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The accumulation of dieldrin and its effects on species composition of benthic algal communities were studied in laboratory streams. Effects of dieldrin on photosynthetic and respiratory rates of the blue-green alga Phormidium retzii were determined using a Gilson Differential Respirometer.

Dieldrin concentrations ranging from 0.05 to 7.0 ppb were maintained in the water of laboratory streams for periods of two to four months. In four experiments dieldrin concentrations in algae ranged from 0.1 to 200 ppm indicating that increases in concentration up to 30,000 times that of the water had occurred. Communities dominated by filamentous algae accumulated greater concentrations of dieldrin than those in which unicellular diatoms were dominant. Algae exported from the streams were responsible for removal of some of the accumulated pesticide from the system.

Regression analysis related the abundance of 15 common taxa of algae to dieldrin concentration, time, and either light intensity or current velocity. The diatoms Synedra rumpens and Achnanthes minutissima were significantly less abundant in streams receiving dieldrin. Changes in the abundance of other algae were related to variations in current velocity, time, and light intensity.

Photosynthetic and respiratory rates of P. retzii were affected only slightly by high concentrations of dieldrin. Photosynthetic rates increased during the first four hours after initial exposure to 148 ppb dieldrin. After 24 hours exposure to the same concentration there was no significant difference between the rates of treated and control material. No differences in respiratory rates were observed during the first five hours after initial exposure to 93 ppb dieldrin. However, after 24 hours exposure the respiratory rate of algae exposed to the same concentration was significantly lower than that of the unexposed material.

Effects of the Insecticide Dieldrin on Benthic Algae in Laboratory Streams

by

Frederick Louis Rose Jr.

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EFFECTS OF THE INSECTICIDE DIELDRIN ON BENTHIC ALGAE IN LABORATORY STREAMS

INTRODUCTION

During the last decade considerable controversy has developed concerning the use and abuse of highly toxic organic chemicals to control populations of plant and animal pests. Carson (9) was the first to draw the public's attention to the problem. Since the late 1940's pesticides have been applied in increasing amounts and are now used in quantities that stagger the imagination (30). The problems that have emerged from the use of pesticides result from their nonselectivity and the fact that stable chemicals accumulate and are transported about in the environment. In addition to the danger of food contamination by these chemicals, their harmful effects on so-called non-target organisms also have been involved in the controversy. Many pesticides are broad-spectrum compounds, show no toxic selectivity, and are therefore lethal to many kinds of organisms. Perhaps the most serious problems develop from chemicals which have great stability, a desirable property in terms of the intended pest. This quality contributes to persistence of such pesticides in the environment and increases the possibility of adverse effects on organisms far removed from the area of application.

One portion of our environment which regularly receives

varying amounts of these translocated chemicals is the aquatic environment. Newsom (31), Breidenbach (5), Grzenda (17), and Hinden (18) are among the numerous authors who have reported the various ways in which pesticides enter aquatic ecosystems. Except in unusual cases, the concentration of pesticide residues in water is very low, usually less than one part per billion. However, certain aquatic organisms can rapidly accumulate and store these chemicals without apparent ill effects. When organisms having such an accumulation are ingested by others, the effect may be one of two kinds: 1) further concentration of pesticide without noticeable effect; or 2) direct harm to the predatory or grazing organism. Hunt (20) termed the concentration process "biological magnification". Several well documented instances of biological magnification have resulted in the death of considerable numbers of organisms representing late or terminal stages of food chains (21, 37). Recently Woodwell (45) reported encountering increasing amounts of pesticide residues in ascending steps of an estuarine food chain. Because victims of the concentrated chemicals are frequently fish or fish-eating birds, special concern has been expressed by fisheries and wildlife biologists.

The chlorinated hydrocarbons comprise one group of toxic compounds that have had extensive application as insecticides. These chemicals include the familiar DDT and its derivatives, and

other compounds such as aldrin, endrin, heptachlor, and dieldrin. Most of the chlorinated hydrocarbons are very stable and persist for varying periods in some portion of the environment. In some instances the original compound undergoes epoxidation to form another toxic substance. Such is the case in the conversion of aldrin to dieldrin and heptachlor to heptachlor epoxide. The chlorinated hydrocarbons tend toward very low solubility in water and high solubility in fats and oils (19). The latter property helps explain the tendency of these chemicals to accumulate in the fatty deposits of organisms.

Dieldrin was the insecticide chosen for the experiments described in this thesis. This particular pesticide has been in use since 1948, and Weaver (44) reported that dieldrin residues have been detected in every major river system in the United States. The compound is very nearly insoluble in water, about 200 parts per billion, and according to Cope (10) is attracted and adsorbed to any exposed surface.

Ecological problems can be approached directly in the field, or alternatively, a segment of nature can be brought into the laboratory for examination in a controlled environment. The field approach offers a more realistic view of nature but is often made impractical by a level of complexity that defies meaningful analysis. The obvious shortcomings of laboratory investigations are that they

occasionally involve conditions too unlike those found in nature, and that information obtained in the laboratory is sometimes difficult to apply to problems in nature. McIntire and Phinney (28) and Davis and Warren (11) have studied aquatic communities in laboratory streams. The results of their experiments have indicated that a similar approach would prove useful in the investigation of pesticide problems in flowing water environments, as these facilities provided the opportunity to study lotic communities with some of the advantages of both the laboratory and field approaches.

Whenever pesticides enter a freshwater ecosystem there is some effect on the flora or fauna. Generally the effects go unnoticed or appear to be insignificant because the affected organisms are of little commercial interest. Only when damage occurs suddenly and very dramatically do we recognize the existence of a pesticide problem. In certain aquatic ecosystems, particularly lotic environments, the benthic algae comprise a major portion of the biomass and may provide substantial surface area for adsorption of dieldrin. Because these algae represent the base of aquatic food chains, any accumulation of dieldrin may be passed on subsequently to other organisms.

The general objective of this investigation was to determine the long-term effects of dieldrin on communities of benthic algae in laboratory streams. Specific objectives of these experiments

included determination of:

1) the extent to which benthic algae accumulate dieldrin;

2) the effect of dieldrin on the species composition of the communities; and

3) the effect of dieldrin on rates of photosynthesis and respiration of the blue-green alga Phormidium retzii (Ag.) Gom.

The investigation was conducted at the Pacific Cooperative Water Pollution Laboratories, Oregon State University, as a cooperative research project involving both faculty and students of the Departments of Fisheries and Wildlife and Botany and Plant Pathology.

APPARATUS AND METHODS

Laboratory Streams

Twelve laboratory streams were housed in a frame building 30 feet wide and 40 feet long. The roof and upper half of the east, west, and south side walls were covered with corrugated translucent plastic. Illumination of the laboratory streams was by sunlight only and therefore followed normal photoperiodic changes. Each stream (Figure 1) consisted of a wooden trough that measured 3 m long, 63 cm wide, and 18 cm deep. A center divider with openings at each end allowed circulation of water around the trough. The troughs were finished with a non-toxic, waterproof white enamel. The substrate in each laboratory stream was taken from a nearby natural stream and consisted of approximately 50 liters of smooth rocks 5-15 cm in diameter, and smaller gravel.

The water source for the laboratory streams was a small natural stream near the laboratory. Water was carried by a wooden flume to storage tanks and subsequently passed through a sand filter before being conducted to the laboratory through polyethylene pipes. The filter removed sticks, leaves, and other coarse debris from the water but allowed passage of smaller suspended matter.

A twelve-port manifold distributed the water through flowmeters to individual streams, and the exchange rate of water for

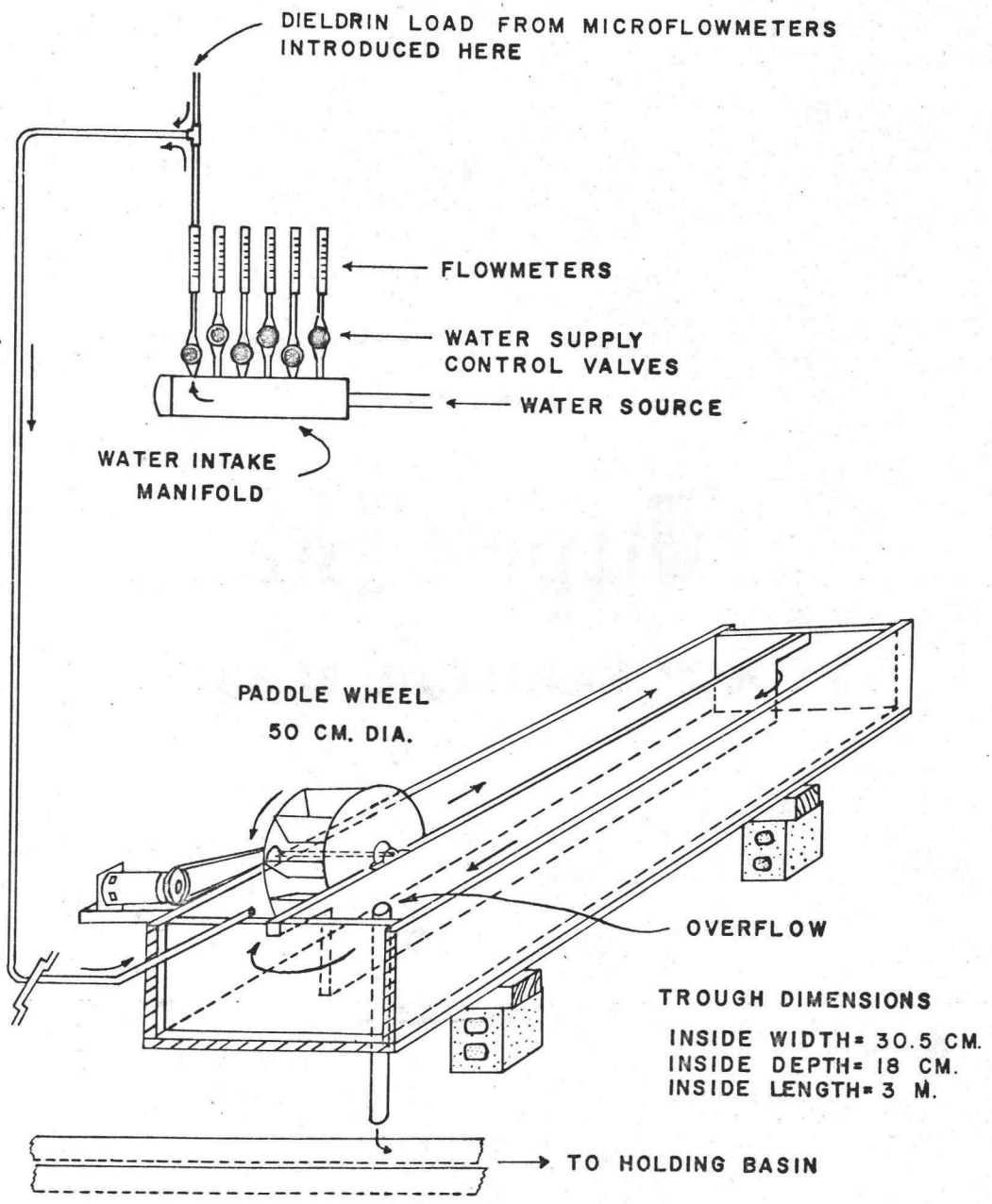


Figure 1. Diagram of one of the laboratory streams.

each stream was controlled by a gate valve. This rate normally was maintained at two l/min, and under such conditions each stream (capacity approximately 250 liters) had 85 percent renewal of the circulating water every four hours. Water depth in the streams was regulated at 16 cm by adjusting the height of overflow standpipes.

Paddle wheels operated by 1/8th horse power electric motors maintained a current velocity of 28 cm/sec and assured mixing of the circulating water. Water temperatures were recorded continuously by a Marshalltown Model 1000 thermograph. The weekly maximum, minimum and mean temperatures have been plotted in Figures 2 and 3. These data apply to all streams, as the source of the influent exchange water was the same for all streams and the water temperature never varied significantly from stream to stream. Water samples were taken from the storage tanks during the fall of 1966 and spring of 1967 for analysis of chemical characteristics (Table 1). Water quality did not change markedly with seasons, although the concentration of dissolved solids and the specific conductance were slightly higher during the fall months.

Dieldrin Introduction

The apparatus employed to introduce dieldrin into laboratory streams is shown in Figure 4. This equipment was designed and

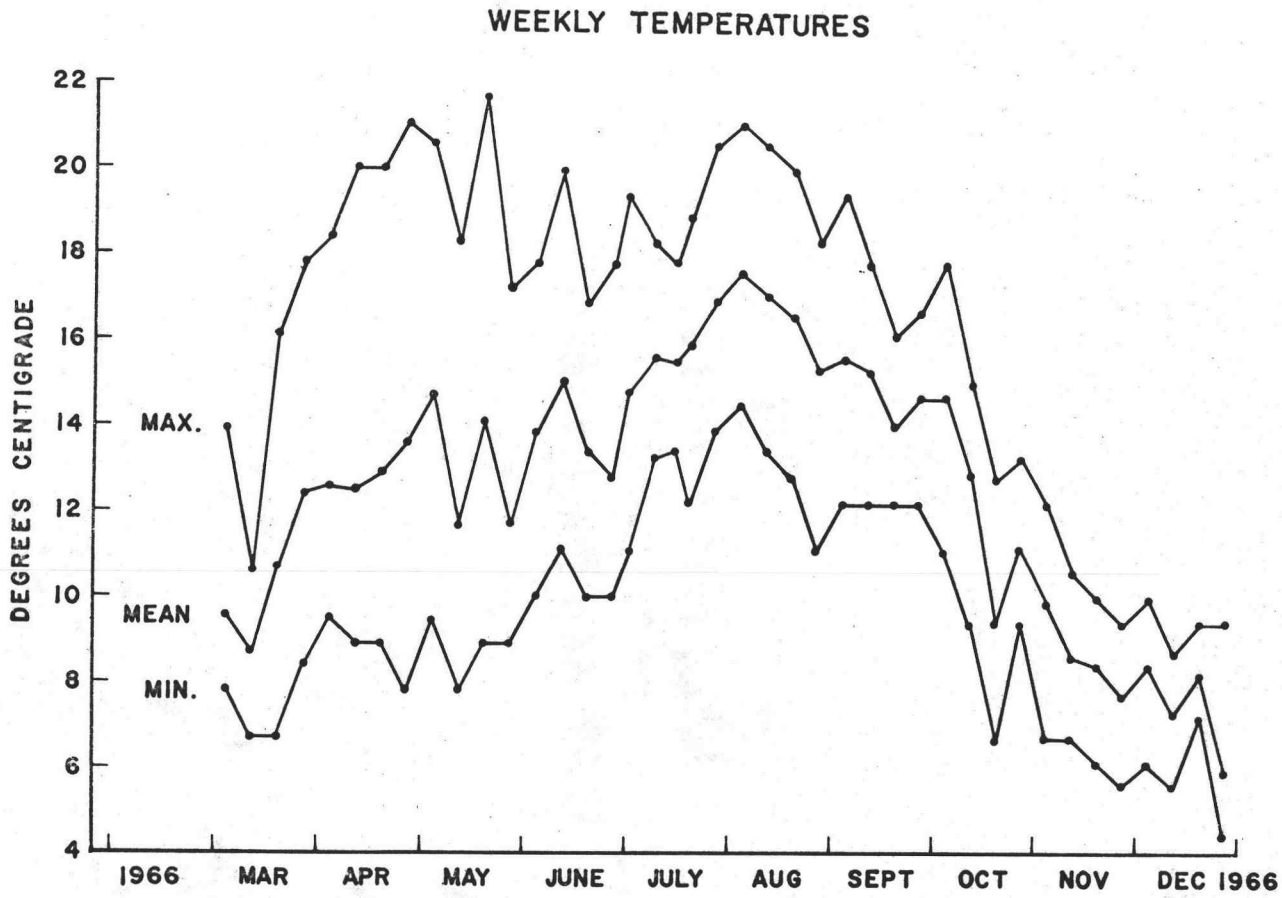


Figure 2. Weekly fluctuations in the water temperatures of the laboratory streams during 1966.

