The Fate of Mercury

in Artificial Stream Systems

Interim Report for

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Henry J. Kania, Robert L. Knight, and Robert J. Beyers

Introduction

The purpose of our research program is to determine the fate of mercury introduced as mercuric ion into artificial stream systems on a continuous basis, and to consider the responses of the biotic communities to this metal. In the past two years, we have worked with water levels of 0.01, 1.0 and 5.0 micrograms Hg per liter. A timetable of major events in the stream research program which includes the dosing levels and periods is given in Table 1. At present we are not inputting mercury but are monitoring the disappearance of this metal from the channels and documenting community changes.

The experimental work is being carried out on the United States Atomic Energy Commission's Savannah River Plant. The stream systems are located several miles from the Savannah River Ecology Laboratory (SREL) of the University of Georgia. Analytical work and sample preparations are performed in a temporary laboratory building located several hundred yards from the stream site.

The artificial stream facility consists of six concrete block channels each three hundred feet long, two feet wide, and one foot deep with concrete block pools at both ends of each stream. The pools and channels are lined with a 20 ml thick black polyvinyl chloride film. Washed builders sand was distributed in the channels to a uniform depth of two inches and water input started in September, 1971. Flows of twenty-five gallons per

minute into each channel, as measured by V-notch weirs, have been maintained since that time except when water pump malfunctions have occured. End plates at the ends of the channels were adjusted to maintain a water depth of eight inches over the sand. Retention times in the streams average two hours.

Biological communities became established in the streams from organisms blown by the wind or carried in by animals. Mosquitofish, Gambusia affinis, were introduced a month before mercury input was begun (Table 1), 400 to each channel.

Water for the channels is pumped from a deep well located near the facility. The water is treated at the stream site by passage through limestone filled tubes in order to increase its hardness and pH and to decrease its free CO_2 content. Because of the large expenses involved, we have not been able to follow through with plans to install a water treatment system which would have also been used to increase the dissolved organic carbon content of the water. We have also been unable to find an economically feasible method of insuring the availability of water at all times. Fortunately, we have had only one extended period without the normal water input during the experimental period. As has been discussed in a previous interim report, the pump failure of July 4, 1972, caused a significant set back in our research program. Any future work with the facility must include

a back-up water supply and also a better means of water treatment.

Water flowing out of the channels is passed through beds of shredded rubber as obtained from a tire recapping firm. We have found this to be a relatively effective method of removing mercury from the effluent water. In June 1972, removal was about 51%. The system was modified and in August, 1972, removal was about 66%. These results are based on two radiomercury uptake studies. Chemical analyses of the water leaving the stream facility after the level increase of August 1, 1973 indicate that about 50% of the mercury is removed by the rubber granules. Approximately 50% of the mercury not removed by the rubber granules is removed in the first 100 yards of the effluent channel (Figure 1).

Our research efforts have been divided into two general areas: (1) to determine the fate of mercury introduced into the channels, and (2) to determine the responses of the stream communities to this metal. Dr. M. C. Ferens was largely responsible for the initial work of determining responses of the stream communities and the research formed the basis for her Ph. D. dissertation, a copy of which is appended to this manuscript. During her work the levels of mercury established were 0.01 and 1.0 ppb. Dr. Ferens decided not to stay with the project after completing her degree, however,

and there was a considerable delay before a qualified replacement was found. This has resulted in discontinuities and delays in several aspects of the program. For example, since sampling by Dr. Ferens was done only during the low level mercury (0.01, 1.0 ppb) inputs, we delayed increasing the levels in the 0.01 ppb channels as long as possible. We had hoped to give her replacement time to become familiar with existing communities before the dose levels in the 0.01 ppb channels were increased. This was not possible because of the time constraints on our project. The increase from 0.01 ppb to 5.0 ppb was necessary in order to establish sufficiently high mercury levels in the experimental channels so that the elimination of mercury could be studied after the inputs were stopped. In many comparisons, channels receiving 0.01 ppb mercury were indistinguishable from controls.

The material that follows is divided into two sections, one of which deals with the mercury levels in the various components of the stream system as a function of time, the other of which deals with the responses of the biological communities to the mercury levels as established in August of 1973. Dr. Ferens' dissertation serves as a summary of work done with respect to community responses prior to the dose increase of August 1, 1973. An interim report issued in January, 1973, for this project summarizes

the data available at that time. Some data contained in that report are not included with this document. The final report that will appear at the end of this project will, of course, synthesize all past work into a single coherent document. All members of the research staff, including Dr. Ferens and her replacement, Mr. Robert L. Knight have been interacting so that species identification difficulties, methodology differences and questions involving statistical handling of the data will be resolved.

Fate of Mercury

This section summarizes data regarding mercury levels measured in various components of the stream systems. In many cases, treatments are lumped so that the variability between channels treated alike, the variability between upstream and downstream locations, the variability between the two walls of the channel, and the effects of periodic minor catastrophes are not evident. The purpose of presenting the material in this manner is to provide a general overview of results. Methods of handling the data in a more sophisticated manner to extract all information are being worked out. As can be seen even in the averages, however, variability tends to be a great problem. We do have, of course, estimates of the variability introduced by our sampling and analytical techniques. We have estimates of the biological variability inherent in some of the materials sampled but these data are by no means complete.

Our measurements of the mercury in various components of the stream systems have been for total mercury. Since organic forms are so important, and all possible pathways seem to exist between mercuric ion, mercury metal, and organic mercury, we have had samples analyzed for methyl mercury by personnel of the EPA's Southeast Environmental Research Laboratory at Athens, Georgia, whenever possible. We have indications that metallic mercury may be important in the transport of mercury between sediments, water and

and air. Methyl mercury results and information implicating metallic mercury as an important transport mechanism are mentioned in the sections that follow.

General Methods and Results

All analyses have been carried out on a Coleman MAS-50 Mercury Analyzer System modified by the addition of a digital read-out device and fused quartz windows on the absorption cell. We have used several different digestion techniques in the past but presently all samples are digested in a mixture of concentrated sulphuric and nitric acids (5:1) and oxidized with a 6% (w/v) potassium permanganate solution. When large particles are involved as with fish, invertebrates and portions of rooted aquatic plants, the samples are allowed to stand in the acids overnight before the permanganate is added. The permanganate mixture is allowed to stand an additional 24 hours before being analyzed. Additional permanganate is added, if required, as indicated by color loss. Samples of fine particulate material, such as periphyton scraped from the various substrates used in the channels and removed from the sediments, are handled in a slightly different manner in that the permanganate is added at the same time as the acids. These procedures have been checked against more complex methods involving refluxing, heat treatments, and additional reagents. We have found

results to be in close agreement. We do not suggest, however, that our methods would be appropriate in more complex systems which might be subjected to mercury releases other than mercuric ion.

Water

The amount of water entering each channel is controlled by a manual valve located just in front of the limestone filled treatment tubes. Flows are checked daily against gradations on a V-notch weir, separating the pool at the head of each channel from the channel. Some variation in flow rate, correlated with pump operation, has been noted.

Mercury was pumped into the channels with a four channel peristaltic tubing pump calibrated daily. Major deviations from the desired input occurred only when the pump failed or pumping tubes were replaced, a monthly procedure.

Prior to August 1, 1973, only occasional mercury analyses were made of water and then only of samples from the 1.0 ppb channels. These analyses showed our water concentrations were close to what was desired, at least in these channels. After August 1, 1973, samples were taken routinely from all channels, including controls.

Water samples were taken in the following manner from two locationsin each channel and also from two areas

of the effluent leaving the stream facility. An acid rinsed 250 ml erlenmyer flask was filled by submersion, the contents checked for any visible particulates and a new sample taken if particles were seen. The clear sample was poured into an acid rinsed polyethylene bottle containing 2.5 ml of concentrated nitric acid. Sampling stations in the channels were located 20 feet from the mercury input tube and two feet from the downstream end. Samples of the effluent water leaving the rubber mercury removal beds were taken near the ends of the channels and 300 feet downstream.

Analyses of 100 ml aliquots of both filtered (0.45 μ Gelman Type A glass fiber filters) and unfiltered water were made. The 100 ml samples were treated with 5 ml concentrated sulphuric acid and 1 ml 6% potassium permanganate and allowed to stand at least 30 minutes before being analyzed. Results using this technique were compared against those obtained from a procedure which included potassium persulphate and a heat treatment. These extra treatments were found to be unnecessary for our samples. The filters used contained less than 0.002 μgs of mercury. Filtration of standards of $HgCl_2$ treated in the same manner as the water samples showed that the filters removed less than 1% of the dissolved mercury from a 1.0 ppb solution.

A summary of mercury analyses of unfiltered water

collected between August 1, 1973 and January 29, 1974 is presented in Figure 1. The data show that the input levels were slightly higher than desired. Figure 1 also shows that the mercury concentrations in the water at the ends of the channels were lower than those at the head. Uptakes based on the figures for unfiltered water were 12.8% and 22.3% in the 1.0 ppb channels and 16.1% and 17.5% in the 5 ppb channels.

Although the distance between the sampling stations in the effluent stream was about the same as between the two channel stations, there appears to have been a greater uptake of mercury (54.6%) from the effluent water even though the residence time of the water in this section was short. The greater uptake might be explained by the high turbulence in this region resulting in greater probability of contact between a mercury ion and some portion of the stream wall. The communities in this region are, of course, quite different from those in the experimental channels.

Starting at noon on January 22, 1974 and continuing for a 24 hour period, water samples were taken every two hours at both stations in those channels receiving a mercury input. Since it requires two hours for a given water mass to traverse the distance between sampling stations, the difference in mercury concentrations between a sample taken near the head of the channel at

one time and at the end of the channel two hours later should give an estimate of the mercury uptake in the channel during that period. These differences as a function of time are shown in Figure 2 for the 1.0 ppb channels and Figure 3 for the 5.0 ppb channels. Since all differences are positive, there is no question but that there is an uptake. However, it is difficult to see any consistent pattern in uptake that could be correlated with biological activity. Based on the results of this intensive sampling, 12.4% and 13.9% of the mercury input was removed in the 1.0 ppb channel; 15.1% and 16.1% of the mercury input was removed in the 5.0 ppb channels. These figures agree with the uptakes mentioned above calculated from several months data. There appears to be a constant proportion of mercury removed, regardless of the input level. This indicates that potential binding sites for mercury are not saturated at the 5.0 ppb levels.

The percent of mercury removed by filtration was calculated for the water samples obtained during the January 22-23, 1974 sampling period. Results are presented in Figure 4. In all cases, the proportion of the total mercury removed by filtration was greater at the downstream ends of the channels than the upstream ends. Based on the results of bacterial counts made June to October, 1972, the results of which are summarized in

Figure 5, it is possible that the mercury removed by filtration is attached to these microorganisms.

At 9 AM, January 29, 1974, the mercury inputs to the channels were turned off. Water samples were taken just prior to this time and every hour for the six hour period following. The results for filtered samples along with results for filtered samples taken three other days during the month of February are shown in Figures 6 and 7. By the end of February, levels were at or below our detection limit of 0.05 ppb.

Air

In the initial stages of this project, we made no plans to monitor mercury losses from the channels at the air-water interface. Because of the acidity of the water (pH 5-6) we believed that formation of volatile forms would be minimal. However, mercury levels in some components of the control streams were high enough to indicate a transfer from the treated channels. The possibility of leaks was remote since each channel is covered with a single continuous sheet of PVC film, and there is no pressure differential across the wall separating adjacent channels.

Air sampling systems as shown in Figure 8 were constructed and the glass boxes suspended by stainless steel straps in all channels. Air was drawn from under

the boxes at the rate of one cubic foot per minute, dried with magnesium perchlorate and then passed through a gas scrubbing bottle containing 100 mls of a 10% (v/v) sulphuric acid, 1% (w/v) potassium permanganate solution. On several occasions, two scrubbing bottles were connected in series. Results indicate a 98% mercury removal in the first bottle. The air was sampled from under the glass collecting boxes for a period of two or three hours. Sampling was done in the early afternoon and only on clear days. After the sampling period, the acid permanganate solutions were transferred to BOD bottles, cleared with hydroxylamine hydrochloride crystals and analyzed, in the usual manner. Analytical results are summarized in Figure 9. Results from the control channels are not shown because they were so low. Figure 9 shows that the releases were erratic, especially while mercury was being input to the channels, and that the air releases continued after the mercury inputs were stopped. There appears to be a positive correlation between total mercury in the sediments and air releases but the analysis of existing data is not complete.

In an attempt to define the form of the mercury released at the air-water interface, a trap consisting of silver plated copper turnings was placed between the drying tube and the gas scrubber containing acid permanganate. Comparing the results from two sampling boxes located

next to each other in the same channel shows the filter effectively removes 97% of the mercury. This type of filter is almost 100% effective in removing metallic mercury from an air stream. We do not as yet know its effectiveness in removing other volatile mercury forms.

During the air sampling we noticed that the amount of mercury collected in the acid-permanganate solutions seemed to be related to the quantity of bubbles rising from the bottom. Samples of this gas were collected and quickly injected into the closed air system of our analyzer. This resulted in a significant absorption of the UV beam and, although we have made no attempt to quantify the results, it suggests the presence of metallic mercury in the bubbles. Samples of gas from the control channels were checked with negative results. It appears that metallic mercury in vapor form may be released from the stream sediments with bubbles rising to the surface. Further work is needed in this area to better define the form of mercury released, to relate the magnitude of the release to other stream properties such as gas production, photosynthesis, respiration and sediment concentrations.

In the intensive 24 hour water sampling mentioned in the previous section, air samples were also taken every two hours. Results are shown in Figures 10 and 11. The results suggest a diurnal release pattern but more data are needed as is a more sophisticated handling of the data.

Fish

In the discussion that follows, all analytical results are presented as means ± 2 standard errors. Except where noted, these results are on a wet weight basis. Because of the small size of mosquitofish (Gambusia affinis), the entire fish was digested for analysis. Analytical results, therefore, are not directly comparable to literature values which are usually based on muscle analyses. In a sample of six dissected mosquitofish, 51 ± 4% of the total mercury in the fish was found to be in the body muscles.

The mosquitofish used in this project were removed from a small pond formed in an abandoned asphalt parking lot. The mercury concentration in these fish was 0.037 ± .013 ppm when they were introduced into the channels and the stainless steel cages located at both ends of each channel. During the study, a number of fish escaped from both the channels and cages when the screens at the ends of the channels plugged and water levels became excessive. The reduced numbers of fish made sampling difficult and also greatly limited the number of samples that could be taken.

Results of analyses of fish collected between May, 1972, and May, 1973, from the 1.0 ppb channels are shown in Figure 12.

In April and May of 1973, all remaining mosquitofish

were removed from the channels and cages and then frozen. Most of these samples have been analyzed. Preliminary calculations with existing data show that the fish in the control channels contained an average of .074 ± .021 ppm mercury after one year, fish in the 0.01 ppb channels, an average of 0.159 ± .021 ppm after one year, and fish in the 1.0 ppb channels, an average of 5.1 ± 1.1 ppm after one year. No difference was found between the caged fish fed with a commercial fish food having a low mercury concentration (0.20 ± .02 ppm Hg on a dry weight basis) and those free living in the channels. This indicates the importance of a direct uptake of mercury from the water by mosquitofish.

Based on two sets of analyses of fish removed from the channels after a one year exposure to water levels of 0.01 and 1.0 ppb mercury, the portion of mercury present as methyl mercury was 28%. The methyl mercury analyses were made by personnel of the Environmental Protection Agency's Southeast Research Laboratory at Athens, Georgia. Because of the importance of organic mercury compounds, especially methyl mercury, with respect to human health, this project would be greatly improved by the inclusion of investigations into the forms of mercury that exist in the various community components.

In May and June of 1973, new mosquitofish were

released into the channels, 400 into each and 40 into each cage. Results of analyses of fish removed periodically are presented in Figure 13 for the 1.0 ppb channels and in Figure 14 for the 0.01 ppb channels. The 0.01 ppb channels were increased in August 1973, to 5.0 ppb. Levels reached in the fish exposed to 1.0 ppb were about the same after seven months as those measured the previous year. Because this second batch of fish was introduced into an already contaminated area, the levels rose more quickly the second year. Figure 14 shows that fish in the channels increased to 5.0 ppb rapidly accumulated mercury after the increase and reached an equilibrium concentration after about four months. The equilibrium level of about 12 ppm wet weight is double that reached by fish in the 1.0 ppb channels.

A single set of methyl mercury analyses was made on fish removed from the channels in mid-August, 1973. Although total mercury levels ranged from 0.04 ppm in the controls to 5.6 ppm in the treated channels, the portion of mercury present as methyl mercury was on the order of 10%. The data are extremely limited, of course, but suggest that the proportion of total mercury present as methyl mercury, may be a function of exposure time.

Wall Communities

Shortly after the water input to the channels was started in September, 1971, 40 plastic strips (10 cm & 20 cm) of the same polyvinyl chloride (PVC) material lining the channels were suspended at upstream and downstream locations in each channel. These strips were removed periodically, scraped and the removed material analyzed for mercury. Biomass determinations were made based on dried and ashed samples of a blended suspension of this material. Sampling of these plastic strips was discontinued in October of 1973, mainly because the large growth of a rooted aquatic plant (Juncus diffussisimus) in the channels interferred with the hanging strips to the extent that they could no longer be considered representative of the channel walls. A method was then devised so that a known area of channel wall could be scraped and all material collected without any holes being cut into the plastic lining.

Results of the biomass determinations made from the PVC strips are shown in Figure 15 and the mercury analyses in Figures 16, 17 and 18. The variability in all these data is primarily due to the very small amount of material that was available for analysis and for biomass determinations. It was not uncommon for the ash free dry weight of the material removed to be less than 20 mgs.

Sediments

Samples of the channel bottom have been obtained in two ways during this project. A specially constructed device which removes frozen cores 14 mm in diameter has been used, and samples have also been removed from the pyrex pans utilized by Dr. Ferens for sampling the benthic invertebrates. The cores provided a means of determining the vertical profile of mercury in the sediments. The results of core analyses show that the mercury in the stream bottom is associated exclusively with the organic portion of the sediment which overlies the sand. The minute amounts of mercury occasionally detected in the lower parts of the core are probably a result of organic matter being pushed down into the sand during sampling.

The levels of mercury measured in the organic portion of the sediment are shown in Figure 19. The sediments in one 0.01 ppb channel were higher in mercury concentration than the controls in all cases. The increase in mercury in the 0.01 ppb channels in August, 1973, was followed by an immediate increase in the sediment levels of mercury (note scale change in Figure 19). These levels appeared to be still increasing when mercury inputs were stopped in January of 1974. There is no way of estimating the equilibrium levels that would have been reached. After mercury shut down, sediment levels in all channels appear to have declined although the data

available at this time are inadequate for estimates of rates of removal of mercury from the sediments.

Large core (23 cm^2) samples were removed from six positions in each channel four days after mercury input was discontinued. The rooted plants were removed, washed, dryed and ashed. The organic portion of the sediments was separated from the sand, homogenized in a measured volume of water, and subsamples taken for biomass and mercury determinations. A summary of results is presented in Table 2. These data show that the biomass of the benthic communities, excluding rooted plants were quite similar, the rooted plants were more common in the control channels than in the treated, and the mercury levels in channel 3 were quite different from channel 6 although these channels were subjected to the same treatment.

Export

We have found no satisfactory method for quantifying the particulate matter leaving the channels and, therefore, the total mercury leaving the systems attached to this material. In any attempt to balance the mercury input with mercury output and storage, therefore, this component of output must be estimated by subtraction. Sampling difficulties have arisen because of the properties of the exported material, variation in these

properties with season (and experimental treatment?), and the fact that transient phenomena, such as heavy rainstorms and high winds, seem to be responsible for breaking loose large portions of the stream communities.

Estimates of the mercury content of the material leaving the channels, based on samples removed from stainless steel screens at the ends, are shown in Figures 20 and 21. Figure 21 shows a rapid increase to what appears to be an equilibrium level in the mercury content of the matter exported from the channels increased to 5.0 ppb mercury in August, 1973.

Rooted Aquatic Plants

In the first summer of channel operation, the bottom communities were relatively simple with no rooted vegetation. By the spring of 1973, however, a great number of small rooted plants were noted, especially in the upstream portions of the outer channels. Although there appeared to be two distinct species, these were later recognized as forms of the same species, Juncus diffusissimus Buckley. The distribution of this plant in April of 1973 in the channels is shown in Table 3. Quite obviously channel one received a much greater input of seeds than the other channels. The upstream-downstream gradient in the distribution pattern may be due to the input of seeds to the pools above the channels. Starting

in June, 1973, samples of Juncus were periodically analyzed for mercury content. Plants were removed from the channels, the roots washed free of sand and separated from the leaves. Both roots and leaves, were weighed and placed in 100 ml volumetric flasks for digestion. Portions of both were also weighed, oven dried at 60°C for 24 hours., re-weighed, ashed at 400°C for 24 hours and again weighed. Ash-free dry weight estimates were made of the portions digested based on the records for the ashed samples. All mercury concentrations were calculated on an ash-free dry weight basis. Analytical results for the roots are given in Figure 22, for the leaves, in Figure 23. Results for the control channels were very low and are not presented. Comparing the figures shows that the roots were much lower than the leaves in all cases, and that the levels were probably still increasing when the mercury inputs were stopped on January 29, 1974.

Invertebrates

Large invertebrates have not been common in the channels so routine samplings for mercury analyses have not been made. The material collected in the screens at the ends of the channels has been saved and examined for invertebrates, however, when time was available. Animals sorted out are identified, blotted dry, weighed, digested and analyzed for total mercury content. The most common

organisms found to date have been nymphs of the dragon fly, Pantala hymenea, two damsel fly nymphs, Argia sp. and Ishnura sp., and various midge larvae. Midge larvae have been recovered in sufficient numbers on several occassions so as to permit mercury determinations on groups of individuals. Based on the results of dissections of mosquitofish removed from the channels, midge larvae form the major food item of these fish.

Levels in the midge larvae have ranged from below detection limits in animals from the control channels to an average of 8.6 ppm wet weight in animals from the 1.0 ppb channels to 20 ppm wet weight in animals from the 5.0 ppb channels. The data are limited and variable but indicate that the midge larvae have a higher level of mercury, on a wet weight basis, than do mosquitofish from the same channel. Analyses of these larvae, of course, include all gut contents. Although it is not clear what "conversion factor" would be appropriate for converting periphyton mercury concentrations from an ash-free dry weight basis to a wet weight basis, it appears that in the food chain leading from the primary producers to mosquitofish, mercury concentration does not occur.

