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THE ROLE OF INSECTS IN SEWAGE DISPOSAL BEDS

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In recent years, the increasing emphasis on the importance of controlling pollution in our natural waterways has led to many studies of the factors involved in the biological stabilization of domestic and industrial waste materials. The present investigation has sought to clarify the part that insects play in two popular waste treatment processes—trickling filters and oxidation ponds.

The larvae of psychodid flies are extremely common in trickling filters, and aid in effecting better stabilized effluents by helping to keep the filter zooglea in a healthy state; the zooglea is responsible for the biological oxidation of organic matter and becomes inefficient if allowed to develop unchecked by scouring organisms. Pilot plant studies are described.

Many species of insects are found in oxidation ponds, the most abundant and important of which are the larvae of midges which burrow into bottom deposits of the ponds. They help to keep the bottom sludges in good condition, aid in removing suspended materials from the substrate undergoing purification, and thereby reduce the biochemical oxidation demand of the final effluent. Laboratory experimental results and field observations are presented.

CONTENTS

PAGE
INTRODUCTION 263
TRICKLING FILTERS 264
ENVIRONMENT 265
THE RICHMOND PILOT PLANT 265
  Results Observed at the Richmond Pilot Plant 266
FIELD OBSERVATIONS AT SAN RAFAEL MEADOWS 269
OXIDATION PONDS 270
LABORATORY INVESTIGATIONS 272
  Results of Laboratory Investigations: Continuous Cultures 274
  Results of Laboratory Investigations: Batch Cultures 277
  Results of Laboratory Investigations: Other Tests 278
FIELD OBSERVATIONS AT CONCORD 280
  Insects of Concord Oxidation Ponds 282
SUMMARY 285
ACKNOWLEDGMENTS 285
LITERATURE CITED 286
FIGURES 287

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THE ROLE OF INSECTS IN SEWAGE DISPOSAL BEDS[1]

R. L. USINGER[2] and W. R. KELLEN[3]

INTRODUCTION

AN INVESTIGATION of the role that insects play in the dynamic process of the biological purification of sewage, both in trickling filters and oxidation ponds, had never been undertaken in this country before the present study was made. Lloyd (1940, 1943*a*, *b*)[4] investigated the ecology of insects in sewage trickling filters in England, but his research was primarily directed toward the problem of inter- and intraspecific competition; the actual effect of insects on the process of sewage purification, or whether or not insects were really involved in the process, was not determined. Lloyd found that the larvae of certain flies of the families Psychodidae and Tendipedidae were abundant in filter beds and that they were physically beneficial to the functioning of the filters. The larvae kept the zoogleal growths on the filter stones in check by their scouring habits and prevented the filters from becoming choked with an overgrowth. Moreover, it was found that competition between the mixed fauna prevented any one species from breeding to excess and thus causing a nuisance. One of the aims of the present project has been to investigate the interrelationship between larval populations and filter efficiency.

The limnological work which has been done recently on the self-purification of streams polluted by domestic and industrial wastes has laid a foundation which is applicable to the very similar conditions found in the oxidation pond method of sewage treatment. The polluted stream and the series of oxidation ponds are essentially parallel in their manner of breaking down and converting the raw sewage to more stable forms. In stream studies, however, the emphasis has been placed on the finding of indicator species by which zones of different degrees of pollution may be recognized. These studies have relegated the role of insects to a passive part in the over-all process of purification. The cause-and-effect picture which has been drawn shows the insect populations qualitatively distributed through an area which ranges from a zone of high pollution serially to a zone of little or no pollution. The cause

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[4] See "Literature Cited" for citations, referred to in the text by author and date.

of this distribution is the lack of dissolved oxygen imposed upon the water by the high biochemical oxygen demand of the influent sewage. The oxidative stabilization of the organic materials requires an adequate availability of oxygen, otherwise the oxygen store is soon depleted and anaerobic conditions, unsuitable for most forms of life, develop.

Although it has been recognized that insect populations are restricted in their distribution by environmental conditions, so that eurytrophic and stenotrophic forms are forced into their respective niches relative to their physiological ability to tolerate the different degrees of pollution, these same populations may nevertheless react to some extent and change the environment in which they live. It has been the purpose of this investigation to determine how the insect populations of oxidation ponds react to their environment and how their presence affects the sewage purification process.

TRICKLING FILTERS

The use of trickling filters for the biological stabilization of domestic wastes is perhaps more prevalent in this country than any other method of secondary sewage treatment. The trickling filter method of sewage treatment consists in applying presettled sewage over a bed of stones. As the sewage percolates through the bed, it flows over the great extent of surface area presented by the stones and comes in contact with the naturally formed zoogleal growth on their surfaces. This zoogleal growth is made up of dense populations of scum-forming bacteria, fungi, and protozoa. The metabolic action of these microorganisms is responsible for the breakdown of the sewage organic matter.

Oxygen availability is primarily through the agency of absorption from the atmosphere as the sewage filters through the bed in the form of a thin film covering the stones. Because of the large surface area in contact with the interstices, the sewage has maximum opportunity for aeration. The process is a function of the size of the stones, which may vary from two to four inches in diameter; their size determines both the amount of surface area available and the magnitude of the interstices. Smaller-sized stones provide greater surface area for aeration and zoogleal action, but the smaller interstices are more easily clogged by the growth of sewage fungi and other organisms that make up the zooglea. Since the presence of oxygen is necessary for aerobic metabolic processes, the flow of air through the interstices of the bed is extremely important. The condition of the zooglea is also of prime importance, for a healthy and rapidly growing and metabolizing zooglea obviously is most efficient in breaking down the organic materials of the sewage.

Algae play a negligible role. Their growth is restricted to the surface, where they have access to sunlight; but they undoubtedly do add a small amount of photosynthetic oxygen to the sewage as it passes over the limited zone of growth. The manner of sewage application also contributes to the oxygenation of the sewage, which falls about ten inches from the distributing arms to the surface of the filter bed and strikes the surface stones with considerable force, breaking into small droplets before passing down through the bed.

Rate of application of sewage to the bed may vary considerably, depending upon the design of the filter. Slow-rate filters may have beds five to ten feet deep, to which settled sewage is applied at rates of one to four mgd (million gallons per day) per acre. High-rate operation is at rates of about sixteen to thirty mgd of settled sewage per acre in beds ten feet deep. In the biofiltration process the rate of application may be even higher, but portions of the effluents are recirculated to the influent at about a 50 per cent ratio; the two filter beds in this case are usually about three feet deep and run in series. With high rates of application, the hydraulic force of the sewage as it flows through the filter may assist in the sloughing of the zoogleal growth and prevent clogging of the interstices. The overgrowth of the zooglea and accumulation of sewage on the surface of the bed (ponding) prevent the penetration of oxygen into the pores of the filter. This may cause anaerobic conditions to develop and prevent proper functioning of the unit.

ENVIRONMENT

Filter beds present a rather narrow, restricted environment; the insect fauna is a highly specialized one, and is almost exclusively dipteran. The zone of habitation is restricted to the zoogleal matrix of the filter stones. To become established here the insects must burrow into the growth to withstand the constant flushing action of the sewage being applied by the distributing arms. Free-living aquatic forms are absent. Insects which find this environment suitable for their life processes have relatively little competition and are particularly able to maintain large, successful populations. The larvae of a small fly belonging to the family Psychodidae are notoriously common in filter beds, and are one of the main biological agencies at work helping to keep the zoogleal growth in check. The larvae of the fly feed upon the zooglea, stimulate the new growth of zoogleal organisms, and prevent the growth from becoming old or so abundant as to clog the interstices of the bed.

THE RICHMOND PILOT PLANT

Two pilot-scale trickling filters were constructed at the University of California Richmond Engineering Field Station in 1953 in order to determine the effect of insects on the zoogleal growth and the resultant effect on the process of sewage purification. The pilot plants consisted of two sheet-metal containers, three feet in diameter and four feet high. Each of these filters was fitted with inverted wire baskets which opened to the outside at three levels (fig. 1).[5] Into each of these baskets was placed a wire drawer which was filled with stones similar to those used in the filter; the stones in the removable drawers were essentially in a continuum with the rest of the filter stones. In this way a drawer could be removed so that the zoogleal growth and the larvae of insects could be examined at different levels of the filters. These sampling drawers were located just beneath the surface, at the middle, and at the bottom of the filters; they extended to the center of the filters and were six inches wide by four inches deep. The stones used in the two pilot

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[5] All figures are grouped at back of this issue.

filters were crushed rock; each piece was approximately three to four inches in size. Because of the characteristic angular shape of the stones it was possible to make estimates of the surface area of the stones examined during a test run and to relate this to the amount of zoogleal growth and larvae present. Thus, a quantitative estimate was obtained of the amount of growth and distribution of the larvae throughout the filters.

The two pilot filters were operated for about a year, using the sewage of the city of Richmond, California. The sewage was first pumped to a primary settling tank, the suspended solids were removed, and the sewage was then pumped to the filters. The filter influent pump was controlled by an electrically operated timer, with which it was possible to control the loading of the filters by varying the duration and interval of sewage application. The sewage was applied by means of two spray nozzles located eighteen inches above the surface of the filters. Each of the filters represented a volume of 4.89×10^{-4} acre-feet. They were seeded with zooglea from a trickling filter, and were operated continuously for an initial two-month period in order to establish a growth on the stones. The various loadings used are shown in table 1.