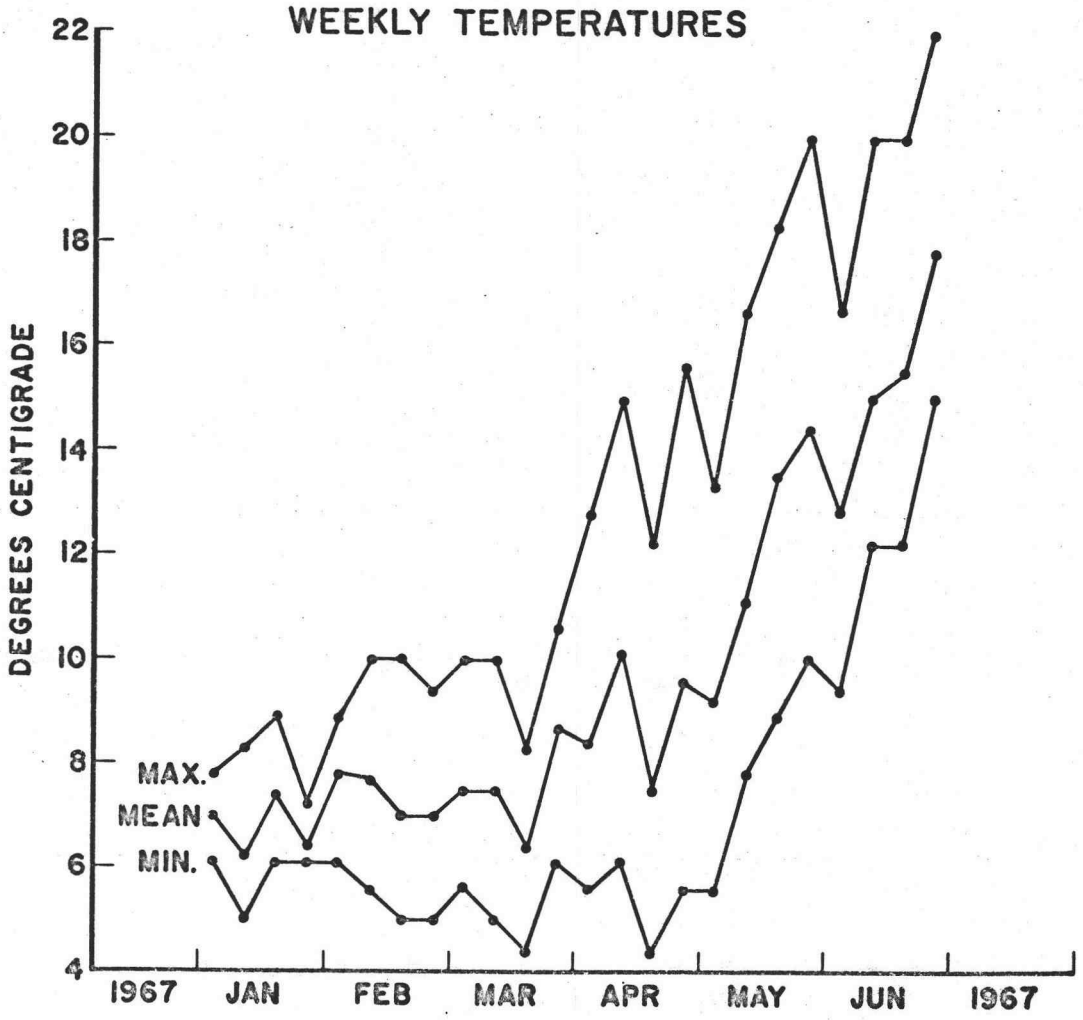


Figure 3. Weekly fluctuations in the water temperatures of the laboratory streams during 1967.

Table 1. Water quality summary for laboratory streams, 1966 and 1967.*

Characteristic or Constituent	Fall 1966	Spring 1967
Sp. conductance (micromhos at 25°C)	214	175
pH	7.3	7.7
Color	5	5
Dissolved solids mg/1	149	124
Hardness mg/1 as $CaCO_3$	98	72
Silica (SiO_2) mg/1	35	32
Iron (Fe) mg/1	--	0.17
Calcium (Ca) mg/1	25	18
Magnesium (Mg) mg/1	8.6	6.7
Sodium (Na) mg/1	9.1	8.8
Potassium (K) mg/1	0.2	0.5
Bicarbonate (HCO_3) mg/1	133	98
Carbonate (CO_3) mg/1	0	0
Sulfate (SO_4) mg/1	0.2	3.6
Chloride (Cl) mg/1	5.5	6.0
Fluoride (F) mg/1	0.1	0.1
Nitrate (NO_3) mg/1	0.3	0.1
Phosphate (PO_4) mg/1	--	--

*These analyses were performed under the direction of G. L. Bodhaine, District Engineer, Water Quality Branch, Geological Survey, United States Department of Interior.

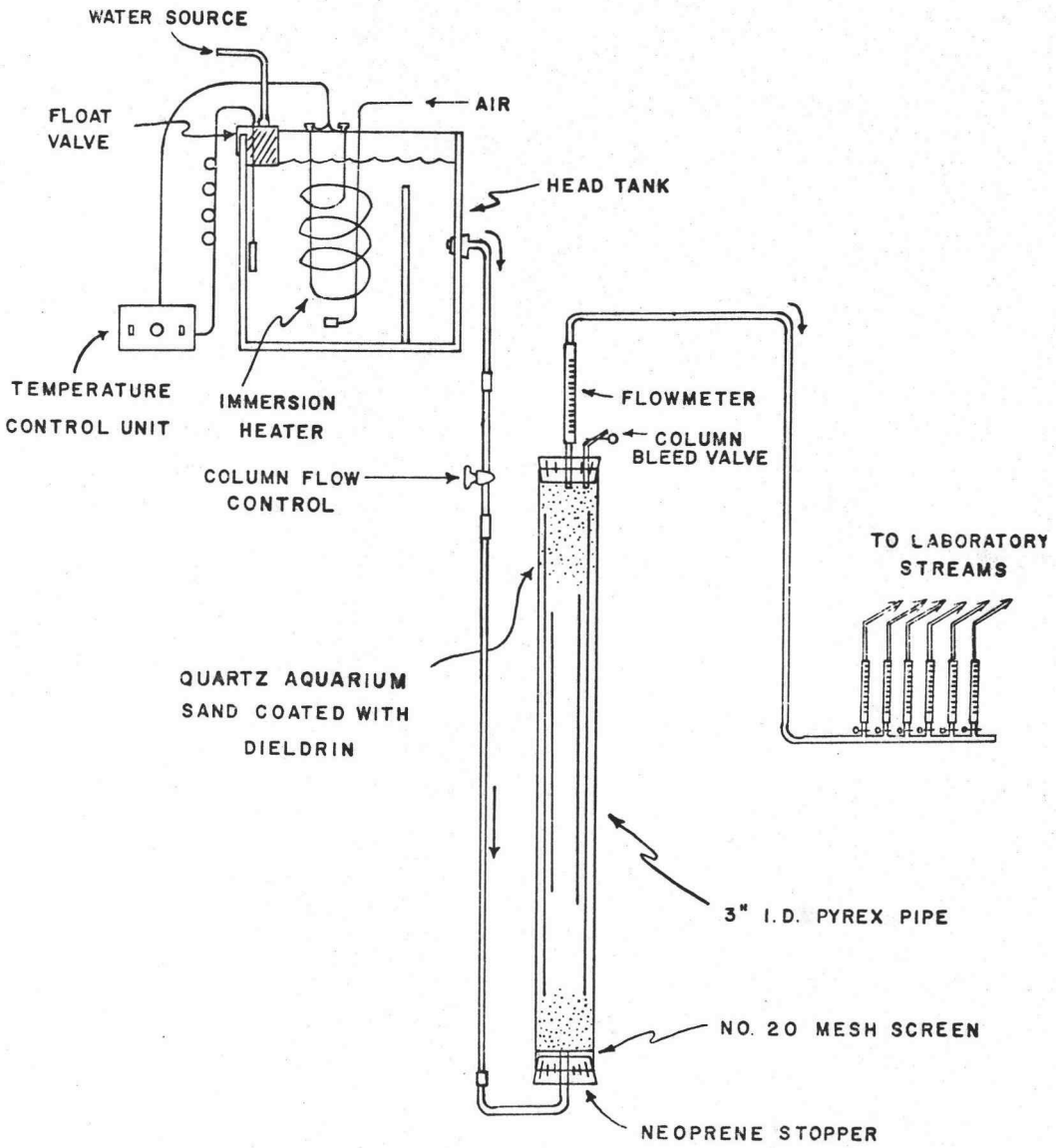


Figure 4. Diagram of the apparatus used to introduce dieldrin into the laboratory streams.

constructed by Mr. George Chadwick, Assistant Professor of Fisheries, Oregon State University. The column consisted of a 3-foot section of pyrex pipe with a 3-inch inside diameter and was filled with smooth, quartz aquarium sand. The sand was retained at each end of the column by neoprene stoppers. The column was filled with a saturated solution of dieldrin in acetone and then drained. This procedure resulted in the deposition of a fine white coating of dieldrin on the surfaces of the sand grains. The column was then connected to an air line for twelve hours to dry this deposit. Before installation, water was passed through the column at a rate of 50 ml/min for four weeks. During this time the concentration of dissolved dieldrin in the water became relatively constant.

Well water was used to introduce dieldrin into the laboratory streams. Water from the well was collected in a wooden tank which maintained a constant head and served as a reservoir. Water temperature in the head tank was maintained at 15°C, and a float valve regulated the height of the water level. Water passed from the head tank through a tygon and glass line to the base of the column. As water moved over the sand, small amounts of the pesticide dissolved. The water then passed through a flowmeter, capacity 100 ml/min, at the top of the column which allowed for rapid visual checks of the flow rate. A tygon line carried the water to a manifold which supplied eight adjustable microflowmeters.

From the microflowmeters, the well water with the dissolved dieldrin was introduced into the influent water lines of the corresponding laboratory streams (Figure 1). Weekly monitoring of the water leaving the column and careful adjustment of flow rates through the microflowmeters allowed maintenance of relatively stable pesticide levels in the streams. The effluent water from all streams flowed into a floor drain and was collected and held in a storage pond below the laboratory. Water loss from this pond was by evaporation and percolation into the ground.

Sampling Laboratory Stream Communities

The communities which developed on the stream substrates were largely a result of natural seeding of cells entering the streams through the water supply. However, in some experiments there was an additional source of organisms. Two experiments were conducted in cooperation with Mr. Charles Keeler who was studying the effects of dieldrin residues on certain aquatic insects, snails, and cottids. At the beginning of these experiments, the laboratory streams were stocked with these animals, at which time algal cells undoubtedly were introduced. The extent to which such introductions influenced the species composition of any given stream was not great, as a comparison of control streams in these experiments with those in other experiments showed a nearly identical species

composition.

During most experiments, portions of the laboratory communities were harvested to obtain estimates of biomass and organic matter per unit area. From these same periodic samples, material for determination of species composition and dieldrin analysis also was obtained. When sampling a stream, the paddle wheel, if present, was stopped to provide a standing water situation. Two watertight wooden panels were then wedged between the sides and gently forced between the rocks to the bottom of the trough. A distance of 25 cm was maintained between the panels, thus enclosing an area of approximately 760 cm^2. This area represented about 1/20 of the surface area of the colonized portion of a stream. All rocks were removed from this area and hand scrubbed to free the attached organisms. The sides of the troughs in this area were also scrubbed, and the water with the suspended material, usually about 11 liters, was siphoned from the enclosed section into a container. The entire sample was passed through a No. 20 silk bolting-cloth plankton net to concentrate the material. After removal of the insects by hand, the algae were returned to the water portion of the original sample and thoroughly mixed in a Waring blender. The volume of this suspension was measured, and subsamples were removed for the determination of biomass, organic matter, species composition, and concentration of dieldrin.

Biomass was estimated by drying and weighing 800 ml of the sample. With the knowledge of the volume of the entire sample, the dry weight of material per unit area of substrate was calculated and reported as grams per square meter. The percentage of organic matter in the biomass was determined by passing 500 ml of the sample through a Foerst continuous flow centrifuge at approximately 100 ml/min. The concentrated material was transferred to platinum dishes, dried, weighed, and incerated for six hours at 550°C, and then reweighed. The weight lost on ignition was divided by the total dry weight to obtain the percentage of organic matter (ash-free dry weight). This percentage was multiplied by the biomass to obtain estimates of organic matter per square meter of substrate. To determine species composition, two subsamples of 2.5 ml were transferred to 2-dram glass vials, and preservative added. These vials were stored until a later date when their contents were observed microscopically. One gallon of the remainder of the sample was transferred to a plastic jug and retained for dieldrin analysis.

In some of the experiments, material exported from the streams was collected by placing a plankton net under the overflow standpipe for 24-hour periods. The material was then removed from the net, dried, and weighed. To estimate the particulate organic matter that was small enough to pass through the net, one

liter of net-strained water was collected and passed through a weighed HA Millipore filter (pore size 0.45 μ). The filter and material were dried, weighed, ignited at 550°C, and reweighed. The export collected by the plankton net was expressed as grams per 24 hours and that collected by the filter as milligrams per liter.

In Experiments 1, 2, and 4, water samples taken for dieldrin analysis were obtained directly from the circulating water. One gallon of water was dipped from each stream and poured through a No. 20 plankton net to remove any free-floating algae which had become dislodged from the substrate. During the third experiment, water samples were collected directly from the influent and effluent lines and passed through the plankton net. While this straining procedure removed most of the suspended algae, it failed to eliminate the smaller particulate organic matter.

Analysis of Samples for HEOD

Dieldrin is the common name for the insecticidal product which contains not less than 85 percent 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene (HEOD) and not more than 15 percent of related compounds, probably aldrin and/or endrin. The analysis of samples for commercial dieldrin involves the determination of the concentration of HEOD (Figure 5). Thus the concentrations of

dieldrin reported in this thesis are actually concentrations of HEOD.

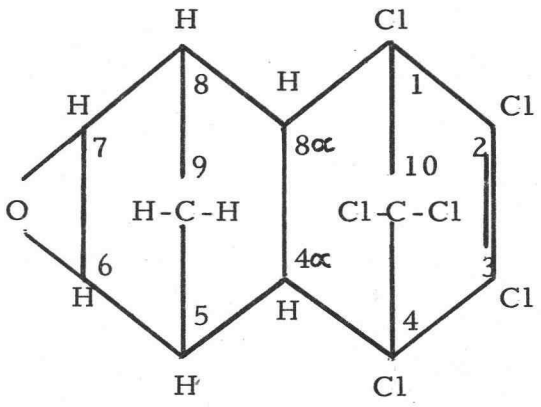


Figure 5. Primary constituent (HEOD) of dieldrin.