Future Work

Routine sampling of selected stream components will be continued as long as differences between mercury levels in the channels are measureable. Appropriate statistical analyses will be made on existing data so as to extract the maximum amount of useful information. In some cases, it has not been possible to get sample sizes large enough to satisfy the requirements of the usual parametric statistical tests so that the less favored non-parametric analyses will be necessary. Laboratory studies suggested by the results from stream analyses, such as determination of the forms of mercury released from contaminated sediments, will be initiated. The formulation of a mass balance model describing the movement of mercury within the channel systems will be attempted.

Community Responses

The study of the algal and benthic insect communities of the artificial stream systems between January, 1972 and April, 1973 was largely the effort of Dr. M. C. Ferens and served as her doctoral research. Her methods for sampling plexiglass plates and benthic dishes are summarized in her dissertation which is appended to this report. All of her algal data are presented as analyses of responses of individual species and no total numbers are given. Shannon-Weaver diversities were calculated for both the algae and insects that she counted.

Between April and October of 1973 there was no quantification of the periphyton and benthic communities. Study of these communities was recommenced in October of 1973, about sixty days after the mercury concentrations were increased to 5.0 ppb in the former low-level streams (0.01 ppb). At that time a study of two substrates in addition to the plexiglass plates was then started in order to analyze time responses of periphyton exposed to mercury. Glass microscope slides were suspended in the channels to study the initial colonization of the periphyton, and small areas of the channel walls were scraped and the periphyton quantified to study a substrate undisturbed since stream construction. Also, taxonomic treatment of the algal species present was continued. Dr. Ferens listed the species she observed in Table 1 of her dissertation (see appendix). A list of the most common

species observed since October, 1973 is given in Table 4 of this report. A complete listing of algal species including the rare forms will be included in the final report.

Methods

Growth on Artificial Substrates Since October, 1973

Three artificial substrates were examined for composition of periphyton communities. Glass microscope slides were suspended in the streams at four different times and changes in the thickness of the algal growth (in two cases) and the species composition (in three cases) were studied. Vertical plexiglass plates placed in the channels by Dr. Ferens in spring of 1973 were examined at four different times for the density and composition of the algal community. Also, at four times, the algae attached to the stream walls were quantified. The last set of data from these artificial substrates was collected after the input of $HgCl_2$ had been ceased.

Five washed microscope slides were suspended in the upper part and five in the lower part of each stream on September 29. Starting on October 9, one of these slides was removed each three or four days for determination of the thickness of the attached communities. This determination was made by use of a water immersion 40x lens and a calibrated fine focus knob. The lens was focused

up until the longest filament or accumulation of cells in the field was in focus and the reading on the knob noted. The lens was then focused down until the cells attached to the slide were in focus. The difference between the numbers on the fine focus knob could be directly read in microns. Ten fields were measured on each microscope slide.

A second set of washed slides was placed in the streams on October 31 with 15 slides at each location (30 per stream). At three to seven day intervals one slide was removed at each location and the thickness of the algal community was determined as above. Then a cover slip was placed on the lower end of the same slide and a count was made of the algae, beginning at a constant distance from the lower end of the slide. Enough areaswas covered to give a count of at least 200 cells although many counts were taken beyond 500 cells. All cells in colonial or filamentous forms were counted individually. The area counted was measured by a stage micrometer and a calibrated ocular micrometer. Numbers were converted to cells per square millimeter. Two more sets of eight glass slides each were placed in the streams on December 7, 1973, and on January 30, 1974. The second set was suspended in the streams at a time when water levels of mercury were less than 0.2 ppb in all of the streams (the mercury input was shut off on January 29, 1974). On these two sets of slides the thickness of the communities was not determined because the more accurate method of directly counting the cells was used.

Plexiglass plates were removed from upstream and downstream locations on four occasions (December 12, January 3, January 31, and February 19). These plates were carefully scraped clean with a rubber scraper and the material removed was diluted into a known volume of water. This water was blended to achieve a homogeneous mixture and subsampled for counting. Slides for counting were made by placing a cover slip over a drop of known volume and the area of the count recorded. The number of cells counted in the given area could be readily extrapolated to an area of the original plexiglass plate.

On four occasions (December 14, January 8, February 5, and March 4) samples were removed from the PVC lining of each stream at upper and lower locations. A known area (59 cm^2) was scraped into a measured volume of water in a three-sided plexiglass box. These were blended and subsampled for counting and the number of cells counted was converted as above to cells per square millimeter.

Benthos

Benthic insects were collected on November 29, 1973, and January 11, 1974, using the some method of removing pyrex dishes as was described by Dr. Ferens (see appendix). The insects were preserved in 80% ethanol until they could be sorted and counted.

Diversity

The Shannnon-Weaver diversity index was calculated using the following formulae given by Peilou (1966):

$$H' = -(n_i/N) \log_2 (n_i/N)$$

$$J = H'/\log_2 S$$

where S is the number of species, N is the total number of cells counted, n_i is the number of cells in the ith species, H' is the Shannon-Weaver diversity, and J is the evenness.

Results

Glass Slides

The data for the accumulation of periphyton on the microscope slides is summarized in Figures 24 and 25. In the first run (Figure 24) the thickness of the algal communities on the glass slides in the control streams and in the low level mercury streams (1.0 ppb) was similar throughout the period of study and still increasing on the 24th day of colonization. This increase was due to the two filamentous algae Oedogonium reinschii and Gloeotila turfosa. In the high-level streams the thickness leveled off after about nine days and did not increase up to the 24th day.

In the second run where the thickness of the algal communities on the slides was measured, (Figure 25), the growth in the control streams was greater than in the low-level streams until November 25 when both showed a

decline in thickness. The high level streams had much less growth throughout the study. In this second run the accumulation of cells seems to be much less than in the first run. This is largely due to the fact that the filaments of Gleotila turfosa were ignored because they were observed to be unattached and would drift about under the water-immersion lens giving highly variable results. The greatest thicknesses seen in the second run are due largely to long filaments of Oedogonium reinschii which are attached to the slide by basal hold-fast cells. This species was abundant in all of the streams receiving a mercury input.

The actual counting and identification of the algae on the slides gives a more reliable picture of the changes in the algal communities that were occuring. The results of two sets of microscope slides that were placed in the streams prior to the shutdown of the mercury input are presented graphically in Figures 26 and 27. These graphs summarize the cell totals, with each point being the average of four counts (two slides from each of two replicate streams). Looking only at these totals the two experiments gave very similar results. In both cases there was an initial period of about 40-50 days when the total number of colonizing algae was increasing and was inversely proportional to the mercury input. The difference between the controls and the low-level slides

was not as pronounced as the difference between the 1.0 ppb and 5.0 ppb treatments. At the end of this initial period all cell concentrations were approximately equal. During the second period the trend observed earlier reversed in that the higher cell counts were found in the channels receiving the 5.0 ppb mercury input. In the second of these experiments the slides were examined once after mercury input was shut off. This last count confused the picture because the algal population on the slides previously exposed to the high mercury levels declined while the other slides continued their trend. These data indicate that the initial rate of colonization of the glass slides was seriously affected by the level of mercury in the water. The second trend seen on the graphs needs further examination.

Figure 28 presents the data for total periphyton cell densities accumulating on glass slides since the shut down of the mercury input. In this data the initial trend of a retardation of growth in the high-level streams is not apparent. However, the second trend seen in Figures 26 and 27 is apparent in this post-mercury experiment. These data lend support to the statement made above that the initial colonization rate on this glass substrate is directly affected by the level of mercury in the surrounding water. It also indicated that the second trend observed earlier is the result of an indirect effect of the original mercury concentrations in the streams. This indirect effect has also been

observed on the more permanent substrates as will be discussed in the next two sections:

Plexiglass Plates

The periphyton present on plexiglass plates located in the artificial streams have been studied since before the mercury input was begun in May, 1972. From May, 1972,until August, 1973,the mercury concentrations in the streams were 0.01 ppb and 1.0 ppb. On August 1,1973, levels in the 0.01 ppb channels were changed to 5.0 ppb and maintained at that level until the mercury input was shut off in January, 1974. Total cell counts are not available for the period prior to December, 1974. However, diversity and biomass values of the data accumulated before that time are available for comparison.

The cell densities on the three sets of plexiglass plates that were examined since the mercury level was increased and the one set taken after the mercury input was shut off are displayed in Figure 29. In all four cases the means of the four counts for each treatment are greater for the highest mercury concentration and lowest for the controls. The differences between the controls and the high-level plates are significant at the 95% confidence interval in all four cases. The controls and the low-level values are not significantly different in any of the counts. Here we see the same trend that was visible in the later part of the glass slide exper-

iments where the amount of algal growth seems to be proportional to the mercury level in the water.

Biomass data collected from these same plexiglass plates is presented in Figure 30 and tends to confirm the results of the direct cell counts. In all samples except one, there was a higher biomass associated with a higher mercury input level. This figure also shows that the biomass was declining during these four winter months. Figure 31 illustrates the total mercury concentrations in these samples on a dry-weight basis. If this figure is compared to the preceeding one, it is observed that as the biomass decreased during this period, the mercury concentration tended to increase. This observation will be discussed later.

The calculated values for the H^1-diversity are presented in Figure 32. This figure shows that the communities receiving the highest mercury levels had the lowest diversity or uncertainty of prediction. The controls were significantly higher than the high level samples in three out of the four cases. This trend is consistent with the data accumulated by Dr. Ferens during the previous year when the mercury concentrations were much lower. The diversity differences were smaller, yet they were significantly lower in the 1.0 ppb streams. The low diversities found in the 5.0 ppb samples indicate that the higher cell densities shown in Figure 29 are the result of the in-

creased dominance of a few species. The species that was largely responsible for this dominance was Oedogonium reinschii, which was also the species that most frequently dominated the glass slides and wall lining during this study. In the previous year, Oedogonium reinschii was found to be most abundant during the months from October to April and to comprise an important percentage of the algal community(about 20% of all of the algae observed). This filamentous alga is well adapted to the initial colonization and continued growth on a substrate because it possesses a basal hold-fast cell and grows rapidly.

Stream Walls

Although strips of PVC plastic identical to the liner of the channels had been harvested during the previous year for biomass and mercury analysis, no quantitative determinations of the algal communities were made until the end of 1973 when two counts were made before the mercury was shut off and two since then (Figure 33). The cell density was found to be consistent at each treatment level during this brief time with the high level streams consistently having a mean 30 times or more greater than the control streams and 10 times greater than the low level streams. The cell densities in the low level streams were significantly higher than the densities in the control streams in all of the four counts.

These data are another example of the trend seen in the glass slides and on the plexiglass plates where the cell densities were greater with higher mercury concentrations in the water.

The H'-diversities for these samples are plotted in Figure 34. It was found that while the mean for the control samples was higher than the mean for the 5.0 ppb samples in 3 of the 4 cases, it was significantly greater in only one case, and in no case was it significantly different from the 1.0 ppb samples. These data indicate that although the cell density was much higher in the high mercury level samples this was not just the result of one species increasing disproportionately in abundance, but the increase in numbers of several species. The two species that were found to be most abundant on the walls in these high mercury level streams were Oedogonium reinschii and Meismopedia punctata.

Benthos

The data describing the two collections of benthic insects is summarized in Figure 35 in the form of H'-diversity and estimated numbers of insects per square meter. Although the mean diversity was lowest in the high level streams in each case, this difference was not significant. As far as the density of the individual insects in each stream, it was found that the low level streams had the most dense populations and the control and high

level streams had similar but lower densities. The results of the work done previous to the increased input of mercury in August, 1973, indicated no treatment affect in upstream samples and an increased H' for the 1.0 ppb treatment in the downstream samples. A more intensive benthic sampling program is needed.

Continuing Work

The intensive gathering of data up to this point, which will continue for the next several months, has not allowed an in-depth analysis of the algal population dynamics. Statistical analysis of differences due to mercury treatment will be carried out on all data. Responses of dominant species to mercury treatment and time of colonization will also be analyzed. It is possible that air releases of mercury may be related to the algae on the bottom of the streams. Total algal counts do not take into consideration species' characteristics; therefore, calculations will be made of total surface areas and volumes for the different species present. Also, it will be possible to calculate several other diversity indices, importance values, and density coefficients.

Several species of algae have demonstrated obvious responses to the treatment levels and consequently need to be investigated in greater detail. The following observations were made: 1) One species of green alga, Microthamnion strictissimum, was found only in channels 3 and 6

which received the high levels of $HgCl_2$ throughout the experiment. Since the mercury input has been shut off, this species seems to be declining in abundance. While no conclusion can be supported without culturing work, there seems to be an obvious effect here; 2) Another green alga, Stigeoclonium elongatum, has been very abundant in the control streams throughout the experiment and noticiably less abundant in the low level streams. It had been almost nonexistent in the high level streams until the mercury input was turned off. It has been a common species in all of the streams on the latest set of glass slides. 3) One species of filamentous algae, Gloetila turfosa, has been abundant in all of the streams during this study. However, morphological changes have been consistently present in the high level streams until input of mercury was turned off. At present the misshapen cells are much less common.

The species mentioned above as well as several other species that have been quite abundant need to be studied in greater detail. Therefore, laboratory culturing of the major species that can be isolated has been started. Once these species have been isolated into pure cultures, the effects of varying mercury concentrations can be observed with a minimum of interference from other factors. Continuing study of the natural algal populations in the streams will include at least one more colonization

experiment with glass slides, and the monthly determination of the populations on the plexiglass plates and PVC wall lining through the spring. At least two more sets of benthic dishes will be sorted to quantify the insect populations during the spring months.

Other projected work includes the identification to species of several algae that have not yet been identified, and the identification of a large number of organisms that have been found in the streams during this study. Specific expertise is necessary for a reliable taxonomic treatment of most of these, so specimens will be sent to experts.

A number of groups have not been considered in detail but may be important in assessing the impact of mercury on stream systems. Also, a more extensive knowledge of the structure of the stream communities will permit more detailed planning for future research projects.

Table 1

Timetable of major events
during the artificial streams mercury project.

September, 1971	Water input started to the channels at 25 gpm.
April, 1972	400 mosquitofish (Gambusia affinis) introduced into each channel and 40 into each cage.
May 15, 1972	Mercury input started to establish water levels of 0.01 ppb in two channels and 1.0 ppb in two channels.
July 4-14, 1972	Water pump out of operation, flows greatly reduced in channels.
May, 1973	Remaining mosquitofish removed from streams and cages. Last sampling of periphyton and benthic communities by Dr. M. C. Ferens. New fish placed in channels and cages.
August 1, 1973	Dose change, 0.01 ppb levels in two channels raised to 5.0 ppb.
October 1, 1973	Mr. Robert L. Knight hired to take over work with community responses.
January 29, 1974	All mercury inputs stopped.

Table 2

Summary of data obtained from six sediment core samples from each channel taken four days after mercury input to the channels was stopped.

Channel Number	Treatment	Mercury concentration in organic portion on ash free-dry wt. basis	Average biomass in organic portion of sediment (ash free dry wt.)	Total mercury in organic portion of sediment	Ash free dry wt. of plants
1	control	1.93 ppm	166 g/m^2	0.018 g	171 g/m^2
2	1.0 ppb	640	178	6.36	71.7
3	0.01-5.0 ppb	1462	149	12.1	52.3
4	control	1.57	170	0.014	96.0
5	1.0 ppb	803	136	6.08	48.9
6	0.01-5.0 ppb	763	165	7.02	72.6

Table 3

Distribution of Juncus diffusissimus Buckley on April 2, 1973, in the artificial streams

Distance from input weir in feet	CHANNEL NUMBER 1 control	2 1.0 ppb	3 0.01 ppb	4 control	5 1.0 ppb	6 0.01 ppb
0-25	453	112	30	170	139	294
25-50	261	131	8	39	10	40
50-75	144	30	8	16	9	20
75-100	93	23	3	10	5	11
100-125	97	13	3	11	8	5
125-150	124	18	11	34	18	13
150-175	222	31	20	33	25	16
175-200	70	13	3	6	1	3
200-225	38	6	0	2	4	2
225-250	26	0	3	7	1	5
250-275	49	17	2	2	12	5
275-300	236	18	5	31	5	9
Total						

TABLE 4: Common algae of the artificial streams from October, 1973 to March, 1974

Chlorophyta

Cholorococcum humicola (Naegeli) Rabenhorst
Cosmarium asphaerosporum Nordstedt
C. laeve var. septentrionale Wille
C. viride var. minor West
Fremosphaera viridis de Bary
Gloeotila turrosa Skuja
Hormidium subtile (Kuetzing) Heering
Microspora quadrata Hazen
Microthamnion strictissimum Rabenhorst
Mougeotia sp.
Oedogonium reinschii
Scenedesmus acutiformis Schroeder
Spondylosium planum W. & G. S. West
Stigeoclonium elongatum (Hassall) Kuetzing

Chrysophyta

Chlorocloster minimus Pascher
Chromulina pseudonebulosa Pascher
Ophiocytium decertum var. minor Prescott
Navicula notha Wallace

Cyanophyta

Anabeana minutissima Lemmermann
Calothrix braunii Bornet & Flahault
Merismopedia punctata Meyen
Oscillatoria geminata Meneghini

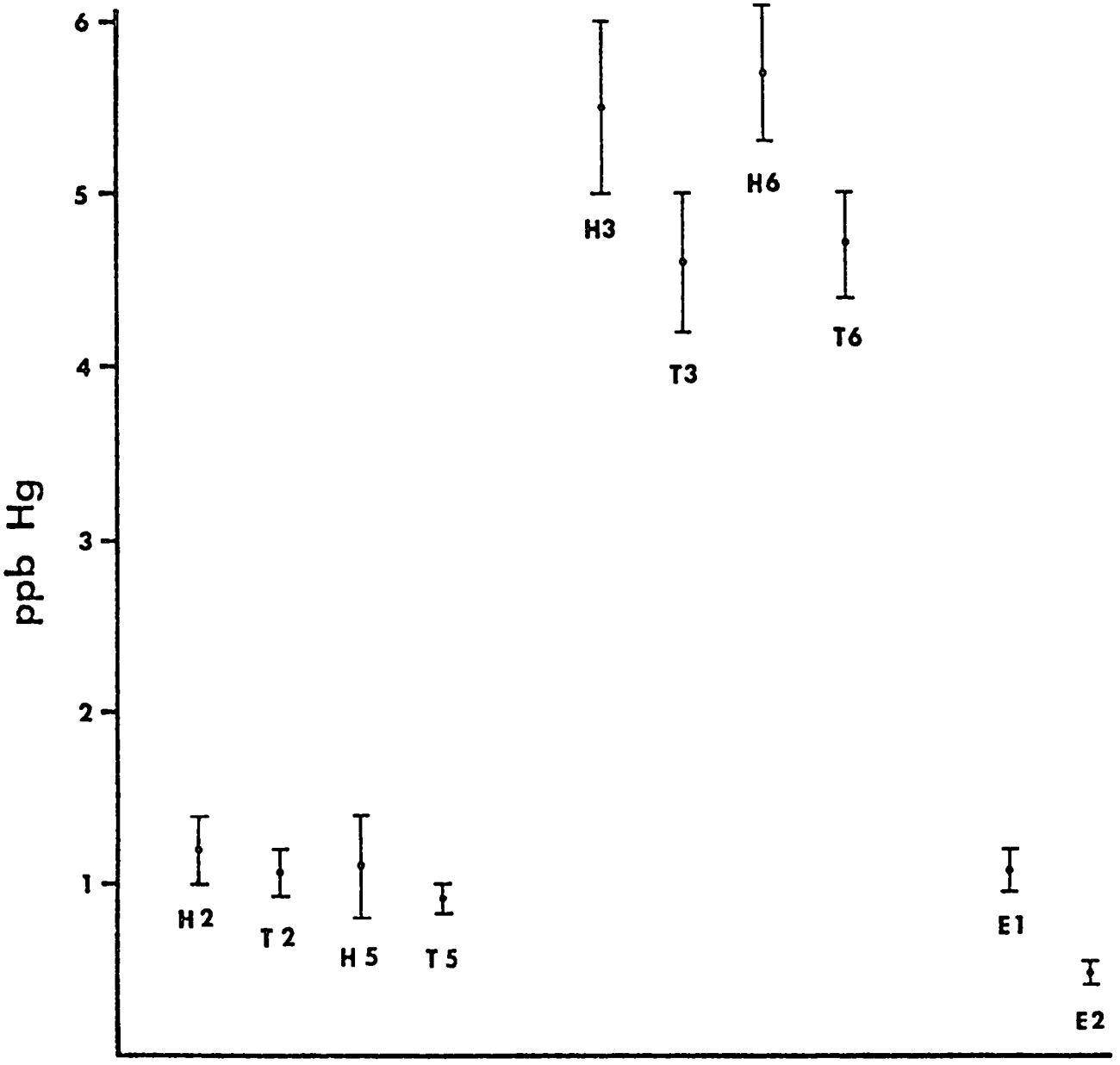


Figure 1. Results of analyses ($\bar{X} \pm 2SE$) of unfiltered water samples collected from upstream (H) and downstream (T) positions in each channel and from two positions in the effluent stream (E) between August, 1973, and February, 1974.

