dead insect, or the head capsule of an insect would be found in the net. These animals were recorded as dead animals. Because an insect need

not drift upon dying, no attempt was made to analyse mortality on a day by day basis. The appearance of molted skins in the nets were also noted.

Following 72 hours exposure to the chemical conditions within the trough the insects were removed by washing each substrate rock; any exuviae left behind by emerged adults were also collected. Insect individuals covered with fungal growths, insect head capsules, and insects that did not show movement upon prodding were sorted out as mortality and preserved in 75% ETOH. All other insects were killed in 95% ETOH and preserved in 75% ETOH. After all four troughs had been emptied in this manner, the troughs were rinsed to remove any remaining sand. The rocks were rinsed with tap water and haphazardly replaced into the troughs. The troughs were then filled with Durham tap water and allowed to rinse for 24-48 hours before being filled with Oyster River water 24 hours prior to the addition of the insects in the next experiment. Following high aluminum-low acidity runs in which much aluminum was precipitated, a 12 hour rinse with acidified (pH 3.5) water preceded the tap water rinse. This removed hydroxides of aluminum from the rocks and trough sides. Despite these rinses, aluminum concentrations in control troughs were higher than aluminum concentrations in water taken from above the dam on the Oyster River, presumably because of residual aluminum from previous runs. Hydrogen ion concentrations in controls matched those of the river.

The preserved insects from each trough were identified using the keys of Hitchcock (1974), Brigham et al. (1981), and Ross (1944). Some identifications were difficult. It is unclear whether the two size classes of Macronema spp. that occurred throughout the experiment represented two cohorts of a single species, or two species. It was also uncertain whether the Macronema species were M. zebratum or M. carolina. The Stenonema spp.

also existed in two size classes. The large individuals were mostly S. modestum. Species identification of small instars was not attempted. When a familiarity with the insects had been developed the fungus encased individuals and head capsules were identified. Because molting insects leave cast skins that preserve identifying characteristics, molted skins and exuviae could be easily identified.

After all dead insects, live insects and molt skins had been identified the following parameters could be computed for each species.

$$(1) \quad \text{drift on day } x \text{ for species } a = \frac{\sum_{k=1}^x \text{\# of species } a \text{ alive in drift on day } k}{\text{\# of species } a \text{ originally in the trough}}$$

Drift is thus the proportion of the total number that had left the trough by the morning of the x^{th} day.

$$(2) \quad \text{mortality of species } a = \frac{\text{\# of species } a \text{ found dead}}{\text{\# of species } a \text{ originally in trough}}$$

$$(3) \quad \text{molt failure rate} = \frac{\text{\# of dead insects in the process of molting}}{\text{\# of cast skins and exuviae found}}$$

A total of 13 chemical treatments were completed, five control troughs were also run, resulting in a maximum of 18 data points. Responses for a given species in a trough were accepted only if nine or more individuals of that species were placed into the trough at the beginning of the experiment. Therefore most species did not have the full complement of 18 data points.

Following the completion of these 18 runs an experiment was performed

to test whether effects due to aluminum concentrations were the result of a response to increased salinity due to potassium ions in the aluminum treatments. To do this, two troughs were treated with $K_2(SO_4)$ solution so as to mimic the rate of addition needed to keep aluminum concentrations at 1.8 ppm Al (if $AlK(SO_4)$ were being added rather than $K_2(SO_4)$). Potassium concentrations increased during these treatments to about 8×10^{-5} M. Since $K_2(SO_4)$ is a neutral salt little hydrogen ion adjustment was necessary; pH remained at about 6.5. Whenever the response of a species was due to aluminum concentrations, the response was predicted from the regression equation for 1.8 ppm Al and pH 6.5. This predicted response was then compared with the response observed in the $K_2(SO_4)$ treatments using the methodology of Neter and Wasserman (1974).

Statistical Analysis

Multiple linear regression was used to analyse the responses of the insects to different chemical conditions. Our use of multiple linear regression followed the methodology of McNeil et al. (1975), in which the regression model is used to identify measured variables that increase the predictability of the response. This method asks the question: "Does the observed response (drift or mortality) increase due to one of the measured variables (aluminum and acidity)?" We did not attempt to find a "best fit" model for our data because of the small data set imposed by the lack of comparability between the spring and winter set of treatments.

Prior to computing the regression models some of the data were transformed. To reduce the dependence of variance and mean found in binomial distributions the responses were transformed using the arcsin of the square root of the response (Sokal and Rohlf, 1969). In order to make the range of values approximately equal for the two independent variables

(aluminum and hydrogen ion concentrations) the hydrogen ion concentrations were transformed using the following equation:

$$(4) \quad [H]_{\text{trans.}} = \log (\text{average } [H] \times 10^7) \\ = 7 - p(\text{average } [H])$$

The response was then regressed using the regression model:

$$(5) \quad \arcsin(\text{response}) \cdot 5 = a_0 + a_1 [Al] + a_2 [Al]^2 + \\ a_3 (7 - p(\text{average } [H])) + \\ a_4 (7 - p(\text{average } [H]))^2 + \\ a_5 [Al] (7 - p(\text{average } [H]))$$

where a_i is a regression coefficient and [Al] is expressed in parts per million. The model was then reduced in complexity by the step-wise removal of the least significant coefficient (the coefficient with the smallest t-test value when testing the hypothesis $H_0: a_i = 0$). The model was reduced in complexity until a model was found containing coefficients that were all significant (not equal to 0 at $p < 0.05$). The model was accepted as a description of the experimental results if the residuals (observed response - response predicted from the regression model) were evenly distributed over the regression surface, and the variance of the response was explained by the regression (the F-test of variance explained by the regression vs. unexplained variance was significant at $p < 0.05$). The MTAB2 computer program was used to compute the regression coefficients and their significance levels. Regressions in which a single data point appeared to have a strong influence upon the regression model were re-examined. If the removal of this data point altered the function describing the data, or removed the significance of the regression, the regression equation and coefficient significance levels were determined without this point. The surface of the regression model was then analysed using calculus to find

slopes and maxima-minima points.

The lack of drift by Stenonema spp. during the main experiment (average drift following 72 hours was less than 1%) surprised us because of the large drift response of Stenonema spp. during the preliminary set of treatments (average drift following 72 hours exposure=33%). This prompted us to investigate the drift responses of this genus further. As mentioned above, the preliminary set of treatments differed from the main experimental set in that they were run in the early winter with younger individuals, and were typified by high variability of chemical conditions. To test whether the drift in early winter was a result of the use of different age classes two sets of data were analysed. The drift of large (over 5 mm from front of the clypeus to the base of the cerci) and small (less than 5 mm in length) individuals following 72 hours exposure were tested for independence using the G-test (Sokal and Rohlf, 1969). Data from a given trough was included in the analysis only if both large and small Stenonema spp. size classes were represented by at least six individuals. Season had no effect on this test because both large and small individuals were present in both winter and spring. To test the effects of season upon drift, the drift response of Stenonema spp. following 72 hours exposure in control troughs of the main experimental set were compared with the drift in the control troughs of the preliminary set of treatments using the G-test. To test whether the increased drift was a response to the varying chemical conditions the drift response following 24 hours exposure was regressed against the range of chemical conditions experienced during these 24 hours (essentially one cycle of chemical conditions, with a period of concentrations above the mean and a period of concentrations below the mean). Again, the response was accepted only if the number of individuals

originally in the trough was nine or more, and was transformed using the arcsin of the square root of the response. The range of hydrogen ion concentrations were transformed to conform to the values of the aluminum concentration range by equation 6.

$$(6) \quad [H]_{\text{trans.}} = \log \left(\frac{([H]_{\text{max.}} - [H]_{\text{min.}}) \times 10^8}{8 - p(\text{range } [H])} \right)$$

Ranges of aluminum concentrations were expressed in parts per million. The model used in the regression was therefore:

$$(7) \quad \arcsin(24 \text{ hour drift}) \cdot 5 = a_0 + a_1([\text{Al}] \text{ range}) + a_2([\text{Al}] \text{ range})^2 + a_3(8 - p([\text{H}] \text{ range})) + a_4(8 - p([\text{H}] \text{ range}))^2 + a_5([\text{Al}] \text{ range})(8 - p([\text{H}] \text{ range}))$$

Where a_i is as in equation 5. Step-wise removal of predictors was then used to search for a regression model fullfilling the same criteria as listed above ($a_i \neq 0$, residuals evenly distributed, significant F-test value). Because drift out of control troughs in the preliminary set of treatments was significantly greater than drift out of controls in the main set of treatments ($G=3.84$, $df=1$, $p=0.05$; see RESULTS for Stenonema spp.) only the results of the preliminary set of treatments were used in this analysis. Similar analyses were performed on the other species tested in the preliminary set of treatments (the same Trichoptera used in the main set of treatments and Ephemerella subvaria).

Multiple linear regression could not be used to test the effects of acidity and aluminum concentrations upon molting success because of the low molting frequency within the troughs. Thus to test the effects of high aluminum concentrations and acidity upon molting success the number of P.

herricki, Plecoptera, and Ephemeroptera that had died in the process of molting, and the total number of attempted molts by these three groups were pooled within the five controls, three high aluminum-low acidity treatments, and three low aluminum-high acidity treatments. Trichoptera were not included in this analysis because no molt attempts were observed in this order. The pooled results for the three insect groups under the three different chemical conditions were then analysed for independence using the G-test (Sokal and Rohlf, 1969).