The algal suspensions were stored in sealed, one-gallon plastic containers and were either frozen or kept at 1-5°C. In preparing an algal sample for HEOD analysis, the suspension was first vacuum filtered. The filtrate was saved and in some instances analyzed for HEOD so that comparisons could be made with water samples taken directly from the laboratory streams. A subsample of moist algae was removed from the filter, weighed, and dried at 110°C to constant weight. This procedure allowed calculation of the dry weight of the algal subsample. The remaining moist algae and filter paper were then weighed, placed in a 400 ml Sorvall omni-mix cup along with 150 ml of 1:1 hexane-isopropanol, and mixed at high speed (6000 rpm) for three minutes. The mixture then was vacuum

filtered, and the filtrate was transferred to a one liter separatory funnel. To insure complete extraction of HEOD from the mixture, the algal suspension was returned to the onmi-mix step and this and the subsequent vacuum filtration were repeated. The isopropanol was washed out by adding 500 ml of distilled water to the separatory funnel and shaking. Because persistent emulsions sometimes formed during this step, only gentle shaking was employed. After draining most of the water from the funnel, the remaining water was separated from the hexane by pouring the mixture through 10 grams of $CaCl_2$. The water-free hexane was reduced to 10 ml by evaporation and added to a clean-up column. Elution with 70 ml of hexane removed any aldrin present. After changing the collection flasks, the HEOD was eluted with 150 ml of 15 percent ethyl ether in hexane (v/v). This fraction contained the HEOD and was ready for analysis on the gas chromatograph. The procedure outlined above required slight modification for some samples, either because of emulsion formation or HEOD levels different from those anticipated.

Water samples were stored at room temperature in one gallon glass containers. A 1500 ml portion of the water sample was transferred to a 2-liter separatory funnel, and 100 ml of redistilled hexane was added. This mixture was placed on a shaker for 10 minutes. After shaking, all but approximately 100 ml of the water was discarded. To break any emulsion, 5 ml of isopropyl alcohol

was added to the remaining hexane and water. The remaining water was then discarded and the hexane fraction was dried by pouring it through 15-20 grams of anhydrous Na_2SO_4. This hexane fraction contained the HEOD and was stored in bottles until it could be analyzed on the gas chromatograph.

Although several gas chromatographs were used, the following specifications represent conditions for a typical analysis. A MicroTek 220 gas chromatograph with an electron capture detector and a 74 by 0.0787 inch I.D. soft glass column was filled as follows: the first four inches--void; the next 45 inches--5% QF1 and the last 25 inches--5% DC11, both on Gas-Chrom Q. The column temperature was 167°C, injection temperature 190°C, and detector temperature 187°C. The detector potential was 20 V; the carrier gas was nitrogen at 40 ml/min; and the standard current was 1920 chart units at 10X1X.

Evaluation of Species Composition

The quantitative evaluation of species composition of benthic algal communities in streams presents some difficult problems. This is especially true when the communities consist of complex mixtures of unicellular diatoms, filamentous diatoms, and filamentous chlorophytes, chrysophytes, and cyanophytes. Patrick and Wallace (33) described a procedure in which large numbers of

individual cells were counted during microscopic examination of permanent mounts. Unfortunately, direct cell counts are very difficult to obtain for some samples of benthic algae because of the filamentous nature and very small size of some common species.

To overcome some of the difficulties imposed by a variety of growth forms encountered in the laboratory streams, a procedure based on collection of "presence or absence" data was developed. This method provided information on the relative abundance of the algal species comprising the major part of the biomass of a community, but fails to quantitatively evaluate the distribution and abundance of rare species.

Measurement of Abundance

Briefly, the procedure involved the preparation of permanent microscope slides of acid-washed diatom frustules from the material preserved in one of the 2-dram vials. This cleaning and mounting procedure has been described in detail by Patrick and Reimer (34). The permanent slides were examined under 200X, 500X, and 1250X magnification, and the diatoms were identified and recorded on data sheets. Species of algae other than diatoms were identified and added to the list after examination of a wet mount of preserved material from the other 2-dram vial. After preparing a second wet mount, the cover glass was sealed with paraffin oil, and the

slide was examined under 500X magnification using both bright field and phase contrast illumination. Fifteen random fields were viewed, and the presence of each species was noted on the data sheet. The same procedure was repeated using a third wet mount until a total of 30 fields had been evaluated for each sample. Abundance of a species was expressed as the proportion of the 30 fields in which it occurred.

The term "abundance unit" was introduced to indicate the presence of a particular taxon in one of the 30 microscopic fields examined during the evaluation of species composition. Therefore, if a certain species was present in 10 out of 30 fields in a particular sample, 10 units of abundance was assigned to that species for that sample. A species composition ratio (Tables 3, 4, and 6) given for some of the algal samples was based on these abundance units. This ratio was calculated by totaling the abundance units for all filamentous algae and dividing by the total number of abundance units of all unicellular diatoms. The resulting values served as an indicator of changes in community structure.

Statistical Procedure

The method of multiple regression has become a standard tool for the analysis of various kinds of biological data. For a detailed description of the applications of multiple regression and the general computing procedure, the reader may refer to any standard textbook

of statistical methods (25, 39). While the method is used to indicate numerically the degree to which variables vary together, caution should be exercised in assuming causal relationships between variables, and the interpretation of results must be based on a sound scientific knowledge and understanding of the particular phenomena involved.

Analysis of the species composition data from Experiments 2, 3 and 4 involved fitting regression response surfaces that related the abundance of each of the different species of algae to time, dieldrin concentration, and either light intensity, or current velocity. Although the general method of multiple regression was used, the fact that abundance was expressed as a proportion of the number of fields in which a particular species occurred rather than as the number of individuals, imposed a constraint which increased the complexity of the analysis considerably.

With data in which abundance of a species is expressed as a proportion, the maximum likelihood estimates of the partial regression coefficients should give predicted values of abundance which are subject to the restriction, $0 \leq \hat{P} \leq 1$. In order to fulfill these constraints without undue difficulty, the observed proportions were transformed into probits, and the analysis of the probit values was conducted. A probit analysis is normally used as a statistical treatment of the sigmoid response curve in bioassay work, but for the

purposes of this analysis it was used only as an arbitrary transformation to remove troublesome constraints. A mathematical treatment of the probit and normit transformations and some convenient conversion tables are available in publications by Finney (14) and Berkson (2).

A normit of the proportion P is defined as the abscissa which corresponds to a probability P in a normal distribution with a mean of zero and a variance of one; a probit is the normit plus five. With this transformation, the observations may assume any value from $-\infty$ to $+\infty$. Frequently, the observed proportions of abundance were either zero or one. When this occurred, these values were approximated before transformation by the expressions

$$0 \sim 1/2\ n \qquad \text{and} \qquad 1 \sim (2n-1)/2n$$

where n was 30, the total number of microscope fields examined.

The entire analysis was performed with a Control Data Corporation 3300 Computer at the Oregon State University Computer Center using a modification of their standard stepwise multiple regression program. Modifications provided for iterative weighted stepwise regression under specified transformation, where the weights are a function of the estimated parameters. This modification was developed by Dr. W. S. Overton of the Department of Statistics, Oregon State University. The program provides a general

analysis of quantal response data under a variety of possible transformations.

After the appropriate model equation was selected, the variables were introduced along with weights and probits obtained from Finney's tables, and the first estimates of the partial regression coefficients were computed by the normal stepwise procedure. The predicted probits ($\hat{Y}$) resulting from the first estimates were converted back to predicted proportions ($\hat{P}$) by the relationship

$$\hat{P}_i = (1/\sqrt{2\pi}) \int_{-\infty}^{\hat{Y}_i - 5} e^{-x^2/2} dx. \qquad (1)$$

Next, a new set of y's (working probits) was calculated from the general expression

$$y_i = \hat{Y}_i + (p_i - \hat{P}_i)/z_i \qquad (2)$$

where p_i was the observed proportion; $\hat{P}_i$ was the predicted proportion; and z_i was the ordinate given by

$$z_i = (1/\sqrt{2\pi})\ e^{-(\hat{Y}_i - 5)^2/2}. \qquad (3)$$

New weights also were calculated from the equation

$$w_i = z_i^2/\hat{P}_i(1 - \hat{P}_i). \qquad (4)$$

The stepwise procedure was then repeated using the new set of y's, the new weights, and the original independent variables, i.e., time, dieldrin concentration, and light intensity or current velocity. The entire procedure was repeated until a total of five iterations had been performed. By the end of the fifth iteration, the partial regression coefficients were considered to be satisfactory estimates if changes from the fourth iteration were slight.

In addition to the partial regression coefficients, the final computer output includes the corresponding t-values for the coefficients, a set of predicted proportions $\hat{P}$, and the coefficient of determination R^2. The t-values may be used to test whether or not the regression coefficients are different from zero at some arbitrarily selected significance level. The significance of each coefficient is based on the amount of additional variation that can be associated with each corresponding variable when the variable enters last in the stepwise procedure. The coefficient of determination R^2 is the square of the multiple correlation coefficient and is equal to the regression sum of squares divided by the total sum of squares. This statistic is of particular interest because it indicates the proportion of variance in the dependent variable which can be associated with variations in the independent variables. All R^2's, variances, and tests were left in terms of the weighted variables.

Manometric Methods

Estimates of the effect of dieldrin on rates of respiration and photosynthesis were based on measurements obtained with a model GRP 14 Gilson Differential Respirometer (16). To estimate the rate of photosynthesis, portions of algal material consisting primarily of Phormidium retzii, but also including some associated diatoms and bacteria, were detached from rocks and transferred to 16 ml reaction vessels containing 5 ml of a 0.05 M $NaHCO_3$ buffer. In four vessels the buffer solution included 148 ppb HEOD, a concentration near saturation. Two vessels without HEOD served as controls. The six vessels were mounted on the respirometer and allowed to acclimate for one hour to the experimental conditions. Changes in gas volume were recorded every 30 minutes for several hours.

For measurements of photosynthesis, the temperature of the water bath in which the reaction vessels were submerged was maintained at 15 ^{o}C. The shaking rate of the respirometer was 110 oscillations/min, and the illumination intensity was 800 foot candles. Since carbon dioxide was supplied as bicarbonate from the buffer, the changes in gas volume were attributed to oxygen evolution and used as estimates of photosynthetic rates. Previous experiments indicated that this buffer was an adequate CO_2 source for the time

period involved in these determinations.

For estimates of rates of respiration, algal material was placed in six vessels containing 5 ml of Millipore filtered (pore size 0.45μ) creek water. Four of the vessels also contained 93 ppb HEOD, and two were left as controls. Two-tenths milliliter of a 10 percent KOH solution and a filter paper wick were added to the centerwell of each vessel to absorb the carbon dioxide released during respiration. After acclimation for one hour, measurements were made in the dark at a temperature of 15°C and a shaking rate of 110 oscillations/min. Manometer readings were recorded at hourly intervals for five hours.

After the measurements were made, contents of the flasks were filtered, dried, weighed, ignited in a muffle furnace at 550°C, and reweighed to determine the ash-free dry weight of material. Rates of photosynthesis and respiration were expressed as microliters of oxygen per hour per milligram of organic matter.

EXPERIMENTAL PROCEDURES

Experiments in the Laboratory Streams

The determination of dieldrin uptake by algae under different sets of physical conditions and the effect of dieldrin on species composition were the principal objectives of the laboratory stream experiments. Four experiments were conducted during an 18-month period extending from December, 1965, to June, 1967. Before the beginning of an experiment, the streams were scrubbed and cleaned, and sufficient time was allowed for the establishment of a benthic algal community. The colonization period varied from four to six weeks depending upon the season. The laboratory conditions during the experiments are summarized in Table 2.

Experiment 1 - Preliminary Study to Determine Rates of Dieldrin Accumulation

Experiment 1 extended from 22 February, 1966, to 18 April, 1966. In addition to the previously stated objectives, this experiment provided the opportunity to test and modify the apparatus, and to determine the feasibility of maintaining low levels of dieldrin in the water for prolonged periods of time. At the start of the experiment each stream was stocked with 100 grams of the snail Oxytrema silicula Gould and an undetermined amount of aquatic

Table 2. Experimental conditions in the laboratory streams during four experiments conducted from December, 1965 - June, 1967.

Experiment	Stream	HEOD concentration of water (ppb)	current velocity (cm/sec)	light intensity
1	21	0.1 - 0.25	28	full sunlight
	24	0.1 - 0.20	28	full sunlight
	22	0.05	28	full sunlight
	25	0.05	28	full sunlight
	23	none added	28	full sunlight
	26	none added	28	full sunlight
2	27	none added	28	full sunlight
	28	0.4 - 0.6	28	full sunlight
	29	none added	0	full sunlight
	30	0.4 - 0.6	0	full sunlight
3	21	0.3 - 0.6	28	full sunlight
	25	0.3 - 0.6	28	full sunlight
	22	none added	28	full sunlight
	26	none added	28	full sunlight
	23	none added	28	shaded
	24	0.3 - 0.6	28	shaded
4	27	none added	28	full sunlight
	28	3.0 - 6.0	28	full sunlight
	29	none added	0	full sunlight
	30	3.0 - 7.0	0	full sunlight

insect larvae. Seven grams of the sculpin Cottus perplexus Gilbert and Everman were introduced into three of the streams and 28 grams into the other three streams. Dieldrin was introduced into four laboratory streams, and two were left as controls. The HEOD concentrations of the water were maintained between 0.1 and 0.3 ppb in two streams and between 0.05 and 0.07 ppb in the other two. The current velocity was 28 cm/sec in all six streams. During the experiment, water samples were taken at weekly intervals, and four samples of the algal community were taken from each stream.

Experiment 2 - Current Velocity and Accumulation of Dieldrin

Experiment 2 extended from August 12, 1966, to December 28, 1966, and was concerned primarily with the effect of current velocity on the accumulation of dieldrin by benthic algae. The paddle wheels were removed from Streams 29 and 30 and the opening between the influent water line and the overflow standpipe was closed. Therefore, the exchange of water was forced to circulate over the entire area of substrate in these streams. The current velocity in Streams 27 and 28 was 28 cm/sec. Although no animal forms were stocked in the streams during this experiment, considerable numbers of insect larvae were observed as the experiment progressed. They were assumed to have developed from eggs

entering the streams through the water supply or from eggs laid directly in the streams by adult insects. The HEOD concentration in the water of Streams 28 and 30 was between 0.4 and 0.6 ppb, and the other two streams served as controls. During the experiment eight water samples and five algal samples were taken from each stream.