TABLE 1

RECORD OF SEWAGE LOADINGS THAT WERE USED IN OPERATING TWO PILOT FILTERS AT RICHMOND, CALIFORNIA

Test series	Rate of application	Gallons per day	Pounds BOD* per day	Gallons per acre-foot per day	Pounds BOD per acre-foot per day
I	60 sec/min	1,350.0	2.82	2,760,000	5,767.0
II	30 sec/min	675.0	1.41	1,380,000	2,883.5
III	15 sec/min	377.5	0.70	690,000	1,441.7
IV	5 sec/min	112.5	0.23	230,000	480.5

* Biochemical oxygen demand.

Each of the tests was continued for about two months in order to allow plenty of time for the filters to reach equilibrium. During this time samples were taken of the sewage as it percolated through the filter, in order to determine the rate of purification. This was done by removing the sampling drawers and inserting beakers to catch the sewage as it flowed through the filter bed. To have a basis for comparison, one of the filters was periodically dosed with DDT at the rate of about 5 ppm for a one-hour period with the influent sewage; this prevented the establishment of psychodid larvae in the filter.

Results Observed at the Richmond Pilot Plant

Figure 2 shows the larval populations at three depths in the untreated pilot filter, plotted against the different rates of sewage application. Under conditions of continuous loading the populations appear at their lowest density, which conforms with the observations that the psychodids are always less abundant in filters which are operated at high rates of sewage application. The high rate of loading apparently prevents the establishment of dense populations, because of the physical action of the rapid flow of sewage over the stones, which tends to flush the adults, eggs, larvae, and pupae through the bed. The adults are particularly vulnerable to this action, since they have no means of withstanding the force except by concealing themselves in protected areas beneath certain of the stones. The physical action of the application is greatest at the top of the bed, and it is there that the lowest populations occur. The density increases toward the middle of the bed and then falls off again at the bottom of the filter.

The zoogleal growth at continuous loading reaches its maximum density when there are no larvae present (fig. 3). The continuous supply of rich nutrients particularly favors the abundant growth of zooglea in the upper portion of the bed; it is about twice that of the middle of the filter and four times as dense as that occurring in the bottom of the filter. As indicated by the curves, the larvae are usually most abundant in the upper half of the filter when the rates are lower, because of the greater amount of food available there.

When the larvae are present under continuous loading the growth of zooglea is greatly reduced; this is not due to the larvae eating the growth, but to the larval movements, which tend to loosen the growth and cause it to slough off more readily. The sloughing caused by the larvae is not a continuous process; dense patches of growth are sloughed off at one time. This unfortunately is not always indicated by the periodic sampling of the effluent on which suspended-solids determinations are made (fig. 4). The suspended-solids reduction is very low under continuous loading, and although much of it is due to the rapid flow-through time of the sewage, some is contributed by the sloughing of the zoogleal growth. This is particularly apparent in the control filter, where a more continuous minor sloughing occurs because of the scouring action of the flow of sewage; the effluent suspended solids are even greater than those of the influent.

A lower rate of application (30 sec/min) produces a reduction in growth in the upper portion of the control bed, and little change in the lower half. Correspondingly, there is an increase in the efficiency of removal of suspended solids, and most of the removal takes place in the lower half of the bed where the growth is less dense. This appears to indicate that zooglea becomes a less efficient tool for sewage treatment when it becomes too abundant. The greater reduction in suspended solids is apparently related to the lower hydraulic loading factor, which produces an increase in detention period of the sewage in the filter and a reduced tendency for sloughed materials to be added to the effluent. The untreated filter develops a higher density of larvae at this application rate than at any other rate. This is due to the reduced flooding tendency of the lower rate of application.

The combined action of the scouring activities of the larvae and the rate of applied nutrients must stimulate the growth of zooglea, for even though the larval density is considerably greater than in the previous case, the weight of zooglea at the three levels of the bed remains about the same. This is especially noticeable in the upper half of the filter, where the larval population is more than twice as dense as the population found in the lower half. Suspended-solids reduction is considerably greater in the filter with larvae than in the control, especially in the upper half of the bed, where suspended solids are reduced equally in amount to the final effluent of the control. This results from the greater efficiency of the more rapidly growing zooglea.

As the rates of application are reduced to 15 and 5 sec/min, the larval populations are also correspondingly reduced. The zoogleal growth in the control filter is reduced because of the competition for the limited amount of available nutrients. The reduction in suspended solids levels off at this point because of the attainment of a maximum relationship between zoogleal activity and its ability to remove solids, as well as sloughing and hydraulic loading. At the lower rates the upper and lower portions of the filter appear to function with equal capacity in relation to solids removal. The larval populations, although considerably reduced, still exert their influence, for the reduction in solids continues to climb and the efficiency of the upper half increases. The zoogleal growth is reduced slightly, but still maintains a greater density in the upper levels.

The reduction in total BOD (biochemical oxygen demand) steadily increases as the rate of application becomes less in both filter beds (fig. 5). The consistent trend is, of course, relative to the pounds BOD applied to the filter per day. It should be noted that the upper halves of the beds are responsible for an increasingly greater percentage of the total reduction as the loading factor is decreased. There appears to be little relation between the amount of zooglea present and its ability to stabilize the organic materials of the sewage; the filter with larvae maintains a constant low density of growth, but it is consistently more efficient in sewage treatment. This can only be attributed to the physiological state of the zooglea. When the larvae actively feed upon the film they remove the old, lower metabolizing organisms and promote the renewed growth of a younger, more efficient zoogleal matrix. The larvae thereby indirectly promote better filter operation.

The supernatant BOD (fig. 6) follows a somewhat similar trend, but the efficiency of removal climbs rather steeply as the rate of application is reduced. As with the total BOD, the upper levels of the filters are responsible for the greater percentage of the reduction. Here again the filter with larvae consistently produces a more stable final effluent.

The pH readings (fig. 7) indicate that a better degree of nitrification is obtained at the rates of application below 30 sec/min. At the higher rates the pH increases from the middle to final effluents, indicating that the ammonia state is being produced. The lower application rates produce final effluents which continue to have lower pH values than those of the middle of the filter, indicating that nitrate production dominates and that it progressively takes places in higher strata of the filter bed; the maximum value obtains at the lowest rate of application. When larvae are present in the filter there is an increased efficiency in this process at all rates of sewage application, but at the lowest rate the hydraulic loading appears to overshadow this difference, and the pH values of the final effluents approach equal values.

The rate of decrease in titratable alkalinity (fig. 8) also indicates that the larvae stimulate the young growth of zooglea; the effect is particularly evident at the lower rates of loading, although the trend is apparent at all rates. When the larvae are present in the filter bed the zoogleal organisms metabolically utilize the salts of the sewage more efficiently, reduce the alkalinity, and cause a reduction in the buffer capacity of the effluent materials. At the high application rates the reduction is least, because of the dense growth and overloading of sewage, for both the control and untreated filters show similar low reductions in alkalinity. The main difference between the action of the two filters appears to be in the greater alkalinity-reducing ability of the lower portion of the filter with larvae; this portion continually accounts for about 50 per cent of the total reduction, whereas most of the reduction takes place in the upper half of the control filter. This may be due to the more even distribution of the larvae throughout the filter at the lower rates of application.

A microscopic examination of the zoogleal growth revealed that the floral and faunal populations in the control filter were not affected by the periodic dosage with DDT. No qualitative determinations of the protozoans were attempted, but in all cases ciliates were the dominant forms present, and their abundance decreased progressively from the top to the bottom of the filter. Moreover, the populations decreased in density as the rate of sewage application decreased. The same general trends held for nematodes also. When the larvae were present the protozoan and nematode populations were usually reduced to about one-tenth of those found in the control filter, and these reduced populations appeared to be more evenly distributed through the filter bed.

FIELD OBSERVATIONS AT SAN RAFAEL MEADOWS

Field observations of a small biofiltration plant at San Rafael Meadows, California, were made in 1953, during the time that the test series was made with the pilot-scale filters at Richmond. This small plant accommodates 200 families, and has two serially operated filters three feet deep and fifteen feet square. The stones of the first filter are about three to four inches in diameter; those of the second are about half this size. Each of the filters is equipped with a separate settling tank, and portions of the effluents of each of these are recirculated (at about a 50 per cent ratio) to the influent sewage being applied to the surface of the beds.

Psychoda alternata Say is by far the most abundant fly living in these beds, but other insects are also present in small numbers. The following is a list of the species found:

- DIPTERA
  - Tipulidae
    - *Limonia simulans concinna* (Williston)
  - Phoridae
    - *Diploneura cornuta* (Bigot)
  - Ephydridae
    - *Scatella stagnalis* (Fallen)
    - *Scatella paludum* (Meigen)
  - Leptoceridae
    - *Leptocera fontinalis* (Fall)
- COLEOPTERA
  - Hydrophilidae
    - *Cercyon fimbriatum* Mannerheim

Box traps were placed on the surface of the beds to catch the emerging psychoda flies so that the population trends could be determined. These general trends are shown in figure 9. The tipulid larval populations were followed by counting the larvae on the walls of the filters, where they were limited in distribution. These predaceous larvae were feeding on the larval psychodids; they were present in the filters only from January to May. The psychodids, however, were always in abundance except during the height of the summer, when the increased growth of zooglea caused ponding to develop; this condition prevented the larvae from continuing in the filter except at extreme edges of the bed.

The high-rate application to the filters apparently favored the overgrowth of zooglea during the summer months, and prevented the larval populations from reaching sufficiently high initial densities to prevent ponding. It should be noted that whenever the biofilters were shut down temporarily, the psychodids increased tremendously in abundance and soon reduced the growth in the beds; this increase was due to the immediate response to the absence of the flushing action of the high sewage application rate.