RESULTS

Insects Showing No Response

With the data available in this study we could not detect a significant increase in drift or mortality due to increased concentrations of aluminum or hydrogen ions for four insect species. The average drift at 24, 48, and 72 hours, as well as average mortality of these four species are shown in Figure 1.

Hydropsyche elissoma (Hydropsychidae)

Hydropsyche elissoma showed no detectable response to chemical changes. Drift was generally low for this species. The average drift following 72 hours exposure was 8%; maximum drift following 72 hours exposure was 25% in a trough experiencing 1.6 ppm Al at pH 6.1. Mortality was also low. Average mortality was 4%; maximum mortality was 18% in a trough experiencing 1.6 ppm Al at pH 4.6. This appears to be a resistant species.

Hydropsyche betteni (Hydropsychidae)

Like its congener, H. betteni also showed no response to chemical alterations. Drift for H. betteni was low. Average drift following 72

hours exposure was 5%; maximum drift following 72 hours exposure was 33% in a trough experiencing 1.1 ppm Al at pH 4.2. Average mortality was 5%; mortality greater than 20% was observed only once, a trough experiencing 0.9 ppm Al at pH 4.5 had a mortality rate of 40%. This species appears to also be resistant.

Chimarra obscurra (Philopotamiidae)

The other trichopteran showing no detectable response was C. obscurra. Drift for this species was unpredictable and low. Average drift following 72 hours exposure was 2%; maximum drift was 22% in a trough experiencing 1.7 ppm Al at pH 6.1. Mortality for C. obscurra was also unpredictable but was high. Average mortality was 19%; maximum mortality was 67% and was observed in two troughs, one was a control the other was a 1.0 ppm Al-pH 5.6 treatment. Many of the C. obscurra individuals making up the set of dead animals consisted of only a head capsule. It is likely that these individuals were captured and consumed by omnivorous hydropsychid caddisflies. Chimarra obscurra is also much less robust than other insects tested and were thus prone to handling damage. It is likely that the combination of hydropsychid predation/competition and rough handling resulted in the majority of C. obscurra mortality.

Ephemerella catawba (Ephemerellidae)

The fourth species showing no significant increase in response due to altered chemical conditions was the mayfly E. catawba. Drift of this species was high, even after only 24 hours the average drift was 38%, after 72 hours average drift was 56%; maximum drift following 72 hours exposure was 85% observed in a trough experiencing 1.6 ppm Al at pH 4.6. Mortality on the other hand was low. Average mortality was 6%; only one trough had a mortality rate higher than 20%, a 1.7 ppm Al-pH 4.7 treatment

produced 67% mortality.

Stenonema spp. (Heptageniidae)

While Stenonema spp. showed no response to increasing concentrations of aluminum and hydrogen ions, an interesting response to variations of chemical conditions was observed. During the main set of treatments drift and mortality were low (the darkened symbols in Figure 2). Average drift following 72 hours exposure was less than 1%; maximum drift following 72 hours exposure was 18% under conditions of 1.6 ppm Al at pH 4.6. Average mortality was 6%; maximum mortality was 29% at 1.2 ppm Al and pH 4.8.

The drift responses observed in the preliminary set of treatments (open symbols of Figure 2) were much higher than those observed in the main set of treatments. Although individuals of two size classes were used in these two set of treatments (one group was longer than 5 mm the other was shorter than 5 mm) size class had no effect upon drift ($G=0.69$, $df=1$, n. s.). Season on the other hand had an effect on drift, winter individuals were more prone to drift than spring individuals (winter control drift=10%, spring control drift=2%; $G=3.84$, $df=1$, $p=0.05$). The effect of the high degree of chemical variability typifying the preliminary set of treatments was tested using the regression model shown in equation 7. A significant regression ($p<0.05$) explained 33% of the variation of drift (Figure 3). The two darkened symbols of Figure 3 show two data points that appeared to be exerting a large influence on the regression equation. If both of these points are removed from the data set the regression is no longer significant. Both points were included in this analysis since the model is not altered, and remains significant if only one point is removed. Thus the higher drift response for Stenonema spp. in the preliminary set of

treatments is found to be a result of seasonal effects and chemical variability.

Insects Responding By Drifting

Potamyia flava and Psephenus herricki responded to increasing aluminum concentrations by increasing drift. The responses were due to the increased salinity of aluminum treatments for P. flava, but P. herricki showed a strong response to aluminum concentrations and little response to the high salinities associated with aluminum treatments.

Potamyia flava (Hydropsychidae)

Drift responses for P. flava were generally low. Average drift following 72 hours exposure was 4%, however a significant regression ($p < 0.05$) explaining 34% of the variation could be fitted (Figure 4). The low response of this species (usually only 1 individual was present in the drift) may have caused artifacts in these results. The drift response as calculated would depend strongly upon the number of individuals originally in the trough, yet the presence of some response in all high aluminum treatments suggests that chemical conditions in the aluminum treatments had an effect upon P. flava drift. However this response is likely due to the increased salinities of aluminum treatments. Drift in the two troughs treated with $K_2(SO_4)$ rather than $AlK(SO_4)$ was not less than that expected from the regression equation; it was in fact significantly greater in the $K_2(SO_4)$ treatments ($K_2(SO_4)$ drift=29%, predicted drift=8%; $t=2.98$, $df=13$, $p < 0.05$). This indicates that potassium has a stronger effect upon P. flava drift than aluminum.

Mortality was low. Average mortality was 6% (standard deviation of the transformed response was 0.24 radians, $N=15$); maximum mortality was

31% under conditions of 0.9 ppm Al and pH 4.5, 30% mortality was also observed under conditions of 1.1 ppm Al and pH 4.2.

Psephenus herricki (Psephenidae)

The drift response of P.herricki was similar for each of the three days and was predictable from aluminum concentrations and the interaction of aluminum and hydrogen. On the first day hydrogen ion concentrations also influenced drift. The regression model of drift following 24 hours was highly significant ($p < 0.01$). This regression explains 79% of the variability and shows increasing drift responses with increasing aluminum concentrations that are most pronounced at low acidities and high aluminum concentrations (Fig. 5). At aluminum concentrations less than 0.8 ppm increasing acidity increased drift. Above this concentration increasing acidity reduced drift. Since most natural snowmelt events rarely raise aluminum concentrations above 0.8 ppm it is likely that under natural conditions increased acidity would have the greatest immediate effect on the drift of P. herricki. The drift response following 48 hours exposure is similar to that for 24 hours, except that acidity alone is no longer a significant component of the model. This regression model explains 53% of the drift variation and is significant ($p < 0.05$). This model shows drift increases with increasing aluminum concentrations, with the greatest response at low acidities and high aluminum concentrations (Fig. 6). The 72 hours drift regression differs in that the response to aluminum is linear rather than exponential (Fig. 7). This regression explains 56% of the variation and is significant ($p < 0.05$). Again, the increased drift due to aluminum is most pronounced at low acidities. The response of P. herricki to increased aluminum concentrations is not due to the increased salinities of high aluminum treatments. When the predicted response of P.

herricki is compared to the observed response in the $K_2(SO_4)$ treatments, the expected response is significantly greater at 24 hours (predicted drift=99%, observed drift in $K_2(SO_4)$ treatments=18%; $t=6.73$, $df=9$, $p<0.01$), and at 48 hours (predicted drift=91%, observed drift=45%; $t=3.49$, $df=9$, $p<0.01$), but not at 72 hours (predicted drift=80%, observed drift=45%; $t=1.63$, $df=9$, $0.20>p>0.10$). Apparently after long periods of exposure to increasing salinities, P. herricki became stressed by high salinities as well as aluminum concentrations. The specificity of the response to aluminum concentrations suggests an explanation for the negative coefficients associated with the aluminum-hydrogen ion interaction term. Hydroxides are the main complexed form of aluminum, especially under alkaline conditions. These hydroxides polymerize and precipitate when OH:Al ratios become high (Hem and Roberson, 1967). To maintain low acidities at high aluminum concentrations K(OH) was added, effectively increasing OH:Al ratio, and promoting the formation of aluminum hydroxide precipitates. It may be these precipitates that are the major cause of P. herricki drift.

Mortality of P. herricki was rare. Average mortality was 2% (standard deviation of the transformed percentages was 0.19 radians, $N=11$); maximum mortality was 20% in a trough experiencing 1.7 ppm Al at pH 4.7. The much higher drift response indicates that mortality is of little importance in the removal of P. herricki from acid perturbed environments.

Insects Experiencing Mortality

Nemoura nigratta and Macronema spp. showed increased mortality when exposed to increased aluminum concentrations. The mortality was higher in $AlK(SO_4)$ treatments than in $K_2(SO_4)$ for both species, indicating that both

species were not responding to the high salinities in the aluminum treatments.