Experiment 3 - Variation in Light Intensity and Accumulation of Dieldrin

Experiment 3 was designed much like Experiment 1 with the exception that light intensity was reduced in Streams 23 and 24. Wooden frames were constructed, covered by several layers of woven plastic screen, and positioned over the surface of these streams to reduce the light intensity. With the screens in place the daily maximum light intensity never exceeded 200 foot candles. In the four unshaded streams (21, 22, 25, and 26) the daily maximum always exceeded 700 foot candles. Each stream received 300 grams of snails and an undetermined quantity of insect larvae. Fish stocking rates were identical to those in the first experiment. The addition of dieldrin was started in the streams January 10, 1967, and continued until March 20, 1967. HEOD concentrations in Streams 21, 25, and 24 were maintained between 0.3 and 0.6 ppb, and Streams 22, 23, and 26 were left as controls. Prior to dieldrin introduction, the

algal communities of all streams were sampled, and during the course of the experiment, ten water samples and three algal samples were collected.

Experiment 4 - Accumulation of Dieldrin at High Concentrations

Experiment 4 was conducted to determine the effect of a high dieldrin concentration on benthic algae. Except for the dieldrin level, this experiment was a replica of Experiment 2. Animals were not stocked in any of the streams. The pesticide was introduced from May 16, 1967, until June 30, 1967. Dieldrin concentration in the water in Stream 28 and 30 normally varied between 3.0 and 7.0 ppb. This concentration range was roughly ten times greater than that of any other experiment. Four water samples and four algal samples were taken from the streams receiving dieldrin. The control streams (27 and 29) were sampled four times for species composition, but a check of the water for the presence of HEOD was made only once.

An experiment was conducted to determine what portion of the accumulated dieldrin might be attributed to adsorption and what importance other processes might have in the concentration scheme. In September, 1966, samples of algae consisting largely of Spirogyra spp. were collected from nearby ponds. The samples

were rinsed in tap water and were hand picked to remove extraneous macroscopic flora and fauna. After drying the samples at 70°C for 24 hours, the material was placed in several bags made from No. 20 silk bolting cloth. These bags were suspended in the laboratory streams for 72 hours, and their contents were then analyzed for HEOD. This experiment was repeated in June, 1967, but was expanded to include bags containing algal samples which appeared to be in an actively growing condition. These samples were suspended in the streams for comparison with the dried material. In addition, a sample of dried and actively growing material was analyzed for HEOD content without any exposure in the laboratory streams.

Warburg Experiments

In August, 1966, several experiments were carried out to determine the effect of dieldrin on rates of respiration and photosynthesis of Phormidium retzii, a blue-green alga common in the laboratory streams. Small rocks bearing gelatinous tufts of this alga were removed from laboratory streams which had received no dieldrin. From some rocks, small portions of the alga were transferred immediately to reaction vessels and mounted on the respirometer. These determinations were conducted to see if changes in respiration and photosynthesis occurred within a short time after exposure to the pesticide. Other rocks bearing the alga were

transferred to beakers containing about 400 ml of buffer or millipore-filtered creek water. One beaker contained an HEOD concentration identical to that used in the reaction vessels, while the other held only creek water or buffer. The samples were retained in the beakers for 24 hours, and then portions of the alga were transferred to reaction vessels for measurements in the respirometer. During the adaptation period an air stone was placed in each beaker to insure adequate aeration and to simulate the turbulent action of the stream water. This treatment allowed comparison of respiratory and photosynthetic rates after 24 hours of adaptation, to those with no previous exposure.

RESULTS

The results of the laboratory studies are presented in three sections: 1) dieldrin accumulation by benthic algae; 2) the effect of dieldrin on species composition; and 3) the effect of dieldrin on respiratory and photosynthetic rates of Phormidium retzii. Studies of dieldrin accumulation by benthic algae and effects of dieldrin on species composition involved four experiments with the laboratory streams, each of two to three months duration. Studies of the effect of dieldrin on respiratory and photosynthetic rates of Phormidium retzii in the Gilson differential respirometer were short term experiments and extended for periods up to 72 hours.

Dieldrin Accumulation by Algae

Experiment 1 - Preliminary Study to Determine Rates of Dieldrin Accumulation

The results of Experiment 1 indicated that the mean concentration of HEOD in the water of Streams 21 and 24 was 0.128 and 0.141 ppb respectively (Table 3). Algal samples from these streams had mean HEOD concentrations of 770 and 775 ppb, which represent an increase in concentration of more than 5,000 times that present in the water. In Streams 22 and 25 which received lower HEOD concentrations (0.05 ppb), the algae never accumulated more than

Table 3. HEOD concentration (ppb) in water and algal samples obtained from six laboratory streams during Experiment 1, February - April, 1966. Species composition is expressed as the ratio of the number of abundance units of the filamentous algae to that of unicellular diatoms.

Stream	Date	HEOD concentration in water (ppb)	HEOD concentration in algae (ppb)	Species composition ratio
21	2/23	0.05	--	--
	2/25	0.09	--	--
	2/28	0.09	--	--
	3/7	0.101	600	--
	3/14	0.168	--	--
	3/21	0.118	580	--
	3/28	--	--	--
	4/6	0.176	940	0.316
	4/11	0.123	--	--
	4/19	0.237	960	0.265
	mean	0.128	770	
24	2/23	0.069	--	--
	2/25	0.112	--	--
	2/28	0.183	--	--
	3/7	0.144	740	--
	3/14	0.149	--	--
	3/21	0.158	780	--
	3/28	0.166	--	--
	4/6	0.134	750	0.341
	4/11	0.162	--	--
	4/19	0.129	830	0.308
	mean	0.141	775	
22	3/7	0.05	50	--
	3/21	0.05	70	--
	4/6	--	70	--
	4/19	0.05	90	--
25	3/7	0.05	60	--
	3/21	0.05	80	--
	4/6	--	60	--
	4/18	0.05	100	--
23 (Control)	3/7	< 0.05	10	--
	3/21	< 0.05	20	--
	4/6	< 0.05	10	--
	4/19	< 0.05	10	--
26 (Control)	3/7	< 0.05	20	--
	3/21	< 0.05	30	--
	4/6	< 0.05	20	--
	4/19	< 0.05	30	--

100 ppb. Water from control streams always had HEOD concentrations less than 0.05 ppb, while the concentration in the algae ranged from 10-30 ppb. Values reported for the water of Streams 22, 23, 25, and 26 were all near the lower limit of detection for the analytical procedure. Values for both water and algae obtained for the control streams probably resulted from errors in the analytical procedure or contamination of equipment in the analytical laboratory.

Experiment 2 - Current Velocity and Accumulation of Dieldrin

Analysis of water and algal samples taken from control streams (27 and 29) during Experiment 2 yielded concentrations of HEOD very similar to those obtained during the first experiment. Values for the control water were less than 0.03 ppb, and the HEOD concentration in the algae again ranged from 10-30 ppb (Table 4). In streams with approximately 0.43 ppb HEOD in the water (28 and 30), algal samples had HEOD concentrations ranging from 730-3580 ppb. These values represented an increase in concentration of several thousand times. However, current appeared to have no direct effect on HEOD accumulation. The HEOD concentration in the algae declined in Streams 28 and 30 as the experiment progressed.

Table 4. HEOD concentration (ppb) in water and algal samples obtained from four laboratory streams during Experiment 2, August - December, 1966. Species composition is expressed as the ratio of the number of abundance units of the filamentous algae to that of unicellular diatoms.

Stream	Date	Current velocity cm/sec	HEOD concentration in water (ppb)	HEOD concentration in algae (ppb)	Species composition ratio
27	8/18	28	< 0.03	10	--
(Control)	10/12	28	< 0.03	20	--
	11/2	28	< 0.03	30	--
	12/8	28	< 0.03	27	--
28	8/18	28	0.41	2,990	0.772
	10/12	28	0.45	2,230	0.239
	11/2	28	0.44	1,750	0.158
	12/8	28	0.44	730	0.061
29	8/18	0	< 0.03	20	--
(Control)	10/12	0	< 0.03	30	--
	11/2	0	< 0.03	20	--
	12/8	0	< 0.03	30	--
30	8/18	0	0.41	3,580	1.775
	10/12	0	0.43	1,210	0.851
	11/2	0	0.42	1,070	0.671
	12/8	0	0.40	940	0.736

Experiment 3 - Variation in Light Intensity and Accumulation of Dieldrin

Results of HEOD analysis of water and algal samples obtained during Experiment 3 (Table 5) varied more than those of other experiments. In all samples except one, the HEOD concentration in the water of control streams was within the range attributed to background material (0.002 - 0.05 ppb). However, on March 27, 1967, the water sample of control Stream 26 had an HEOD concentration of 0.14 ppb. When the HEOD concentration of the corresponding algal sample from Stream 26 was compared with those from other control streams it must be concluded that the reported value for the water was in error. Also, HEOD concentrations in algae from Stream 24 on January 27, 1967 and Stream 21 on March 7, 1967 were unusually low, about 50 ppb, as concentrations in other samples from these streams ranged from 1,000 to 2,400 ppb. Possibly, a change in laboratory technicians and modifications in the analytical procedure which occurred during this experiment were responsible for these anomalies. Ignoring these unusual values, it appeared that light intensity had little or no effect on dieldrin uptake by the algae.

Table 5. HEOD concentration (ppb) in water and algal samples obtained from six laboratory streams during Experiment 3, December, 1966 to March, 1967.

Stream	Date	HEOD concentration in water (ppb)	HEOD concentration in algae (ppb)	Light intensity
22	1/27	<0.04	149*	full sunlight
(Control)	3/7	--	10.8	full sunlight
	3/20	--	48.4	full sunlight
26	1/27	--	--	full sunlight
(Control)	3/7	0.14*	2.6	full sunlight
	3/20	0.002	15.2	full sunlight
23	1/27	<0.04	3.2	shaded
(Control)	3/7	--	--	shaded
	3/20	--	37.3	shaded
24	1/27	0.56	< 50*	shaded
	3/7	0.48	1,090	shaded
	3/20	0.32	804	shaded
21	1/27	0.64	2,060	full sunlight
	3/7	0.40	53*	full sunlight
	3/20	0.23	2,280	full sunlight
25	1/27	0.53	1,430	full sunlight
	3/7	0.67	1,070	full sunlight
	3/20	0.33	2,370	full sunlight

*Results of these analyses differ markedly from expected values. Possible sources of error are explained in the text.

Experiment 4 - Accumulation of Dieldrin at High Concentrations

The concentrations of HEOD in water and algal samples taken from streams which received dieldrin during Experiment 4 (Table 6) were much higher than for those of any other experiment. HEOD concentrations in the water from Stream 28 varied from 0.35 to 6.69 ppb and those for Stream 30 from 3.09 to 7.28 ppb. In corresponding algal samples the concentrations of HEOD ranged from 10,200 to 205,000 ppb. The latter represented an increase in concentration of nearly 30,000 times. Control streams were sampled only once, and HEOD concentrations of both water and algae were similar to those of control streams in other experiments.

Dieldrin Accumulation by Algae Suspended in Bags

Results of HEOD analysis of algae placed in bags and suspended in streams indicated that considerable accumulation of the pesticide occurred in both living and dead material (Table 7). In September, 1966, only dried algae were used. Although the HEOD concentration of the water in both streams was approximately 0.4 ppb, concentrations in the algae were 1,100 ppb from the stream with a current of 28 cm/sec but only 160 ppb in standing water. The procedure was repeated in June, 1967, and the effect of current on accumulation of dieldrin by algae was again pronounced. In this

Table 6. HEOD concentration (ppb) in water and algal samples obtained from four laboratory streams during Experiment 4, May - June, 1967. Species composition is expressed as the ratio of the number of abundance units of the filamentous algae to that of unicellular diatoms.

Stream	Date	Current velocity cm/sec	HEOD concentration in water (ppb)	HEOD concentration in algae (ppb)	Species composition ratio
27 (Control)	6/1	28	0.022	67	--
28	5/23	28	0.35	10,200	0.815
	6/1	28	3.04	31,600	0.692
	6/13	28	2.76	25,800	0.387
	6/30	28	6.69	32,000	0.218
29 (Control)	6/1	0	0.03	30	--
30	5/23	0	6.40	103,000	3.050
	6/1	0	3.09	24,700	2.730
	6/13	0	7.28	205,000	2.950
	6/30	0	4.28	10,300	1.526

Table 7. Comparisons of HEOD accumulation in living and dried algal masses suspended in the laboratory streams.

Date	Condition	Current velocity cm/sec	HEOD concentration in water (ppb)	HEOD concentration in algae (ppb)
9/6/66	dried	28	0.44	1,100
9/6/66	dried	0	0.38	160
6/28/67	living	28	6.69	40,200
6/28/67	dried	28	6.69	75,000
6/28/67	living	0	4.28	5,150
6/28/67	dried	0	4.28	500
6/28/67	dried	not suspended in a stream		17

case living algae had an HEOD concentration of 40,000 ppb after exposure in a stream with a current and 5,150 ppb after exposure in standing water. The dried algae had even greater differences in concentration of HEOD. Dried material suspended in a stream with a current had a concentration of 75,000 ppb, while the concentration in material suspended in standing water was only 500 ppb.

Total Dieldrin Accumulation and Export in Laboratory Streams

An estimate was obtained for the total pesticide contained by the benthic algae of a given stream for each sampling date. The total pesticide content was calculated by the expression

$$P = D \times B \times A \qquad (5)$$

where P is the total pesticide content of the algae (mg/stream); D is the HEOD concentration in the algae (ppm); B is the biomass (gm/m^2); and A is the surface area of the stream (m^2). The amounts of pesticide which accumulated in algae during Experiments 1 and 2, expressed as mg/stream appear in Table 8. These values for streams receiving dieldrin varied between 1.25 and 2.64 mg/stream in Experiment 1 and between 0.53 and 2.41 in Experiment 2. Because the surface area of the streams were identical, the change in the total amount of pesticide for a given stream

Table 8. Biomass (g/m^2), HEOD concentration in the algae (ppb), and the total quantity of HEOD associated with the periphyton community in selected streams during Experiments 1 and 2.

Experiment	Stream	Date	Current velocity cm/sec	Biomass g/m^2	HEOD concentration in algae (ppm)	Total HEOD mg/stream
1	21	3/7/66	28	769.8	0.64	1.55
	24	3/7/66	28	581.8	0.74	1.25
	21	3/21/66	28	861.2	0.58	1.68
	24	3/21/66	28	693.4	0.78	1.81
	21	4/6/66	28	770.1	0.94	2.42
	24	4/6/66	28	650.5	0.75	1.63
	21	4/19/66	28	819.0	0.96	2.64
	24	4/19/66	28	669.4	0.83	1.86
2	28	8/18/66	28	117.9	2.99	1.18
	28	10/12/66	28	230.6	2.23	1.72
	28	11/2/66	28	410.2	1.75	2.41
	28	12/8/66	28	378.3	0.73	0.93
	30	8/18/66	0	143.4	3.58	1.72
	30	10/12/66	0	302.6	1.21	1.22
	30	11/2/66	0	149.5	1.07	0.53
	30	12/8/66	0	172.5	0.94	0.54

resulted from changes in the dieldrin concentration in the algae and/or changes in biomass.