During the period of ponding there was a noticeable reduction of the suspended solids in the effluent (fig. 10). When the larvae again established themselves the solids increased; the peak reached in January and February was probably due to the sloughing of some of the ponded materials which were built up during the summer. The pH (fig. 11) of the effluent material remained fairly constant through the year, but the alkalinity continually rose during the period of ponding, and appeared to reach a minimum during the period of larval abundance. The BOD of the influent (fig. 12) showed considerable variation because of the very nature of the material. (The effluent, however, should be fairly homogeneous because of the mixing which occurs in the settling chambers; therefore, the time of sampling should not be too critical.)

During the period of unloading of the ponded material in winter there was an increase in the effluent BOD, but it dropped off during the following months when the larvae became established and the suspended materials decreased. During the period of ponding there was a gradual trend for increase of BOD because of the decreased efficiency of the filters, but the increase did not become very great; apparently the recirculation of the effluents and the capacity of the filters were adequate to produce fairly stable effluents, even during the periods of inefficient operation.

OXIDATION PONDS

The use of oxidation ponds as a method for the secondary treatment of sewage has been rather widely adopted in the West because it represents one of the most economical means known for sewage treatment, availability of land and climatic conditions permitting. Essentially, this sewage treatment process is effected by a series of shallow basins, the size and number of which may vary, depending upon the capacity for which the plant is designed. After primary settling to remove suspended solids the sewage is allowed to flow through the ponds for a definite period of time. During this time the organic material is stabilized by bacterial action. Primary treatment usually removes about 45 per cent of the initial sewage BOD; treatment in the oxidation ponds usually removes about 80 per cent of the remaining BOD, and produces a final effluent with a BOD of about 20 ppm without further treatment.

Oxidation ponds may vary in number and size, depending upon the situation. They may cover more than thirty acres of land, but are always relatively shallow (up to three feet deep), to allow for aeration and penetration of sunlight. Naturally, the operation of the ponds is greatly influenced by seasonal climatic conditions, such as temperature, rainfall, hours of sunlight, etc. Where a number of ponds are normally used, say six, it is possible that some of the end ponds may be by-passed during optimal periods of treatment, and/or portions of the effluent may be rerouted to the influent to dilute the initial organic loading of the ponds and to supply oxygen where it is most needed.

The breakdown of the organic materials of the sewage in oxidation ponds is accomplished by the action of aerobic bacteria that are indigenous to sewage. The time that the sewage is held in the ponds is the detention period; detention is important because sufficient time must be allowed for bacterial action to take place. The detention period is dependent upon such factors as the nature of the sewage, volume of sewage, size of the ponds, and the prevailing climatic conditions.

Bacterial breakdown of sewage provides a rich source of nutrient materials and an abundant production of carbon dioxide as end products of bacterial metabolism. Under these conditions rich blooms of unicellular algae appear, converting the CO_2 and nutrients into cellular material in the presence of sunlight and releasing oxygen in their photosynthetic processes.

Papenfuss and Silva (1952), in their systematic study of the algae occurring in oxidation ponds, report more than twenty-six species of algae inhabiting the oxidation ponds of the San Francisco Bay area and the San Joaquin and Imperial valleys of California; they also report the abundance and seasonal incidence of some of these forms. These unicellular plants contribute greatly to the oxygenation of the ponds, and undoubtedly are beneficial in supplying oxygen for aerobic bacterial action during the daylight hours; however, the algae also compete with the bacteria for oxygen during the night when photosynthetic activity ceases. Moreover, under conditions of long detention (periods of three to six weeks), such as would exist in the latter ponds of a series, these algal cells are potentially unstable organic materials. The organic content of the ponds in the form of algal cell material may be many times greater than that of the sewage present, which is almost completely stabilized.

Ludwig *et al.* (1951), investigating the symbiotic relationship between algae and bacteria in oxidation ponds, report: "The principal work of treatment is accomplished by aerobic bacteria, which oxidize the organic carbon contained in the sewage to carbon dioxide. The algae, through photosynthesis, convert much of this carbon dioxide to algal cell material. Therefore, the pond effluent may contain as much organic material as the sewage entering it. . . . 'Old' [*Euglena*] cells are large and fat, are yellowish in color due to lack of chlorophyll, are reproducing and growing very slowly, produce less oxygen than they respire, and tend to settle out and adhere to bottom surfaces." This algal senescence and settling is constantly occurring in oxidation ponds; the ultimate fate of these cells is opposed to the basic function of the entire purification process, for they add to the organic content of the ponds at a point when the sewage materials have already been stabilized.

Papenfuss and Silva (1952) also recognize the organic load in oxidation ponds in the form of algae; they report: "The effect of the algal population on the BOD of the sewage oxidation ponds and on that of the stream receiving the final effluent is important. A living algal cell exerts only a slight BOD, that which is necessary for respiration. When the algal cell dies, however, its body is added to the load of unstable organic matter that exerts a full BOD. A final effluent that has been detained beyond the optimum period will seriously increase the BOD of the receiving stream because of the large number of dead cells." From this it appears obvious that any agency operating toward the removal of this algal cell material will be directly beneficial to the operation of sewage oxidation ponds.

Since oxidation ponds are basins hollowed from the earth, and have abundant algal plant food available, it is not surprising that certain insect forms become established in them. Qualitative studies undertaken by this research project have revealed about fifty species of insects inhabiting oxidation ponds. By far the most abundant forms present are, in number of individuals, members of the dipterous family Tendipedidae (Chironomidae). The larval stages of these flies are aquatic and live in small tubes constructed in the superficial layers of the bottom material. In certain areas during the summer months they may reach a density of twenty to thirty larvae per cubic inch of bottom material. The larvae have been found to occur most abundantly in the zones of recovery in oxidation ponds, i.e., in the zones where algal populations are most dense. Moreover, these larvae are omnivorous and feed directly upon the algae, and undoubtedly are the primary agency removing algae from the system. Weston and Turner (1917), in a study on the digestion of a sewage-filter effluent by a small stream, report that they found great numbers of these flies breeding in the bottom materials and realized that they must be exerting a beneficial influence. They state: "From the omnivorous habits of the larvae, their value as food for fish, and the number of mature insects which leave the water entirely, it is obvious that *Chironomus* is an important factor in the removal of organic matter."

The purpose of the present investigation has been to determine how tendipedid larvae effect this algal removal, and the influence the larvae have on the BOD during the process of sewage purification under controlled conditions.

LABORATORY INVESTIGATIONS

Two distinct methods were employed in the laboratory to investigate the effects of insects on sewage purification. These were continuous cultures and batch cultures. The important difference between the two methods is the detention period. In continuous cultures a dynamic third dimension is introduced by operating the culture tube, or growth unit, at a certain predetermined detention period, i.e., a given amount of effluent is withdrawn each day and an equal amount of fresh influent is added. In this way daily analyses can be made of the effluent material from the growth unit, and a continuous record of the changes taking place can be traced as the forces involved strive for equilibrium. This technique closely approaches the physical operation of oxidation ponds in the field. Moreover, it involves the most important physical variable determining oxidation pond operation—the length of time available for bacterial action. Also, the detention period may impose limitations on the algal growth by decreasing available nutrients at long detention periods, or by restricting algal populations at short detention periods by rapid removal of cells.

Batch cultures, on the other hand, have a theoretical detention period of infinite time, since nothing is added or removed during the course of operation. Batch cultures have the advantage of expressing direct relationships in terms of simple time variables. It is difficult, however, to compare directly one set of cultures with another. This can be solved by employing controls with each series and comparing the separate series in terms of the differences expressed by their respective controls.

The continuous growth units used in this investigation were six vertically mounted glass tubes eighteen inches long and three inches in diameter. Each was mounted six inches from a single thirty-watt daylight-type fluorescent tube, from which it received continuous lighting. For temperature control, water from a constant-temperature water bath of 25° C. was passed through a tube running the entire length of the unit. Since it was found necessary to employ some kind of mechanism for the purpose of mixing the solution so that algal population counts could be made, another tube was introduced from the top of each unit and extended to within one inch of the bottom. Air supplied by a small aquarium pump was bubbled into the growth units through these tubes at the rate of 100 cc per minute. The bubbling invalidated any attempt to make determinations of dissolved oxygen, but because of the continuous lighting and presence of algae the dissolved oxygen was always above the saturation point, owing to photosynthetic oxygenation.

So that the larvae which were to be introduced into these units would have a solid substrate in which to burrow, a half inch of coarse sand previously washed free of soluble materials was placed in the bottom of each. All units were autoclaved before starting a series of tests.

A total volume of 500 ml of dilute sewage was employed in each test using continuous growth units, regardless of the detention period. The substrate used in all tests was a synthetic sewage adapted from a formula developed by L. W. Weinberger (1949). This sewage had the following characteristics: 5-day BOD (20° C.) = 210 ppm; pH = 7.5; total titratable alkalinity = 165 ppm.

Twenty species of bacteria from pure cultures and common to normal sewage were used in all the test runs. A single species of alga, *Scenedesmus obliquus* (Turp.) Kutz, also from pure culture, was used in these tests to duplicate the algal growth normally found in oxidation ponds. By consistently using the same species of bacteria and the single species of alga, it was hoped to keep the biological variables at a minimum.