Nemoura nigratta (Nemouridae)

The mortality of N. nigratta could be significantly regressed upon aluminum concentrations (Figure 8), thus explaining 50% of the variation in mortality. Moreover, the mortality predicted at an aluminum concentration of 1.8 ppm is significantly greater than that observed in the two troughs treated with $K_2(SO_4)$ (predicted mortality=13%, observed mortality in $K_2(SO_4)$ treatments=0%; $t=3.05$, $df=6$, $p<0.05$) indicating that N. nigratta is resistant to high salinities, but has an adverse physiological reaction to high concentrations of aluminum. Since increasing acidity did not reduce mortality at any given aluminum concentration (as evidenced by the lack of an aluminum-hydrogen interaction term), precipitated hydroxides of aluminum are apparently not more toxic than dissolved forms of aluminum.

Drift for N. nigratta was low. Average drift following 72 hours exposure was 2% (standard deviation of the transformed values=0.16 radians, $N=8$); maximum drift was only 11% under conditions of 0.9 ppm Al at pH 4.5. The low drift response shows that although N. nigratta become stressed at high aluminum concentrations, they do not respond to this stress by leaving their habitat.

Macronema spp. (Hydropsychidae)

Macronema spp. mortality could also be regressed upon aluminum concentrations (figure 9), thus explaining 33% of the variation of mortality. Like N. nigratta, Macronema spp. suffered higher mortality in aluminum treatments than in high salinity treatments lacking aluminum (predicted mortality at 1.8 ppm Al=21%, mortality observed in $K_2(SO_4)$ treatments=11%; $t=4.18$, $df=13$, $p<0.01$). Also like N. nigratta, increasing

acidity did not mediate the stress of high aluminum concentrations, indicating that aluminum hydroxide precipitates are not the main cause of Macronema mortality.

Macronema spp. drift was high and not related to chemical concentrations, but like Stenonema spp., Macronema spp. drifted in response to the range of aluminum concentrations during the first 24 hours of exposure. Average drift following 72 hours exposure in the main set of treatments was 21% (standard deviation of the transformed values=0.20 radians, N=15); maximum drift was 56% and took place in a (control) trough experiencing 0.4 ppm Al at pH 6.8. No regression model could be found to predict drift when only mean concentrations of aluminum and hydrogen ions were used as predictors. However, when Macronema spp. drift following 24 hours exposure in the preliminary set of treatments was regressed against the range of chemical variation a significant regression ensued (Figure 10). This regression explains 62% of the drift variance. This model does not result from the analysis of the complete data set. Only the results from the preliminary set were analysed because drift following 24 hours exposure in the controls of the main set of treatments (28%) was higher than drift following 24 hours exposure in controls of the preliminary set (14%) (though not significantly so; $G=2.79$, $df=1$, $0.05 < p < 0.10$). Also, one data point (the darkened symbol of Figure 10) appeared to greatly influence the model. With this point included, the response to aluminum remained significant, but became curvilinear and an influence of hydrogen ions became evident. Rather than let one data point influence the results, this point was removed from the data set prior to the final analysis. The high drift response of Macronema spp. in controls of the main experimental set is anomalous to the finding that aluminum range can be used to estimate

Macronema spp. drift, apparently some other important (unmeasured) factor affects the drifting behavior of Macronema spp.

Effects of Aluminum and Acidity Upon Molting Success

A test of independence between molting rate observed in troughs experiencing different chemical conditions is shown in Table 1. The molting success in controls did not differ from the molting success in high aluminum-low acidity treatments ($G=0.05$, $df=1$, n. s.), but molting success in controls did differ from molting success in low aluminum-high acidity treatments ($G=8.88$, $df=1$, $p<0.01$). This result is consistent with the findings of Bell (1971) that molt failure is an important component of insect mortality under acidic conditions and of Hall et al. (1980) that insect emergence was reduced 37% following the acidification of a stream. However, Sanderson et al. (1982) found that aluminum inhibited chromosomal puffing (associated with RNA synthesis) at ecdysterone activated sites of the polytene chromosome of Simulium vittatum. Since ecdysterone is an important molting hormone of insects one might postulate that aluminum may influence molting ability. Our results show that if there is such an effect it is non-acute, or requires an exposure period longer than three days.

DISCUSSION

Effects of Snow Melts upon Organisms

The rapid increase in drift of Psephenus herricki to increased aluminum and hydrogen ion concentrations is one of the most pronounced results of this experiment. Psephenus herricki, commonly known as water penny beetles, are grazers and are closely compressed to the substrate.

Strong sprays of water do not remove them from a stone's surface; while collecting insects from a trough at the termination of a treatment P. herricki had to be pried from the stone's surface with forceps. Thus the increased response of this species represents a distinctive behavioral change. The higher response of P. herricki in the presence of $\text{AlK}(\text{SO}_4)$ than in $\text{K}_2(\text{SO}_4)$, and the negative effect of increasing acidity at high aluminum concentrations imply that the drifting response is caused by hydroxides of aluminum that precipitate at the high OH:Al ratios of high aluminum-low acidity treatments. Hydroxides of aluminum have been found to cause mortality in fish due to the formation of a yellow mucus on the gills that interrupts respiration and ionic regulation (Driscoll et al. 1980). It is unclear whether P. herricki drift is due to interference with respiratory surfaces as in fish, or if it is due to the ingestion of aluminum hydroxide polymers which may be toxic or distasteful to P. herricki.

Potamyia flava, the other insect displaying a drift response did not respond as rapidly and appeared to respond to the high salinities in aluminum treatments. Potamyia flava is a filter feeding caddisfly that spins a net to capture detritus. To enter the water column as drift P. flava individuals must climb out of this net. Thus drift represents an active change in behavior. The response of P. flava to increasing aluminum concentrations is not as rapid as that of P. herricki. This is indicated by the lower slope of the P. flava regression.

Both Nemoura nigratta and P. herricki responded to aluminum sulfate salt additions more than potassium sulfate salts. Whereas P. herricki drifted, N. nigratta individuals died as a result of the aluminum. The low drift rates of N. nigratta suggests it has little behavioral response to

increased aluminum induced stress. Since it is a grazer that walks about on exposed rock surfaces N. nigratta should be more likely to appear as drift when stressed. This makes the lack of a drift response even more indicative of no behavioral response to aluminum stress by N. nigratta. The lack of a mediating effect from hydrogen ions implies that the mortality may not be due to hydroxide polymers of aluminum. Thus even though they are both grazers living in the same community, the effects of acid and aluminum on P. herricki and N. nigratta are different.

Macronema spp. also suffered higher mortality in aluminum treatments than in potassium treatments. Macronema spp. are net spinning caddisflies, but its cases are more robust than other hydropsyhids tested, and during the collection of insects at the termination of a treatment Macronema spp. individuals were loath to leave their cases. The effect of high aluminum ranges upon drift of Macronema spp. indicate that Macronema spp. individuals respond to stream chemistry alterations by leaving their cases. Since aluminum concentrations are reduced as stream order increases (Johnson et al., 1981), this drift behavior of Macronema spp. may allow them to escape from habitats where aluminum has increased markedly, before they are physiologically stressed.

It was not surprising that the two Hydropsyche species studied were resistant. Bell and Nebeker (1969) as well as Bell (1970) found that H. betteni is very resistant to high acidities. The resistance to aluminum would allow Hydropsyche spp. to inhabit riffle areas where P. flava and Macronema spp. would be stressed.

The lack of a significant response in Ephemerella catawba was unexpected. It has been found that mayfly densities are reduced in streams with high acidities (Peckarsky and Cook, 1981; Harriman and Morrison, 1982)

and are stressed by high acidities (Bell, 1969; Bell and Nebekar, 1971). However, Fiance (1978) found that Ephemerella funeralis was relatively unaffected by increased stream acidities. Since Stenonema spp. also showed little response it appears that these mayflies are resistant to short term changes in water chemistry.

It was striking that no species suffered mortality as a result of high acidity alone. Hydrogen ions have been found to cause ionic imbalances by disrupting sodium/chloride regulation in aquatic insects (Stobbart, 1967; 1971; Wright, 1975; van Genechten et al., 1980). Other aquatic crustacea have also been found to have ionic imbalances when exposed to high acidities (Potts and Fryer, 1979; Malley, 1980; Morgan and McMahon, 1982; Havas and Hutchinson, 1983). Apparently the 72 hour exposure period was not long enough, nor the number of samples large enough, to detect any hydrogen ion effect upon mortality of the test organisms. Also, the acidities tested may not have been low enough to detect any differences in mortality; Bell and Nebekar (1969) found that after 96 hours exposure, of the ten aquatic insects studied by them, only Pteronarcys dorsata (Pteronarcyidae: Plecoptera) and Ephemerella subvaria showed noticeable mortality above pH 4.5.

Effects of Weather and Habitat upon Community Perturbations

Since not all of the test insects responded to the increases in aluminum or acidity in the same manner, all organisms in all habitats will not be affected by an acid snow melt. Weather conditions accompanying the snow melt and the location of the habitat will strongly influence the organisms affected by the snow melt.

Acute responses to aluminum in acidified snow melts may be present in restricted areas. To leach out a large amount of aluminum the melting snow

must be highly acidic and be allowed to percolate through the soil. This percolation is only possible if the soil is unfrozen. There are two ways in which melting snow may overlies defrosted soil: a thick snow cover will insulate the soil below and promote warming due to biological activity; the melting snow may be from a heavy snowfall late in the spring following the defrosting of the soil. The effects of the resulting percolate upon P. herricki populations would be rapid. If the snow melt continued so that high aluminum conditions persist, effects upon N. nigratta, P. flava and Macronema spp. would become evident.