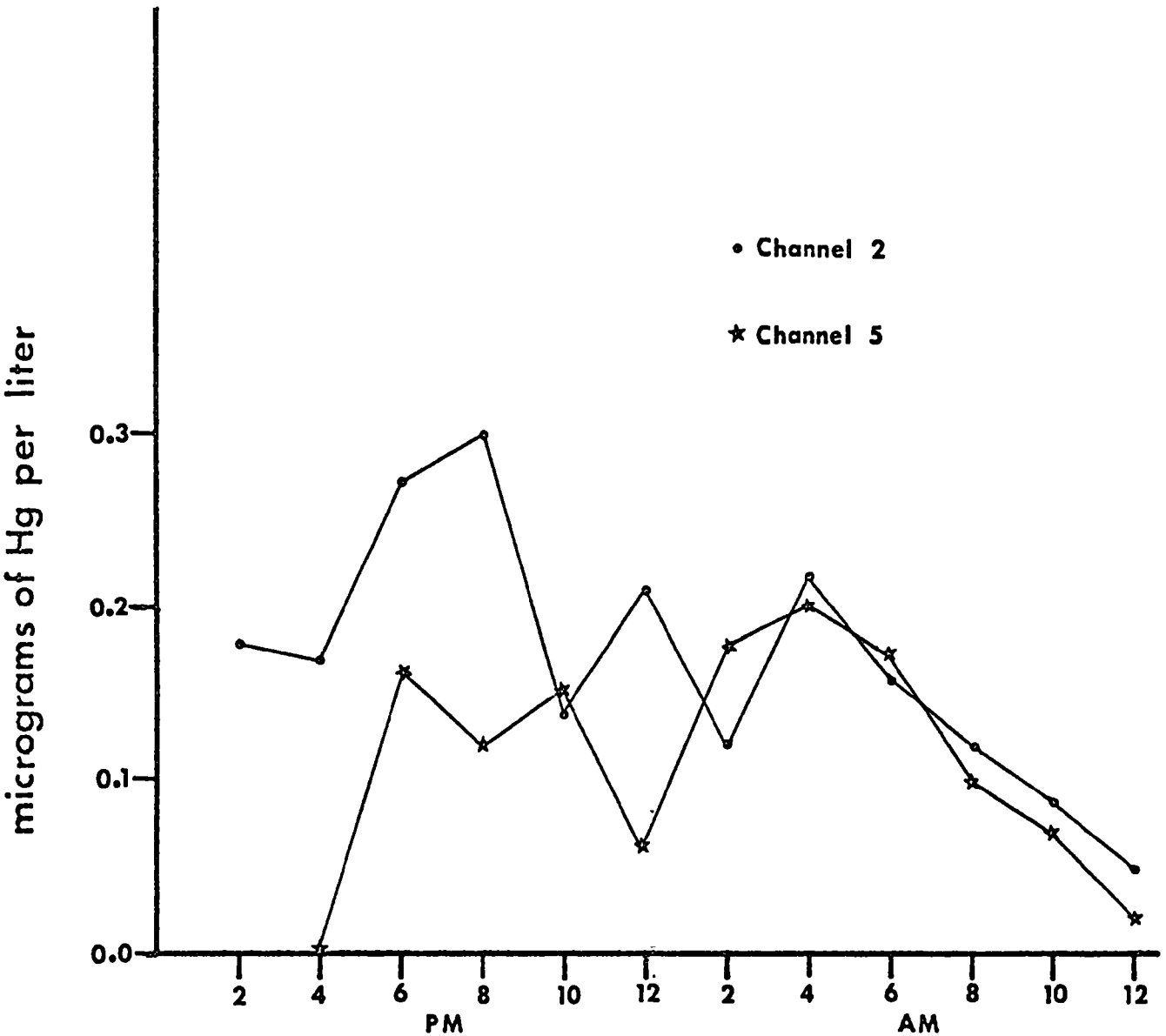


Figure 2. Mercury uptake January 22-23, 1974, in channels receiving an input of 1 ppb Hg as determined by upstream-downstream differences in water concentrations.

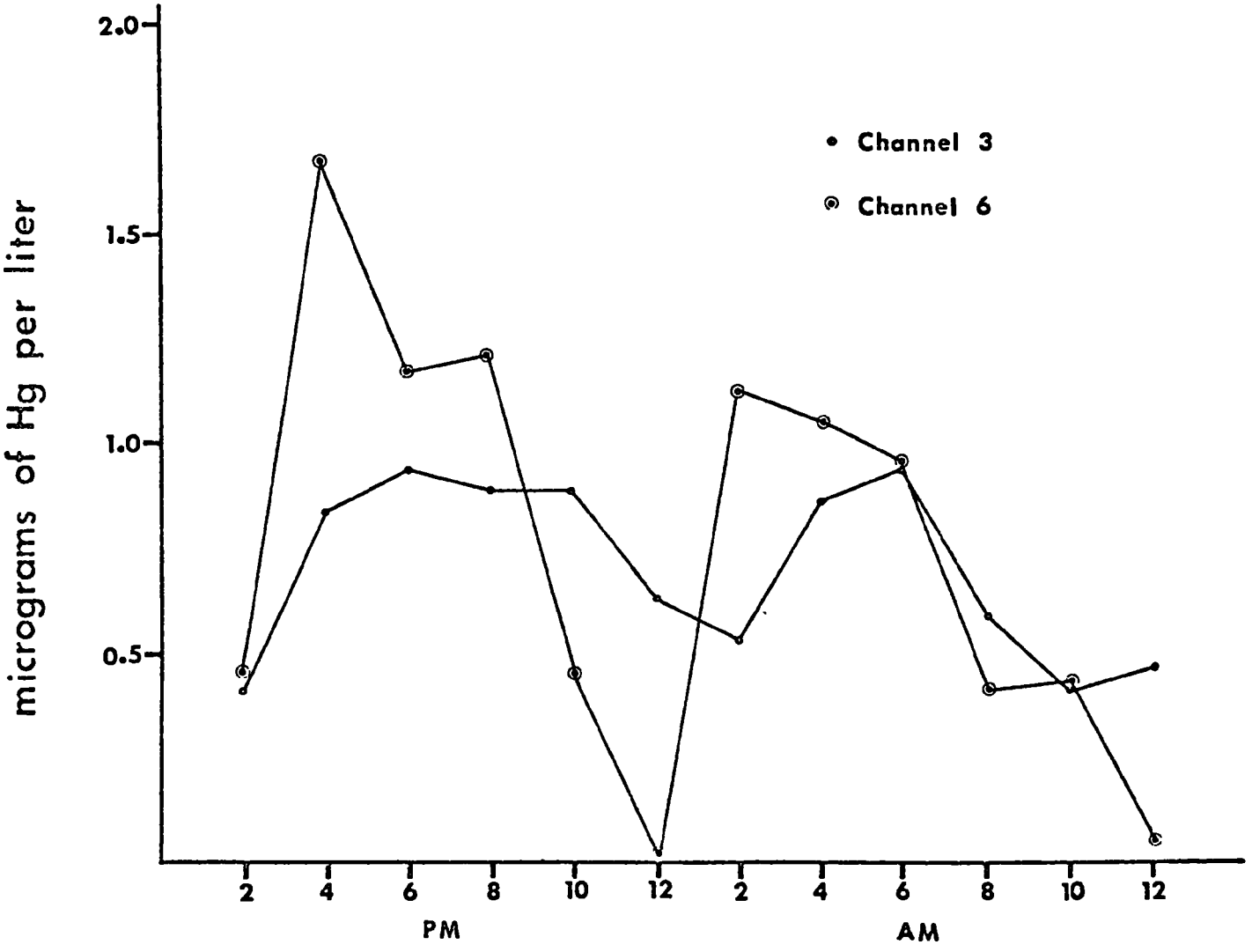


Figure 3. Mercury uptake January 22-23, 1974, in channels receiving an input of 5 ppb Hg as determined by upstream-downstream differences in water concentrations.

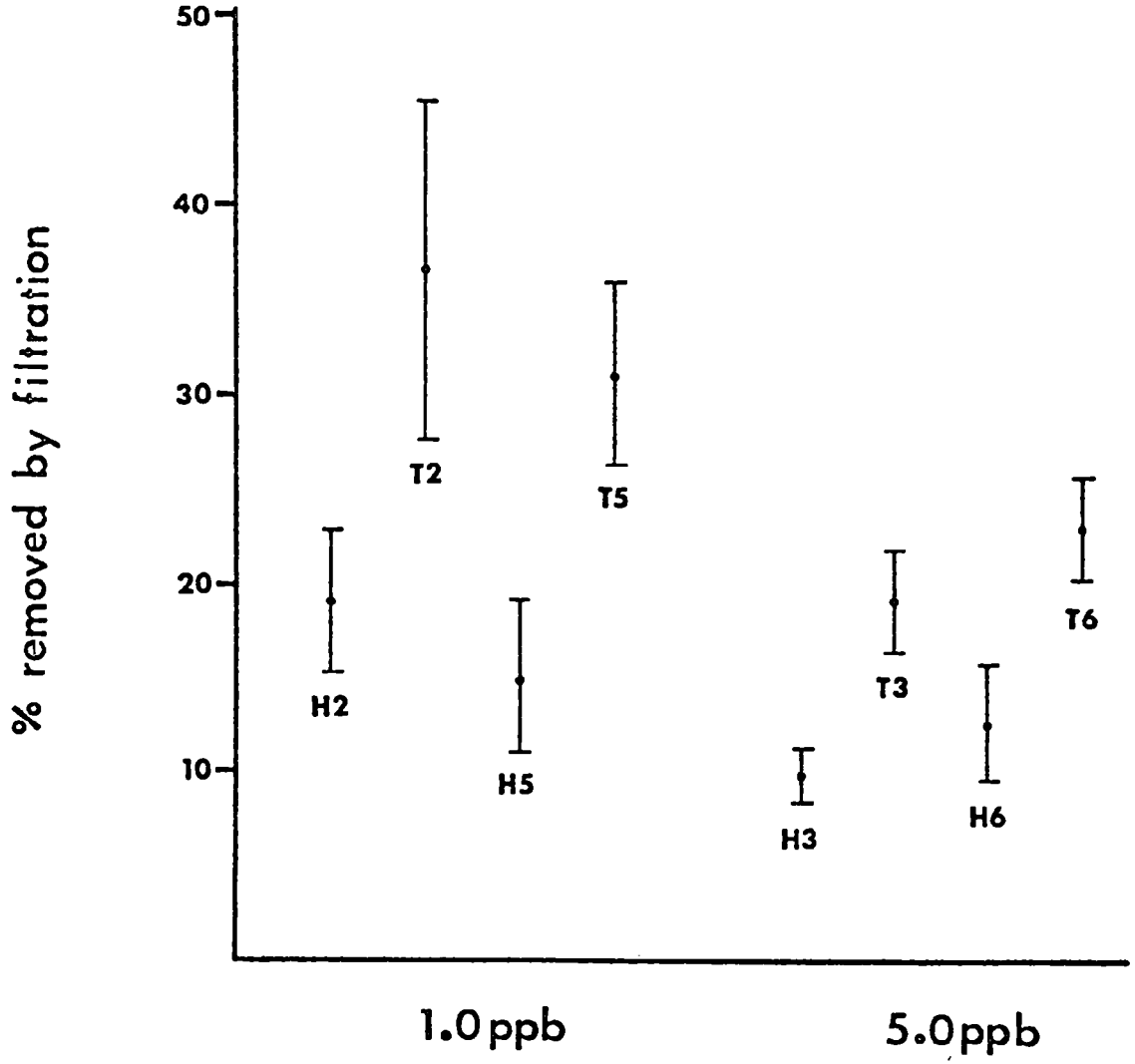


Figure 4. Removal ($\bar{X}$ ± 2SE) of mercury from water by filtration at upstream (H) and downstream (T) locations in channels receiving a mercury input.

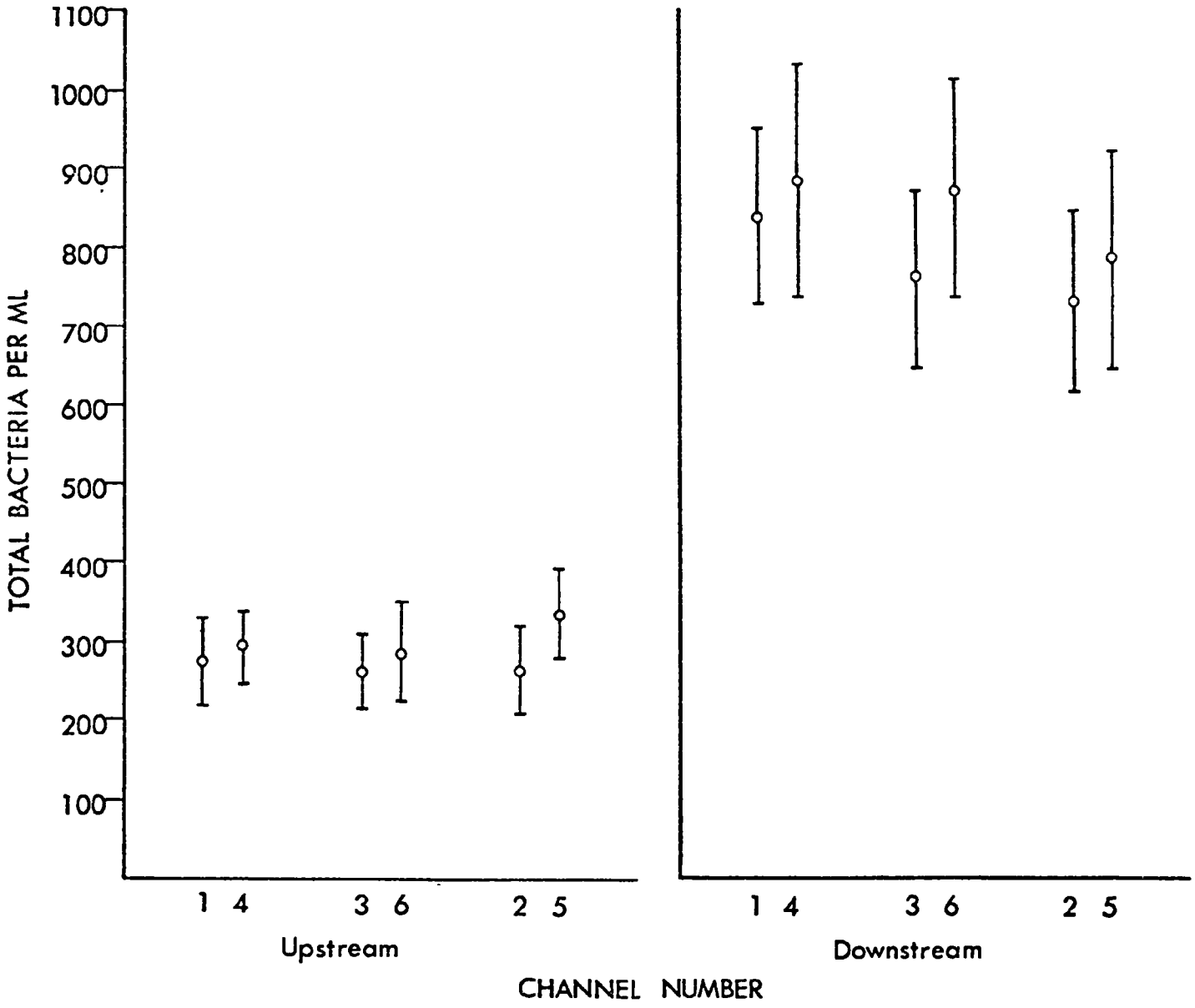


Figure 5. Total bacteria per ml (X ± 2SE) at two locations in the stream channels, based on results of daily determinations made June 1, 1972, to October 10, 1972.

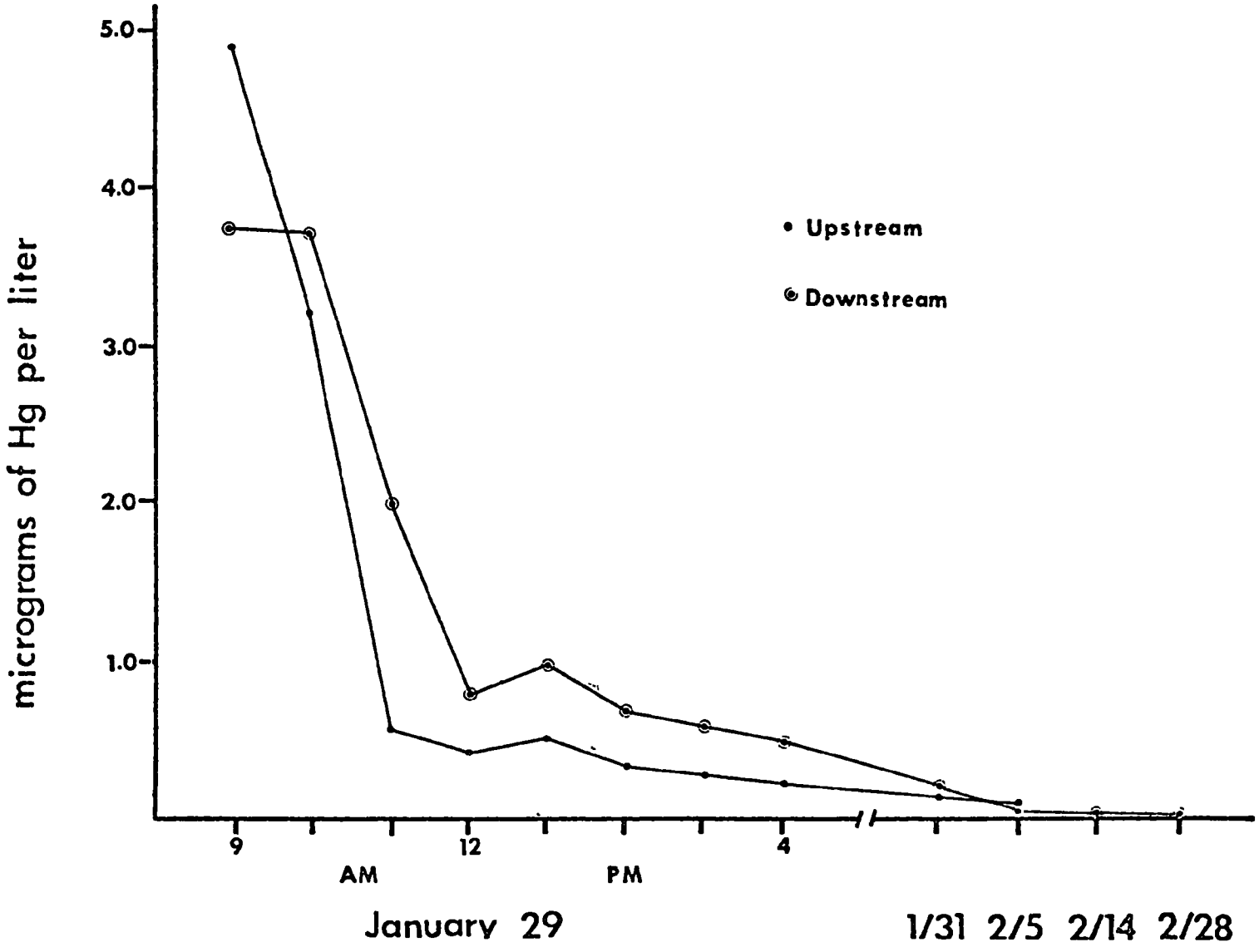


Figure 6. Decrease in water concentrations of mercury at upstream and downstream locations in channels having received an input of 5 ppb Hg until 9 AM, January 29, 1974.

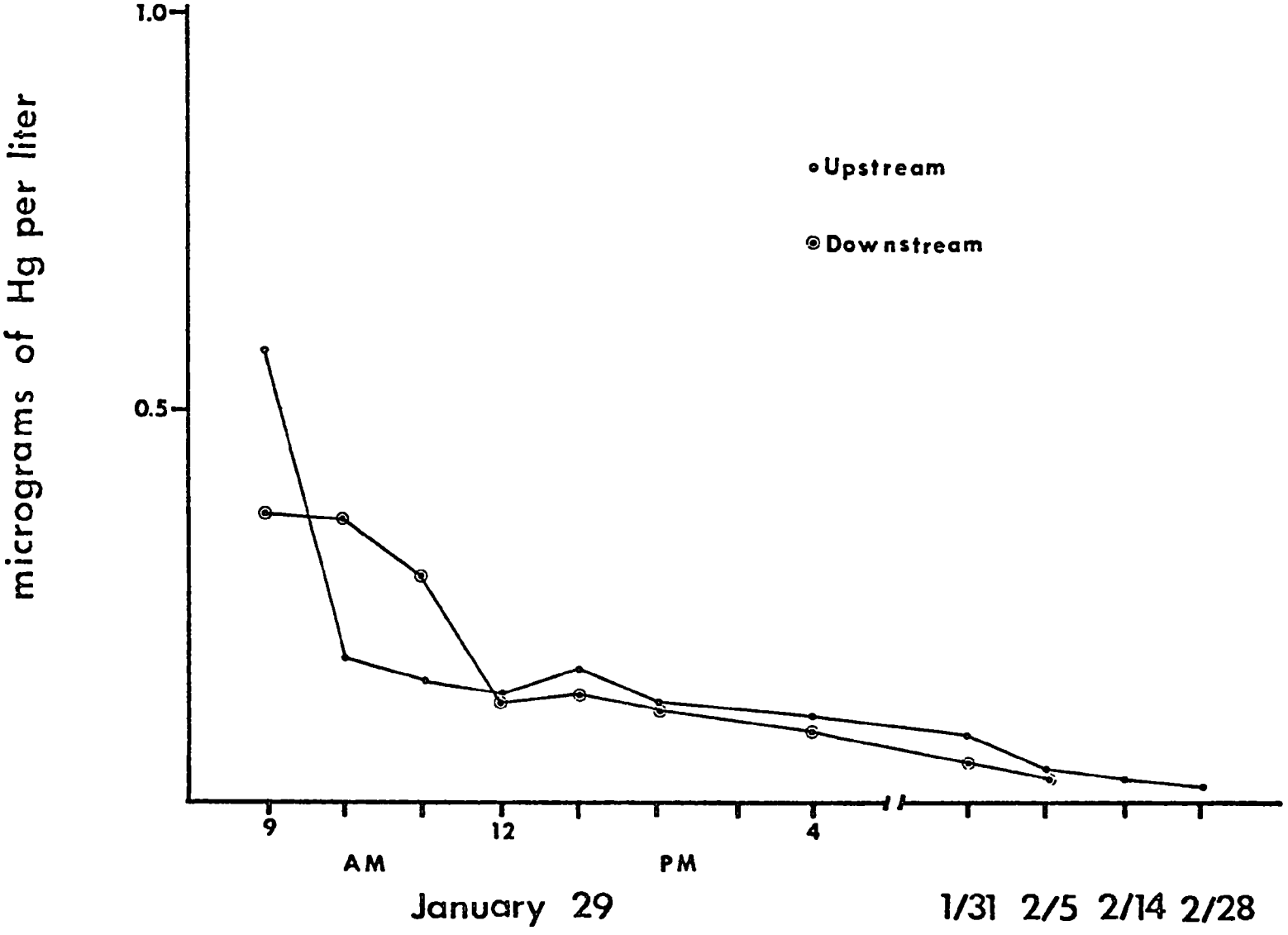


Figure 7. Decrease in water concentrations of mercury at upstream and downstream locations in channels having received an input of 1 ppb Hg until 9 AM, January 29, 1974.