To initiate a series of tests, a mixture of bacteria was first inoculated into a small amount of synthetic sewage and a film of algae was added. The algae and bacteria were maintained in pure cultures grown on nutrient agar. The initial mixture was allowed to grow for three or four days until a dense growth of algae had developed. At this time 100 ml of synthetic sewage was added to the growth units, together with 20 ml of algal-bacterial inoculum. As the algal growth developed in the growth units, new sewage was added each day until a total volume of 500 ml and an algal population of about 6×10^6 per ml had been obtained. At this time a complete transinoculation was made of all six units, larvae were added, and the fixed detention period was started.

Mature larvae of *Glyptotendipes barbipes* (Staeger), about 1.5 cm long, were used throughout the entire investigation. They were first thoroughly washed by repeatedly passing them through successive changes of distilled water to remove any associated protozoans. Individuals which pupated during the course of the tests were removed and replaced immediately by new larvae.

Algal cell counts were made with a haemocytometer. pH readings were made on a Beckman pH meter; total titratable alkalinities were also determined on the pH meter by titrating the sample to a pH of 4.3 with standard acid. Total BOD samples were first heated to 100° C. to kill the algae, and then treated in the normal manner according to the "Standard Methods" (American Public Health Association, 1946) technique of the dilution-bottle method of BOD determination.

Results of Laboratory Investigations: Continuous Cultures

Figures 13 and 14 show graphically the growth curves of algal populations with respect to detention period[6] and larval action in the continuous growth units. The effects of detention alone upon the "control" units show that total algal populations increase greatly as the detention period becomes longer. In the shorter period (three days) the obvious limiting factor is the physical removal of the cells with the large volume of effluent; the cells apparently are unable to multiply rapidly enough to reach higher densities, even though nutrients are abundantly available. At the longest detention period (twenty days) the total populations are highest. Under these conditions the algae are no longer limited by their rate of removal with the effluent, but are limited by their nutritional requirements. These cells are what might be called "old" cells, for they are multiplying slowly and have a greater tendency to settle out of suspension. The comparative rate of algal multiplication under extremes of detention period can best be appreciated by considering the yield of algal material, i.e., by dividing cell populations by their respective detention periods. This shows that the algae at the shorter detention periods are actually multiplying twice as fast as those at the longer periods. They are, therefore, the most vigorous and most rapidly assimilating factors in the production of algal cell material.

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[6] The interpretation of "detention period" data in figures 13 through 20 will be facilitated by reference to the following formula:

$$\text{Detention period} = \frac{\text{Total volume}}{\text{Daily influent volume}} = \text{Time (days) required theoretically to replace total volume.}$$

Detention periods of five and nine days express intermediate conditions of algal growth as compared with the two extremes. Theoretically, these algae are existing under what may be considered optimal conditions, since neither detention periods nor available nutrients should be limiting. They are obviously multiplying more slowly than those of shorter detention periods, but are removed by their effluents rapidly enough so that they do not become "old" cells characteristic of longer detention periods.

Because of the continuous lighting, algal populations in the growth units are greater than those which generally occur under field conditions; however, comparable densities have been observed in certain restricted zones in the field, especially along the edges of the ponds.

The effect of the presence of larvae on algal populations at all detention periods is an obvious one: they reduce algal densities in all cases, even to the point of completely eliminating them. From the evidence it appears that the rate of removal by the larvae is greatly influenced by the detention period. At the shorter detentions the rate of removal seems to be considerably increased by the volume of the effluent. This is especially noticeable after the algal populations have been reduced to a low level beyond which they are unable to maintain themselves further and are very rapidly removed from the system. This influence of the detention period appears to overshadow the comparative rates of removal by the different numbers of larvae used, for in detention periods up to nine days a few larvae appear to cause as much reduction in algal populations as do many larvae. At the longest detention period, however, the differential influence of the number of larvae is apparent, since each unit volume is in association with the larvae for a longer period of time and therefore reflects their influence more strongly; moreover, the physical influence of the detention period is lowest and does not overshadow that of the larvae. The rate of removal at a twenty-day detention period is lower than at shorter periods because of the higher initial algal population. Also, the sudden drop in population does not occur, which supports the observation that the higher effluent volumes are mainly responsible for the rapid decline in populations at shorter detention periods, and that the larvae are acting primarily as trigger mechanisms by reducing the algal populations to a point where they can no longer compete with the detention.

The biochemical oxygen demand (BOD) test is a measure of organic material as expressed by the amount of oxygen consumed by the bacteriological oxidation of the material at 20° C. for a five-day period. Since this investigation considered algal cell material as a potential load caused by the ultimate death of the cell, all effluent materials on which BOD determinations were made were first heated to 100° C. to kill the algal cells, so that they would be attacked by bacteria and their total BOD would be expressed. This technique gave a common denominator to all such BOD determinations.

Figures 15 and 16 show the results of BOD determinations performed on the growth-unit effluents. The "control" units again show the striking influence of detention upon algal populations, but these populations are expressed in terms of their organic material (plus that portion of the substrate which had not been stabilized previously by bacteria). In general, however, the BOD's approach the actual growth curves of the algae themselves, reaching higher peaks as the detention becomes longer and the algal population becomes higher.

The general effect of the larvae is to lower significantly the BOD's at all detentions. At a detention of three days the BOD remains at a relatively high level, even though the algal cells have been greatly reduced; this BOD is attributed to the sewage, which is only partially stabilized at this short detention, although larval excretory products are undoubtedly also adding to it. The intermediate detentions (five and nine days) appear to give the lowest BOD's; they also produce this low level in the shortest time. This coincides with the observation that these intermediate detentions are apparently also optimal for algal growth. However, after the growths have been reduced, the sewage exhibits a very low BOD, showing that it (plus any larval end products) has had sufficient time to be almost completely oxidized by the bacteria. The BOD's of the "control" are therefore almost entirely the results of algal cell material. The highest BOD's of the series occur in the growth units operating with the longest detention periods, paralleling the highest algal density.

The effects of the larvae are very clearly seen to duplicate the curves of associated algal populations, which likewise show the quantitative effects of the larvae. As with the algal populations, the quantitative effects of the larvae do not express themselves at the shorter detentions, but in general follow the algal population curves which are the major contributors to the BOD. However, the various factors involved apparently reach a relatively common low level of equilibrium at the end of each series, for the curves approach a common point.

Sewage undergoing bacterial action typically has its pH reduced initially by first-stage oxidation with the production of organic acids; later, the pH rises with the further breakdown of the organic acids. However, when algae are present in the system, pH always appears to be on the basic side. Total titratable alkalinity always runs inversely to algal populations, for the algae abstract carbon from the alkalinity of the sewage in accordance with the reaction:

$$HCO_3^- = CO_2 + OH^-.$$

These released hydroxyl ions, plus the removal of CO_2 by the algae, could well explain the rise in pH. As shown in figures 17 and 18, pH varies over rather wide limits, for the buffer capacity of the sewage becomes very weak after it has been associated with the algal population, as is indicated by the titratable alkalinity.

Apparently, the general trend is for the pH values to be higher in the units operating under the shorter detention periods; this is because of the greater amount of bicarbonate alkalinity made available by the higher influent volumes. Therefore, since the algal cells are multiplying more rapidly in these systems and attacking this alkalinity more vigorously, they are releasing the hydroxyl ions to solution in greater amount. Alkalinity at all detentions falls rapidly to very low levels, but because of the different physiological states of the algae at short and long detentions and the differences in the influent volumes, the mechanisms producing this lowered alkalinity are qualitatively different.

On the other hand, when larvae are present in the continuous growth units, curves for alkalinity and pH are about the same as for the "control" units until the algal populations begin to decline in density (figs. 19 and 20). Then there is an immediate increase in the total alkalinity, showing the very marked relationship between algae and alkalinity and the suppressing effect which the algae exert. At detention periods up to nine days the alkalinity recovers quite rapidly, for the natural reduction in alkalinity by bacterial oxidation of the sewage is not increased by the algal growth; also, larval end products are adding to the titratable alkalinity. At the longest detention the alkalinity does not recover at the end of the test series, for the algal populations are greater and the influent volume is small; however, at the end of the series a slight rise in alkalinity does begin to express itself, paralleling the late decline in algal populations at this detention.

A single series of tests was also run, using hydrophilid beetles instead of tendipedid larvae. These beetles are common in oxidation ponds, are omnivorous in their feeding habits, and probably feed on algae to some extent in their natural environment. It was decided to see what effect they would have on algal populations when algae were the only source of food available to them. These tests were at a detention of five days with increments of five and ten beetles. Figures 21 and 22 show the results.

Apparently, the beetles do not very effectively remove algae from suspension. This may result from their mode of feeding, for the algae would have to settle out in order to be effectively available to them. Since the algae were deliberately kept in suspension in these tests, undoubtedly the cells were not so available to the beetles as they would have been in the field, where the natural tendency to settle occurs. The tendipedid larvae, on the other hand, spin webs of silk in forming their tubes, and by their undulatory movements within them cause currents of algae to pass through and adhere to the tubes. The larvae then literally eat part of their tubes to obtain the algae. They also, of course, feed upon algae which have settled from suspension. The beetles, however, did lower the algal population to some degree, and correspondingly also lowered the BOD of the substrate. At the end of the test the growth unit with five beetles showed a BOD higher than the control, even though the algal population was lower; this could not be explained on the basis of the fecal material (which was considerable) added to the solution, for ten beetles produced a much lower BOD. This test was not duplicated, so it is possible that the final BOD determination was for some reason invalid. pH and alkalinity curves for beetles followed courses similar to those which were obtained with larvae, i.e., pH's were consistently lower and total titratable alkalinities were slightly higher because of the lower algal populations.