It should be emphasized that the highest aluminum concentrations tested in this experiment represent rare or extreme events in nature. The low acidities and slow melting of snow packs typical of most springs should cause only small increases in aluminum concentrations and should produce only small changes in community structure. For example, a snow melt event described by Johnson et al. (1981) showed aluminum concentrations of 0.65 ppm at pH 4.75 on March 6, 1979, in the headwaters of a small New Hampshire stream. Our models predict that three days exposure to these conditions would produce 15-20% drift losses of P. herricki, but would result in less than 5% losses of other test organisms. Thus the effects of a single snow melt event may not cause obvious changes in a community. However, the effects of repeated or continuous chemical alterations will compound these small changes in community structure and may result in long-term alterations in insect communities.

Because the acidity of the snow melt water is a main factor affecting the response of P. herricki effects should be apparent for this species under different conditions than for those species affected solely by aluminum. For maximum acidity of meltwater, little ion exchange within the

soil must take place. Little ion exchange takes place when percolation is minimal, as when soil is frozen or is overlaid by a layer of ice. If highly acidified snow melt water runs into streams the effect upon P. herricki would be apparent.

The effects of increased salinities upon P. flava suggests that a different type of snow melt event may affect this species. Runoff from roads treated with halite (used to melt road ice) may be affecting this species adversely.

The effects of the variation in aluminum concentrations upon drift of Stenonema spp. and Macronema spp. following one cycle of conditions suggests that these genera respond to an unusual type of snow melt. These genera may be affected by rapid melting of snow high in heavy metals, followed by cessation of melting and decreased ion input. These conditions would be present at high altitudes where high diurnal insolation is followed by nocturnal radiative cooling, and watersheds are small. Heavy metals and other ions have been found to be at high concentrations in high altitude snow and hoarfrost (Schlesinger and Reiners, 1974; Reiners et al., 1975). Alpine streams affected by industrial pollutants may be adverse environments for Stenonema spp. and Macronema spp.

While both of the communities supplying insects are relatively unaffected by snow melt induced chemical alterations, the North River community appears more prone to disturbance. The strong effect of P. herricki to short term acidity increases indicates that this species may be regularly stressed by acidic events. The long term effects of increasing aluminum upon N. nigratta indicates that this species may also be removed from the North River when aluminum concentrations increase. This would promote competitive release for the resistant grazing insects such as

Stenonema spp. and E. catawba. Since P. herricki and N. nigratta are at high densities in the North River (personal observation) this effect may be great.

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SUMMARY AND CONCLUSIONS

By subjecting stream insect species to altered aluminum concentrations (0.03-2.5 ppm) and pH levels (4.3-6.9) we were able to identify conditions that will cause community changes in riffle populations and the responses that cause those changes. Three of the caddisflies (Hydropsyche betteni, H. elissoma, and Chimarra obscurra), and two of the mayflies (Ephemerella catawba and Stenonema spp.) did not appear to be physiologically stressed and did not leave a habitat in response to short term alterations in aluminum and hydrogen ion concentrations. Mortality of the caddisfly Macronema spp. and the stonefly Nemoura nigratta was induced by high aluminum concentrations. Because the increase in mortality was not great, these organisms would make poor indicators of acid rain perturbed environments. The much more dramatic drift response of the beetle Psephenus herricki to increased acidity and aluminum concentrations would make this species a good indicator of acid rain perturbed environments. Our models estimate that 15-20% P. herricki drift would result from

previously recorded acid snow melts. The low drift response of the caddisfly Potamyia flava to increased salt concentrations within the trough suggest that this species may be affected by the increased salinity from roadside salts as well as acidic snow.

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FIGURE HEADINGS

Figure 1-Average drift and mortality (± 1 standard deviation) of H. elissoma, H. betteni, C. obscurra and E. catawba. 24 hour drift=circles, 48 hour drift=squares, 72 hour drift=hexagons, mortality=triangles.

Figure 2-Average drift and mortality (± 1 s. d.) for Stenonema spp. Symbols are as in figure 1 except, drift in preliminary set of treatments=open symbols, drift in main set of treatments=filled symbols.

Figure 3-Results of regression analysis of Stenonema drift following 24 hours exposure in the preliminary set of treatments vs. range of aluminum concentrations. Regression equation is: $\arcsin(\text{drift}) \cdot 5 = 0.27^* + 0.11^* ([Al] \text{ range})^2$ (F=6.44, df=1,13, p<0.05). Symbols indicate treatments in which the p(range [H]) was less than 5 (circles), between 5 and 6 (triangles) and above 6 (squares). *significantly different from 0 at p<0.05, **significantly different from 0 at p<0.01.

Figure 4-Results of regression analysis of P. flava drift following 72 hours exposure vs. aluminum concentrations. Regression equation is: $\arcsin(\text{drift}) \cdot 5 = 0.07 + 0.13^* ([Al])$ (F=6.86, df=1,13, p<0.05). Symbols indicate treatments run at below pH 5 (circles), between pH 5 and pH 6 (triangles) and above pH 6 (squares). *significantly different from 0 at p<0.05, **significantly different from 0 at p<0.01.

Figure 5-Results of the regression analysis of P. herricki drift following 24 hours exposure vs. aluminum concentrations. The regression equation is: $\arcsin(\text{drift}) \cdot 5 = -0.08 + 0.63^{**} (7-\text{pH}) + 0.66^{**} ([Al])^2 - 0.76^{**} ([Al])(7-\text{pH})$ (F=8.96, df=3,7, p<0.01). Surface contours are shown for pH 6.5, pH 5.5 and pH 4.5. Symbols are as in figure 4.

Figure 6-Results of the regression analysis of P. herricki drift following 48 hours exposure vs. aluminum concentrations. The regression equation is: $\arcsin(\text{drift}) \cdot 5 = 0.43^{**} + 0.31^* ([Al])^2 - 0.19^* ([Al])(7-\text{pH})$ (F=4.6, df=2,8, p<0.05). Surface contours are shown for pH 6.5, pH 5.5 and pH 4.5. Symbols are as in figure 4.

Figure 7-Results of the regression analysis of P. herricki drift following 72 hours exposure vs. aluminum concentrations. The regression equation is: $\arcsin(\text{drift}) \cdot 5 = 0.27^* + 0.55^* ([Al]) - 0.17^* ([Al])(7-\text{pH})$ (F=5.09, df=2,8, p<0.05). Surface contours are shown for pH 6.5, pH 5.5 and pH 4.5. Symbols are as in figure 4.

Figure 8-Results of the regression analysis of N. nigratta mortality vs. aluminum concentrations. The regression equation is: $\arcsin(\text{mortality}) \cdot 5 = 0.07 + 0.21^* ([Al])$ (F=5.97, df=1,6, p=0.05). Symbols are as in figure 4.

Figure 9-Results of the regression analysis of Macronema spp. mortality vs

aluminum concentrations. The regression equation is:
 $\arcsin(\text{mortality})^{.5} = -0.01 + 0.27^* ([Al])$ (F=6.6, df=1,13, p<0.05).
Symbols are as in figure 4.

Figure 10-Results of the regression analysis of Macronema spp. drift following 24 hours exposure in the preliminary treatments vs. aluminum ranges. The regression equation is: $\arcsin(\text{drift})^{.5} = 0.38^* + 0.16^* ([Al \text{ range}])$ (F=17.5, df=1,11, p<0.01). Symbols are as in figure 3.

Table 1-Molt failure rate of P. herricki, Plecoptera, and Ephemeroptera in three types of chemical treatments.

treatment	# of molt attempts	molt failure rate
control	N=40	r=.05
high aluminum	N=19	r=.11
high acidity	N=16	r=.375