Attempts to budget the pesticide introduced into the laboratory streams were not entirely successful. During Experiment 3 the monitoring of the water included both influent and effluent samples from those streams which received dieldrin (Table 9). With the exceptions of Stream 21 on January 13, 1967, and all streams on March 2, 1967, the effluent water had slightly higher HEOD concentrations than the influent water. Since on most sampling dates more pesticide was leaving the streams in the effluent water than was entering in the influent exchange water, it was probable that the additional pesticide was adsorbed on particulate organic matter which became suspended in the water as it passed over an established periphyton community. The export of particulate organic matter small enough to pass through a No. 20 plankton net was measured by Millipore filtration during Experiment 2 (Table 10). The mean values of this filter-collected fraction of the effluent water expressed on an ash-free dry weight basis, were 0.94 and 1.36 mg/l for Streams 27 and 28 and 0.58 and 0.44 mg/l for Streams 29 and 30. Assuming that the HEOD concentration of the particulate organic matter was the same as that in the algal samples, these suspended particles could account for the higher HEOD concentrations in the effluent water.

Table 9. Concentrations of HEOD in influent and effluent water of selected streams during Experiment 3, December, 1966 - March, 1967. (All concentrations expressed in ppb.)

Date	Stream	Influent water	Effluent water
1/13/67	21	0.84	0.70
1/13/67	24	0.49	0.62
1/18/67	24	0.43	0.48
1/18/67	25	0.56	0.63
1/31/67	21	0.55	0.71
1/31/67	24	0.51	0.54
1/31/67	25	0.42	0.47
3/2/67	21	0.44	0.31
3/2/67	24	0.48	0.43
3/2/67	25	0.67	0.60

Table 10. Estimates of export of periphyton from the laboratory streams during Experiment 2. Data for the net collected material is expressed as grams dry weight per 24 hours, and that for particulate organic matter collected on a Millipore filter as mg ash-free dry weight per liter.

	28 cm/sec current				standing water			
	Stream 27		Stream 28		Stream 29		Stream 30	
Date	Net	Filter	Net	Filter	Net	Filter	Net	Filter
8/18	8.20	--	4.31	--	0.05	--	0.21	--
8/23	0.64	0.40	1.44	0.60	0.03	0.80	0.29	0.20
8/30	0.66	--	1.42	2.00	1.68	0.37	0.23	--
9/7	0.87	1.60	2.57	0.43	0.11	0.60	0.17	0.50
9/14	1.36	0.70	1.16	--	1.71	--	0.05	0.70
9/21	0.42	1.50	1.20	1.40	0.23	0.80	0.08	0.70
9/28	0.87	0.70	0.87	0.30	0.25	0.53	0.39	0.60
10/5	0.66	0.60	0.44	0.90	0.05	0.53	0.12	0.35
10/12	0.58	0.70	0.78	0.20	0.05	0.20	0.04	0.30
10/20	0.10	--	0.30	5.50	0.11	--	0.03	0.13
10/26	0.89	2.30	2.72	3.00	0.03	--	0.03	--
11/10	0.09	0.10	0.60	0.20	0.09	--	0.03	--
11/16	0.33	--	0.37	--	0.25	--	0.07	--
11/23	0.51	--	0.58	0.75	0.35	--	0.03	--
11/30	0.48	--	0.29	--	0.11	--	0.11	--
12/22	2.22	0.80	0.40	1.00	0.02	0.80	0.04	--
means	1.18	0.94	1.22	1.36	0.32	0.58	0.12	0.44

As the algal communities aged, chunks of material became dislodged from the substrate and were transported out of each stream through the overflow standpipes. During Experiment 2 the export of this material was monitored weekly by placing a No. 20 plankton net under each outlet for a 24-hour period. In Streams 27 and 28 (current velocity 28 cm/sec), the mean dry weights of this fraction were 1.18 and 1.22 g/24 hours, respectively, while in Streams 29 and 30 (standing water), the means were only 0.32 and 0.12 g/24 hours (Table 10). The unusually high values for Streams 27 and 28 on August 8, 1966, resulted from the rapid sloughing of an algal bloom which occurred in these streams in early August.

In order to estimate the role that dislodged algae play in translocation of accumulated pesticide, the mean rate of net-collected export from Stream 28 (Table 10) was multiplied by the HEOD concentrations of algal samples taken from Stream 28 during the same experiment (Table 8). Assuming that the HEOD concentration of the dislodged material was the same as that in the algal samples, the exported material was responsible for the removal of approximately 1-4 μg (1 - 4 ppm/g dry weight) of HEOD per 24 hours. On a particular date (8/18/66) the rate of HEOD transported in this manner theoretically could have been as high as 13 μg/24 hours.

Effect of Dieldrin on Species Composition

A list of algal taxa recorded during the preliminary examination of all samples taken over the 18 months when experiments were conducted is presented in Table 11. A subjective evaluation of their abundance is also included. Of the 66 taxa the majority (54 taxa) were members of the Division Bacillariophyta, the diatoms. Identification of diatoms was based on the taxonomic concepts presented by Schmidt et al. (38), Hustedt (22, 23), Patrick and Reimer (34), Van Heurk (42), and Sovereign (40). Although the diatoms had by far the largest number of taxa, one or more species of filamentous chlorophyceans, cyanophyceans, or xanthophyceans occasionally dominated the communities. These algae were identified with the aid of the manual by Prescott (36). Without doubt, the 66 taxa did not represent all the species, as the method used in this study was designed to evaluate the abundance of only those taxa which contributed significantly to the community biomass.

A total of 15 algal taxa were considered in the regression analysis. Diatoms included were Achnanthes lanceolata (Bréb.) Grun., A. minutissima Kütz., Cocconeis placentula var. euglypta (Ehr.) Cl., Epithemia sorex Kütz., Nitzschia linearis (Ag.) W. Smith, the lanceolate Nitzschia as a group, Nitzschia dissipata (Kütz.) Grun. and N. columbiana Sov. together, Melosira varians Ag.,

Table 11. List of algal taxa recorded for samples obtained from the laboratory streams, December 1966 through June 1967. Abundance is expressed as dominant (d), abundant (a), occasional (o), rare (r), or exceptional (e).*

Taxon	Abundance
Division Bacillariophyta	
Achnanthes exigua var. heterovalva Krasske	o
Achnanthes lanceolata (Bréb.) Grun.	d
Achnanthes minutissima Kütz.	d
Amphiprora ornata Bailey	r
Caloneis ventricosa var. subundulata (Grun.) Patr.	r
Cocconeis placentula var. euglypta (Ehr.) Cl.	d
Cymbella ventricosa Kütz.	r
Diploneis elliptica (Kütz.) Grun.	o
Epithemia sorex Kütz.	d
Epithemia turgida (Ehr.) Kütz.	a
Epithemia zebra (Ehr.) Kütz.	a
Eunotia pectinalis var. minor (Kütz.) Rabh.	r
Fragilaria vaucheriae (Kütz.) Peters.	a
Gomphonema acuminatum var. coronata (Ehr.) Rabh.	o
Gomphonema angustatum (Kütz.) Rabh.	a
Gomphonema angustatum var. aequalis Greg.	r
Gomphonema dubravicense Pant.	r
Gomphonema gracile Ehr.	r
Gomphonema lanceolatum Ehr.	r
Gomphonema olivaceum (Lyngbye) Kütz.	o
Gomphonema parvulum (Kütz.) Rabh.	a
Gomphonema subclavatum Grun.	a
Gyrosigma obtusatum (Sulliv. & Wormley) Boyer	e
Melosira varians Ag.	d
Meridion circulare (Grev.) Ag.	a
Navicula cryptocephala Kütz.	a
Navicula cryptocephala var. veneta (Kütz.) Rabh.	a
Navicula elginensis var. rostrata (A. Mayer) Patr.	r
Navicula heufleri Grun.	r
Navicula minima Grun.	a
Navicula minuscula Grun.	e
Navicula radiosa Kütz.	a
Navicula salinarum var. intermedia (Grun.) Cl.	a
Navicula tantula Hust.	o
Navicula tripunctata var. schizonemoides (V.H.)Patr.	e

Continued

Table 11. Continued.

Taxon	Abundance
Nitzschia acicularis W. Smith	d
Nitzschia amphibia Grun.	r
Nitzschia columbiana Sov.	a
Nitzschia dissipata (Kütz.) Grun.	a
Nitzschia frustulum var. perminuta (Rabh.) Grun.	r
Nitzschia linearis (Ag.) W. Smith	a
Nitzschia oregona Sov.	d
Nitzschia palea (Kütz.) W. Smith	a
Pinnularia maior (Kütz.) Rabh.	e
Rhoicosphenia curvata (Kütz.) Grun.	a
Rhopalodia gibba (Ehr.) O. Müller	a
Rhopalodia gibberula (Ehr.) O. Müller	r
Stauroneis kriegeri var. kriegeri Patr.	r
Stauroneis phoenicentron Ehr.	r
Stauroneis phoenicentron var. brunii (M. Perag. & Herib) Voigt	e
Surirella angustata Kütz.	a
Surirella ovata Kütz.	o
Synedra rumpens. var. familiaris (Kütz.) Hust.	d
Synedra ulna (Nitz.) Ehr.	d
Division Chlorophyta	
Closterium sp.	r
Oedogonium sp.	a
Scenedesmus sp.	r
Stigeoclonium subsecundum Kütz.	d
Ulothrix variabilis Kütz.	o
Division Chrysophyta	
Tribonema minor (Klebs) Hazen	d
Division Cyanophyta	
Anabaena variabilis Kütz.	d
Calothrix parietina (Naeg.) Thuret	a
Nodularia harveyana (Thw.) Thuret	d
Nostoc microscopicum Carmichael	o

Continued

Table 11. Continued.

Taxon	Abundance
Phormidium retzii (Ag.) Gom.	a
Schizothrix calcicola (Ag.) Gom.	a

*Abundance is expressed as:

dominant (d) - occurring in 50 percent or more fields in at least one sample;
abundant (a) - abundant enough to occur in some samples during examination of 30 fields, but always in less than 50 percent of the fields;
occasional (o) - regularly observed during preliminary examination of material, but usually not observed in one of the 30 fields;
rare (r) - not regularly observed during preliminary examination of material, but more than two specimens found during the experiment;
exceptional (e) - not more than two specimens observed during the experiment.

Synedra rumpens var. familiaris (Kutz.) Hust. and S. ulna (Nitz.) Ehr. Some species of Nitzschia were evaluated quantitatively in two groups because of the difficulty in distinguishing between these at 500X magnification. Algae other than the diatoms included in the analysis were two species of Cyanophyta, Anabaena variabilis Kutz. and Nodularia harveyana (Thw.) Thuret; two species of Chlorophyta, Stigeoclonium subsecundum Kutz. and Spirogyra sp.; and one filamentous xanthophycean, Tribonema minor (Klebs) Hazen.

Ratios of the abundance of filamentous algae to unicellular diatoms (species composition ratio) were calculated only for streams which received dieldrin, and the values varied considerably during the different experiments (Tables 3, 4, and 6). Unfortunately, the contents of some of the 2-dram vials obtained during Experiment 1 were accidentally destroyed. Values which were calculated during this experiment indicated that the ratios remained relatively constant. However, in subsequent experiments, marked changes in relative abundance of these forms took place as the experiments progressed. During Experiment 2 the species composition ratio in Stream 28 (current velocity 28 cm/sec) decreased from 0.775 to 0.061, indicating that unicellular diatoms became the dominant organisms. Values for Stream 30 (standing water) decreased from 1.775 to 0.736 by the end of the experiment. No species composition ratios were compiled during Experiment 3. During Experiment

4 the values for Stream 30 (standing water) were the highest, ranging from 3.050 to 1.526, while in Stream 28 (current velocity 28 cm/sec) the range was much lower, varying from 0.815 to 0.218.

Regression Analysis of Selected Species

In three experiments a regression analysis was performed to determine the influence of experimental variables on the abundance of the dominant taxa. From Experiment 2 seven diatom taxa, one chlorophyte, one cyanophyte, and one xanthophycean were chosen for the analysis; eight diatoms were selected from Experiment 3; and from the Experiment 4, eleven taxa were selected, of which six were diatoms, two cyanophytes, two chlorophytes and one a xanthophycean. Geometrical representations relating the abundance of selected taxa to the experimental variables are shown in Figures 6, 7, 8, 9, and 10, and the constants for the regression equations are given by experiments in Appendix Tables I, II and III.

The regression model used for the analysis was

$$\hat{Y} = b_0 + b_1 T + b_2 C + b_3 D , \qquad (6)$$

where $\hat{Y}$ was the predicted abundance expressed as a probit; T was the time (days); C was the current velocity (cm/sec); D was the HEOD concentration of the water from which the sample was collected; b_0 was the intercept term; and b_1, b_2, and b_3 were the

corresponding partial regression coefficients. In the regression equation for Experiment 3, the light intensity experiment, current velocity (C) was replaced by the light intensity (L) in foot candles. Equation (6) represented an estimate of the extent to which each of the experimental variables influenced the abundance of the dominant taxa during the experimental periods. Obviously, this equation was valid only for the laboratory streams under the range of conditions in effect during these experiments.

During Experiment 2, only two diatom taxa showed significant differences (5 percent level or better) in abundance associated with the presence of the pesticide (Appendix Table I). Both Achnanthes lanceolata and Cocconeis placentula (Figure 6) were more abundant in streams receiving dieldrin. In addition, the regression analysis indicated that the abundance of several taxa was significantly different in relation to the other independent variables. Both Synedra ulna and Epithemia sorex increased in abundance as the experiment progressed and the filamentous forms, Stigeoclonium subsecundum and Tribonema minor, decreased. The effect of current velocity was evident with four taxa. Melosira varians, Tribonema minor (Figure 7), and Anabaena variabilis were less abundant in 28 cm/sec current than in standing water. On the other hand, Achnanthes minutissima, Stigeoclonium subsecundum (Figure 8), and Epithemia sorex were significantly more abundant in the streams

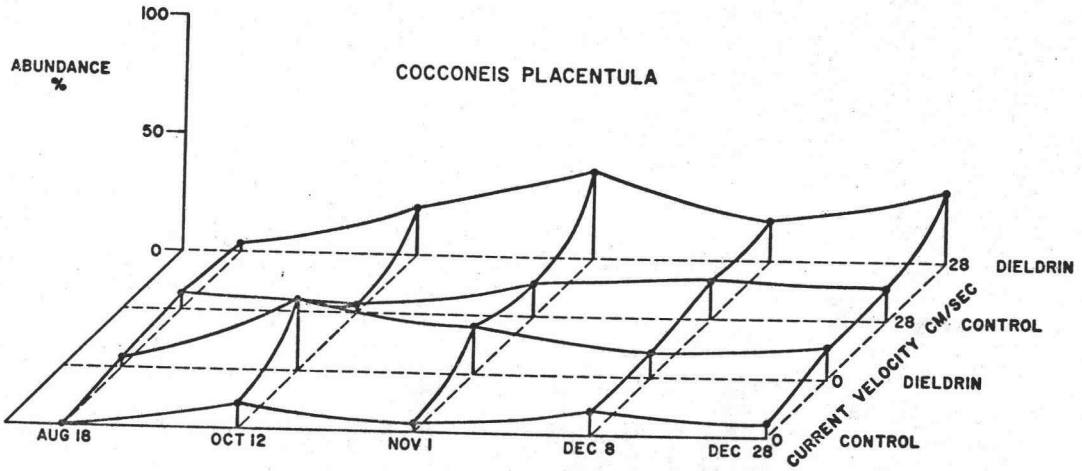


Figure 6. Abundance of Cocconeis placentula in four laboratory streams during Experiment 2, August to December, 1966.