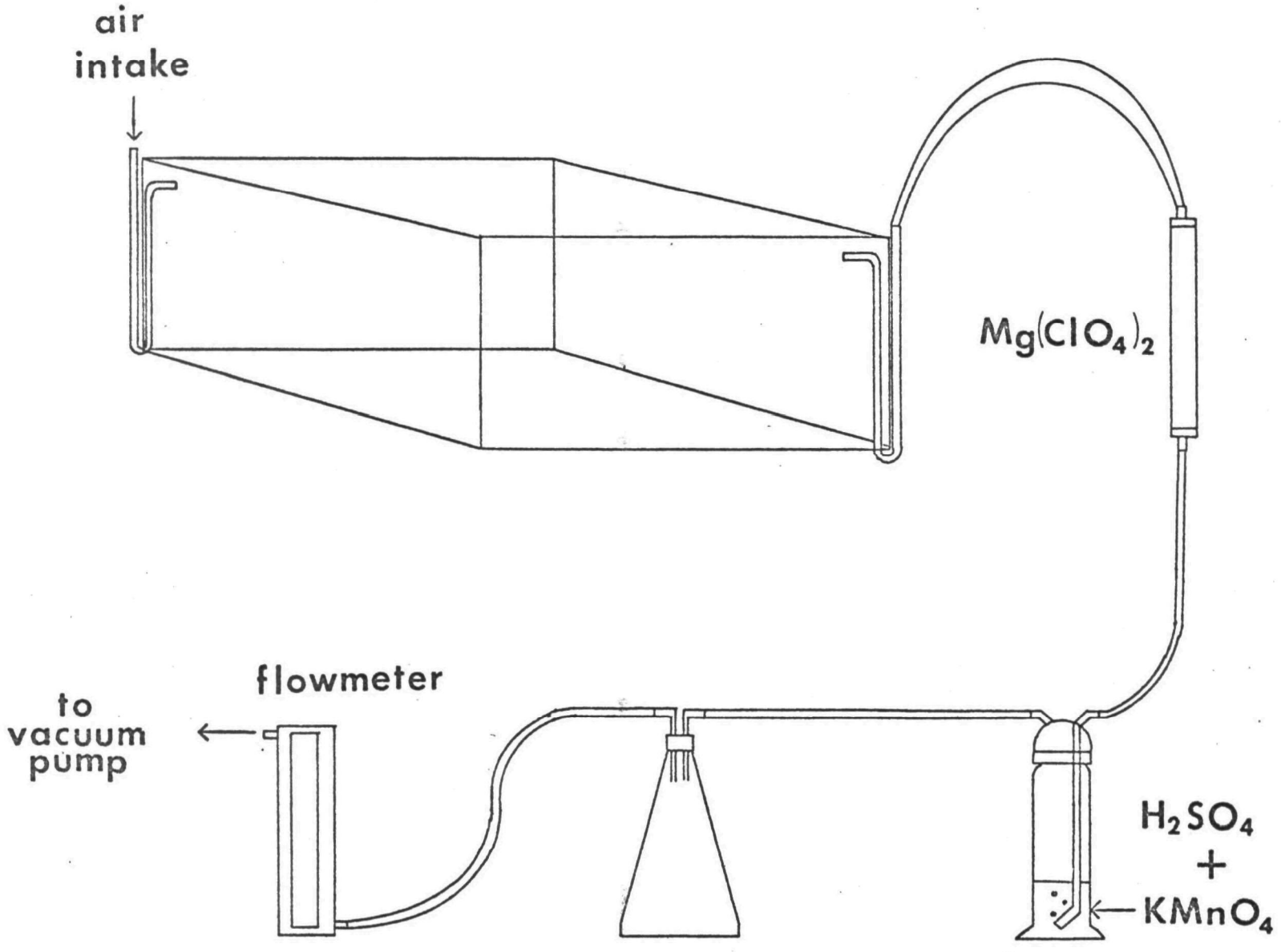


Figure 8. Air sampling system.

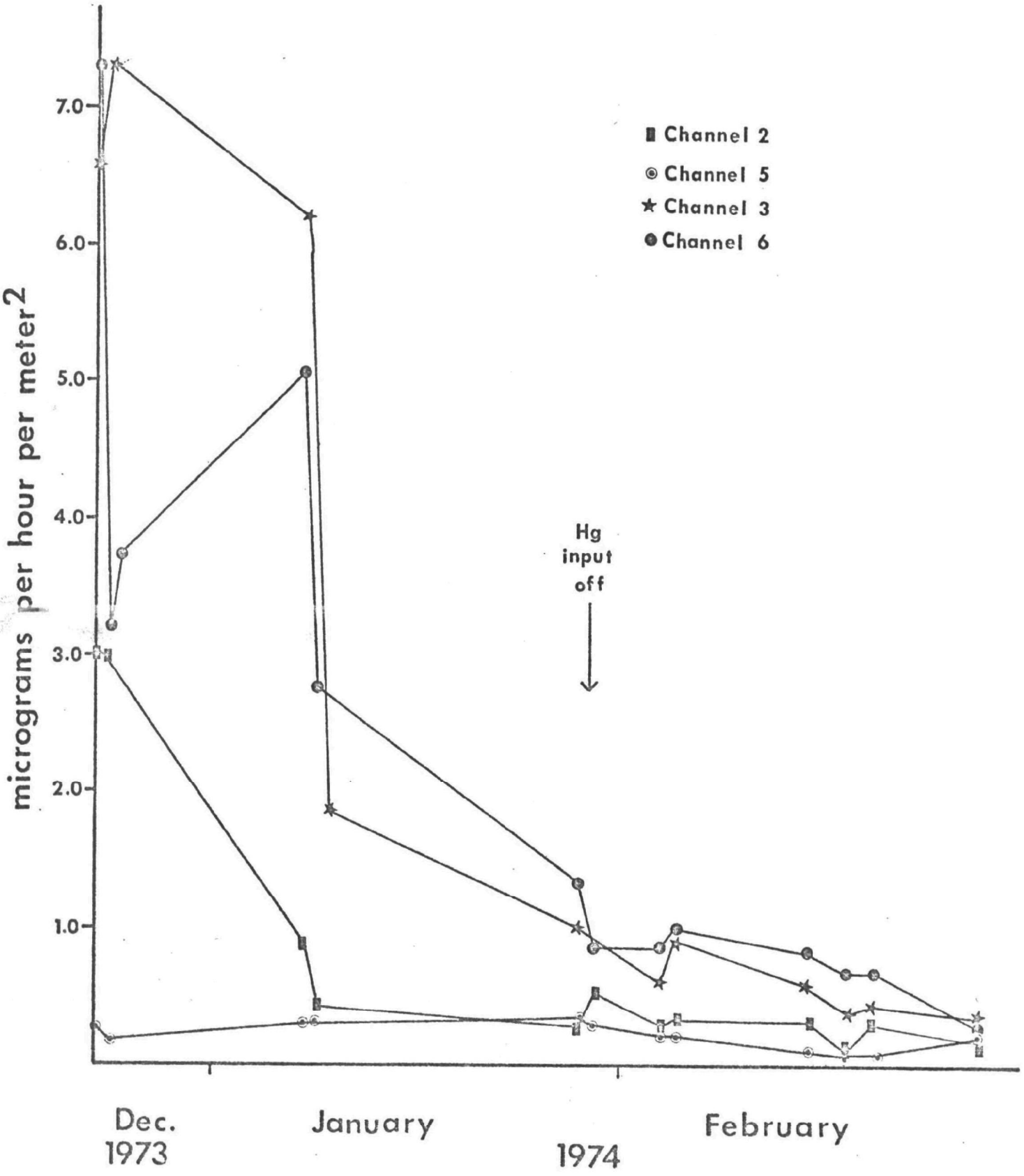


Figure 9. Mercury releases at the air-water interface of the channels receiving a mercury input.

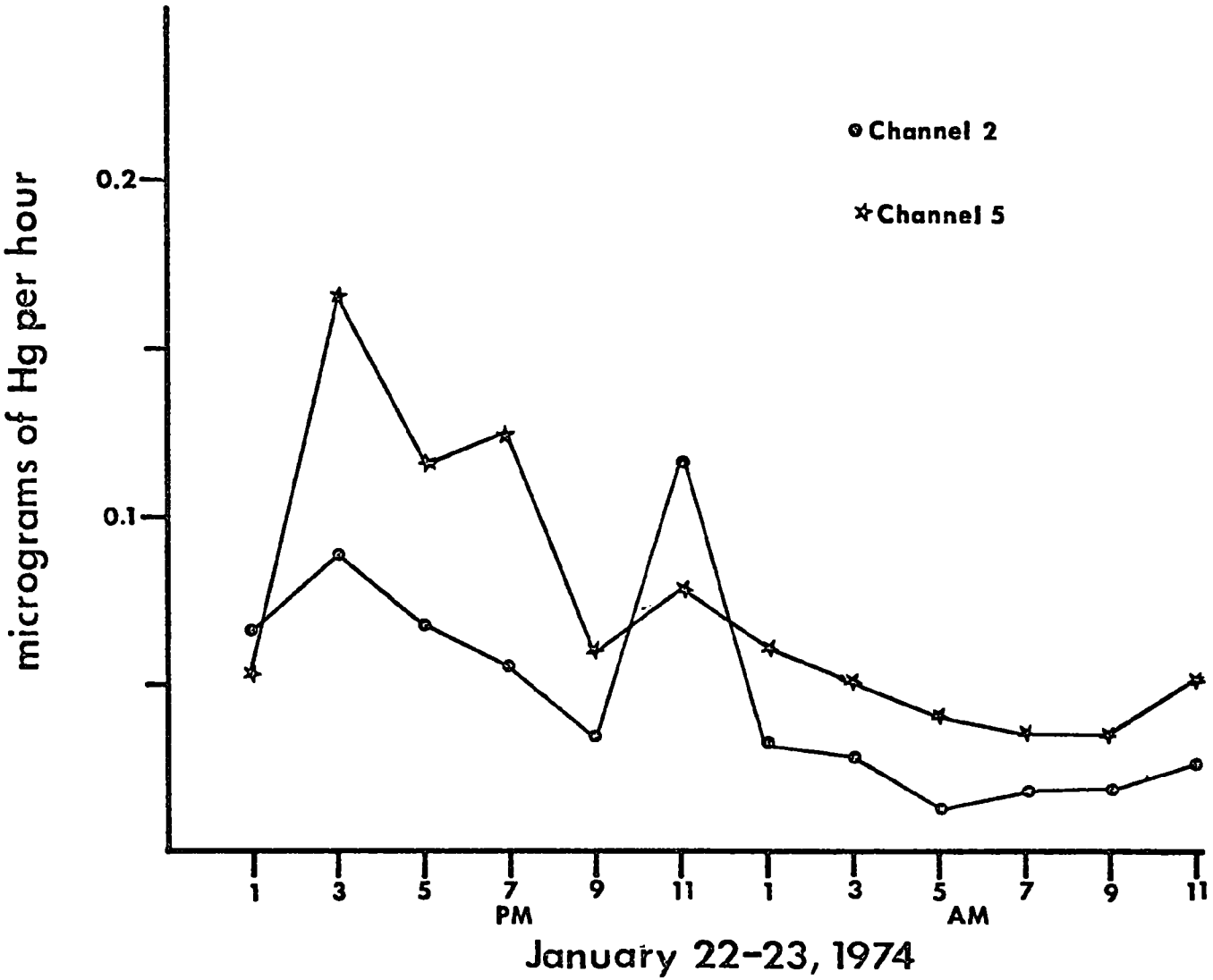


Figure 10. Mercury releases in a 24 hour period over a two square foot area of stream surface of channels receiving a input of 1 ppb Hg.

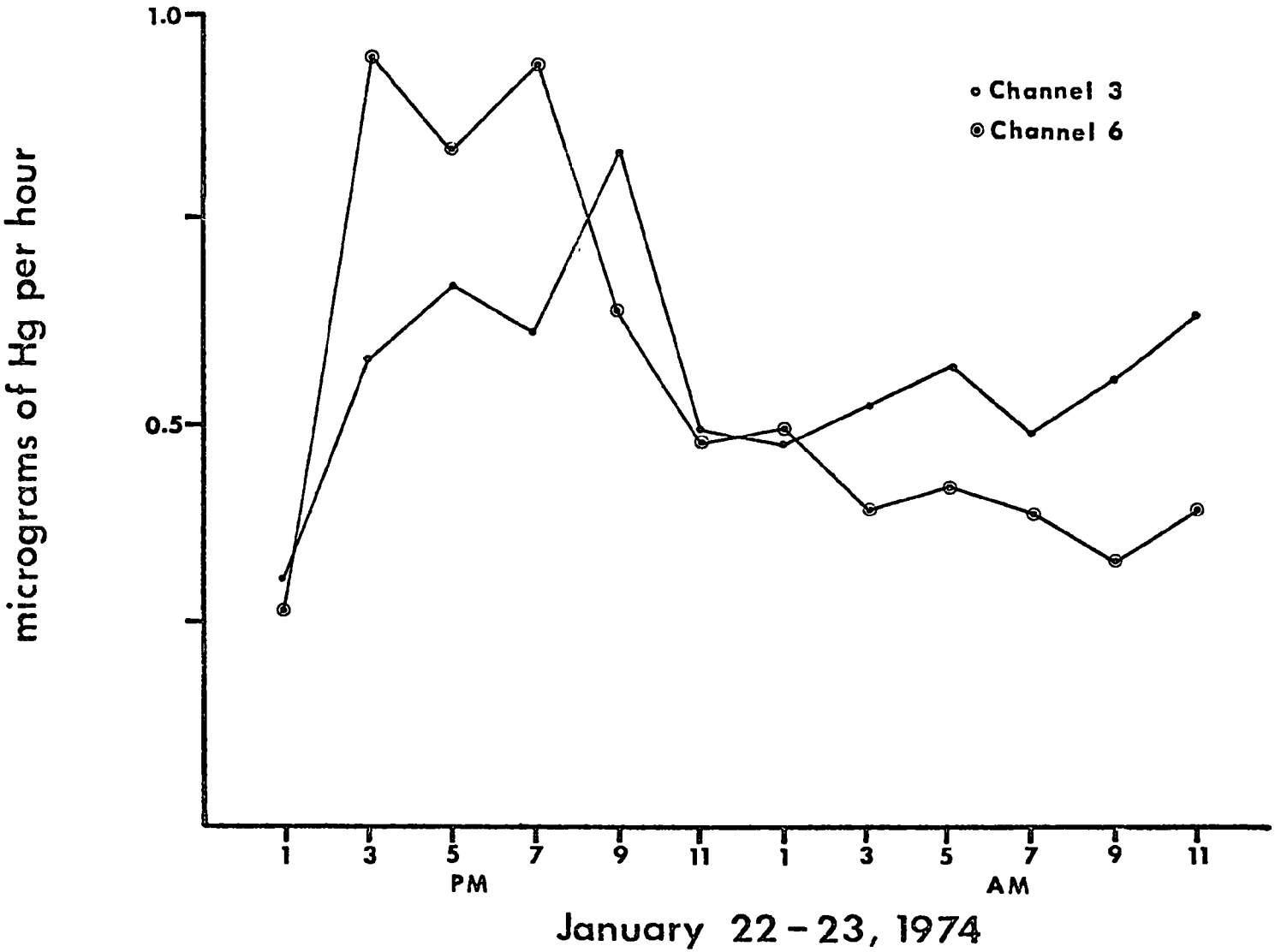


Figure 11. Mercury releases in a 24 hour period over a two square foot area of stream surface of channels receiving an input of 5 ppb Hg.

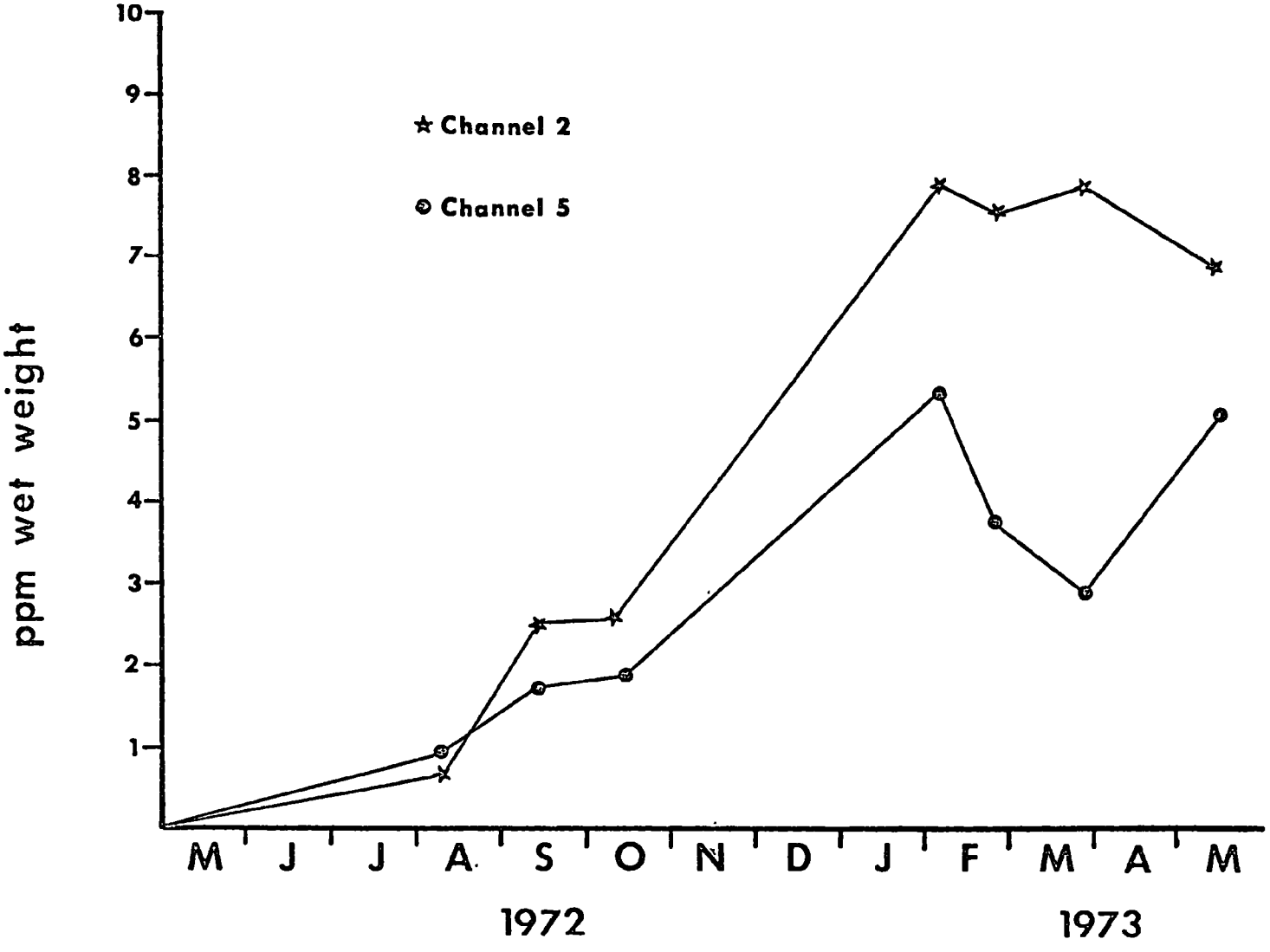


Figure 12. Levels of mercury in mosquitofish removed during the first year from the channels receiving a mercury input of 1 ppb.

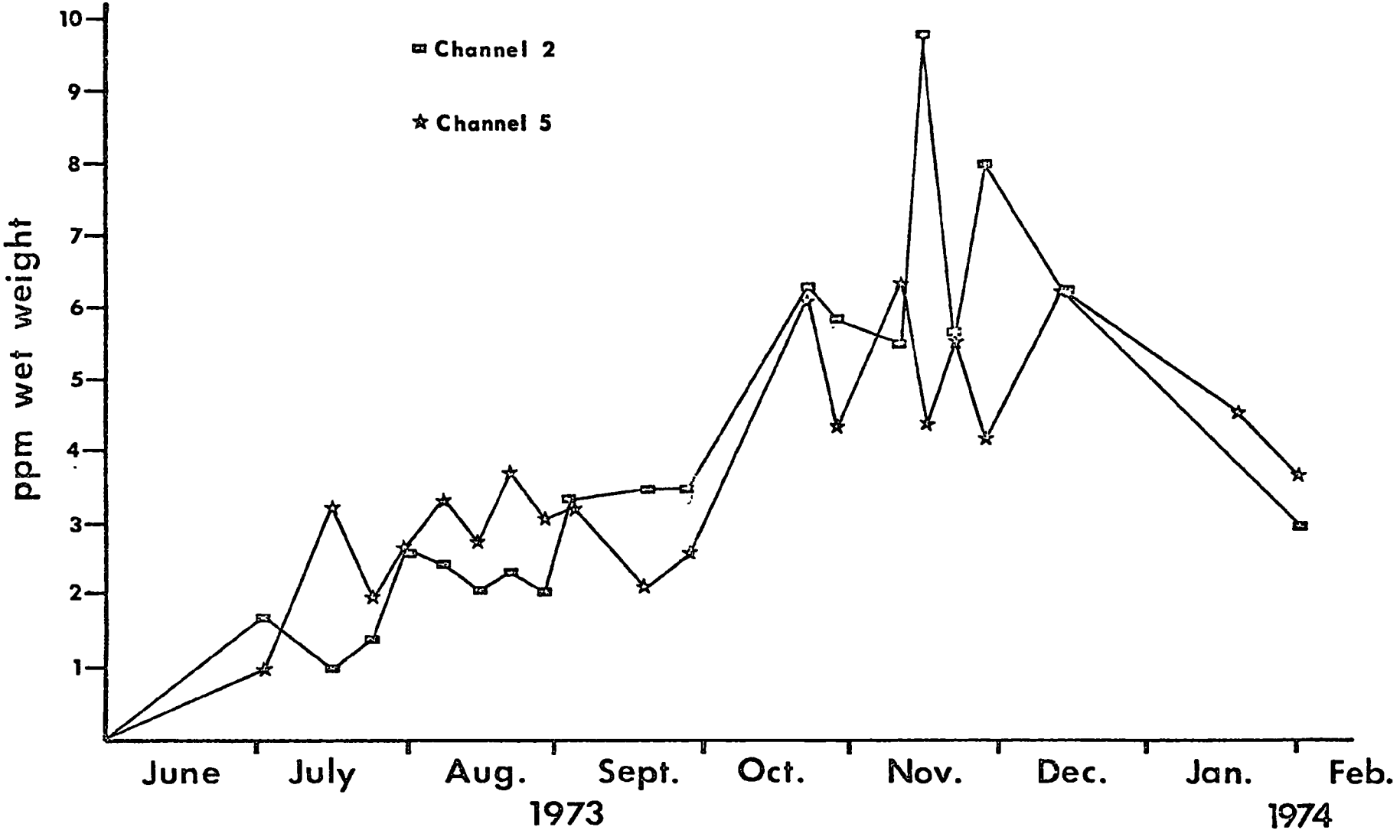


Figure 13. Levels of mercury in mosquitofish removed during the second year from the channels receiving a mercury input of 1 ppb.