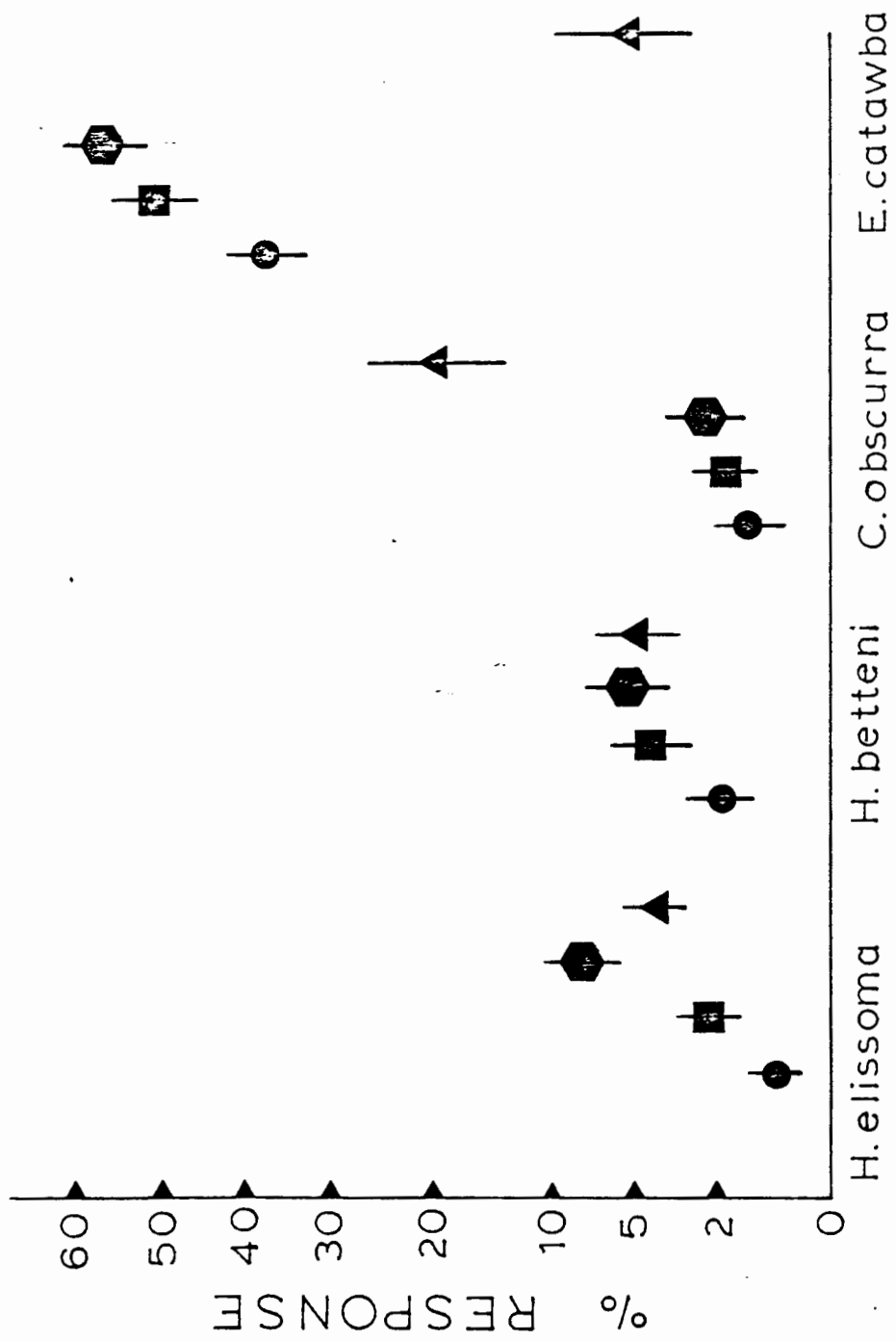


Fig. 1.