Results of Laboratory Investigations: Batch Cultures

Altogether, thirty-six separate 100-ml batch cultures were performed, using the synthetic sewage, bacteria, and algae as before; twelve of these were controls and the others were carried on in two separate series, each with ten larvae present. These tests were run for different lengths of time, ranging from three to twelve days. The differences expressed between individual controls and those with larvae present were then plotted by using a base line to represent the controlled conditions, and plus and minus values to represent points of departure from these controlled conditions (figs. 23 and 24).

In the batch cultures the reduction of BOD caused by the larvae very clearly becomes greater when the larvae are in association with the culture for longer periods of time; at the end of twelve days the differences between the controls and the cultures with larvae are about 100 ppm. The reduction in actual cellular material also declines steadily as time increases, reaching its lowest point in twelve days (when the series was terminated). At these longer periods the potential BOD of the cells becomes apparent, for although they are no longer multiplying very rapidly, they are still increasing their BOD by becoming senescent and accumulating larger quantities of fat.

pH values are consistently lower than those of the control cultures. Total titratable alkalinities, on the other hand, average higher than the controls; this supports observations made on the continuous growth units. The expressed values in the batch cultures are generally of less magnitude than those of the growth units; these differences obviously result from the influence of detention. The data supplement and support those of the growth-unit series, but are not directly comparable because of the important physicochemical differences of different detention periods. It is interesting to note that the batch-culture results approximate most closely those expressed by the longest detention (twenty days); this is to be expected, since detention is exerting its minimal influence at its longest period.

Results of Laboratory Investigations: Other Tests

A second series of somewhat similar tests was performed with the growth units, but mechanical stirring was employed for mixing instead of air-bubbling. Furthermore, the detention period was determined in a different manner; the growth units were run in series, so that the effluent of each preceding unit was introduced to the succeeding unit as influent, i.e., the effluent of the first unit was used as influent for the second unit, the effluent of the second became the influent of the third, etc. The only unit receiving fresh synthetic sewage was the first one; all others received the spent substrate of the preceding unit plus the algae the substrate contained. The detention periods obtained in this series of tests were two, four, six, twelve, and eighteen days.

The serially arranged growth units were first allowed to come to equilibrium without the presence of larvae, so that the direct effect of detention on the algal populations could be observed; figure 25 illustrates the density of the algae. At the shortest detention (two days) the algae were limited in density by the rate of removal by the effluent volume; these cells were multiplying more rapidly than at the other detentions, for the algae received a fresh supply of nutrients each day. As expected, there was an increase in density at the four-day detention, but since the initial density of the first unit was introduced to the second, the increased density indicated that the cells continued to multiply, but at a somewhat lower rate. The decline in algal reproduction was due to the lowered nutrient value of the substrate. At detention periods up to twelve days there was little change in the algal density; the cells discontinued multiplying. These algae showed evidence of becoming "old" cells, and they were no longer typically elongated, but were rounded and vacuolated. At the longest detention period there was a decrease in cell density; the algae tended to be rounder and more highly vacuolated; others became lysed.

Under the conditions of this test the algae apparently were exposed to optimal growing conditions at the shorter detention periods where the available stubstrate nutrients stimulated rapid cellular reproduction and a healthy physiological state. The reduced BOD (fig. 26) during these detention periods indicates that the synthetic sewage was partially stabilized, but because of the rapid turnover, bacterial action could not go to completion. When the algae exhibited reduced physiological activity at six days and longer, the cells apparently began to secrete certain carbohydrates and fats which were added to the substrate (figs. 27 and 28). These added materials of course added to the BOD of the substrate. Although the amount of fats leveled off at the longest detention, the carbohydrate concentration rose sharply. At the longer detentions, therefore, the BOD of the subtrate became greater than that of the original synthetic sewage.

The total effluent BOD (fig. 29), which included the cellular material, followed the same trend as the supernatant, but at a higher level, indicating that most of the unstable materials of the long periods of detention were in the liquid phase. It should be noted, however, that the BOD of the algal cell material continued to increase at detention periods from six to twelve days, even though the population density remained constant; this increase is attributed to increased stored products in the individual cells. Moreover, when the algal density declined at eighteen days the relationship between total and supernatant BOD remained the same; the algae, though less dense, exerted a BOD equal to that of higher densities because of the stored products which increased as the cells became older. The stored materials were probably of the same nature as those which were secreted.

Determinations of the dissolved and volatile solids in the supernatant (fig. 30) also illustrate the increase of the secreted cellular substances as the algae became older at the longer detention periods; the suspended solids, however, followed a trend similar to the algal populations (fig. 31). The cells were the sole contributors to the suspended material, but whereas the algal density declined at eighteen days, the weight of suspended material remained at the same level as the higher density, again illustrating the increased weight of the older algal cells owing to stored products. The rise in dissolved solids supports the previous determinations of increased fat and carbohydrate concentrations secreted by the older cells and the correspondingly increased BOD.

The evidence obtained under the conditions of the above experiments supports the hypothesis that the presence of physiologically old algae is unfavorable to sewage treatment; the algae promote an increase of unstable organic materials in the substrate and defeat the purpose of the treatment process.

Twenty larvae were introduced to the growth units operated at detention periods of two, four, and six days, in order to determine the influence they would exert on the algal populations and on the stabilization of the substrate. Because of the high secondary organic loading of the longer detention periods, the dissolved oxygen content of the substrate was exhausted and the larvae were unable to survive. At short detentions, however, the young algae produced photosynthetic oxygen and maintained a level of dissolved oxygen of about 50 per cent saturation. This indicates that young, actively metabolizing and photosynthesizing algae are beneficial to the treatment process by supplying critically needed oxygen for the aerobic bacterial stabilization of the organic material.

The effect of the algal-feeding larvae on the algal populations is apparent (fig. 25); the larvae reduced the initial density of the cells in the first growth unit and thereby reduced the number of cells introduced to the second unit. The algal density continued to decrease in the second and third units because of the further removal by the larvae. Reduction of suspended and dissolved solids followed similar trends, since the source of these materials was dependent upon the algal density. The reduced BOD of the whole substrate and the supernatant illustrates that the stabilization of the organic materials proceeded along a more normal course; as the detention increased, there was increased time for bacterial action to attack and stabilize the organic load. Moreover, little added organic material was contributed to the initial synthetic sewage loading, indicating that the algae remained physiologically young and did not add secreted products to the substrate.

FIELD OBSERVATIONS AT CONCORD

A series of field observations of oxidation ponds was undertaken at Concord, California, in 1952–1953. At Concord there is a series of six oxidation ponds, the first two of which are composed of two ponds each, run in parallel (fig. 32). The settled domestic sewage is passed from a clarifier directly into pond I, from which it flows successively through the remaining five ponds; after a total detention period of about forty days the stabilized effluent is finally discharged into Walnut Creek. Since the chemical composition of sewage is different at any given time, the conditions produced in each of the ponds may vary to some degree, but usually the first two ponds are in a septic or near-septic condition. The water is slightly milky in color, and there is an evident odor of hydrogen disulfide produced by the anaerobic bacterial action that is taking place. Floating patches of black, gelatinous sludge are found along the edges. The influent portion of pond III is similar to pond II, but during the detention period of this pond a heavy bloom of algae is developed. The latter portion of pond III is usually very green with algae, and long streams of the unicellular plants can be observed passing into the effluent pipe.

The last three ponds are very similar in appearance; all have the same heavy bloom of algae, about equal to that found in the latter part of the third pond. No other kinds of aquatic plants are present. When flow into the ponds is stopped for any length of time the algal bloom also develops to an equal degree in the first two ponds, but when flow is resumed the septic conditions quickly develop again. The total algal bloom in each of the ponds appears to be highly variable, for the first two ponds may occasionally take on a greenish color, and at other times all ponds may lose a large part of their algae during a comparatively short period. This fluctuation must be the result of the interaction of many variable factors acting on the total environment, but the chemical composition of the sewage and the activities of algal-feeding organisms are probably the major ones.

The availability of dissolved oxygen is the first and foremost single factor to be considered in any system designed to stabilize biologically a continuous and heavy organic loading. In oxidation ponds, algae constitute the most important agency at work supplying this much-needed oxygen. The oxygen produced by algal photosynthetic activity greatly exceeds the individual metabolic requirements of the algae, and is readily available to the bacteria for oxidation of the sewage organic materials. It naturally follows, however, since photosynthetic oxygenation is dependent upon sunlight, that there must be great fluctuations in dissolved oxygen during any twenty-four hour period. In a zone of high algal density the oxygen productivity during sunlight hours is very great, but during the night the algae compete with the bacteria for oxygen. Moreover, in zones of low BOD and high algal content the oxygen used at night would be about the same as in zones of higher BOD and lower algal density.

To determine precisely how the dissolved oxygen does fluctuate from day to night, a test was performed on the last four oxidation ponds at Concord from 5 P.M. to 11 A.M., September 26–27, 1952 (figs. 33 and 34). Samples were taken at the effluent ends of the ponds at a depth of six inches. The dissolved oxygen content of the first two ponds was continually zero; this is not surprising, since the ponds received the greatest load of BOD from the influent sewage, and algae were absent. Figure 35 shows that the largest percentage of BOD removal occurred before the sewage reached pond III; the drop of initial BOD which began in November, 1952, resulted from the beginning of continuous 50 per cent recirculation of pond III effluent to the influent of pond I.