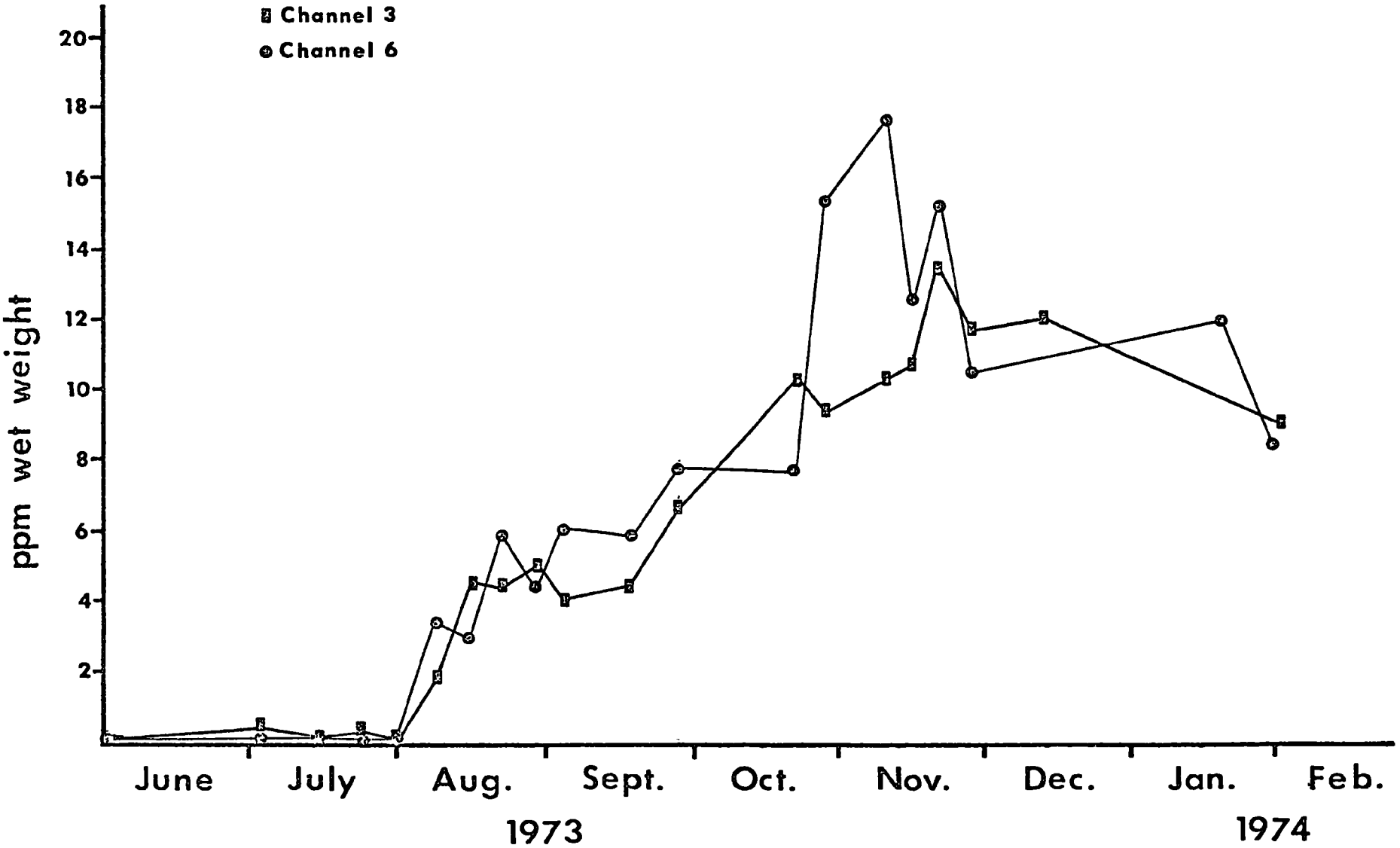


Figure 14. Levels of mercury in mosquitofish removed during the second year from the channels receiving a mercury input of 0.01 ppb until August, 1973, when the levels we increased to 5.0 ppb.

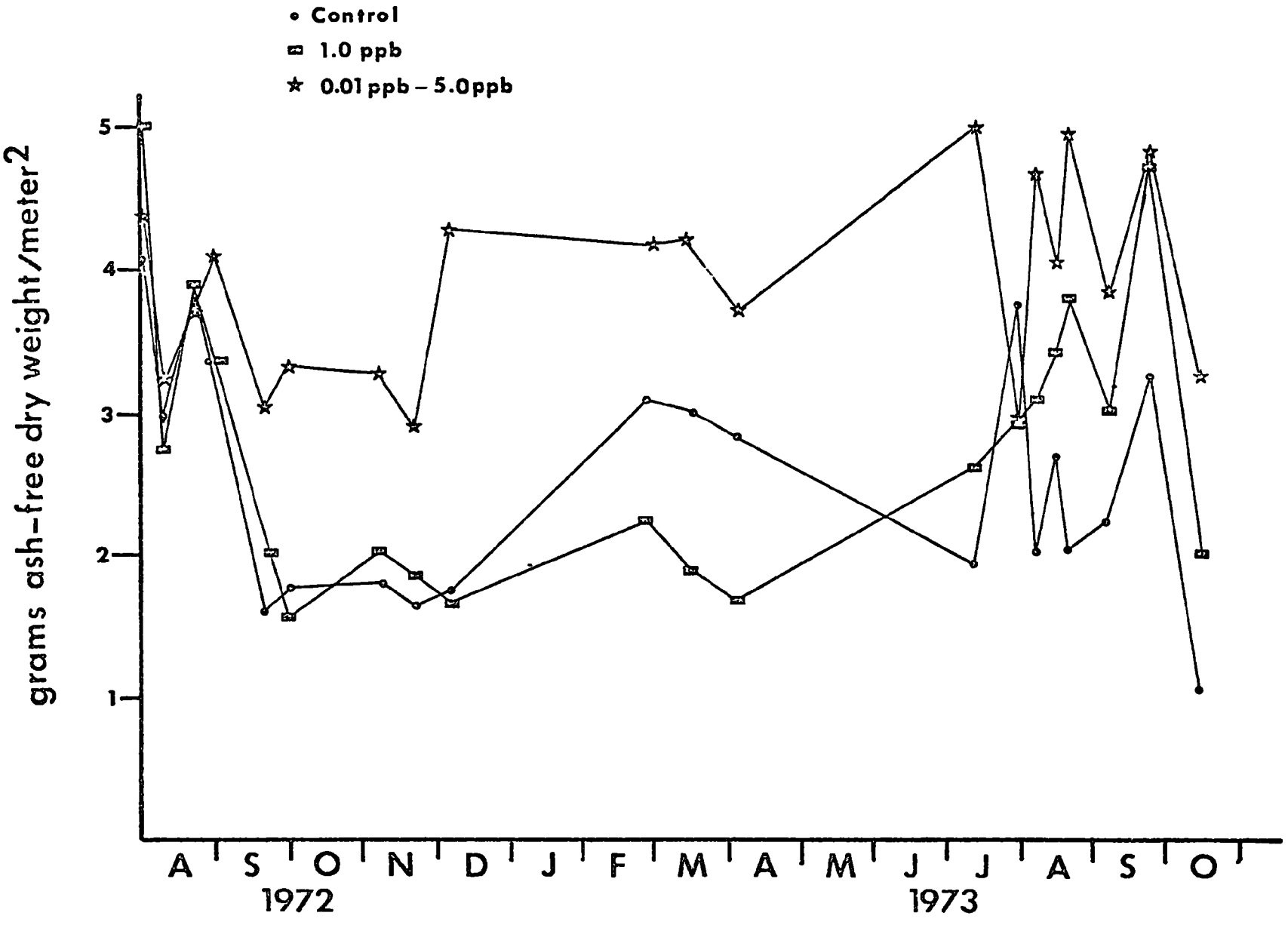


Figure 15. Channel wall biomass estimates based on material scraped from PVC strips.

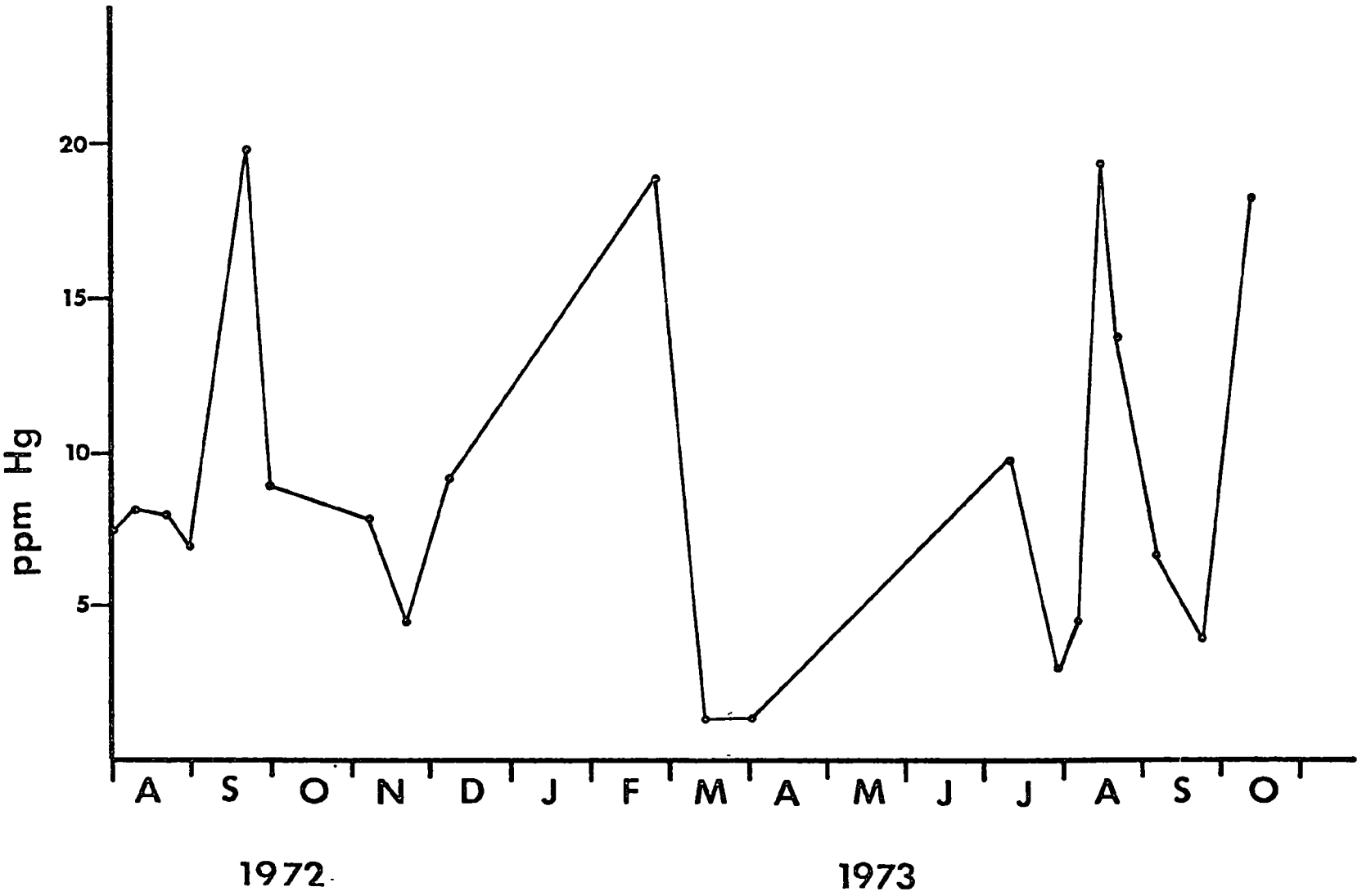


Figure 16. Average mercury concentrations, on an ash-free dry weight basis, in communities scraped from PVC strip suspended in the control channels.

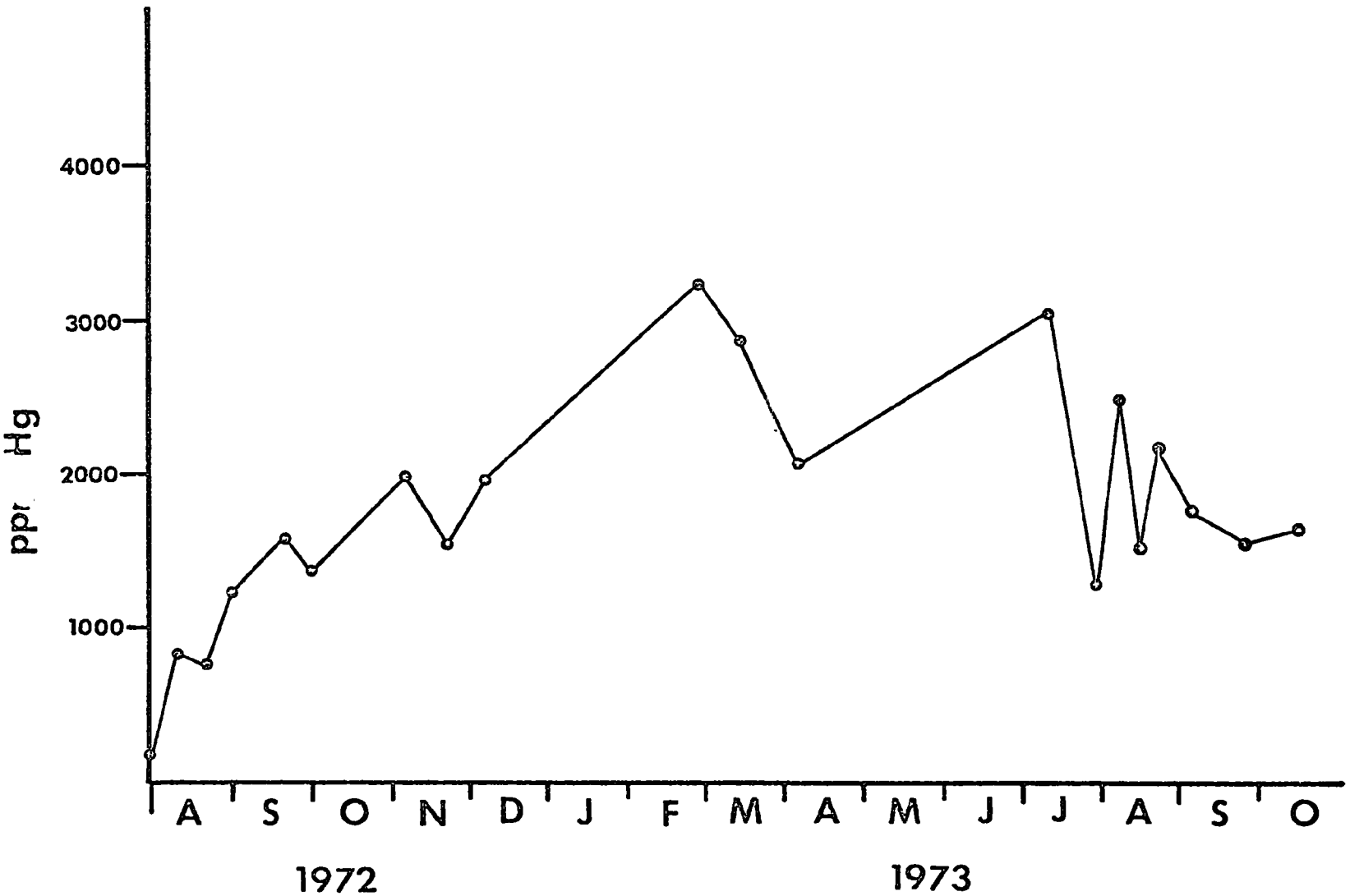


Figure 17. Average mercury concentrations, on an ash-free dry weight basis, in communities scraped from PVC strips suspended in channels receiving a mercury input of 1.0 ppb.

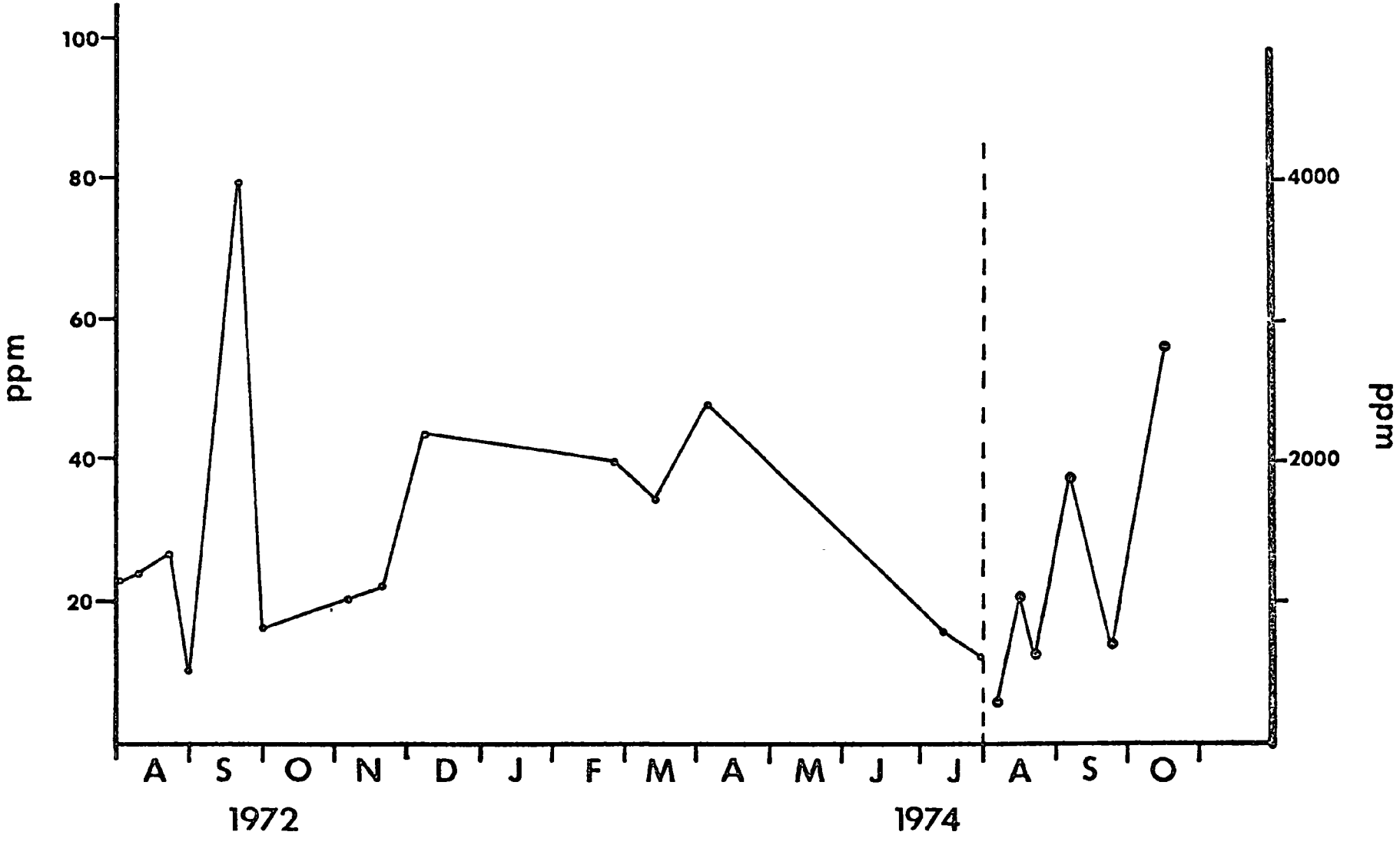


Figure 18. Average mercury concentrations, on an ash-free dry weight basis, in communities scraped from PVC strips suspended in the channels receiving a mercury input of 0.01 ppb until August 1, 1973, and an input of 5.0 ppb after this date.