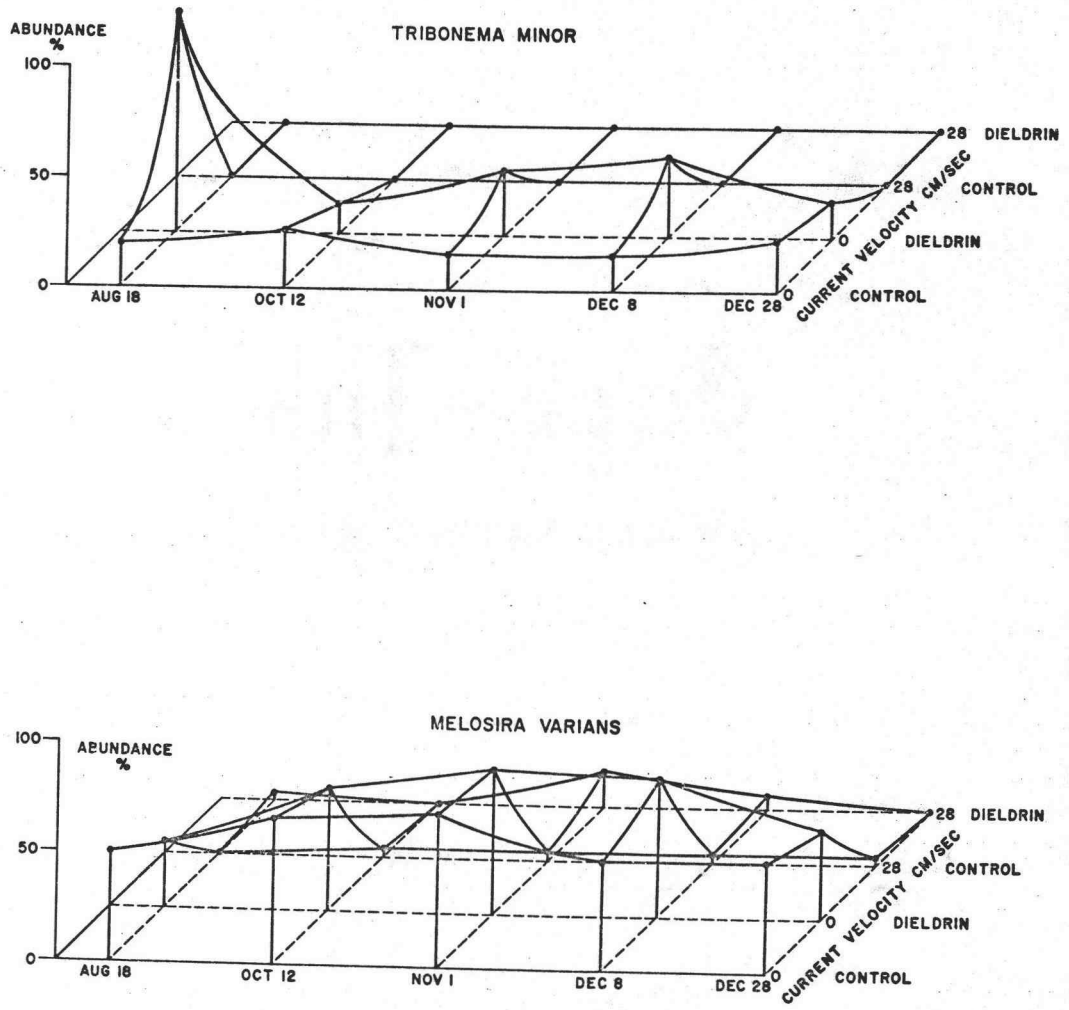


Figure 7. Abundance of Tribonema minor and Melosira varians in four laboratory streams during Experiment 2, August to December, 1966.

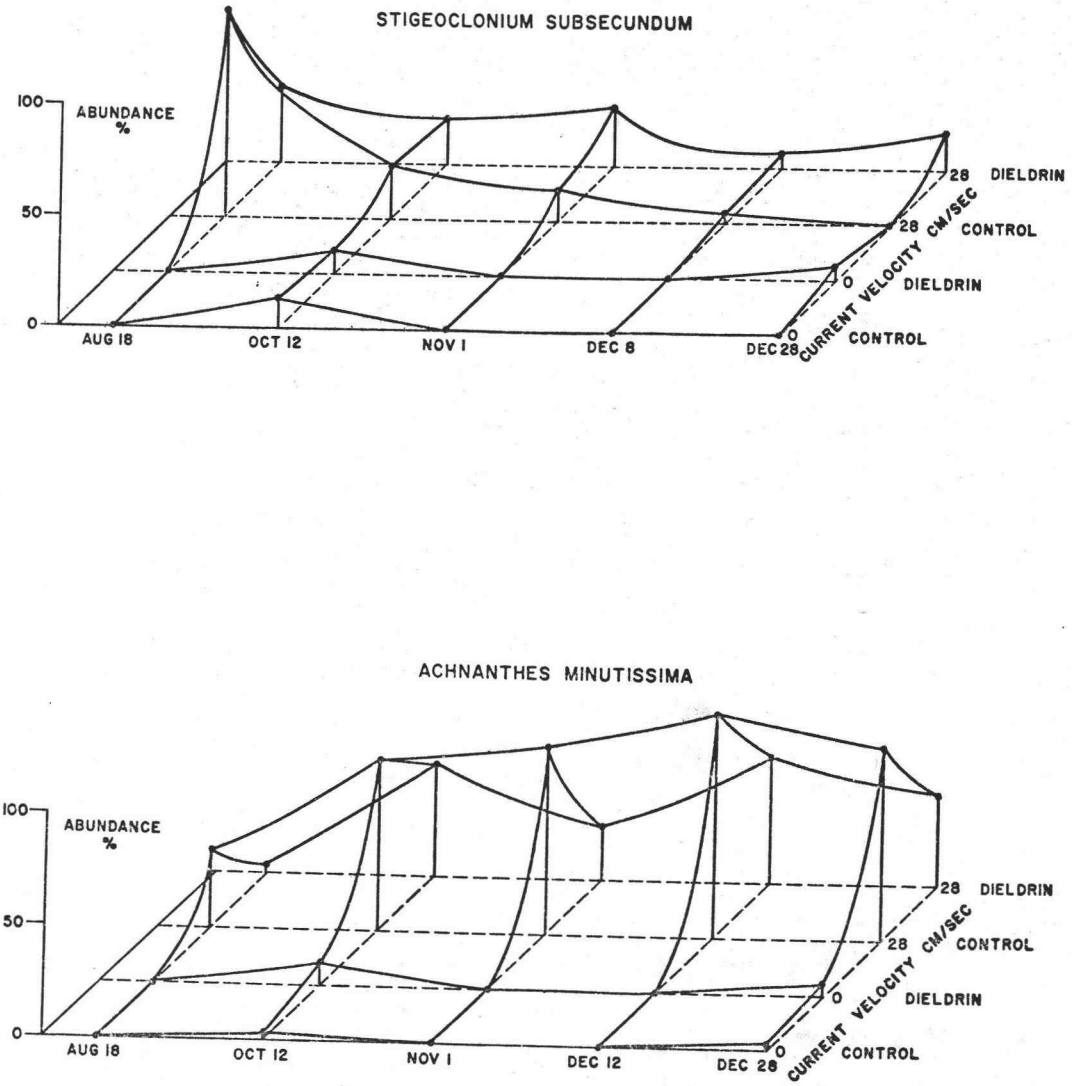
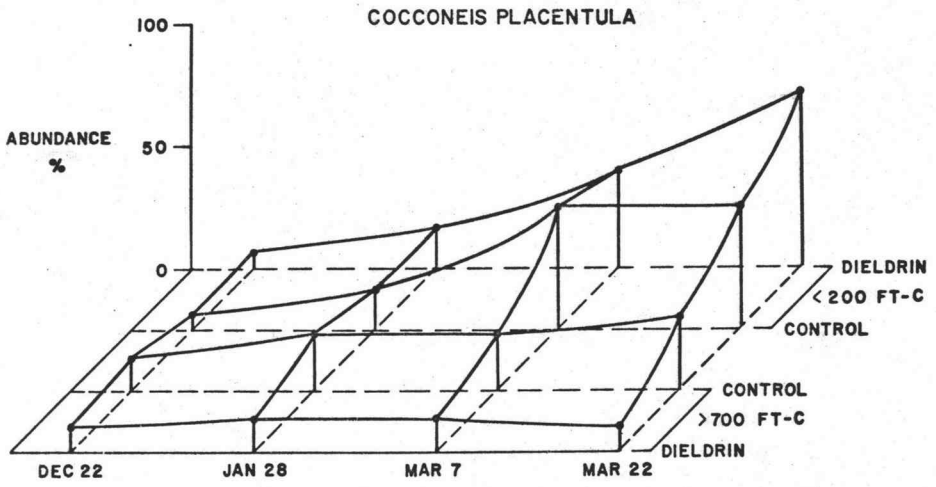


Figure 8. Abundance of Stigeoclonium subsecundum and Achnanthes minutissima in four laboratory streams during Experiment 2, August to December, 1966.

with a current. The remainder of the species apparently were current indifferent.

In Experiment 3, no filamentous algae were abundant enough to be included in the regression analysis (Appendix Table II). Both Achnanthes lanceolata and Epithemia sorex (Figure 9) were significantly more abundant in streams receiving dieldrin. Only Cocconeis placentula (Figure 9) increased significantly with time during the experiment, while the abundance of both groups of Nitzschia, the lanceolate group (N. oregana and N. palea) and the N. dissipata-columbiana group, decreased with time. Light intensity was not an important variable during this experiment, as most taxa appeared to be indifferent to the effects of shading. However, Achnanthes minutissima and Epithemia sorex were more abundant in streams receiving full sunlight than in shaded streams, but only Cocconeis placentula (Figure 9) was more abundant in the streams with less than 200 foot candles as the daily maximum light intensity.

In Experiment 4, Synedra rumpens (Figure 10) was significantly less abundant in both Stream 28 (28 cm/sec current) and Stream 30 (standing water), the streams receiving high dieldrin concentrations (Appendix Table III). Stigeoclonium subsecundum was less abundant in Stream 28 (with dieldrin) than in Stream 27 (control). In contrast, Synedra ulna was more abundant in Stream 28 than in Stream 27. Stigeoclonium subsecundum, Synedra ulna, and Nitzschia



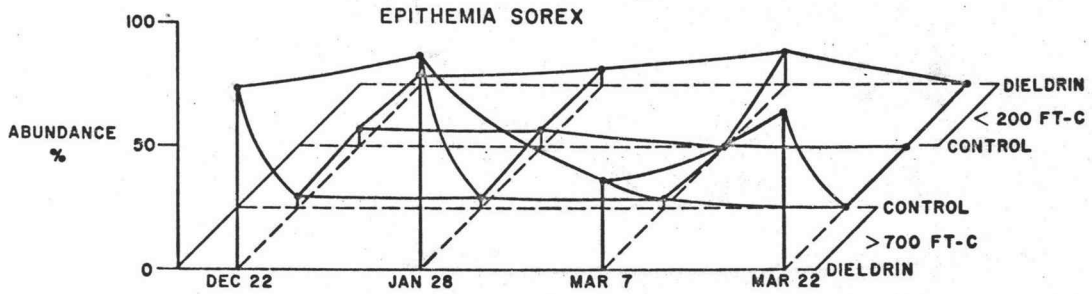


Figure 9. Abundance of Cocconeis placentula and Epithemia sorex in four laboratory streams during Experiment 3, December, 1966 to March, 1967.

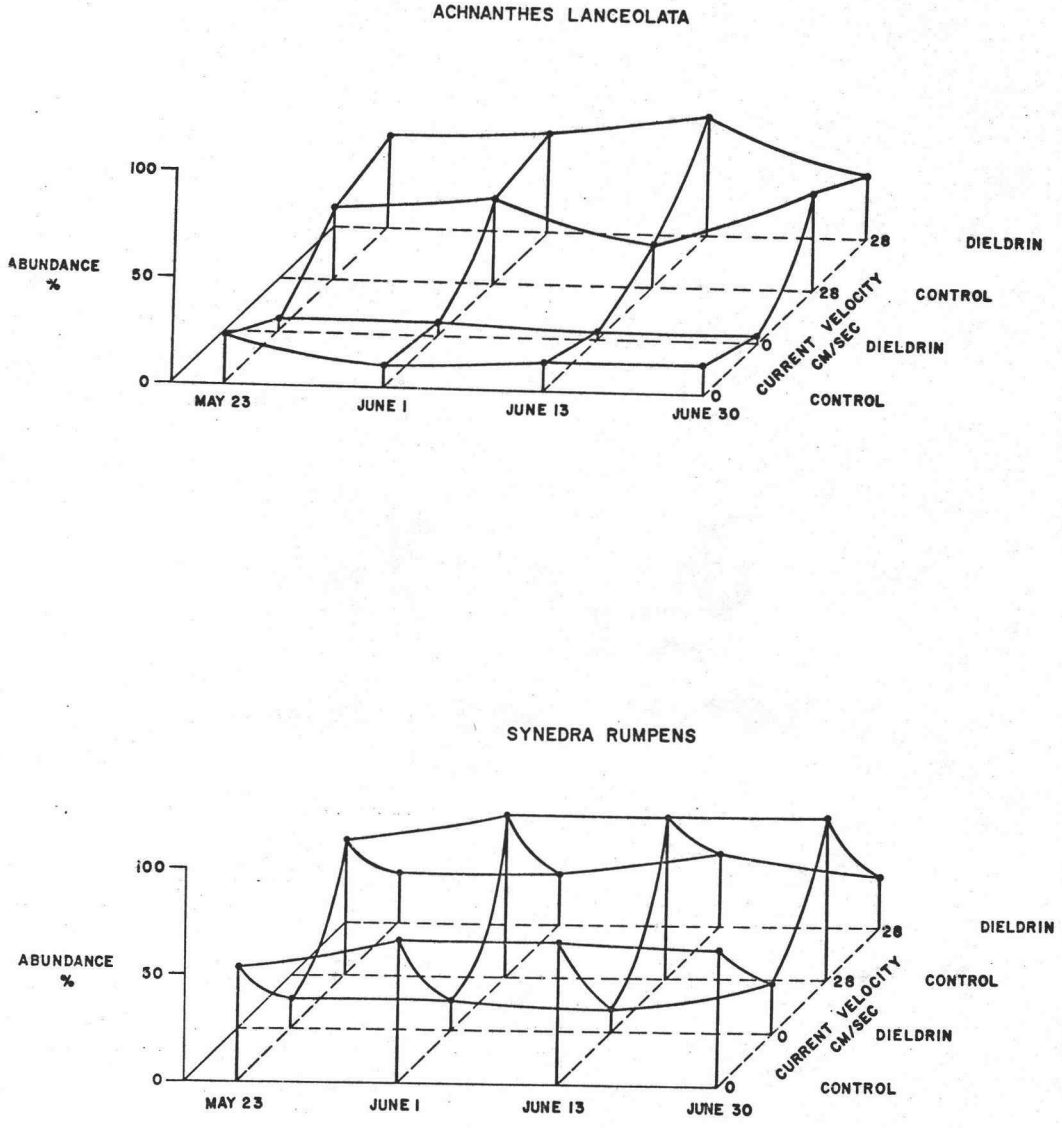


Figure 10. Abundance of Achnanthes lanceolata and Synedra rumpens in four laboratory streams during Experiment 4, May to June, 1967.

linearis increased significantly as the experiment progressed, while only Anabaena variabilis decreased with time. Seven taxa including Achnanthes lanceolata (Figure 10) showed significant responses to the effects of a current. Stigeoclonium subsecundum, Synedra ulna, S. rumpens, Nitzschia linearis, and Achnanthes lanceolata were all more abundant in the streams with a current than in those with standing water. Two filamentous algae, Tribonema minor and Spirogyra sp. were more abundant in the streams with standing water.