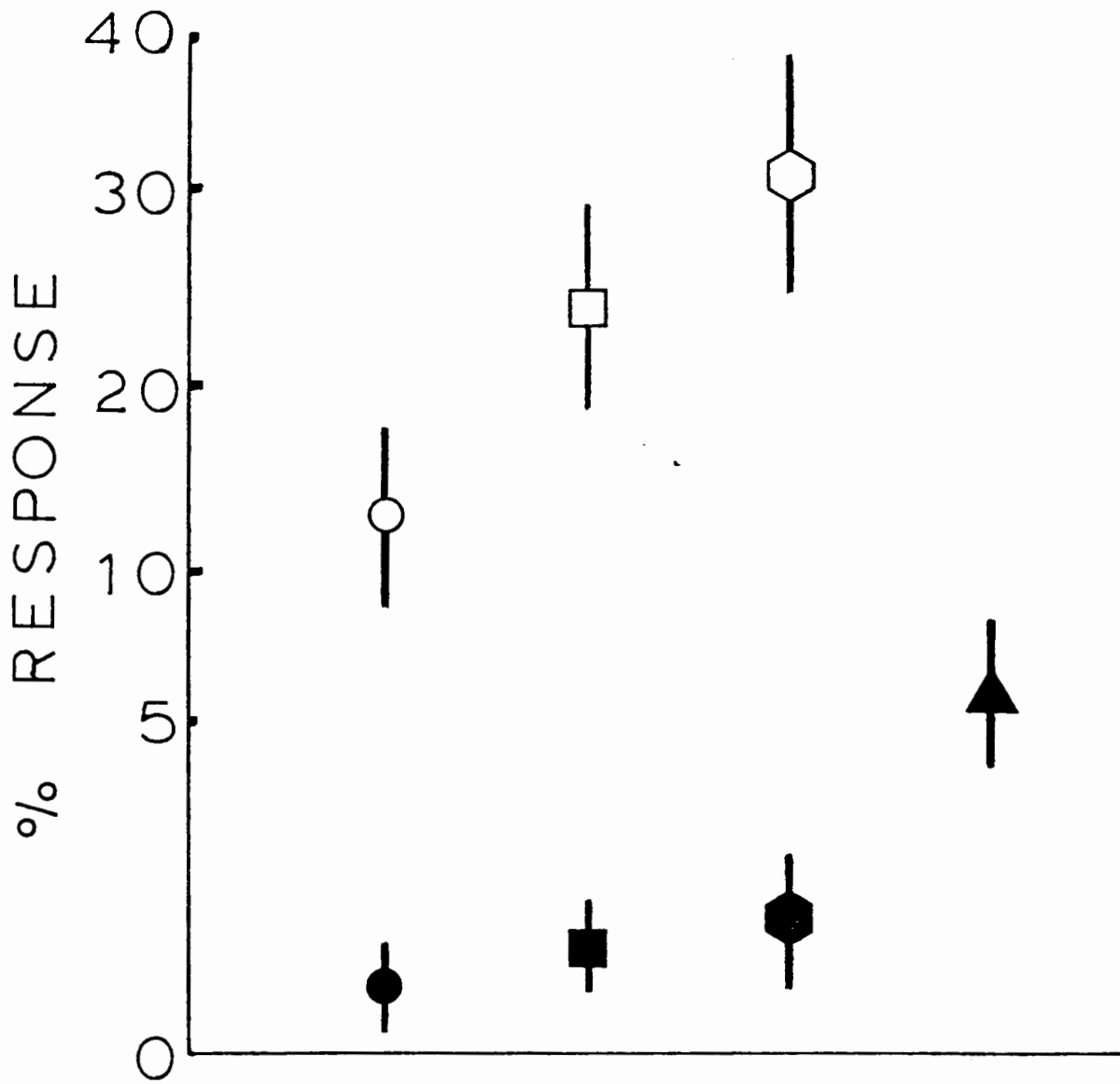


Fig. 2

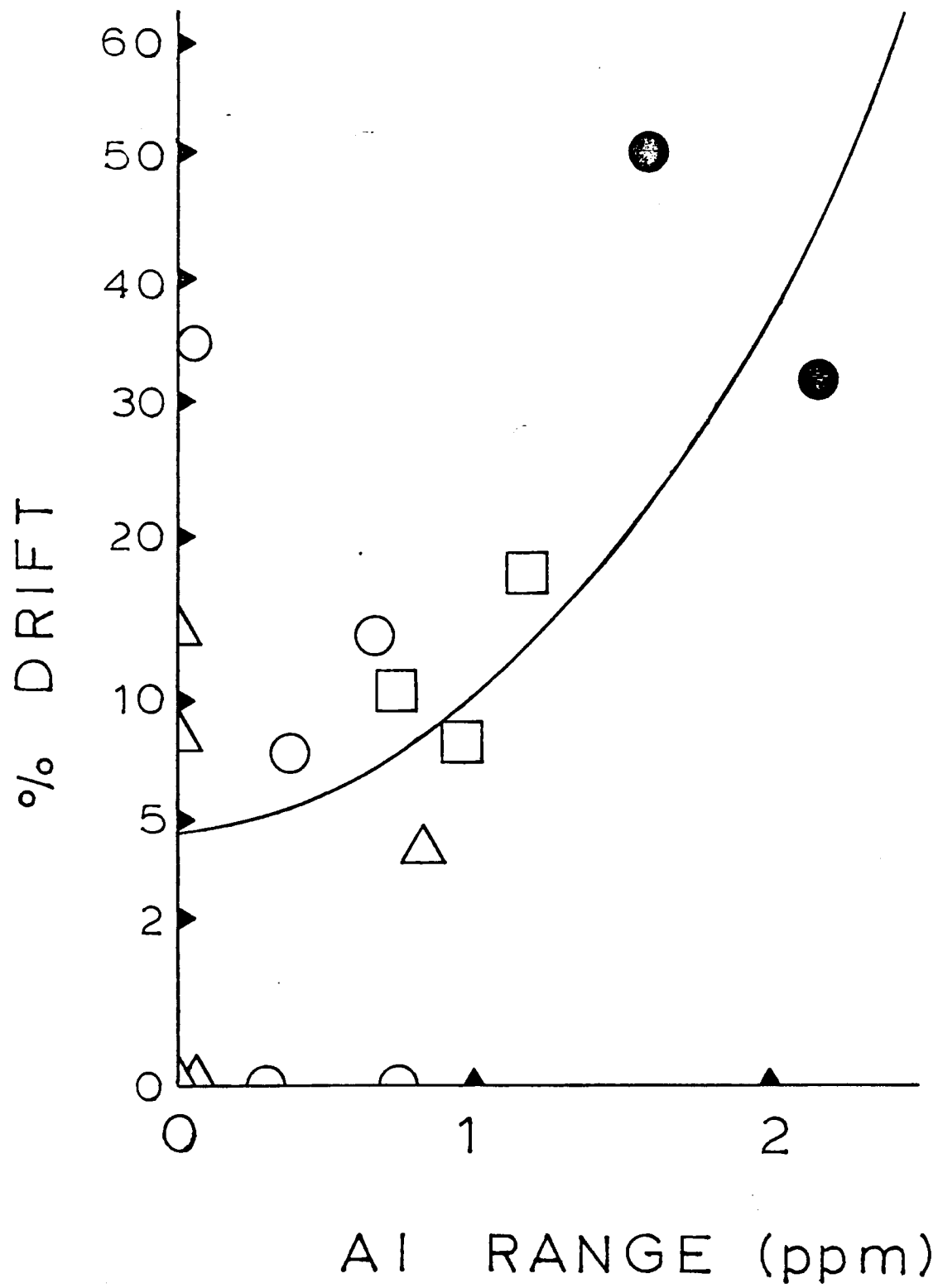


Fig. 3

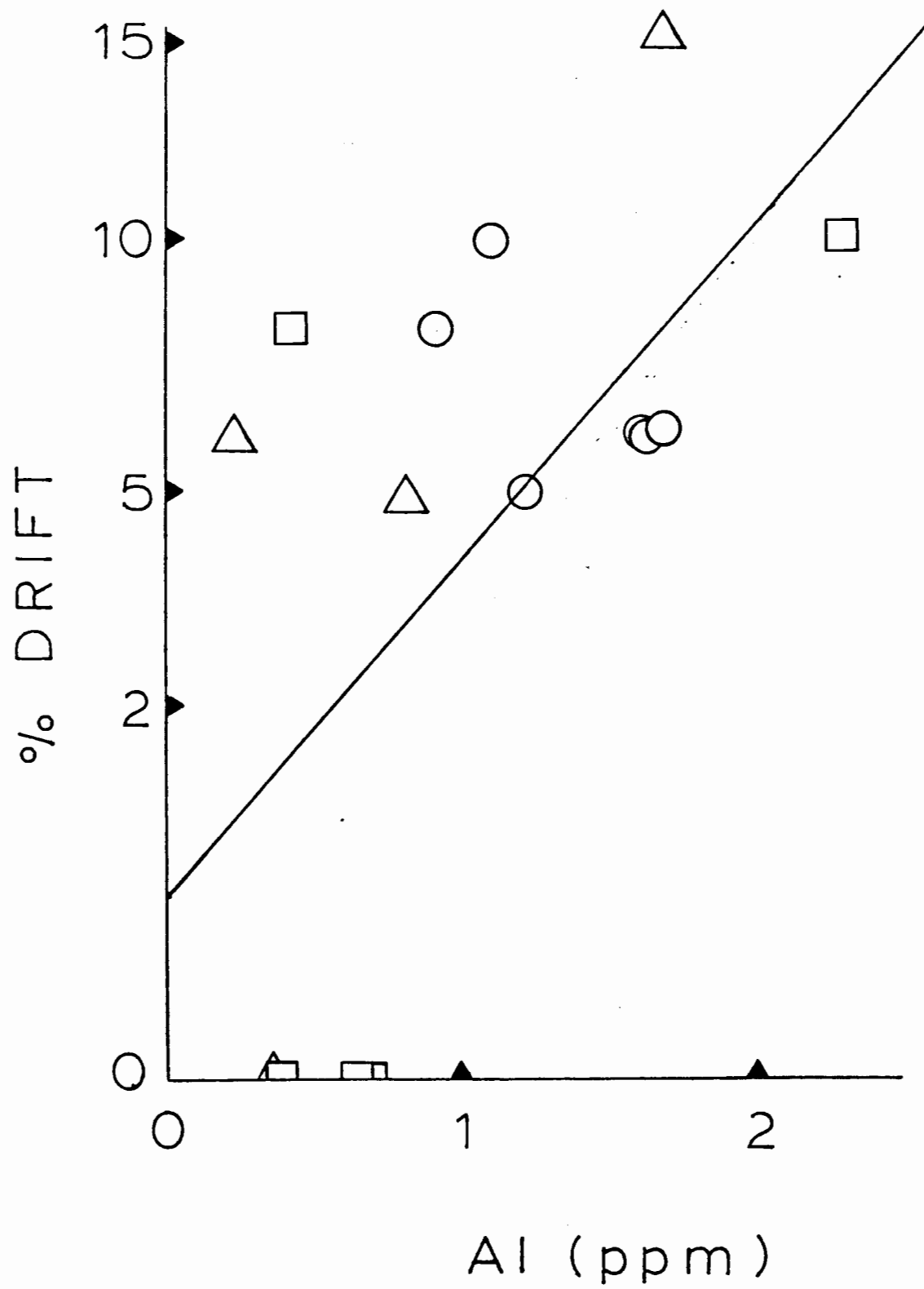


Fig. 4

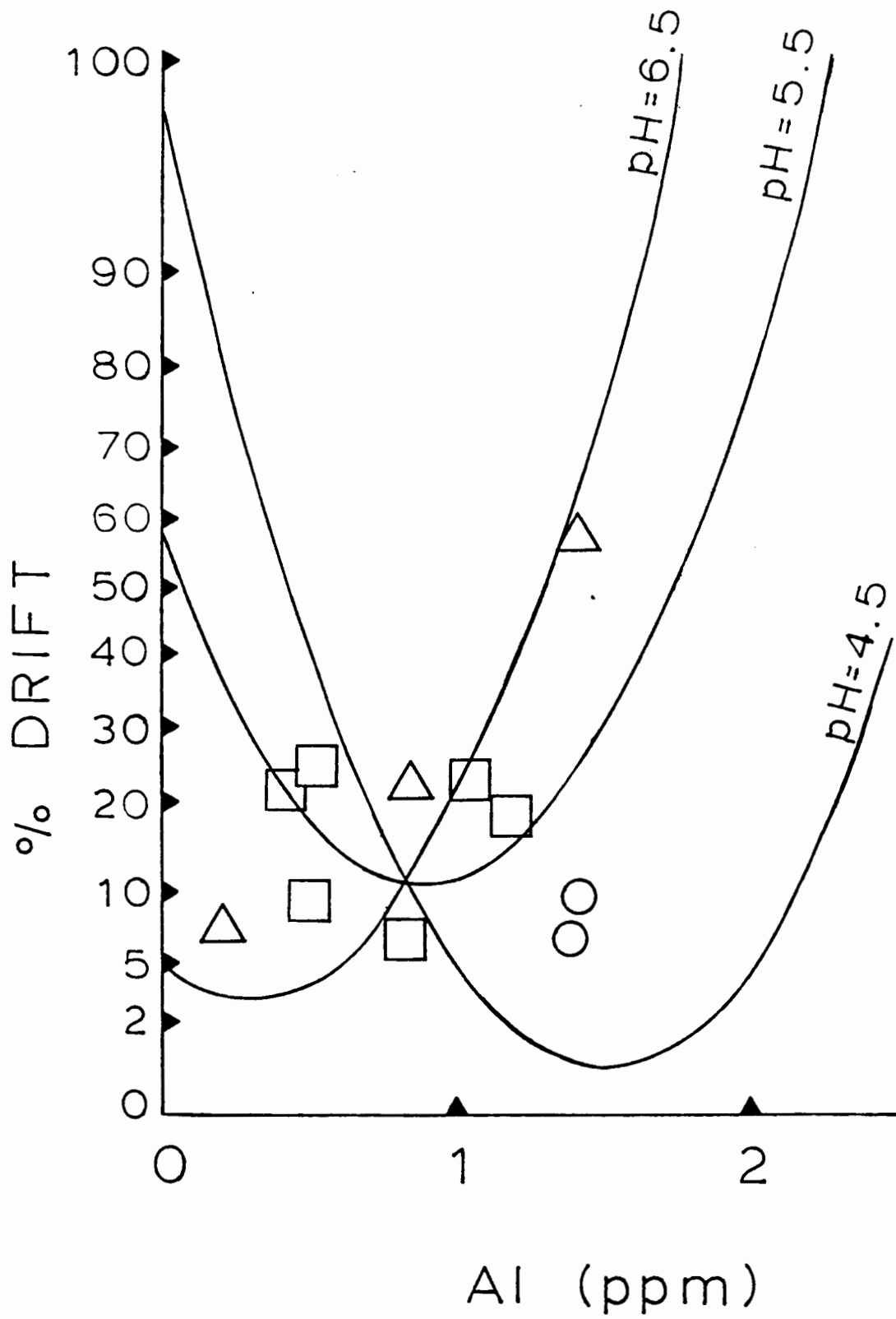