Figure 33 shows that pond VI had the highest degree of supersaturation of dissolved oxygen, and also that DO declined most rapidly in this pond; pond VI consistently had the most dense algal populations. Pond IV was the only one to reach zero DO, probably because of the combination of BOD and insect and algal respiration. It is interesting that the lowest DO's occurred just at sunrise and that recovery occurred very rapidly from that point, showing the very dramatic influence of photosynthetic oxygenation.

Another series of samples was taken at the edge of pond V, where algae and nymphal corixids were very abundant (fig. 34). In this local zone the DO dropped much more rapidly and remained at a low ebb until morning. Although determinations at the lowest points read zero, it seems probable that micro-amounts of dissolved oxygen were still present, but could not be measured by standard methods.

In the oxidation ponds the beneficial mixing of the water by free-swimming insects, causing diffusion of the oxygen to the deeper zones where it is most needed, must certainly outweigh the small amount of oxygen that the insects use and remove from solution. The surface strata to a depth of one foot or more are always supersaturated during the sunlight hours, but at the bottom (three feet deep) the DO may be relatively low, about 3 or 4 ppm or less. Here the tendipedid larvae are active; they aid in supplying oxygen for aerobic bacterial action by burrowing into the bottom materials and bringing a constant flow of water from the upper zones by their undulatory motions.

Figures 36, 37, and 38 show the effective mixing and turbulence created by a tendipedid larva—*Glyptotendipes barbipes* (Staeger). For this study a larva was placed in a short glass tube which substituted for the tubes the larvae normally construct by cementing together bits of organic matter and algae. The tube containing the larva was placed in a shallow pan of water and a grid of ink droplets was introduced. The currents created by the larva are shown by the displacement of the ink droplets. The function of larval undulatory action is to cause a current of oxygen- and food-containing water to pass through the burrow. The oxygen is absorbed through the integument of the larva for respiratory needs. The suspended food particles are snared in a filter of silk spun by the larva in the posterior end of the tube, and after a period of undulatory motion the larva reverses its position and eats the collected materials. A new filter is spun to replace the eaten one, and the process is repeated.

Insects of Concord Oxidation Ponds

A qualitative investigation of the insect fauna revealed a total of fifty-two species represented in the ponds (table 2).

The insect fauna of the first two ponds is almost identical. Most of the forms belong to the families Syrphidae and Ephydridae (Diptera), the larval stages of which have long been recognized for their ability to live in septic environments. The larvae are equipped with anal respiratory tubes for breathing atmospheric oxygen; they are found buried in the floating masses of sludge or in the mud at the edge where they feed on the available organic materials. The only other insects found are a single species of predaceous aquatic beetle belonging to the family Dytiscidae and occasional large numbers of springtails of the family Proisotomidae (Collembola). These latter insects cannot rightly be considered aquatic, for they live mainly at the edge of the pond feeding upon decaying vegetable materials, but because of their extremely small size they are also capable of venturing onto the surface of the water, where they may gather in dense patches. Parkinson and Bell (1919) found other members of this order to be very important in preventing ponding in trickling filters in England.

Midges of the family Tendipedidae are frequently found dead on the surface of the first two ponds, and during the time the flow is stopped and a bloom of algae develops, eggs of these flies are found near the edge; but no larvae were ever recovered from the ponds.

The third pond is the turning point for the development of a more favorable environment for insect life, for the pond is able to support a more mixed population than the preceding two ponds. Altogether, forty-eight species were collected from pond III in contrast to the thirteen species found in the second pond. Practically all these species, however, were taken in the latter

TABLE 2

INSECTS FOUND IN OXIDATION PONDS I TO VI AT CONCORD, CALIFORNIA

Order	Family	Genus and species	Ponds
Collembola	Proisotomidae	*Proisotoma aquae* (Bacon)	I, II, III
Odonata	Coenagriidae	*Enallagma carunculatum* Morse	III, IV, V, VI
	Aeschnidae	*Aeschna multicolor* Hagen	III, IV, V, VI
		Anax junius (Drury)	III, IV, V, VI
Hemiptera	Saldidae	*Saldula interstitialis* (Say)	III, IV, V, VI
	Gerridae	*Gerris incognitus* Drake and Harris	III, IV, V, VI
	Notonectidae	*Notonecta kirbyi* Hungerford	IV, V, VI
		N. unifasciata (Guerin)	III, IV, V, VI
		N. undulata Say	VI
		N. shooteri Uhler	III, IV, V, VI
		Buenoa scimitra Bare	III, IV, V, VI
	Corixidae	*Hesperocorixa laevigata* (Uhler)	III, IV, V, VI
		Sigara mackinstryi Hungerford	III, IV, V, VI
		Cenocorixa wileyae (Hungerford)	III, IV, V, VI
		C. blaisdelli (Hungerford)	III, IV, V, VI
		Corisella inscripta (Uhler)	III, IV, V, VI
		C. decolor (Uhler)	III, IV, V, VI
Coleoptera	Dytiscidae	*Laccophilus decipiens* LeConte	III, IV, V, VI
		L. atristernalis Crotch	III, IV, V, VI
		Rantus anisonychus (Crotch)	III, IV, V, VI
		Hygrotus lutescens LeConte	I, II, III, IV, V, VI
		Agabus lutosus LeConte	III, IV, V, VI
		A. disintegratus Crotch	III, IV, V, VI
		Eretes sticticus (L.)	III, IV, V, VI
		Bidessus subtilis (LeConte)	VI
		Hydroporus axillaris LeConte	III, IV, V, VI
		Thermonectus basillaris (Harris)	III, IV, V, VI
		Colymbetes strigatus LeConte	III, IV, V, VI
		Acilius semisulcatus Mannerheim	V
		Cybister explanatus LeConte	III, IV, V, VI
		Dytiscus marginicollis LeConte	III, IV, V, VI
	Gyrinidae	*Gyrinus punctellus* Ochs	III, IV, V, VI
	Hydrophilidae	*Tropisternus californicus* (LeConte)	III, IV, V, VI
		T. lateralis (Fabricius)	III, IV, V, VI
		Berosus ingeminatus D'Orchymont	III, IV, V, VI
		Hydrophilus triangularis Say	III, IV, V, VI
Diptera	Psychodidae	*Psychoda alternata* Say	I, II, III
	Culicidae	*Culex pipiens molestus* Forskal	I, II, III, IV
		C. stigmatosoma Dyar	I, II, III
	Tendipedidae	*Glyptotendipes barbipes* (Staeger)	III, IV, V, VI
		Tendipes decorus (Johannsen)	III, IV, V, VI
		T. stigmaterus (Say)	III, IV, V, VI
		Pelopia punctipennis Meigen	III, IV, V, VI
		Cricotopus brunnescens Walley	III, IV, V, VI
	Syrphidae	*Tubifera aeneus* (Scopoli)	I, II, III, IV
		T. tenax (L.)	I, II, III, IV
		Helophilus mexicanus Maquart	I, II, III, IV
	Ephydridae	*Paracoenia bisetosa* (Coq.)	I, II, III, IV, V, VI
		Scatella paludum (Meigen)	I, II, III, IV, V, VI
		S. stagnalis (Fallen)	I, II, III, IV, V, VI
		S. laxa Cresson	I, II, III, IV, V, VI
	Leptoceridae	*Leptocera fontinalis* (Fall)	I, II, III, IV, V, VI

portion of pond III where the algal bloom is indicative of the fact that the septic conditions no longer exist and the DO level of the water is on the increase. The improved environment is also borne out by the types of insects present, for aside from there being more species than in the two previous ponds, the species belong to entirely different groups, almost to the exclusion of those found in the septic ponds. Adults of the syrphids and ephydrids are found along the edges, and they breed in the corners of the pond where septic conditions may develop; but these forms are not the dominant feature of the fauna of the pond. Collecting from the bottom materials of pond III reveals that one of the outstanding features of the pond is the great abundance of larval tendipedids. These are established only in the latter half of pond III where the dissolved oxygen reaches a level capable of meeting respiratory requirements, for the earlier portion of the pond which receives the effluent of pond II is as devoid of larvae as the second pond itself. The larvae absorb the oxygen directly from the water, and some species are unique in having haemoglobin in their system. Walshe (1950) has shown that members of this family are able to live under conditions of very low oxygen concentration and are capable of recovering from complete anaerobiosis in water with only 7 per cent air saturation. From this evidence the larvae should be able to penetrate zones of near-septic conditions. However, although certain areas may be suitable for larval habitation at a given time, because of the varying conditions already indicated, minimal requirements for the larvae may not be met for a period equal to their life cycle; this would prevent the larvae from becoming established in a particular region. A case in point is the finding of eggs in pond II, whereas larvae were never recovered.

Other forms found in the third pond which are also dependent upon dissolved oxygen for respiration are the naiads of Odonata, three species of which were taken; naiads are predaceous upon larval tendipedids and other soft-bodied insects. Adults of other species of odonates are often seen near the ponds, but their immature forms are not found in them.

Six species representing the hemipterous family Corixidae were collected from pond III, but only two of them, *Corisella inscripta* (Uhler) and *Corisella decolor* (Uhler), actually breed in the pond. Corixids feed on algae and other small organisms gathered with the algae; they also readily attack and suck the body fluids from larval tendipedids.

The aquatic beetles appear to be the most diverse group found in pond III, and they are the most numerous in number of species. The family Dytiscidae is represented by fourteen species, Hydrophilidae by four species, and Gyrinidae by one species. The larval and adult stages of dytiscids and gyrinids are predaceous; the hydrophilid larvae are also predaceous, but the adults are vegetable scavengers.