The Effect of Dieldrin on Rates of Photosynthesis and Respiration of Phormidium retzii

Curves showing the effect of dieldrin on rates of photosynthesis of Phormidium retzii and associated organisms appear in Figure 11. Each point on the curve for the dieldrin vessels is a mean of four replications and for the control vessels a mean of two replications. The results of this experiment indicated that the photosynthetic rate was significantly higher (5 percent level) during the first few hours of exposure to dieldrin at a concentration of 148 ppb than in the buffer solution without dieldrin. However, after 24 hours exposure to the same concentration of dieldrin, there was no significant difference in photosynthetic rates of the dieldrin and control vessels. During both experiments, the pH changed from 7.9 to

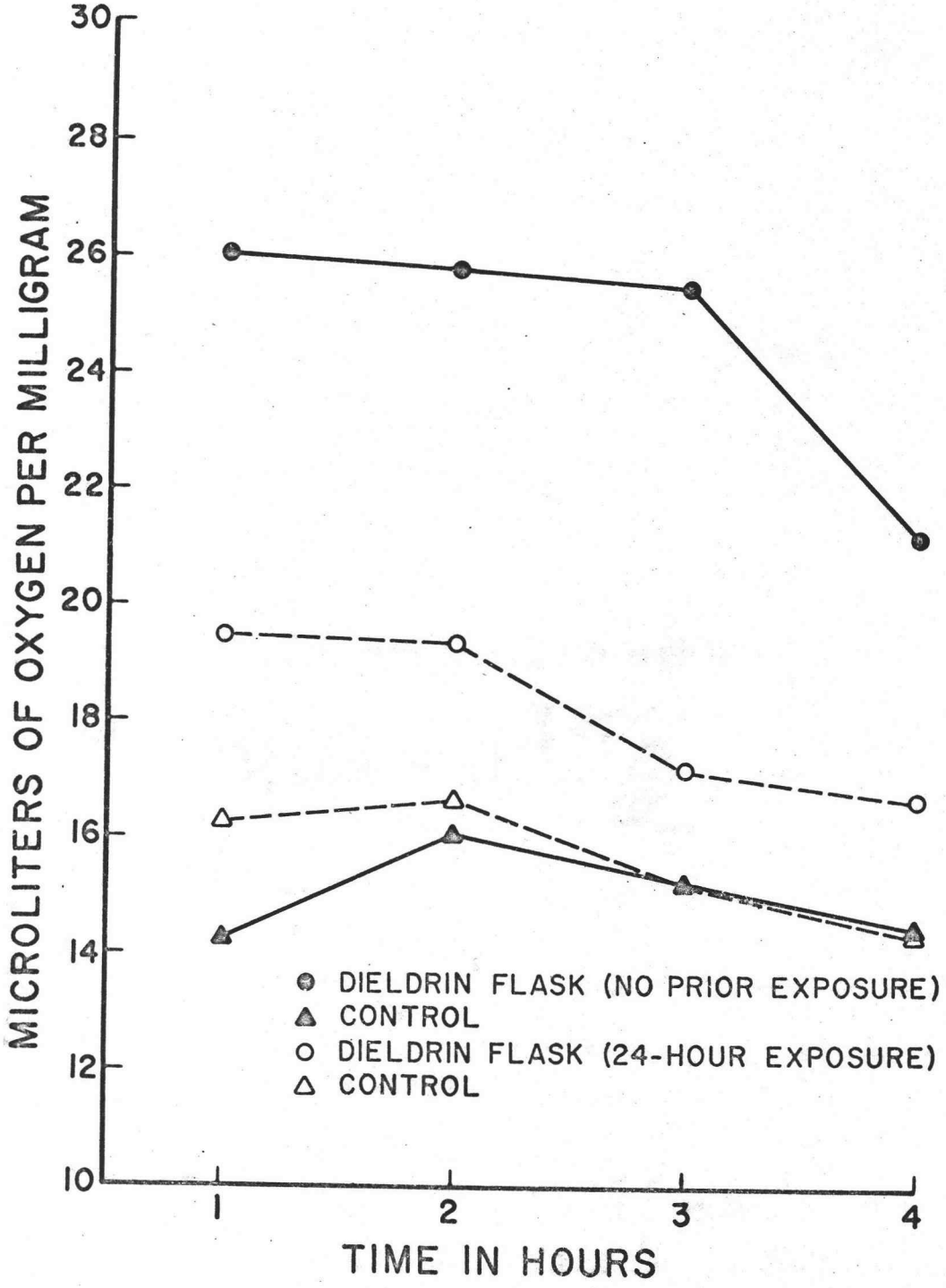


Figure 11. Effect of dieldrin on photosynthetic rates of Phormidium retzii and associated organisms, expressed as $\mu l\ O_2$/mg ash-free dry weight, for samples exposed for 24 hours to dieldrin (98 ppb HEOD) and samples with no prior exposure.

9.0 in the reaction vessels.

The effect of dieldrin on rates of respiration of P. retzii and associated organisms is given in Figure 12. Again, each point on the curve representing the dieldrin vessels is a mean of four replicates and those representing the control vessels a mean of two replications. With no previous dieldrin exposure, rates of respiration were approximately the same in the dieldrin and control vessels. However, after 24 hours exposure to 98 ppb dieldrin, the mean oxygen uptake in the dieldrin vessels was significantly lower (5 percent level) than that in the control vessels. The pH remained between 6.8 and 6.9 during these experiments.

An additional experiment was conducted to determine the effect on photosynthetic rates of longer exposure (72 hours) to dieldrin. The results of this experiment were inconclusive. Rates in both control and dieldrin vessels were markedly depressed, indicating that the conditions under which the samples were held during the 72-hour exposure period were inadequate.

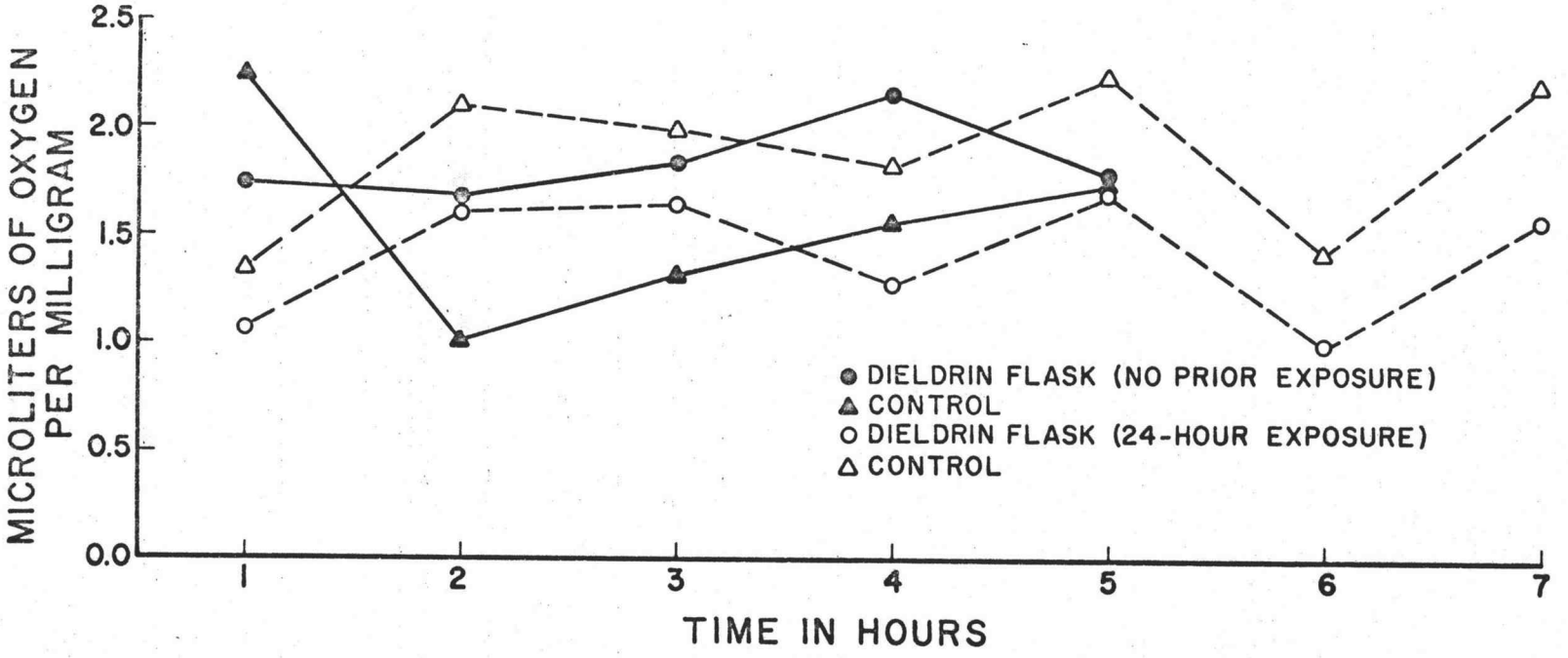


Figure 12. Effect of dieldrin on respiratory rates of Phormidium retzii and associated organisms, expressed as µl 0_2/mg ash-free dry weight, for samples exposed for 24 hours to dieldrin (98 ppb HEOD) and samples with no prior exposure.

DISCUSSION

The range of HEOD concentrations introduced in the laboratory streams during the first three experiments (about 0.5 ppb) was well within the range of chlorinated hydrocarbon residues that have been found in natural streams (6, 18, 19, 43, 44). The extent to which the periphyton community accumulated HEOD in these experiments ranged from 100 to about 30,000 times the concentration in the surrounding water. Keith and Hunt (24) have reported concentrations from 10,000 to 100,000 times in particulate matter of natural aquatic ecosystems, and Warnick et al. (43) and Holden (19) have encountered concentrations 1000 times greater in algae than in the water in which the algae was growing.

When comparing the accumulations of pesticides that have occurred in natural aquatic communities with those in the laboratory streams, some important differences should be kept in mind. In the experiments conducted during this study, concentrations of dieldrin in the water were maintained at or near some constant level for the duration of the experiment. Under natural conditions, it seemed more likely that pesticides would reach a watercourse in intermittent pulses, each followed by a period of relatively low introduction. Dustman and Stickel (12) noted that heavy rains or flooding can carry away significant amounts of pesticides with

transported surface soils. Irrigation run-off water with its pesticide load or heavy precipitation and subsequent run-off shortly following the application of a pesticide probably have contributed much more to pesticide pollution than the leaching process. The latter appears to be mediated by a wide variety of soil factors. Bowman et al. (4) found that from 1 to 65 percent of applied dieldrin may be leached from soils, depending on the soil type. A more complete discussion of the factors affecting persistence of pesticides in soils has been presented by Lichtenstein (26).

The chlorinated hydrocarbons have very low solubilities in water and are classified as hydrophobic compounds by Fleck (15). Cope (10) has noted that dieldrin is attracted and adsorbed to any exposed surface and tends to remain associated with such surfaces. The speed at which this accumulation occurs is surprising. Dustman and Stickel (12) reported that radioactive DDT residues were detected on vegetation and mud of a Colorado farm pond within one-half hour after treatment. Moreover, Meeks and Peterle (29) reported that algae had concentrations ranging up to 245 ppm in three days following DDT treatment of a marsh at the rate of 0.2 pounds per acre.

The mechanisms responsible for the accumulation of dieldrin in algae, i.e., adsorption and absorption, were of particular interest and needed to be clarified. While we have no conclusive

evidence that adsorption was entirely responsible for the accumulation of HEOD, there was indication that this phenomenon played a major role. This was best shown by the HEOD analyses on living and dried algal samples suspended in the streams in bags (Table 7). Results of the first of these experiments clearly showed that a current enhanced dieldrin accumulation by dried algae. A considerably greater volume of water passed by the algal sample suspended in the current than over that suspended in standing water. Although the dieldrin concentration of the water was very nearly the same in both streams, more of the pesticide was available for adsorption in the stream with a current. Since the algae had been dried prior to exposure, the mechanism of uptake was undoubtedly simple adsorption. When the procedure was repeated in June, 1967, using dried and living algae, the samples exposed in the 28 cm/sec current again had higher HEOD concentrations than algae which had been suspended in standing water.

Accumulation of dieldrin by algae was affected by both the age and structure of the community. Therefore, those factors which influenced community structure directly, such as current velocity and light intensity, also exerted an indirect influence on the capacity of an algal community to concentrate the pesticide. The ratio of the abundance of filamentous algae to unicellular diatoms was always greater in the streams with standing water than those with a

current. Where filamentous forms comprised the greater portion of the biomass, their loosely woven nature in standing water or long oscillating threads in a current presented a considerable surface area upon which pesticides could adsorb. In contrast, a community dominated by diatoms was usually characterized by a dense, felt-like growth covering the surface of the substrate (27, 28). Although the number of individual cells was often greater in a community dominated by diatoms, their very compact arrangement probably resulted in an actual decrease in surface area available for adsorption. Therefore, accumulation of dieldrin could be greater in communities dominated by filamentous algae. On the other hand, some pesticide might actually be absorbed into the interior of the algal cells. Since dieldrin is soluble in fats and oils, there is a possibility that HEOD also accumulates in the stored oils of diatoms. Perhaps experiments using a radioactive labelled pesticide and an autoradiographic process would furnish additional information about uptake mechanisms.

Community age was a factor contributing to the differences in pesticide accumulation. The results of Experiment 2 indicated that the concentrations decreased as the communities aged, while in Experiment 1, pesticide accumulation did not vary markedly from a mean value. During Experiment 1, the benthic communities were well established and had existed in the laboratory streams for more

than two months prior to the start of the experiment. Thus, the relative abundance of the different species did not change appreciably while the experiment was in progress. Experiment 2 was begun only three weeks after the streams were filled with creek water, at which time filamentous forms were very abundant. However, shortly after the experiment began, most of this material was exported from the streams, especially from those having a current velocity of 28 cm/sec. Table 4 shows a concurrent decrease in the species composition ratios. The effect of current on HEOD accumulation by algae during this experiment was obscurred because of the persistance of copious amounts of filamentous algae in the streams with standing water and also because the communities were undergoing a seasonal breakdown. This case provided a striking example of the manner in which pesticide accumulation was influenced by an interaction of both physical and biotic factors.

A relationship between community structure and accumulation of dieldrin also appeared to exist in Experiments 3 and 4. In Experiment 3, the HEOD concentration in algal samples did not change markedly as the experiment progressed. Because filamentous algae were nearly eliminated as a result of the feeding by snails, the communities were composed almost entirely of diatoms, and the stable HEOD concentrations therefore were expected. During Experiment 4, the very high HEOD concentrations in the

algae probably were due to a combination of the high concentration of dieldrin in the water and the great abundance of filamentous algae, especially in the standing water.