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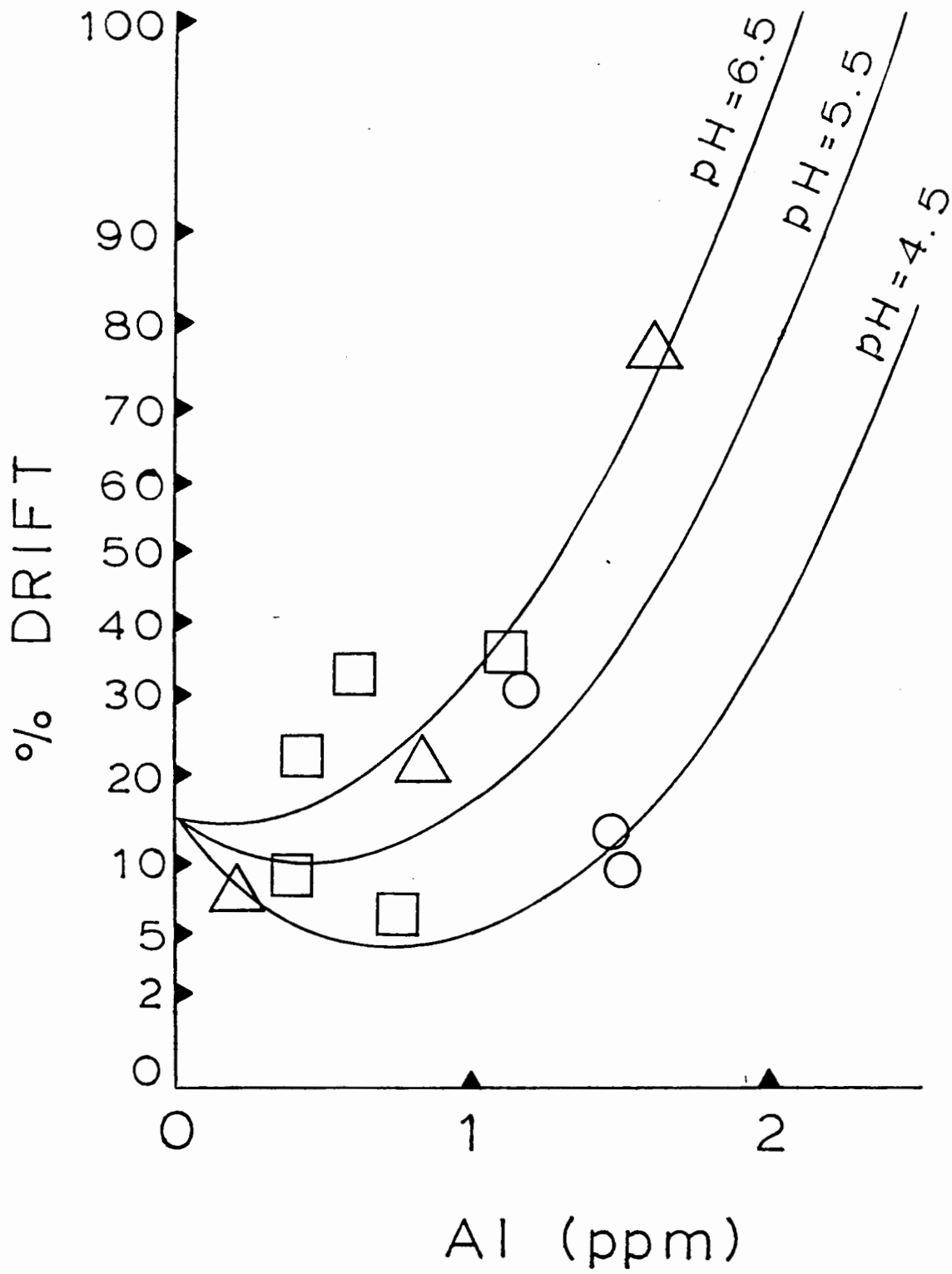


Fig. 6

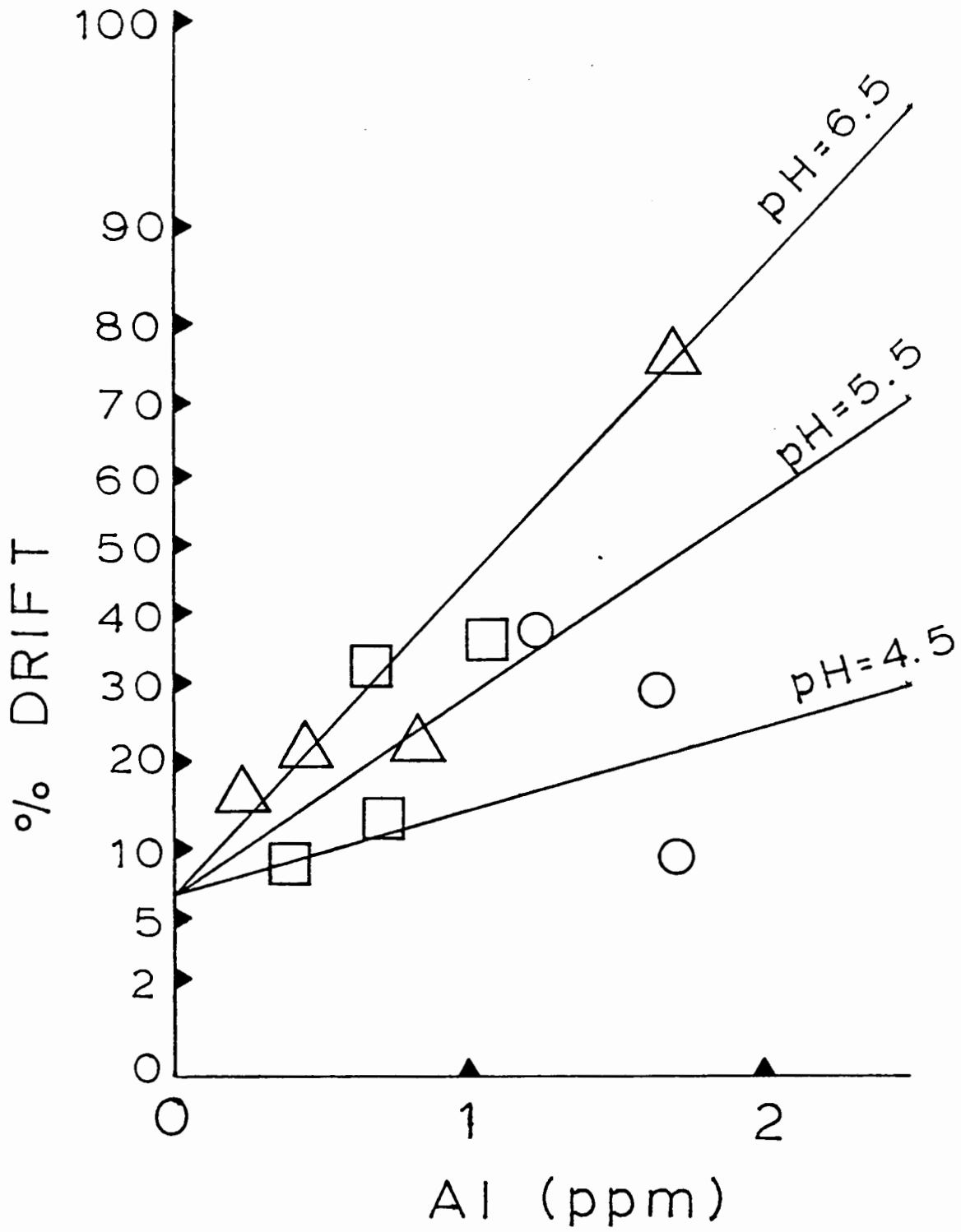


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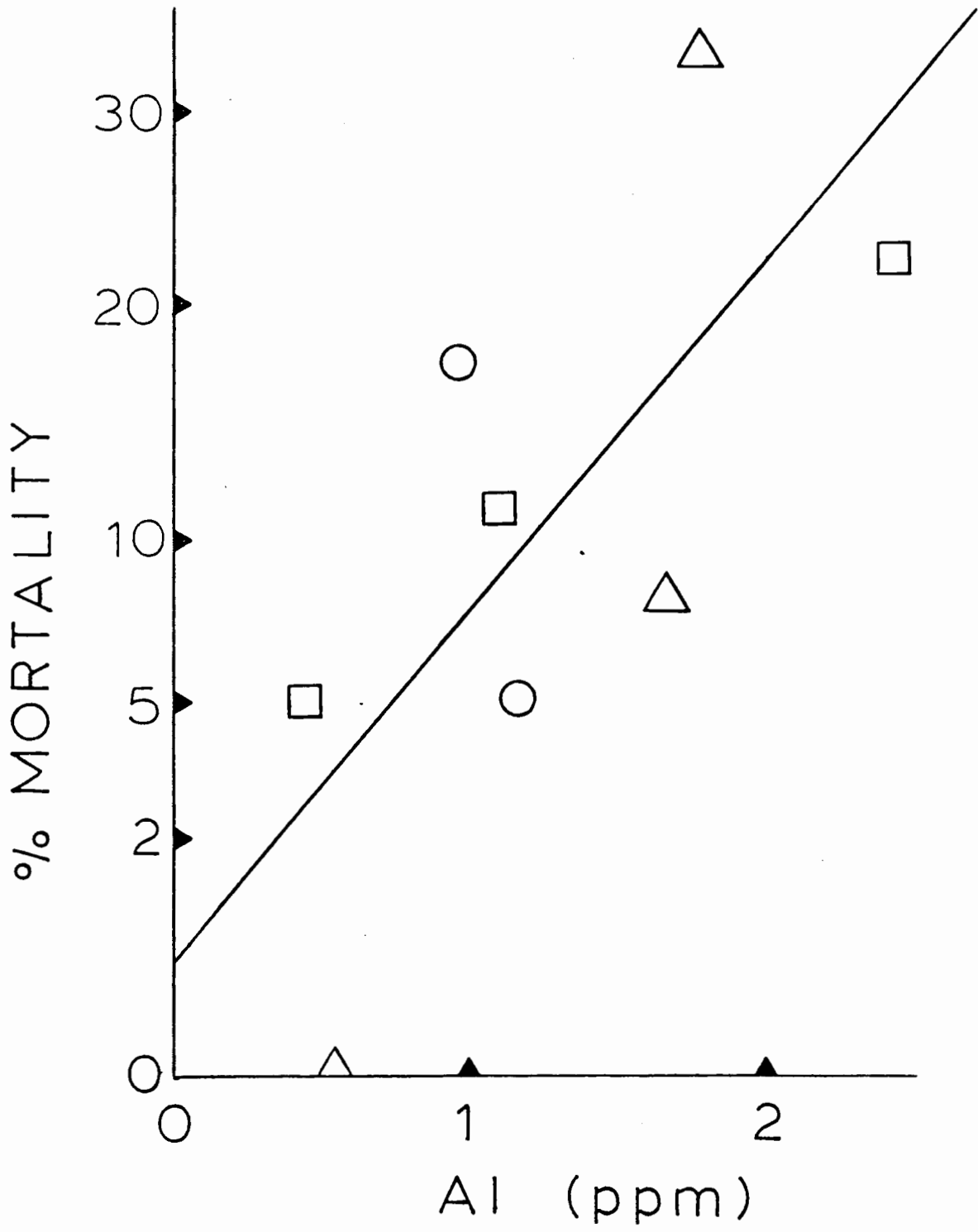