Collembolans of the same species recorded from the first two ponds were also found at the edges of the third pond, but they occurred only in small groups. Numerous adult psychodid flies were found in the weeds surrounding the ponds, and sampling of the edge materals revealed the larval and pupal stages of the flies. As indicated by table 2, the insects of all species found in ponds IV to VI were qualitatively similar to those of pond III.

To determine the relative quantitative abundance of some of the species of insects living in the oxidation ponds, bottom sampling for larvae and subaquatic-trap sampling for free-swimming forms were carried out (figs. 39 through 50). The relative densities of the various forms are shown to vary considerably from pond to pond and from month to month. Although climatic conditions of the region (figs. 51 and 52) control the broad tendencies of the population densities, intra- and interspecific competition undoubtedly also exert strong influences. The successive seasonal change of dominant larval tendipedids in the bottom of the ponds illustrates how the rhythms of the populations are coordinated so as to produce a minimum of competition between the forms; each reaches a maximum density and is then replaced by another form.

The decrease in the corixid and beetle populations during the second season was caused by the great increase of mosquito fish in all the ponds; an examination of the stomachs of the fish showed that they were feeding on the nymphal and larval forms of the insects. During the first season the fish were present only in pond III, which accounts for the more rapid decline of the populations during that period. Larval tendipedids were little affected, since the larvae burrow into the bottom materials and are protected. Moreover, the higher reproductive capacity of the tendipedids apparently balanced the loss of first-instar larvae, which were eaten before they were able to establish themselves in the bottom of the ponds.

SUMMARY

The purpose of this investigation has been to determine the role of insects in the process of sewage purification. Trickling filters are very widely used for sewage treatment; psychodid flies breed in the filters and feed on the zoogleal growth. By reducing the growth on the filter stones the larvae prevent clogging and permit a more vigorous and efficient growth to develop. In this way the larvae promote more efficient sewage treatment. Pilot-scale filters are described. Maximum larval density occurs when intermediate sewage loading is applied. At higher rates of application the insects are prevented from reaching high populations because of the flooding effects; at low application rates there is not enough growth to support higher populations. Maximum stabilization of effluents occurs at the lowest rate of loading. Field observations are given.

Oxidation ponds are widely used for secondary sewage treatment in areas of mild climate. Algal blooms occur in the ponds and contribute photosynthetic oxygen for aerobic bacterial stabilization. Algae also add to the organic loading when they become senescent. Insects occur abundantly in the ponds, and qualitative and quantitative determinations are given. The larvae of tendipedids are beneficial because they remove algae from the system and cause mixing at the mud–water interface. Controlled laboratory experiments are described.

ACKNOWLEDGMENTS

Appreciation is extended to Mr. W. J. Oswald, Research Engineer; his cooperation and suggestions greatly aided the progress of the project.

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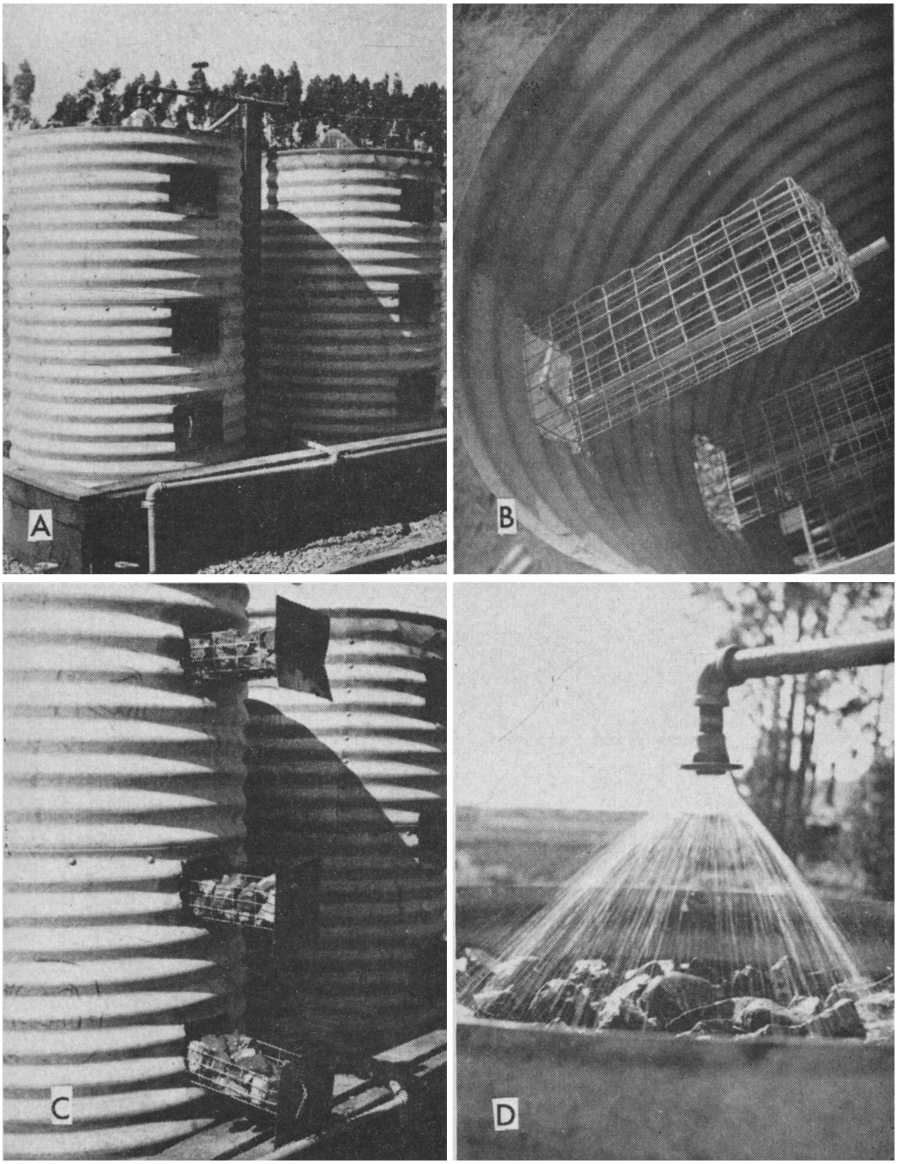


Fig. 1. *A*, two pilot-scale trickling filters tested at Richmond, California; *B*, supports for removable sampling drawers; *C*, sampling drawers removed, showing filter stones; *D*, spray nozzle, with sewage being applied to surface of filter bed.

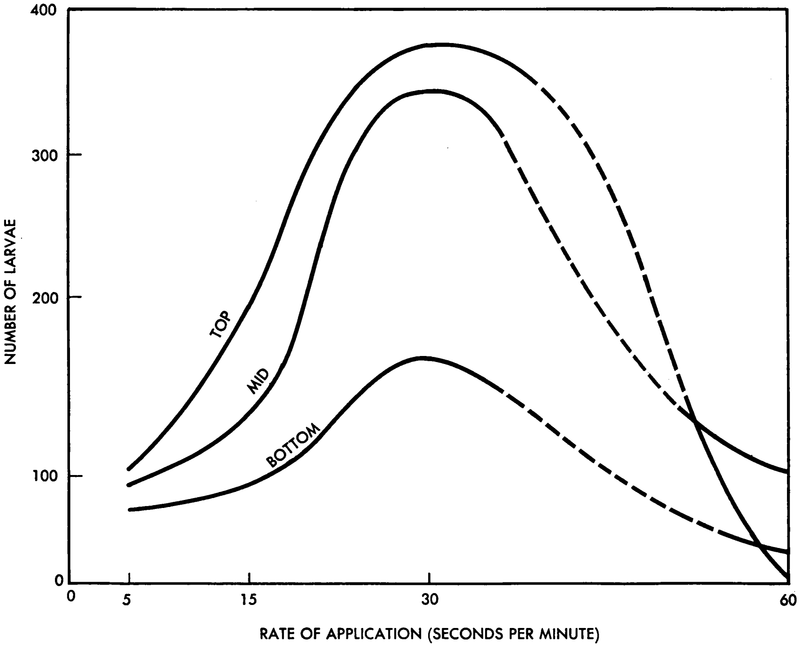


Fig. 2. Larvae per 100 cm² of filter stone surface observed at Richmond pilot plant.

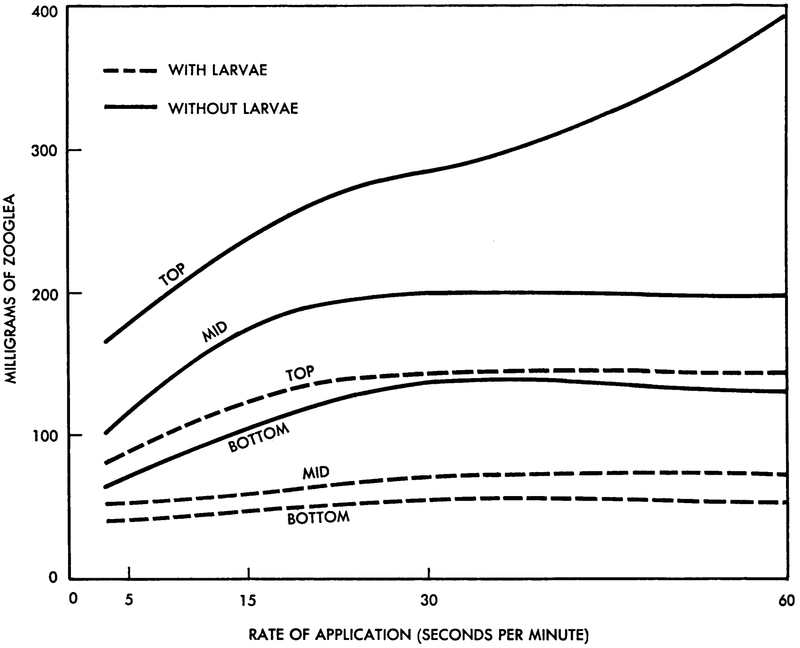


Fig. 3. Dry weight zooglea per 100 cm² of filter stone surface observed at Richmond pilot plant.