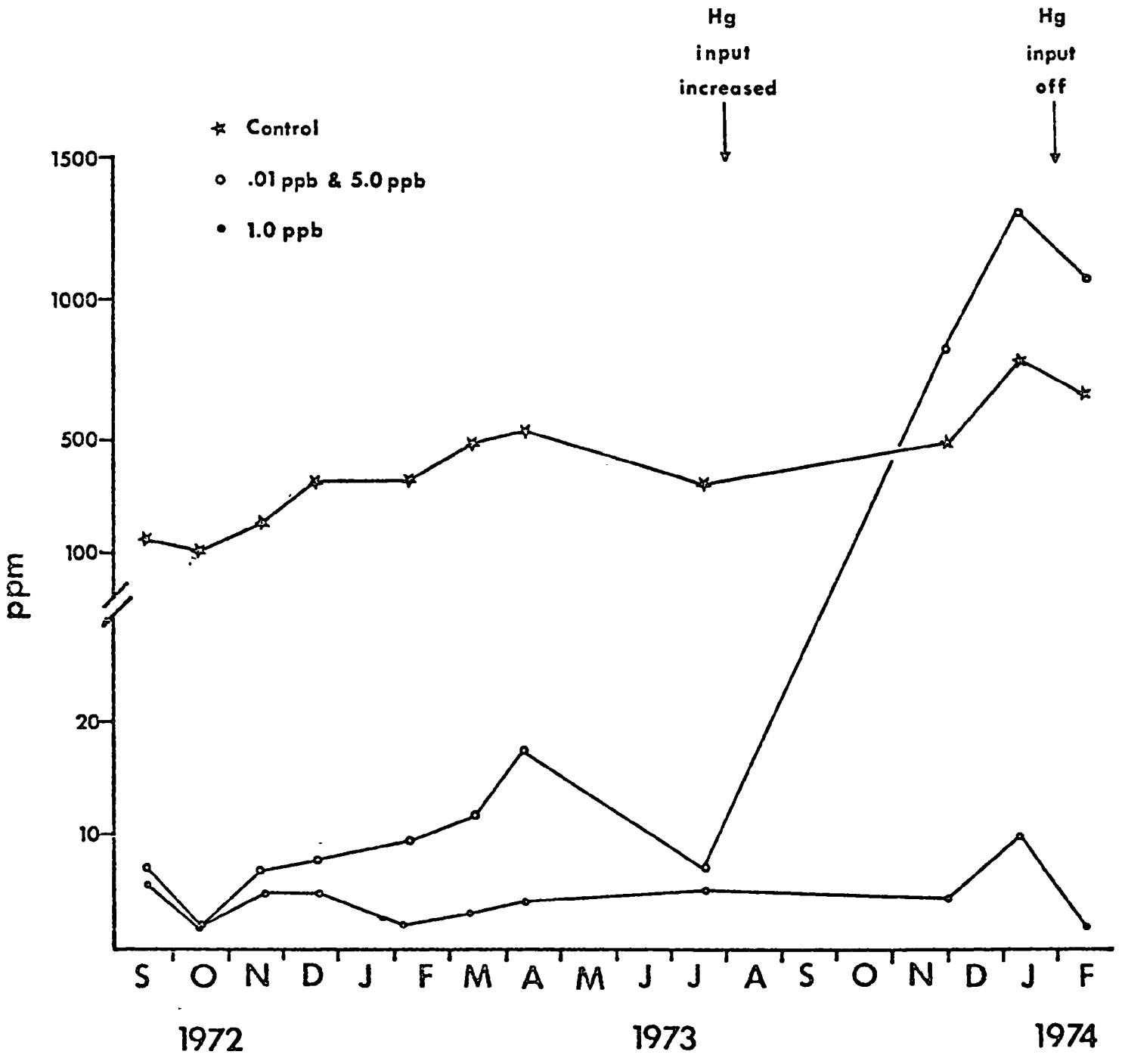


Figure 19. Mercury levels, on an ash-free dry weight basis, in the organic portion of the channel sediments.

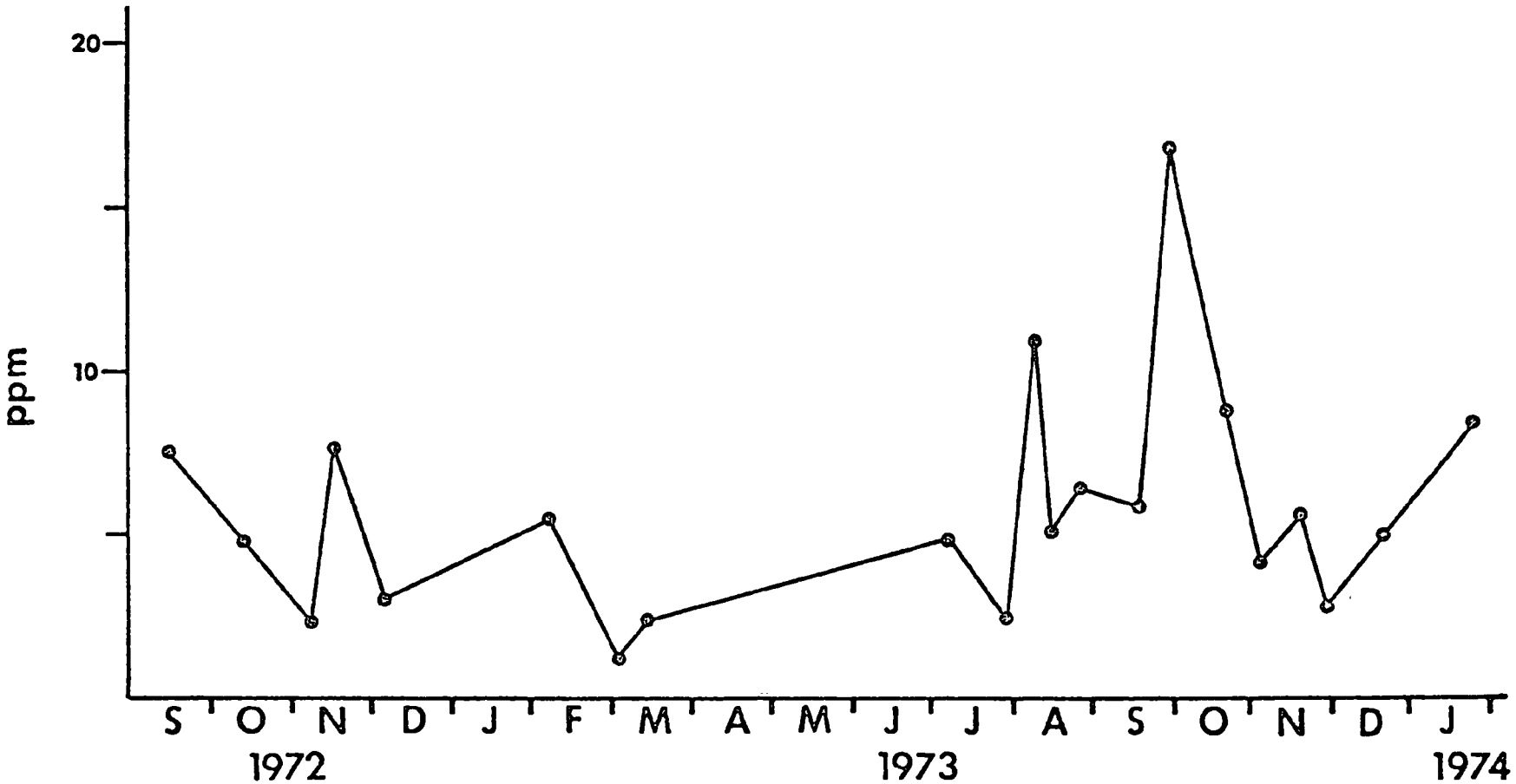


Figure 20. Average mercury content, on an ash-free dry weight basis, of material collected from the end screens of the control channels.

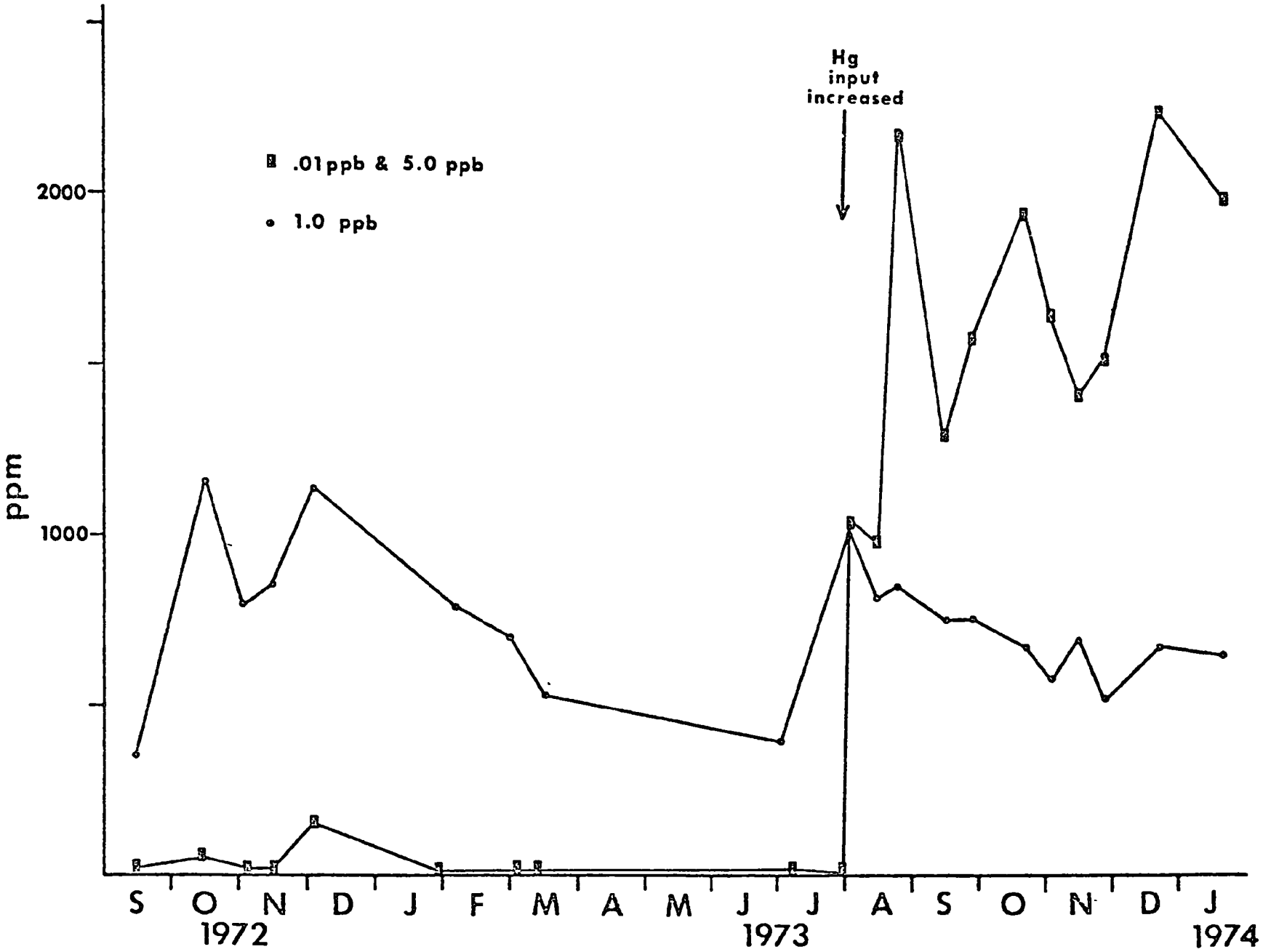


Figure 21. Average mercury content, on an ash-free dry weight basis, of material collected from the end screens of channels receiving a mercury input.

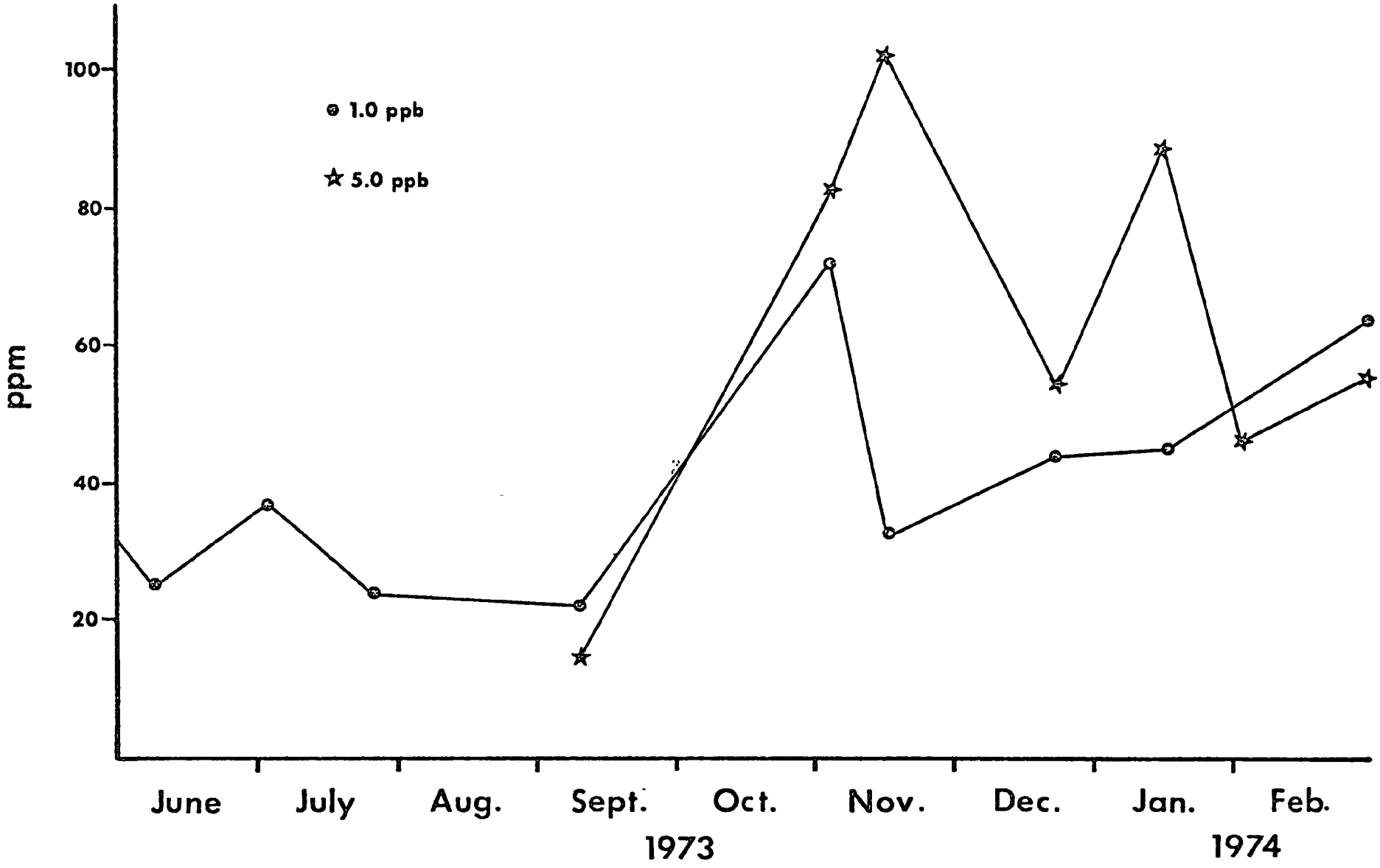


Figure 22. Mercury concentration, on an ash-free dry weight basis, in roots of Juncus diffusissimus collected from the channels receiving mercury inputs.

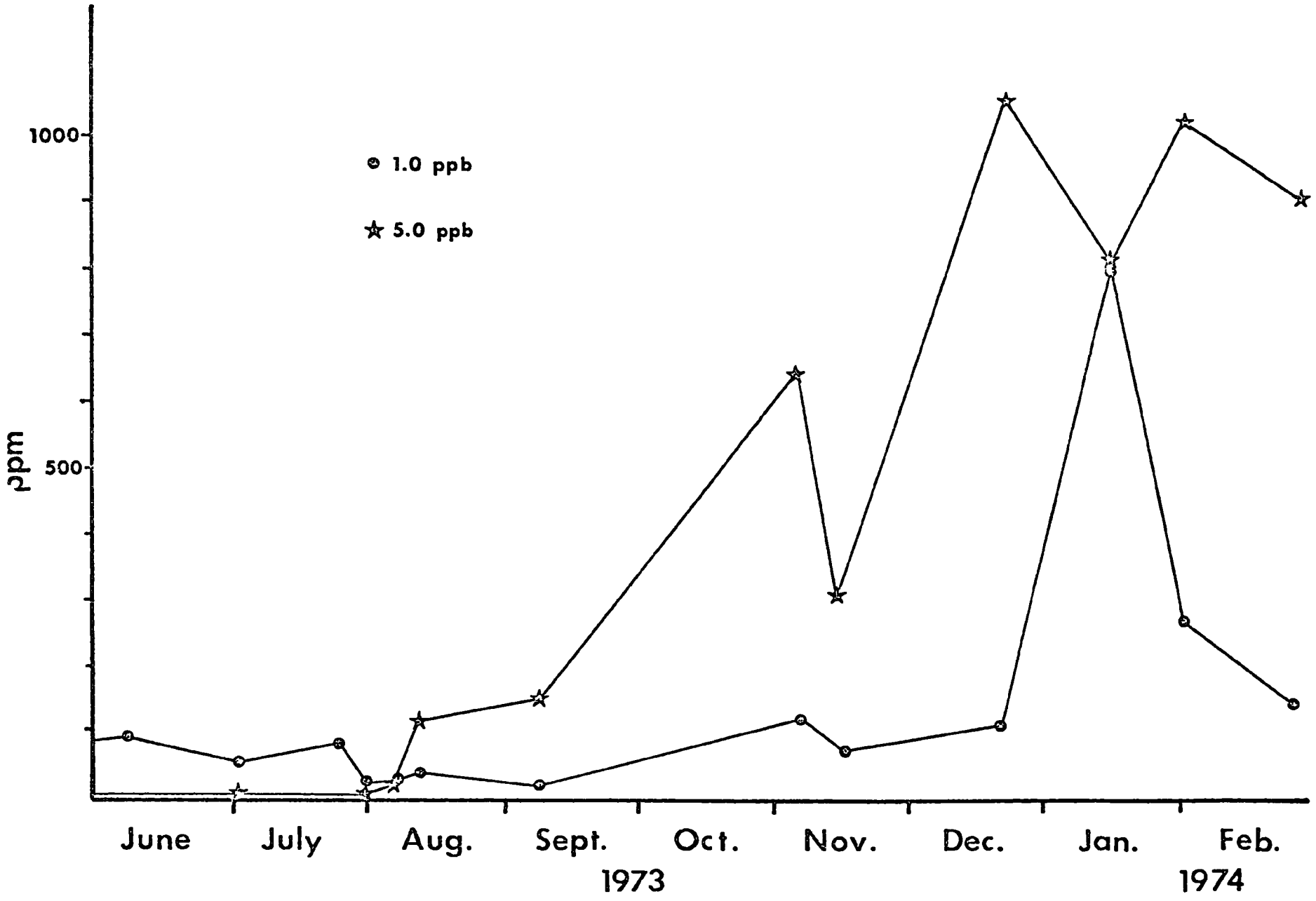


Figure 23. Mercury concentration, on an ash-free dry weight basis, in leaves of Juncus diffusissimus collected from the channels receiving mercury input

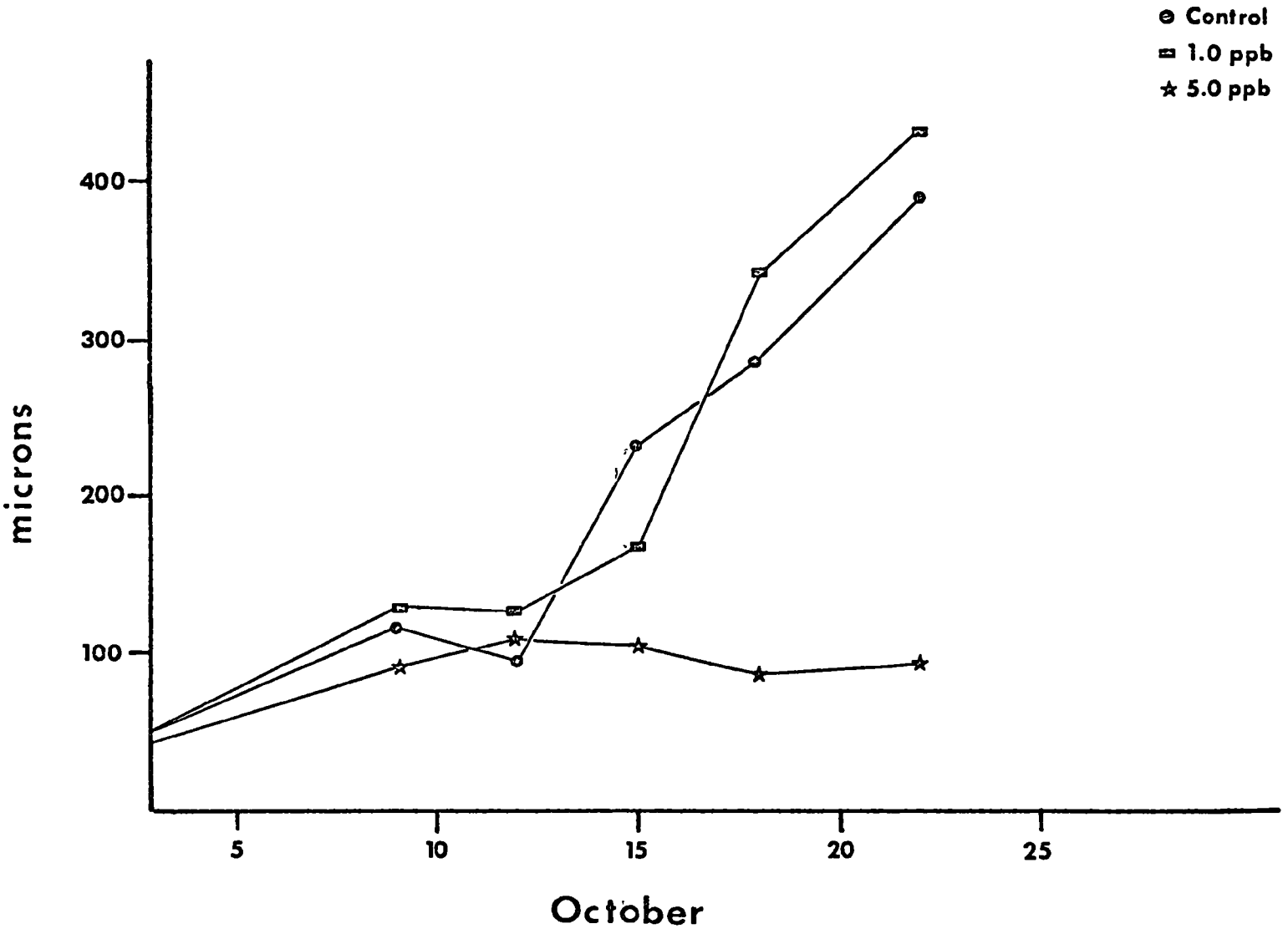


Figure 24. Changes in thickness of periphyton on glass slides exposed to different mercury levels, started September 29, 1973.