Fig. 8

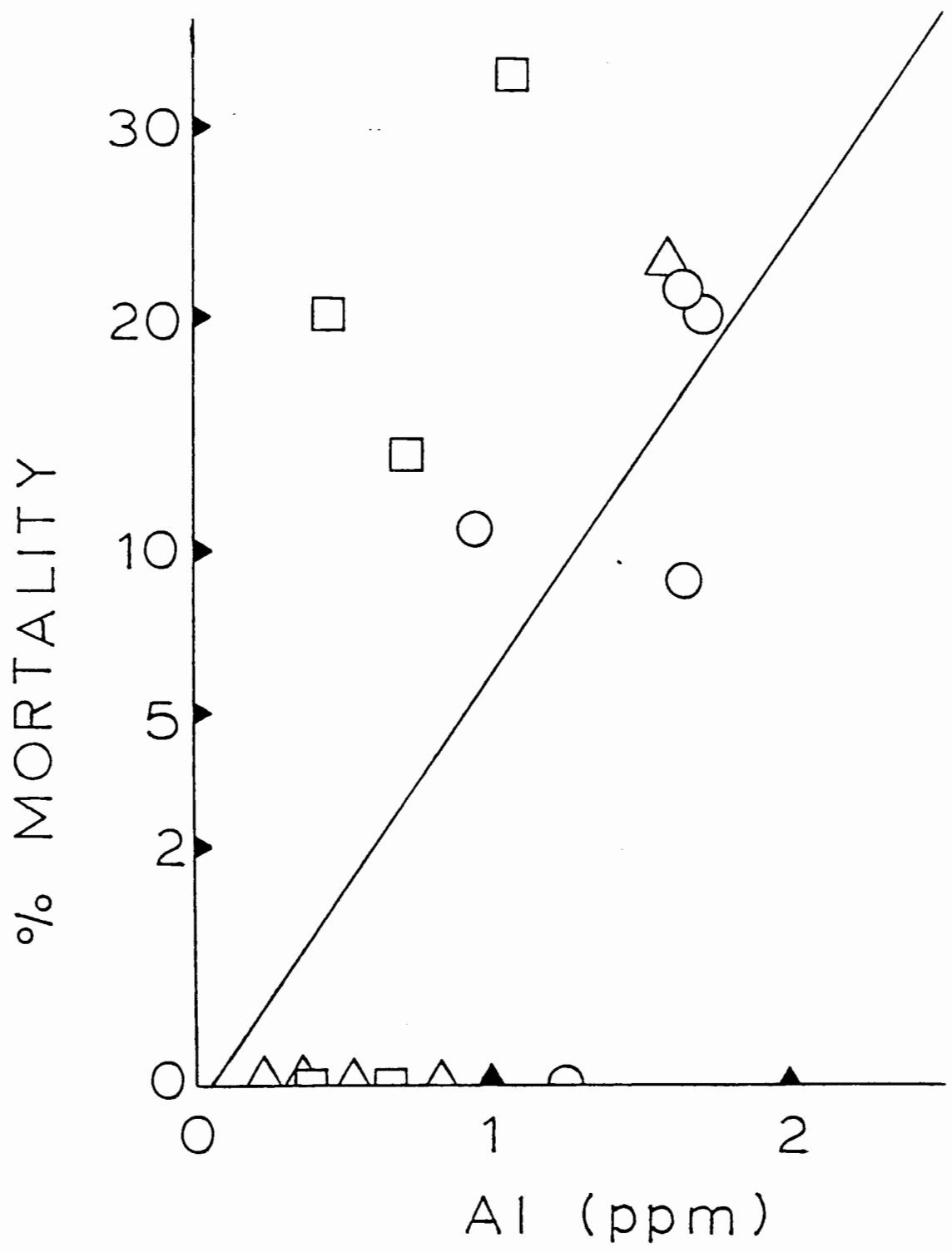


Fig. 9

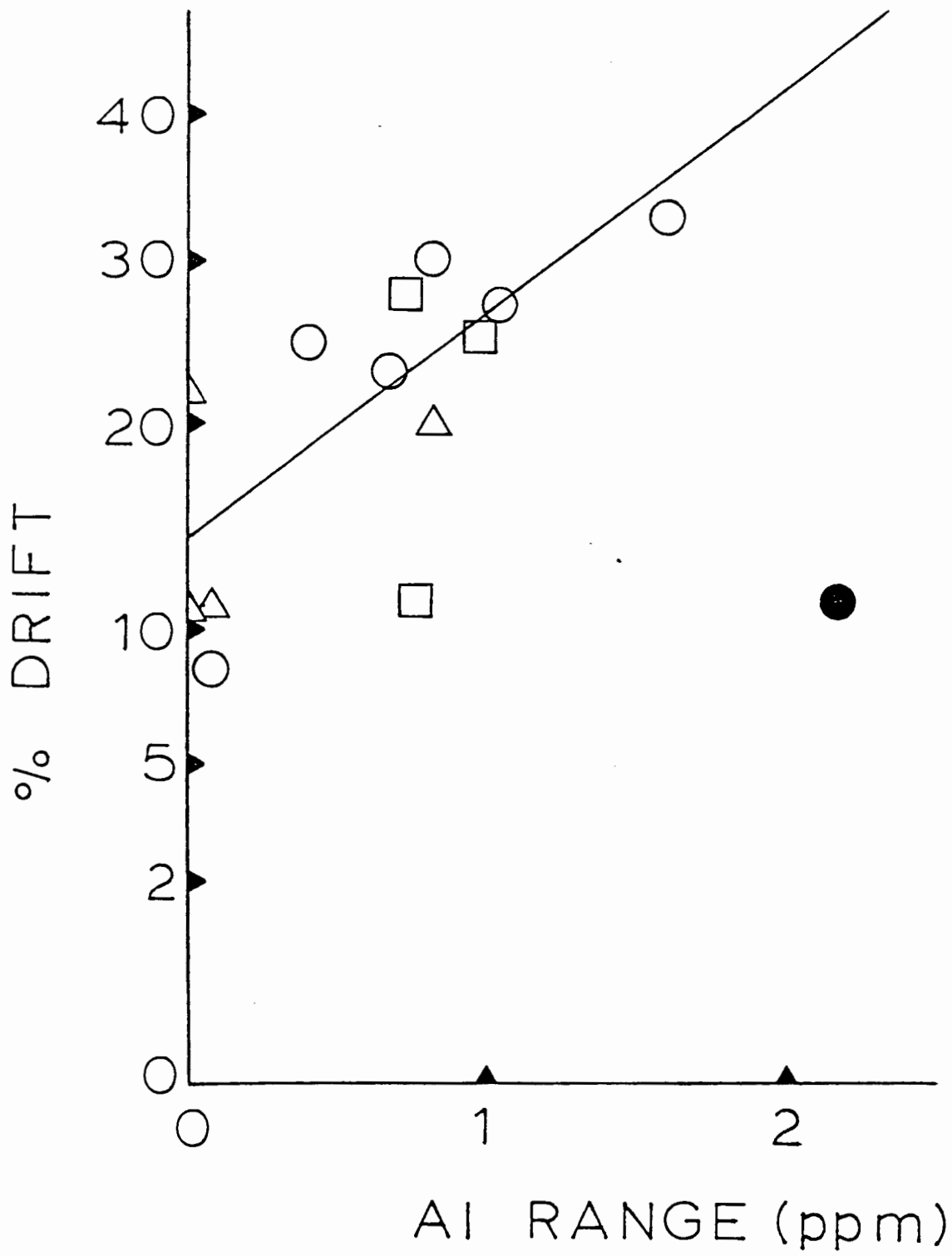


Fig. 10