If dieldrin were actually absorbed, and stored in algal cells in very large amounts, the HEOD concentration in the algal samples should have continued to increase as an experiment progressed. Furthermore, if dieldrin had accumulated in the oil reserves of diatoms, HEOD concentrations would have been higher in samples from those communities with lower species composition ratios. Since HEOD concentrations in the algae either remained the same or decreased during the experiment and concentrations were higher in samples with the higher species composition ratios, these observations appear to support the hypothesis that dieldrin accumulated on algae by adsorption to the surface of the cells.

Very little information is available in the literature concerning the effect of pesticides on the productivity and physiological processes of populations of micro-organisms. Bishop (3) noted that naturally occurring phytoplankton populations in lakes and marshes were reduced following DDT treatment at the rate of 0.2 - 0.5 pounds per acre. Tarzwell (41) reported that toxaphene, another of the chlorinated hydrocarbons, had severe toxic effects on aquatic life of all kinds when applied at 0.1 pounds per acre, and that reduced plankton populations persisted for at least three weeks after

treatment. More recently, Butler (7) reported an inimical effect of dieldrin on a natural phytoplankton community. Based on C^{14} uptake, he noted that dieldrin caused an 84.8 percent decrease in productivity during a 4-hour exposure to 1.0 ppm. At lower concentrations, productivity increased, a fact which he attributed to the toxic effect on only the animal (grazers) portion of the community. In the same investigation DDT was reported to have caused a 77.2 percent decrease in productivity after four hours exposure to a concentration of 1.0 ppm. Unfortunately it was not clear whether 1.0 ppm referred to the DDT concentration in the phytoplankton or in the surrounding water.

The experiments to determine the effect of dieldrin on photosynthetic rates indicated neither the mechanism nor the biochemical process that was involved. Such questions must be answered by a physiologist or biochemist. However, there was little question that the concentration of dieldrin (148 ppb) employed in these studies enhanced photosynthetic rates of Phormidium retzii for at least five hours after exposure. This effect was not detectable after 24 hours exposure. Since the algal sample included some diatoms and bacteria, other organisms may have influenced the results. Furthermore, the dieldrin concentration used in this work was near saturation for the buffer and was far greater than any concentration which might be expected to occur in nature.

Although rates of respiration of Phormidium retzii and associated organisms were significantly lower after 24 hours of exposure to dieldrin, the range of values was about the same as that for samples with no previous exposure. It was possible that the organisms selected for these experiments, primarily P. retzii, did not accurately reflect responses by the other community constituents. Dykstra (13) pointed out that differences in individual sensitivities make it extremely difficult to predict the severity of effects of toxic chemicals upon an entire community. In the search for effects of dieldrin on respiration of soil organisms, Bartha (1) found that carbon dioxide production was inhibited by 24 percent. However, this reduction in CO_2 liberation occurred only when dieldrin was present at a concentration of 250 ppm, a level which greatly exceeded the recommended agricultural application.

The investigation of changes in species composition of communities of micro-organisms in relation to prolonged exposure to pesticide residues has not been attempted previously. Also, there is a complete absence of information concerning the effect of chlorinated hydrocarbons on the abundance of individual species of algae in natural streams. Butler (8) did report that the productivity of unialgal cultures of Platymonas sp. declined during a 4-hour exposure to 1.0 ppm DDT.

Before discussing the observed changes in abundance of

individual algal taxa it is necessary to comment on the general structure of the laboratory communities. The total number of species of algae in a laboratory stream is usually slightly less than that found in natural, unpolluted streams. By controlling some of the physical factors in the environment, species diversity, i.e., the ratio of the number of species to the number of individuals, is reduced. Patrick (32, 35) has observed that when a reduction in the number of diatom species occurs in natural streams there is a concomitant increase in the numbers of individuals of certain remaining species.

In general, the results of the regression analysis indicated dieldrin had little or no effect on the species composition of benthic algae in the laboratory streams. Of the few organisms which were sensitive to dieldrin, Synedra rumpens was the best example. During Experiment 4 this organism was one of the more abundant species in both control streams, but it was markedly less abundant in the streams receiving dieldrin. Achnanthes minutissima also was less abundant in streams receiving the pesticide during Experiment 2. However, during the third experiment there was some indication that this taxon was more abundant in streams with dieldrin (see Table 8). Such differences in the abundance of this organism indicated that other environmental factors were involved in a complexity of interactions that defied analysis.

Before Experiment 3, about 300 grams of the snail Oxytrema silicula were introduced into each of the six laboratory streams (ca. 1000-1200 animals per stream). Since these organisms fed on the benthic algae and bacteria, the effect of grazing was very noticeable, and the high density of snails resulted in a marked decrease of filamentous algae in the streams. The feeding behavior of this snail involved a scouring of the substrate with a rasp-like organ, the radula. As a result, much of the filamentous material was not actually ingested by the animal, but was exported by the action of the current after it was dislodged by the snails. Thus, it appeared that the effect of grazing was the most important factor influencing the benthic communities during Experiment 3.

These studies have emphasized that communities of attached algae in streams can accumulate rapidly relatively high concentrations of dieldrin without any dramatic effect on species composition and abundance of the individual taxa. Consequently, these communities provide a contaminated food source for animals that normally graze on stream periphyton. Subsequently, the process of biological magnification can lead to a substantial accumulation of toxic chemicals in the tissues of animals that are of aesthetic and commercial value to man.

Considerable amounts of dieldrin and other pesticides also enter the bodies of animals through epithelial surfaces. In aquatic

organisms this may include passage through the skin, the digestive tract, and the gill surfaces. The toxic chemicals then are commonly accumulated in the fatty deposits of these animals. The amount of pesticide adsorbed on algae and other aquatic vegetation is not available for incorporation into animals by this process. Therefore, in this manner the algae may actually "compete" with animals for the pesticide, thereby effectively reducing the immediate danger.

Results of the export studies in the laboratory streams suggest a way in which algae could play a role in removing pesticide from a particular section of a stream. The periphyton community in the laboratory streams and in natural streams is continuously changing and operates under some sort of steady state wherein algal material is dislodged and transported down stream by action of the current and such material is continuously replaced through the normal growth processes. Material that is dislodged and exported from a stream should contain concentrations of pesticide similar to that of the standing crop. Thus, these events could represent an effective way of removing accumulated chemicals from a particular section of a stream. In the experiments carried out in this study, the pesticide was continuously introduced in low concentration with the result that new algal cells immediately accumulated pesticide. However, in a natural stream where pesticide residues are more likely to occur in intermittent pulses rather than in sustained

concentrations, the removal of accumulations by this mechanism could play an important role. Although removal of pesticide accumulations in this manner might reduce concentrations in one portion of a stream, the process also would result in the translocation of toxic chemicals to areas downstream. In these areas, however, the incorporation of pesticide into animal tissues would be regulated entirely by the amount of the chemical transferred through the food chain.

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APPENDIX

Appendix Table I. Constants of regression equations relating the abundance of ten taxa of benthic algae from Experiment 2 to time (days), current velocity (cm/sec), and HEOD concentration (about 0.5 ppb).[t]

Taxon	Y intercept	R^2	Multiple correlation coefficient	Independent variable	Regression coefficient	Significance
Synedra ulna	4.434565	0.408162	0.638874	age	0.010042*	0.01 < P < 0.02
				current vel.	0.020760	0.05 < P < 0.1
				dieldrin	0.099362	0.8 < P < 0.9
Melosira varians	5.060877	0.812479	0.901376	age	0.000893	0.7 < P < 0.8
				current vel.	-0.066510*	P < 0.01
				dieldrin	-0.083645	0.8 < P < 0.9
Achnanthes lanceolata	3.132349	0.289552	0.538100	age	0.002145	0.4 < P < 0.5
				current vel.	0.009680	0.2 < P < 0.3
				dieldrin	1.047529*	0.02 < P < 0.05
Achnanthes minutissima	2.445713	0.456999	0.676020	age	0.010176	0.1 < P < 0.2
				current vel.	0.085008*	P < 0.01
				dieldrin	-1.833519	0.1 < P < 0.2
Cocconeis placentula	3.128167	0.456460	0.675620	age	0.004162	0.05 < P < 0.1
				current vel.	0.009249	0.1 < P < 0.2
				dieldrin	0.988558*	0.01 < P < 0.02

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Appendix Table I. Continued

Taxon	Y intercept	R^2	Multiple correlation coefficient	Independent variable	Regression coefficient	Significance
lanceolate Nitzschia	4.456734	0.123714	0.351727	age	-0.001078	$0.7 < P < 0.8$
				current vel.	0.003305	$0.7 < P < 0.8$
				dieldrin	0.638767	$0.3 < P < 0.4$
Epithemia sorex	2.262176	0.566221	0.752477	age	0.021532*	$P < 0.01$
				current vel.	0.069334*	$P < 0.01$
				dieldrin	-0.675572	$0.4 < P < 0.5$
Stigeoclonium subsecundum	3.952445	0.428574	0.654658	age	-0.012369*	$0.02 < P < 0.05$
				current vel.	0.044170*	$0.01 < P < 0.02$
				dieldrin	-0.161868	$0.8 < P < 0.9$
Anabaena variabilis	4.428150	0.445469	0.667440	age	0.004623	$0.1 < P < 0.2$
				current vel.	-0.027486*	$P < 0.01$
				dieldrin	-0.315390	$0.5 < P < 0.6$
Tribonema minor	4.767775	0.514980	0.717617	age	-0.007769*	$0.02 < P < 0.05$
				current vel.	-0.111071*	$P < 0.01$
				dieldrin	1.111637	$0.05 < P < 0.1$

*Significant at 5% level or better.

[t]The equations predict the abundance of each taxon in terms of probit values which can be converted to proportions using the tables of Finney (14) or equation (6) in the text. Significance of the partial regression coefficients is expressed as the probability (P) of having its t-value as large or larger by chance.

Appendix Table II. Constants of regression equations relating the abundance of eight taxa of benthic algae from Experiment 3 to time (days), illumination (full sunlight or shaded), and HEOD concentration (about 0.5 ppb or control).[t]

Taxon	Y intercept	R^2	Multiple correlation coefficient	Independent variable	Regression coefficient	Significance
Synedra ulna	4.986112	0.367697	0.606378	age	-0.002373	0.5 < P < 0.6
				light intensity	0.056639	0.05 < P < 0.1
				dieldrin	0.883695	0.1 < P < 0.2
Melosira varians	3.03906	0.391037	0.625326	age	-0.001001	0.8 < P < 0.9
				light intensity	0.076405*	0.02 < P < 0.05
				dieldrin	0.950757	0.1 < P < 0.2
Achnanthes lanceolata	4.531923	0.416420	0.645305	age	-0.003820	0.1 < P < 0.2
				light intensity	0.002266	P < 0.9
				dieldrin	1.027575*	0.02 < P < 0.05
Achnanthes minutissima	3.814549	0.505416	0.710927	age	-0.002063	0.3 < P < 0.4
				light intensity	0.055455*	P < 0.01
				dieldrin	0.442249	0.1 < P < 0.2
Cocconeis placentula	4.033289	0.625251	0.790724	age	0.011056*	P < 0.01
				light intensity	-0.050646*	0.02 < P < 0.05
				dieldrin	-0.294733	0.4 < P < 0.5

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Appendix Table II Continued.

Taxon	Y intercept	R^2	Multiple correlation coefficient	Independent variable	Regression coefficient	Significance
lanceolate Nitzschia	5.396596	0.553138	0.743730	age	-0.010300*	P <0.01
				light intensity	-0.012869	0.5 < P <0.6
				dieldrin	-0.547812	0.1 < P <0.2
Nitzschia dissipata-columbiana	4.835879	0.537505	0.733146	age	-0.011023*	P <0.01
				light intensity	-0.052306	0.05< P <0.1
				dieldrin	-0.502563	0.3 < P <0.4
Epithemia Sorex	2.180047	0.465385	0.682193	age	-0.006349	0.4 < P <0.5
				light intensity	0.165310*	0.02< P <0.05
				dieldrin	3.454958*	0.02< P <0.05

*Significant at 5% level or better.

[t]See footnote Appendix Table I.

Appendix Table III. Constants of regression equations relating the abundance of 11 taxa of benthic algae from Experiment 2 to time (days), current velocity (cm/sec), and HEOD concentration (about 5.0 ppb).[t]

Taxon	Y intercept	R^2	Multiple correlation coefficient	Independent variable	Regression coefficient	Significance
Synedra rumpens	5.227083	0.941009	0.970052	age	0.004201	$0.2 < P < 0.3$
				current vel.	0.010565*	$P < 0.01$
				dieldrin	-0.253758*	$P < 0.01$
Synedra ulna	2.156134	0.924043	0.961279	age	0.052734*	$P < 0.01$
				current vel.	0.062486*	$P < 0.01$
				dieldrin	0.069714*	$0.02 < P < 0.05$
lanceolate Nitzschia	3.035504	0.719402	0.848173	age	0.004308	$0.5 < P < 0.6$
				current vel.	0.045678	$P < 0.1$
				dieldrin	0.045385	$0.2 < P < 0.3$
Nitzschia linearis	2.070252	0.751842	0.867090	age	0.021338*	$P < 0.01$
				current vel.	0.042760*	$P < 0.01$
				dieldrin	0.069628	$0.05 < P < 0.1$
Epithemia sorex	2.943455	0.340383	0.583421	age	0.014719	$0.1 < P < 0.2$
				current vel.	0.012668	$0.1 < P < 0.2$
				dieldrin	0.062487	$0.2 < P < 0.3$
Achnanthes lanceolata	3.826799	0.725671	0.851866	age	-0.003666	$0.5 < P < 0.6$
				current vel.	0.036539*	$P < 0.01$
				dieldrin	-0.009770	$0.7 < P < 0.8$

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Appendix Table III Continued.

Taxon	Y intercept	R^2	Multiple correlation coefficient	Independent variable	Regression coefficient	Significance
Nodularia harveyana	4.271259	0.122419	0.349883	age	0.004858	0.5 < P < 0.6
				current vel.	-0.005789	0.4 < P < 0.5
				dieldrin	0.052440	0.2 < P < 0.3
Anabaena variabilis	5.569378	0.533914	0.730692	age	-0.031939*	P < 0.01
				current vel.	-0.000092	0.8 < P < 0.9
				dieldrin	0.010938	0.8 < P < 0.9
Tribonema minor	4.412126	0.606234	0.778608	age	0.011053	0.1 < P < 0.2
				current vel.	-0.111534*	P < 0.01
				dieldrin	-0.026544	0.4 < P < 0.5
Spirogyra sp.	4.918904	0.738750	0.859507	age	0.006068	0.2 < P < 0.3
				current vel.	-0.124778*	P < 0.01
				dieldrin	-0.025175	0.3 < P < 0.4
Stigeoclonium subsecundum	1.460862	0.890123	0.943460	age	0.008337*	0.01 < P < 0.02
				current vel.	0.107019*	P < 0.01
				dieldrin	-0.077763*	P < 0.01

*Significant at 5% level or better.

[t] See footnote Appendix Table I.