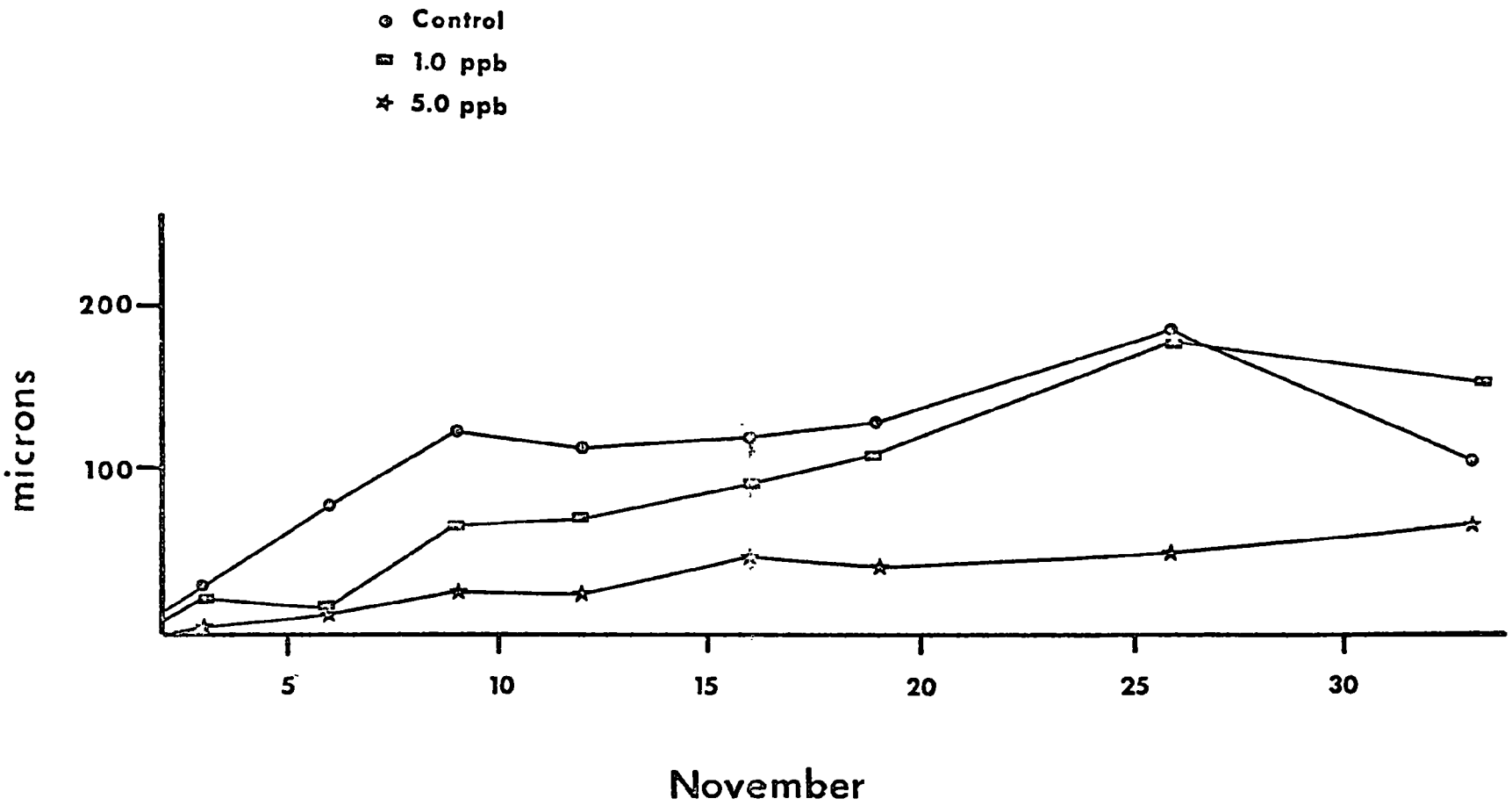


Figure 25. Changes in thickness of periphyton on glass slides exposed to different mercury levels, started October 31, 1973.

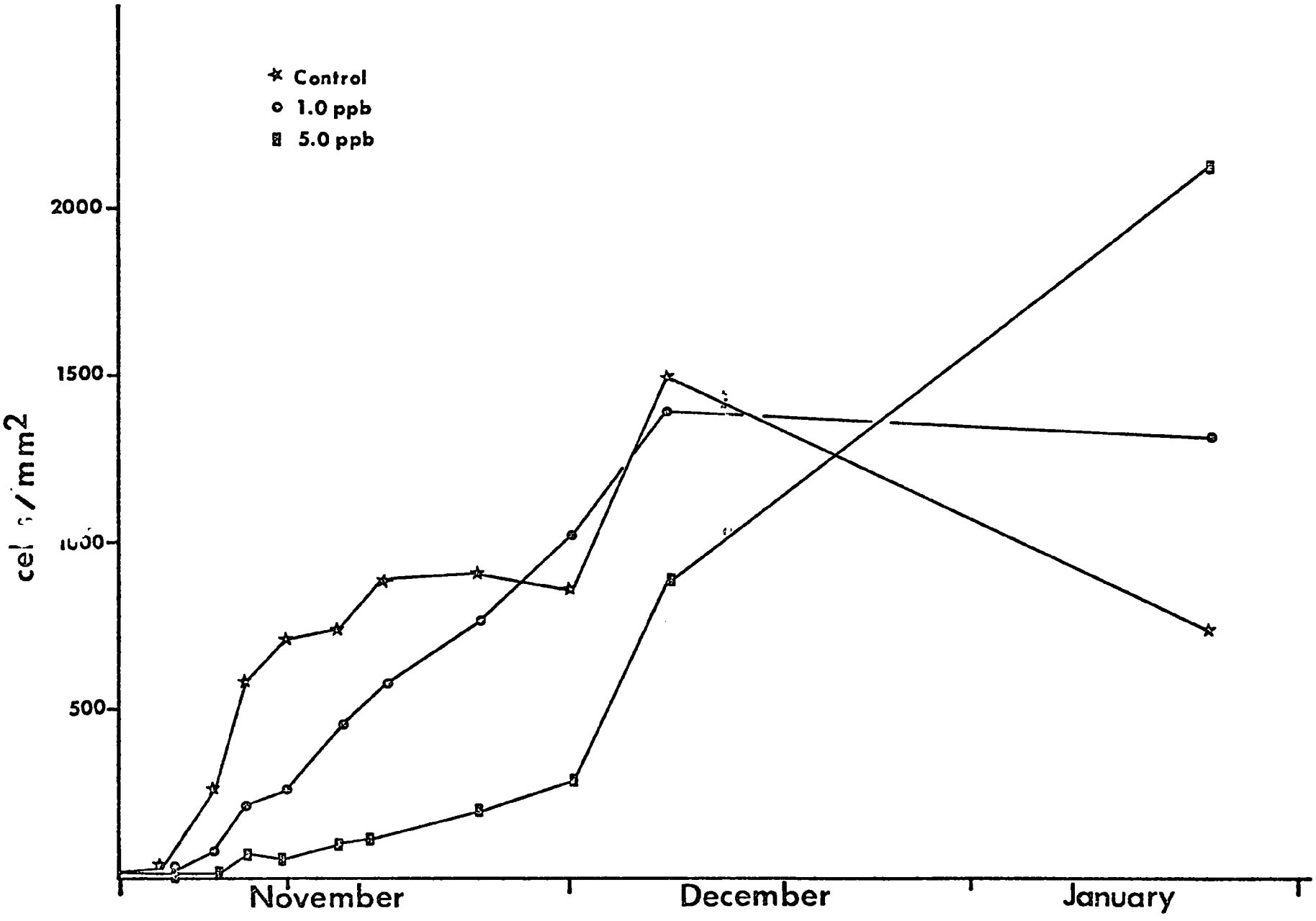
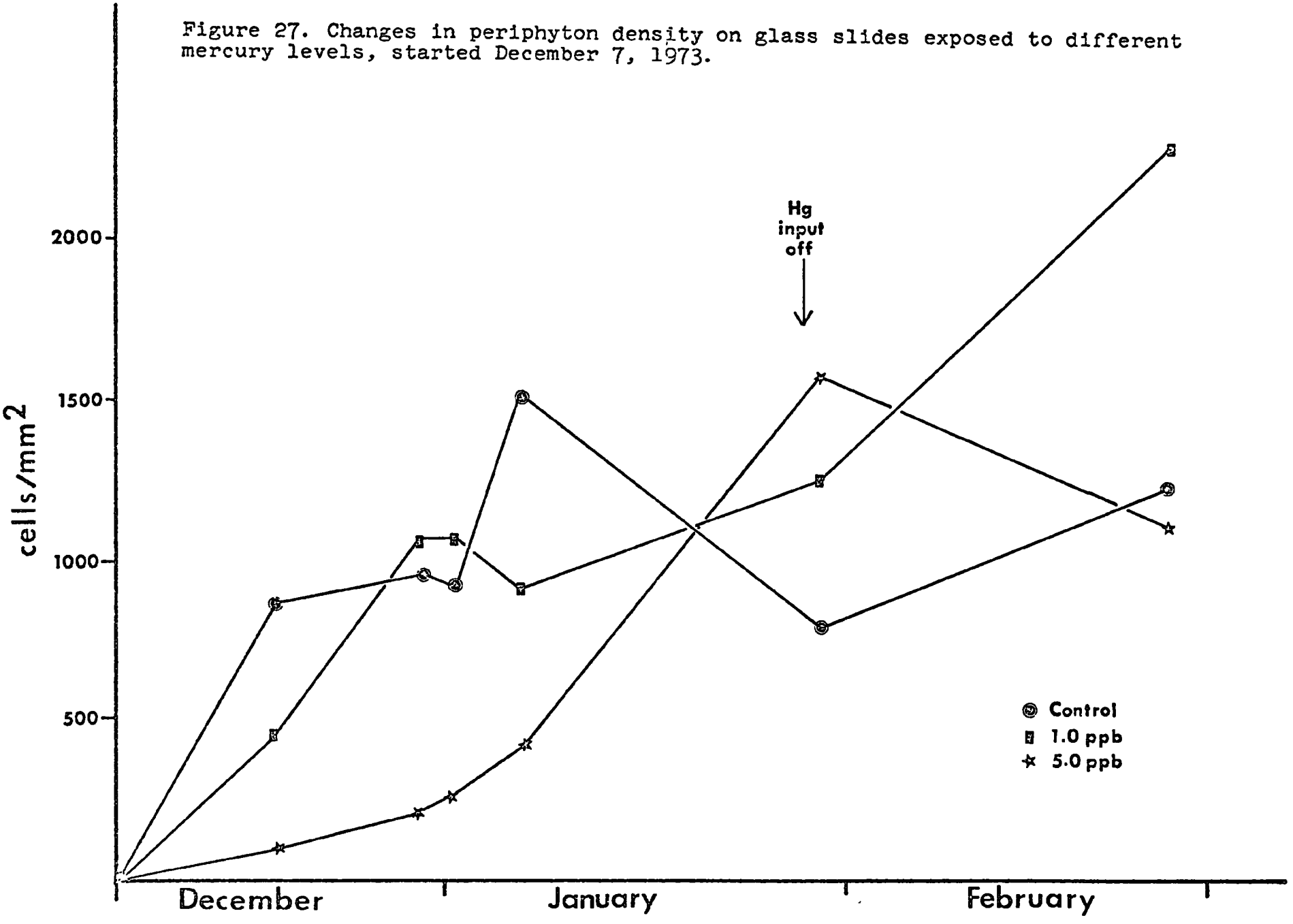


Figure 26. Changes in periphyton density on glass slides exposed to different mercury levels, started October 31, 1973.

Figure 27. Changes in periphyton density on glass slides exposed to different mercury levels, started December 7, 1973.



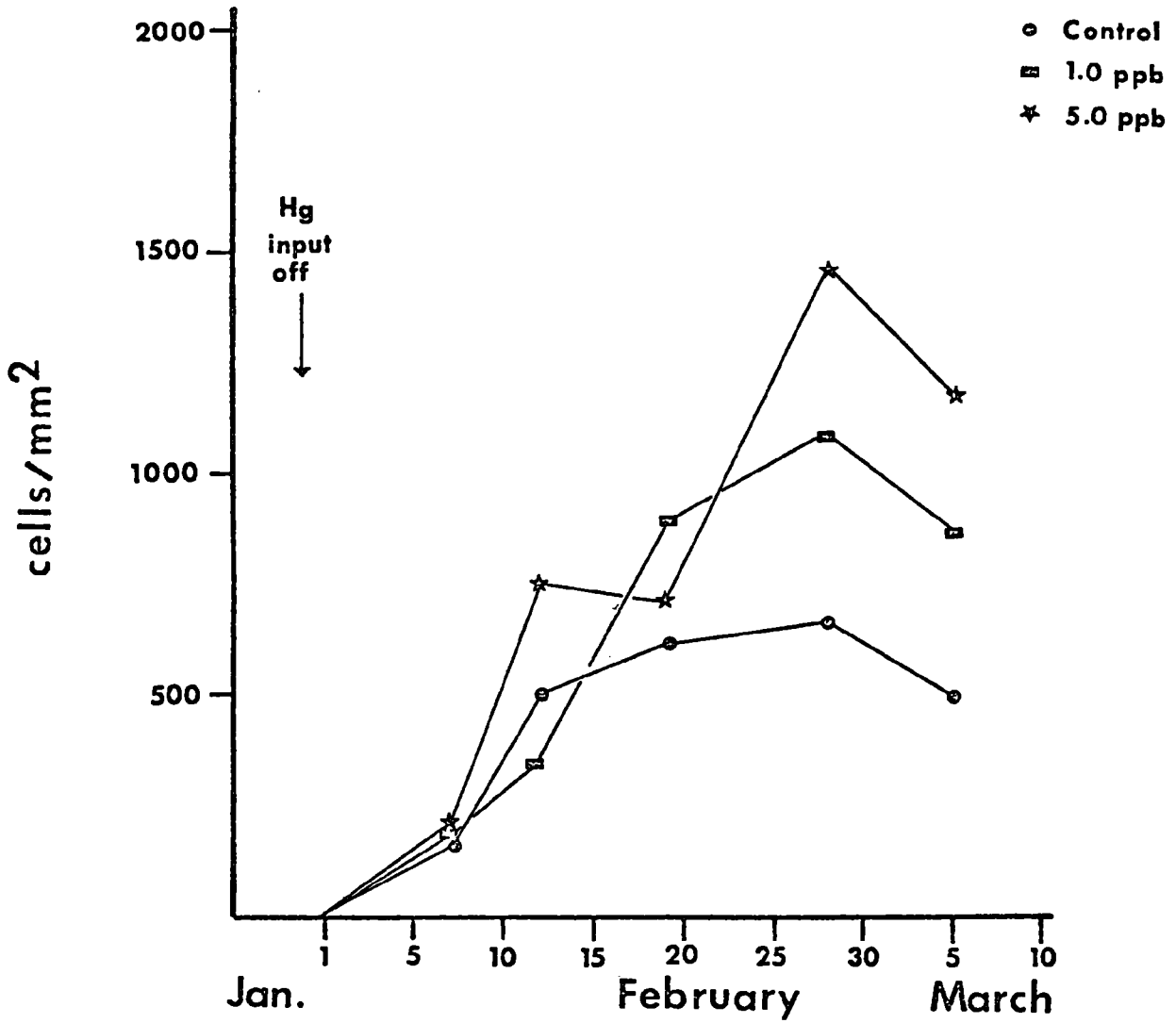


Figure 28. Changes in periphyton density on glass slides after mercury input was stopped, started January 30, 1974.

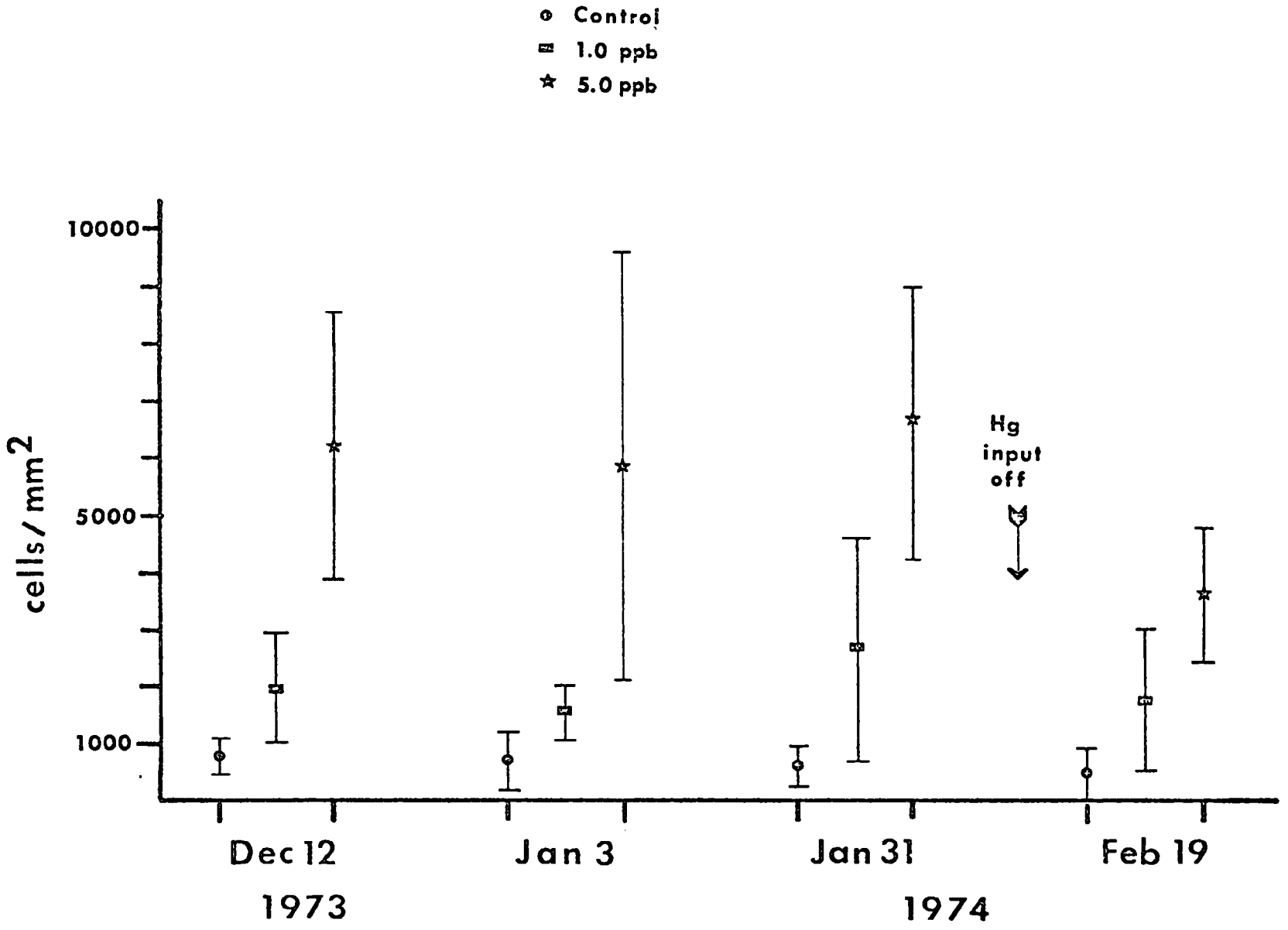


Figure 29. Periphyton density ($\bar{X}\pm 2SE$) on plexiglass plates exposed to different mercury levels.

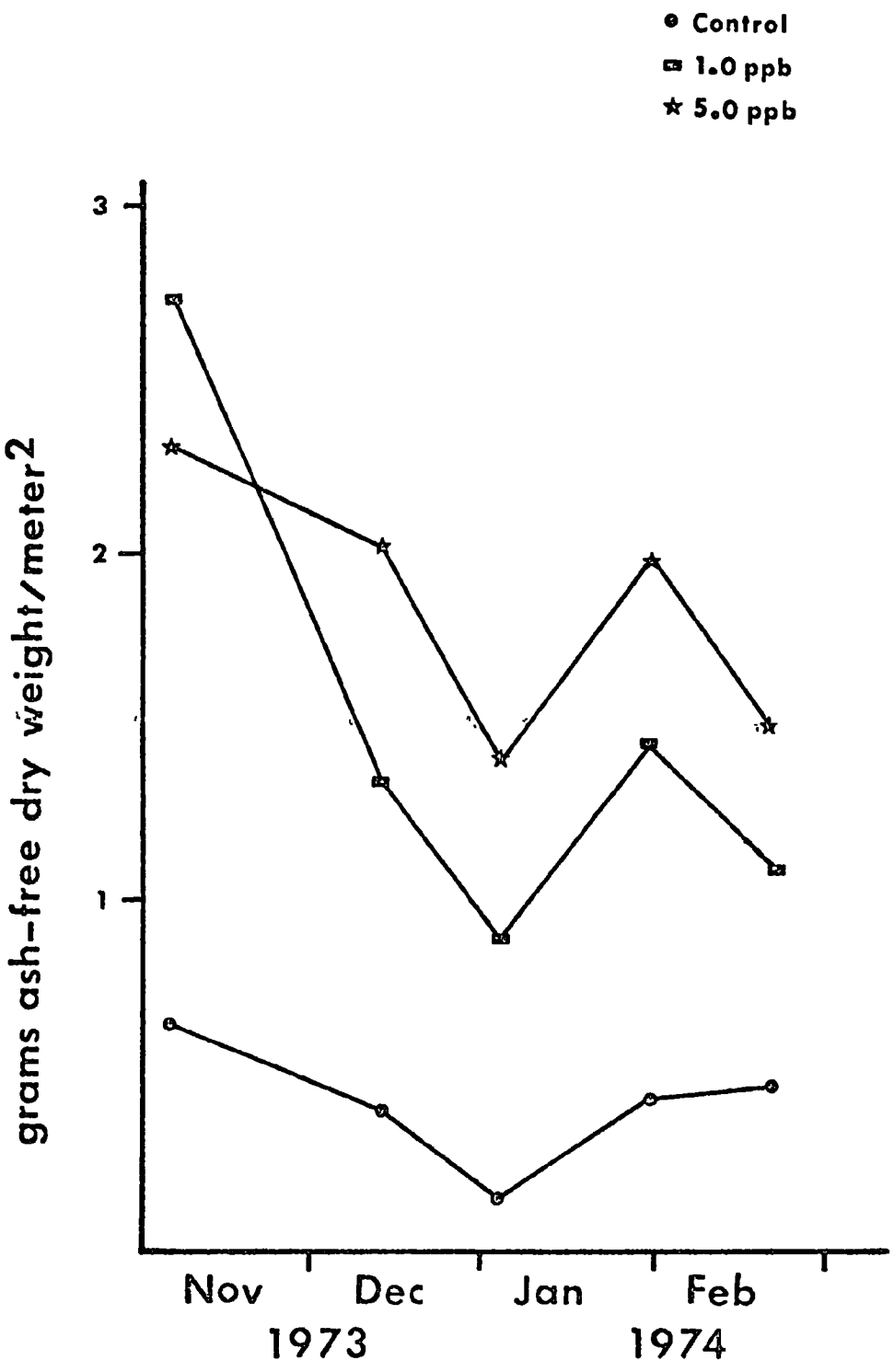


Figure 30. Periphyton biomass on plexiglass plates exposed to different mercury levels.

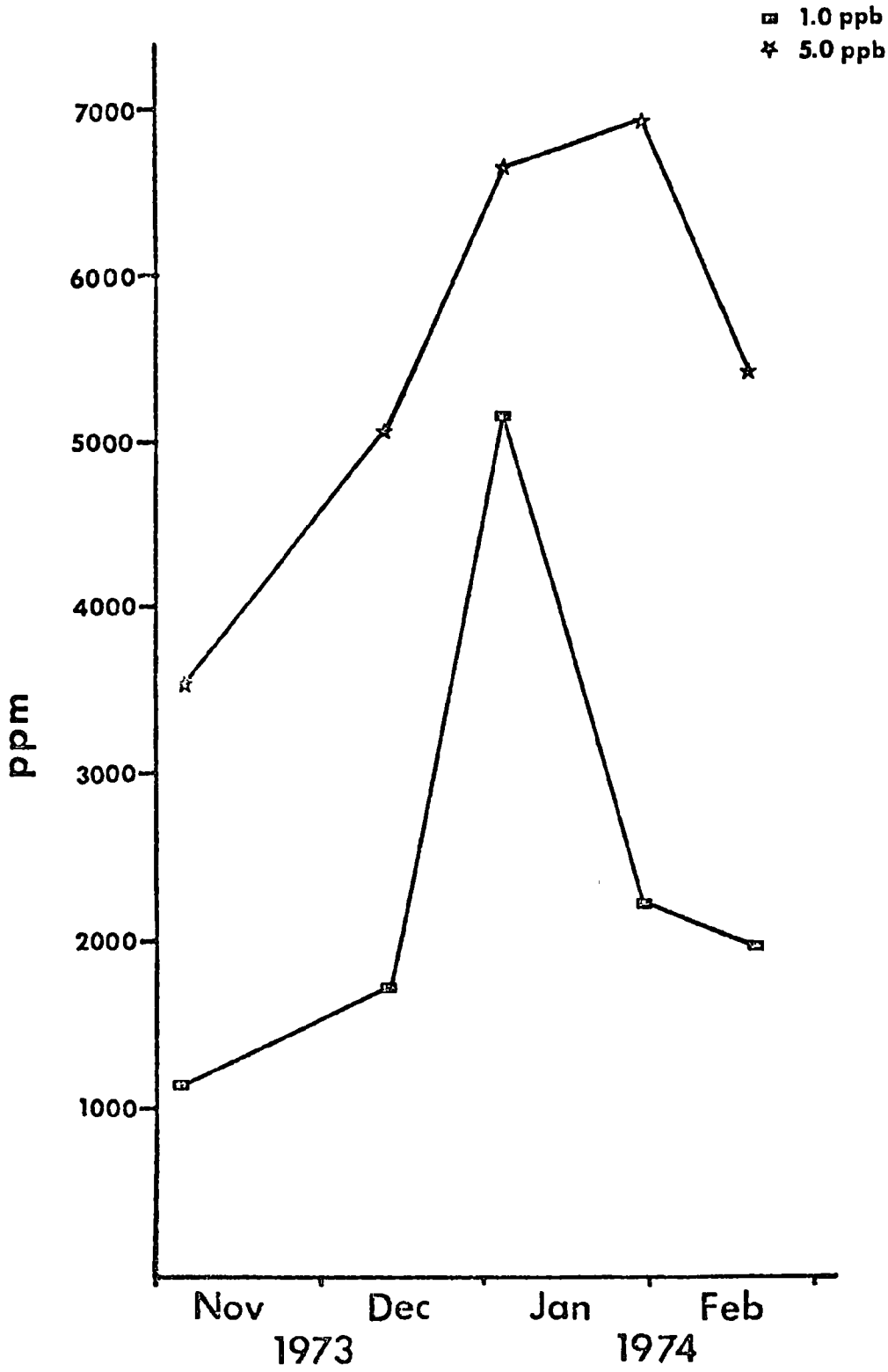


Figure 31. Concentration of mercury on an ash-free dry weight basis of periphyton scraped from plexiglass plates exposed to different mercury concentrations.

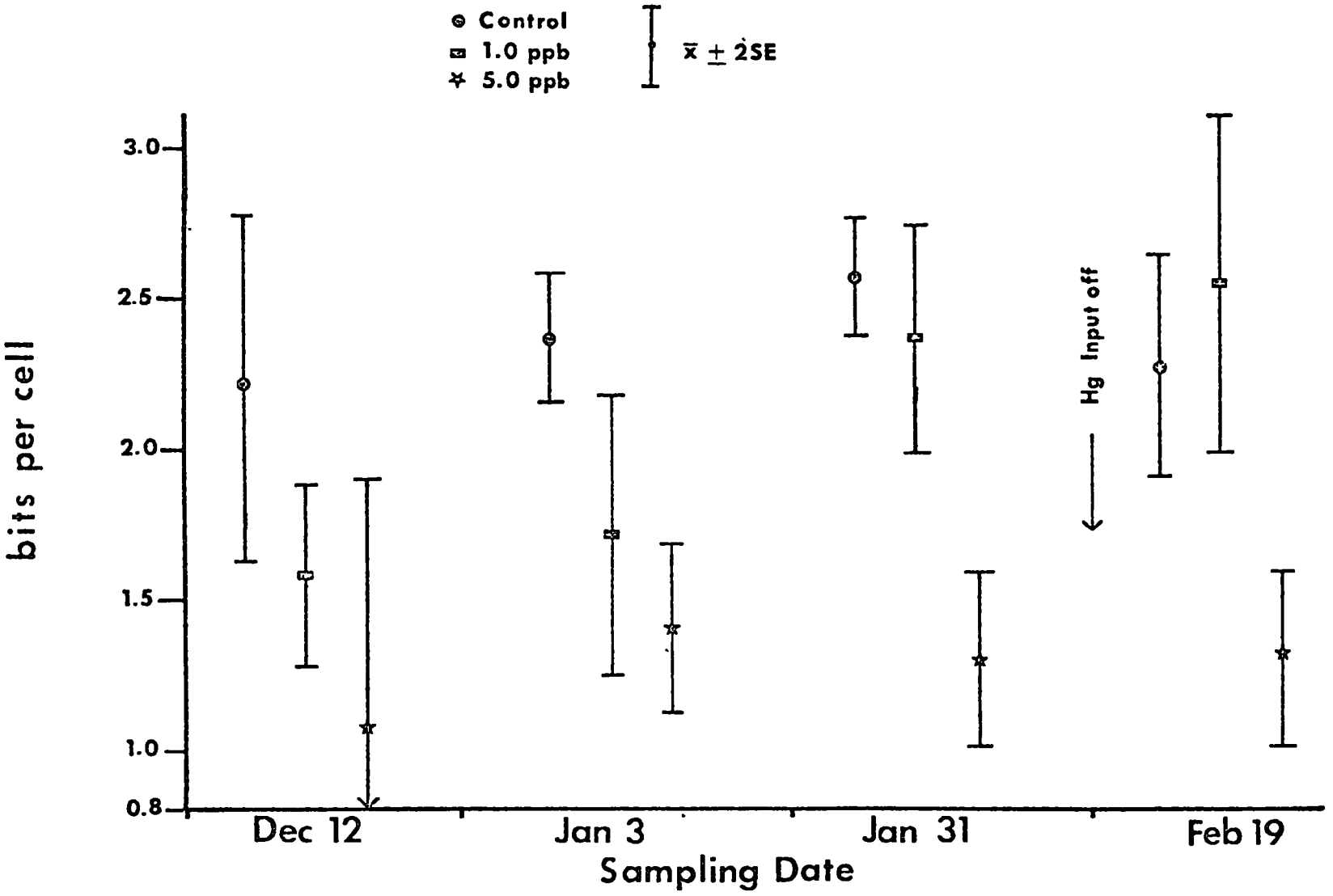


Figure 32. H' diversity ($\bar{X}$±2SE) of periphyton communities inhabiting plexiglass plates exposed to different mercury levels.

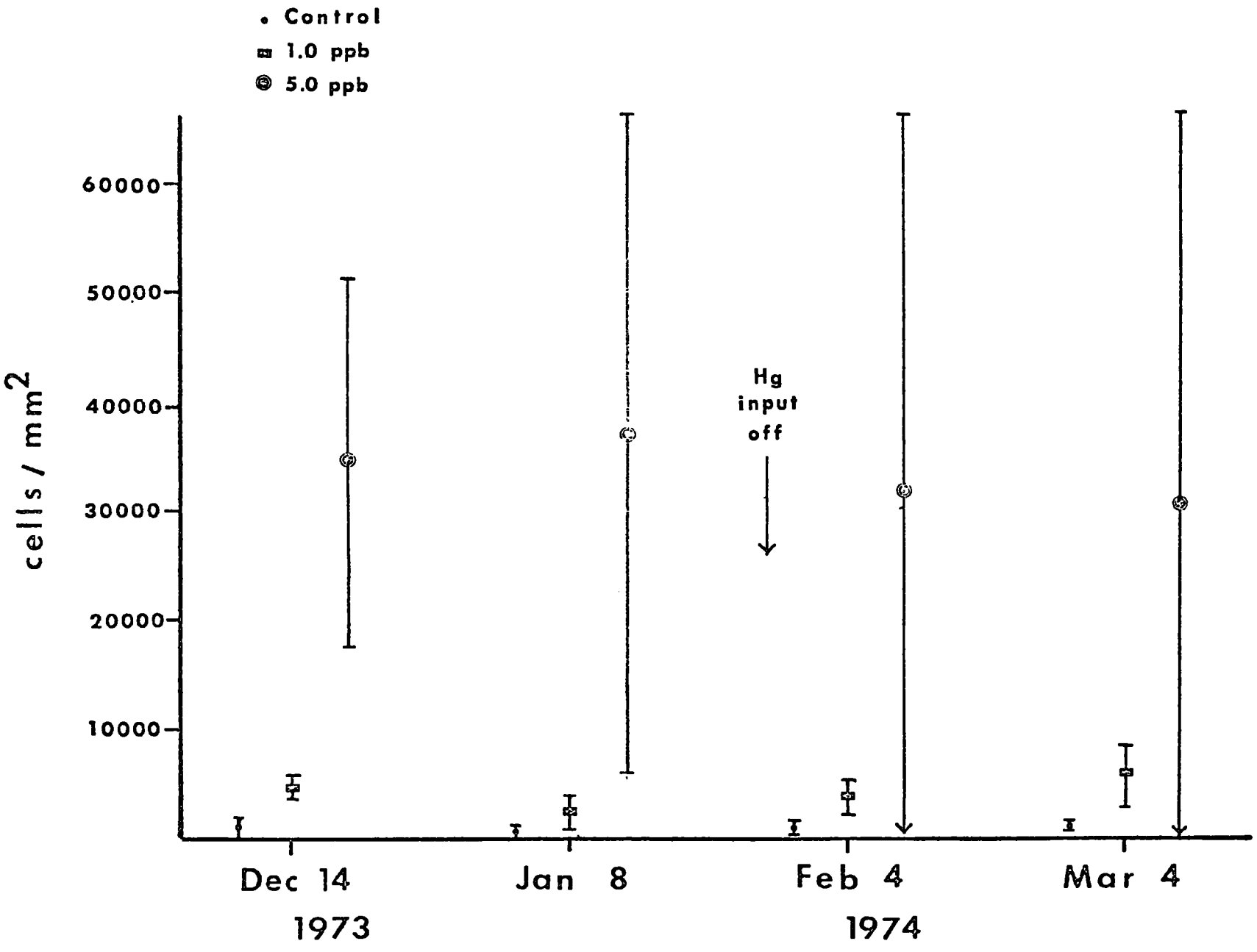


Figure 33. Periphyton density ($\overline{X}\pm 2SE$) on channel walls exposed to different mercury levels.

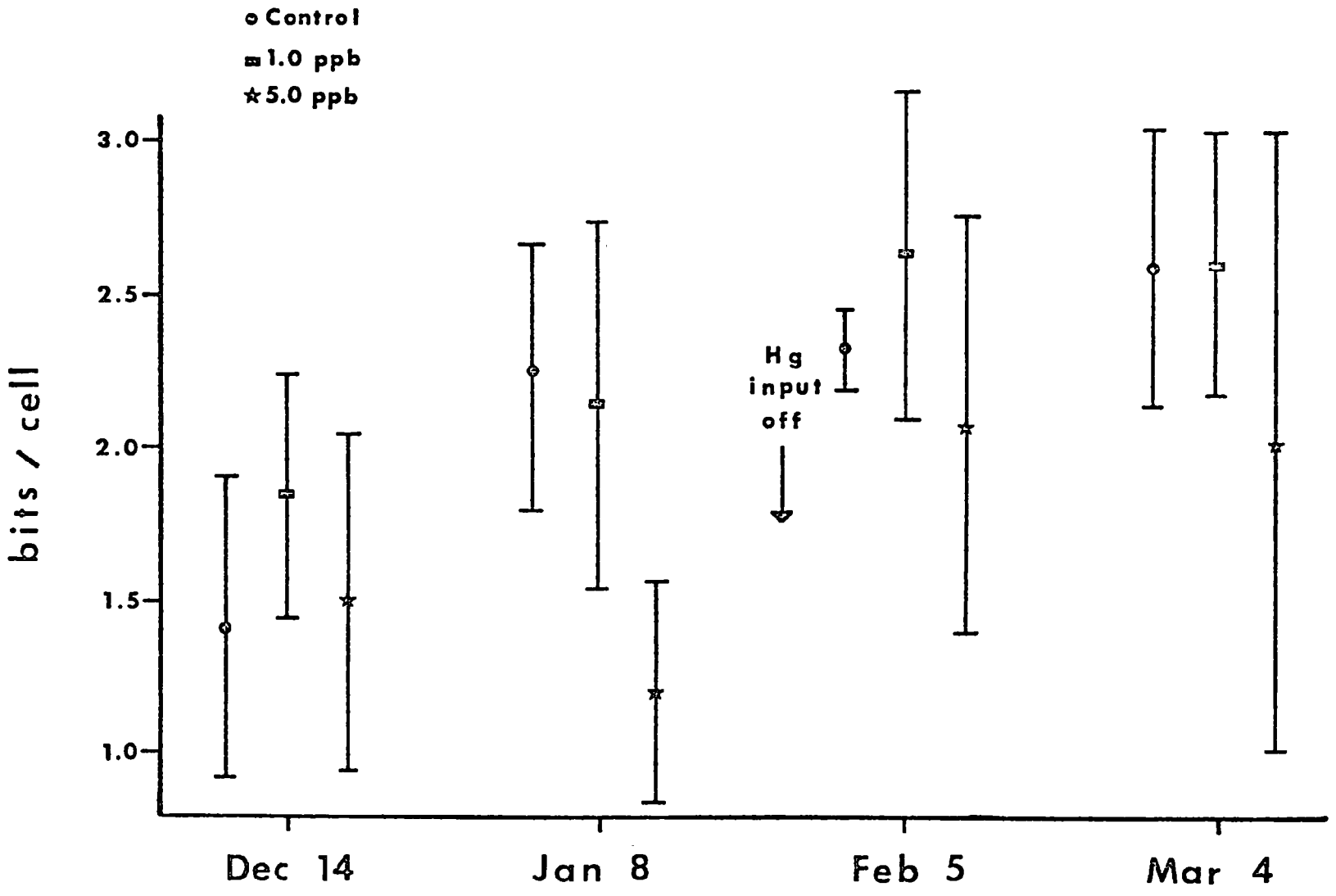


Figure 34. H' diversity ($\bar{X}$±2SE) of periphyton communities inhabiting channel walls exposed to different mercury levels.

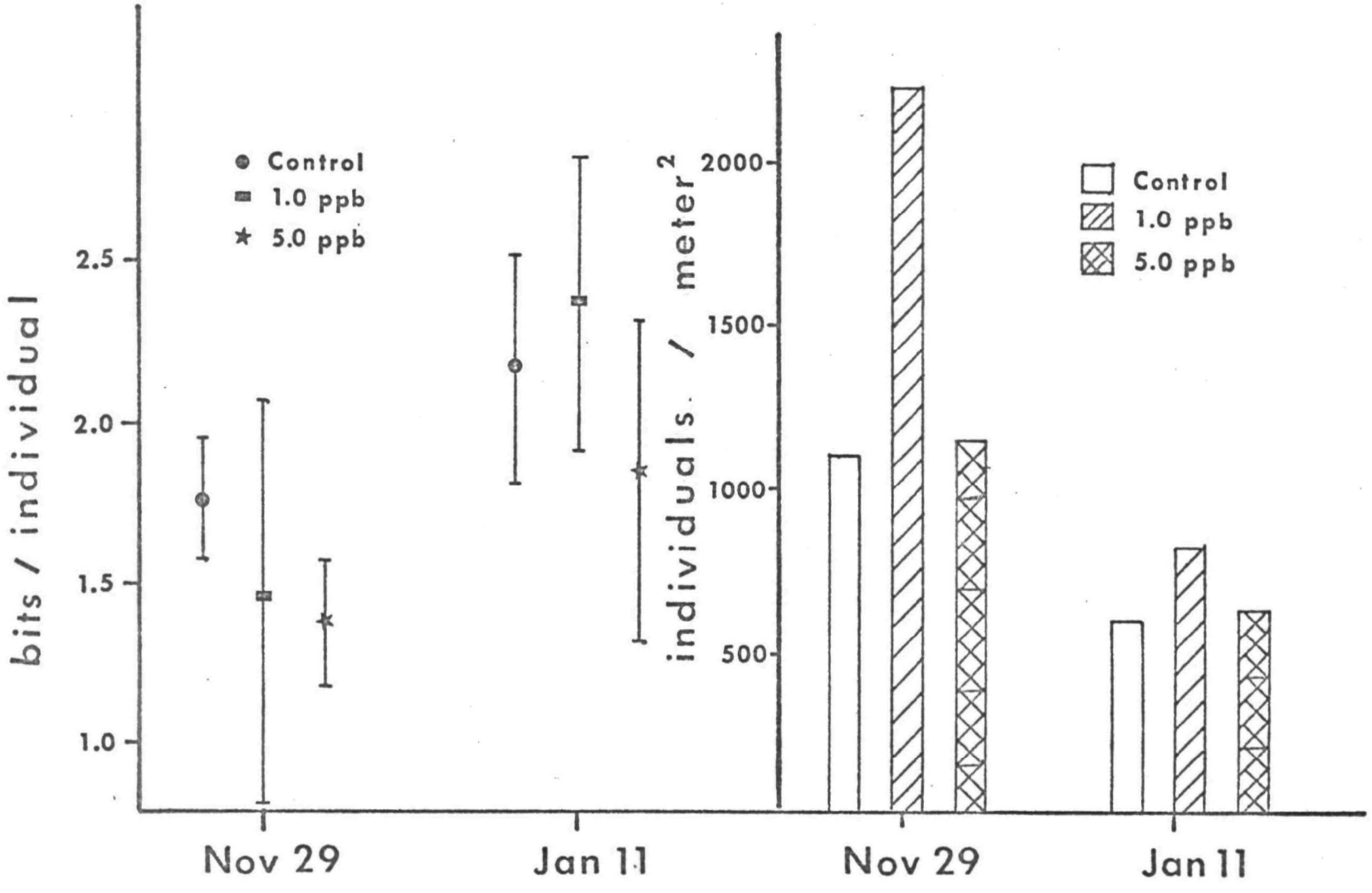


Figure 35. H' diversity ($\bar{X}\pm 2SE$) and density of benthic insects collected from channels receiving different mercury levels.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
SOUTHEAST ENVIRONMENTAL RESEARCH LABORATORY
ATHENS, GEORGIA 30601

June 13, 1974

Mr. Elton Homan
Office of Toxic Substances (WH-557)
U. S. Environmental Protection Agency
Washington, DC 20460

Dear Mr. Homan:

Enclosed is an Interim Report on "The Fate of Mercury in Artificial Stream Systems," EPA Research Grant #R800510, by Kania, Knight, and Beyers, University of Georgia. This report presents data on the transport and fate of three levels of mercuric ion (0.01, 1.0, and 5.0 ppb) introduced continuously into our artificial stream channels located at the Savannah River Plant, AEC, Aiken, South Carolina. The report presents data on the transport to and accumulation of Hg in the following areas:

- Hg removed from the water
- Hg loss at the air-water interface
- Bioaccumulation of Hg by mosquitofish
- Bioaccumulation of Hg by attached wall growths
- Accumulation of Hg in organic portion of sediments
- Accumulation of Hg on exported detrital material
- Bioaccumulation of Hg by roots of aquatic plant
- Bioaccumulation of Hg by leaves of aquatic plant

- Changes in periphyton density due to Hg inputs
- Diversity H' of periphyton communities exposed to two levels of Hg
- Diversity of aquatic insects exposed to two levels of Hg.

This information will be published as a part of the final grant report next spring; however, because of the urgent need for this type fate data we are distributing a few copies of this interim report now. I would appreciate it however if you would respect the prerogative of the authors until the information is published.

We also have a paper in progress by Dr. Holm et al. of our staff, giving both total and methyl mercury concentrations in several food chain organisms. This work was done in cooperation with Kania et al. and the paper is expected to be completed in the near future.

Hope you find the material useful.

Sincerely,

Walter M. Sanders III, Ph.D.
Chief
Freshwater Ecosystems Branch

Enclosure