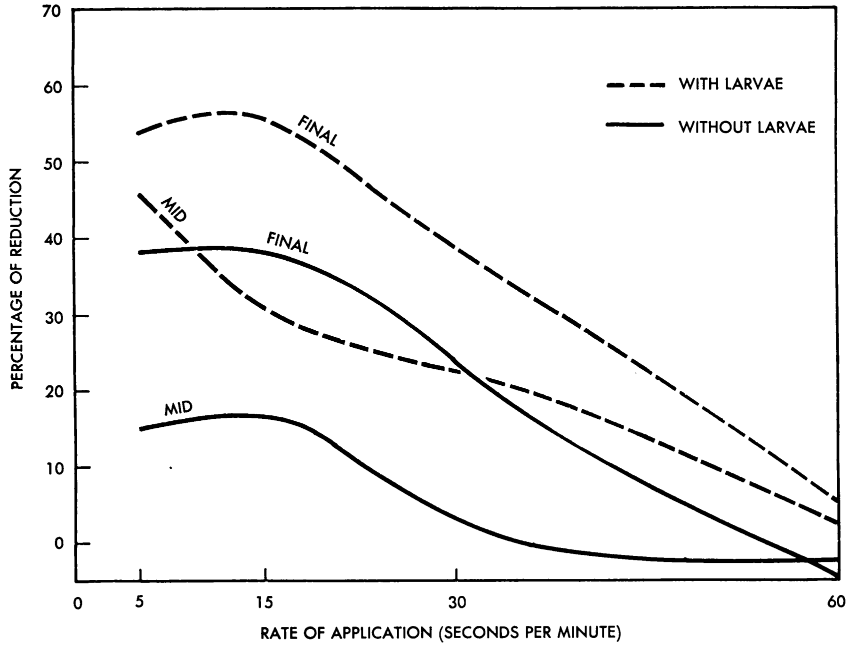


Fig. 4. Percentage of reduction of suspended solids observed at Richmond pilot plant.

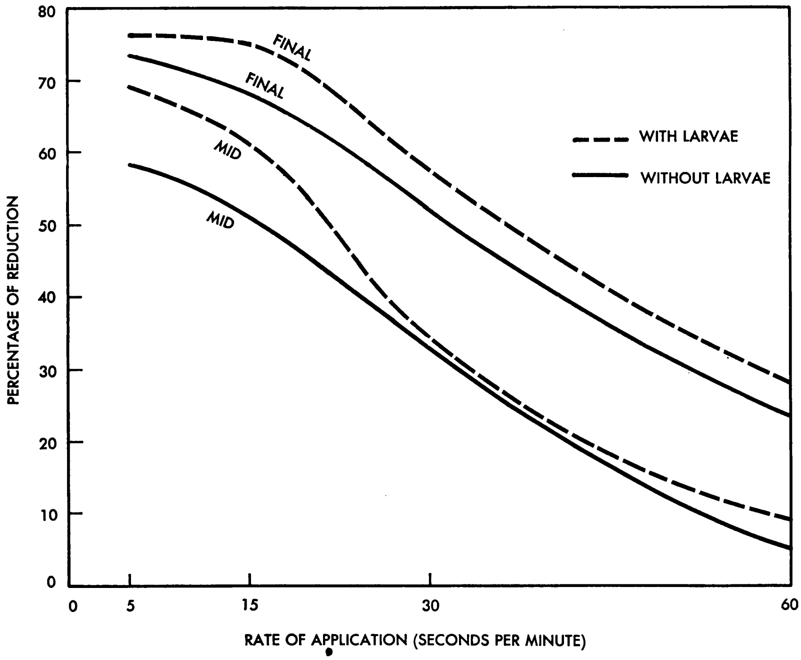


Fig. 5. Percentage of reduction of total biochemical oxygen demand observed at Richmond pilot plant.

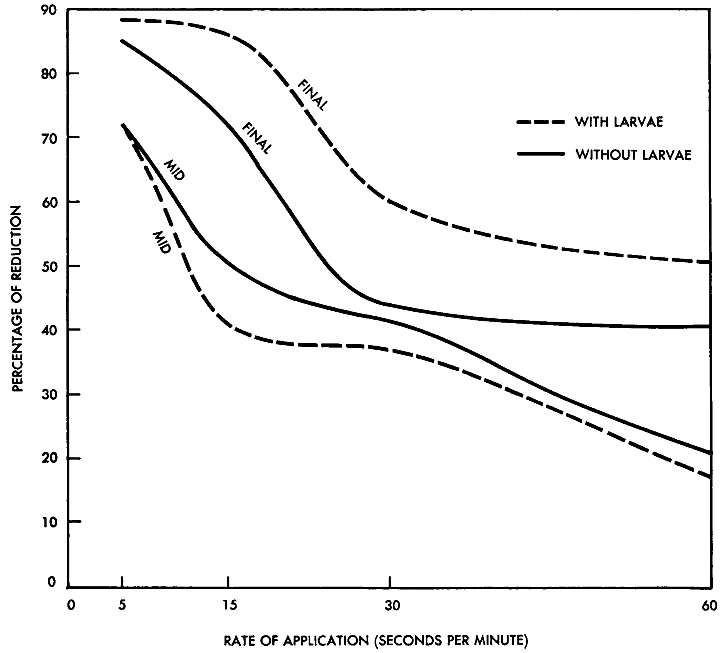


Fig. 6. Percentage of reduction of supernatant biochemical oxygen demand observed at Richmond pilot plant.

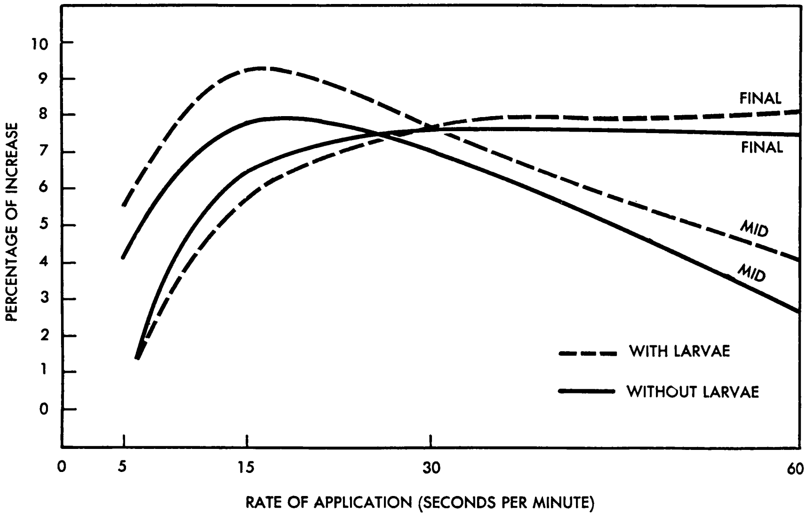


Fig. 7. Percentage of increase in pH observed at Richmond pilot plant.

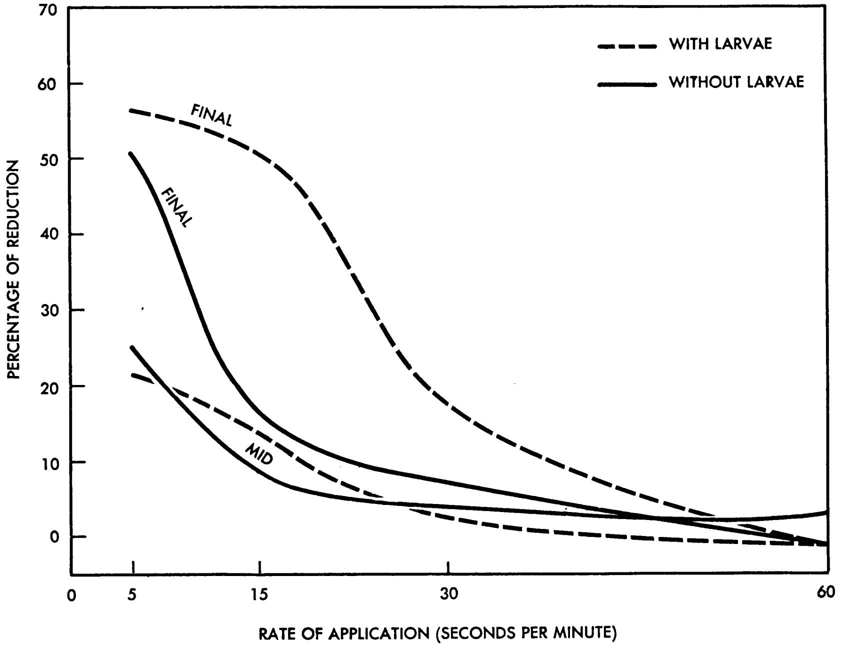


Fig. 8. Percentage of reduction of total alkalinity observed at Richmond pilot plant.

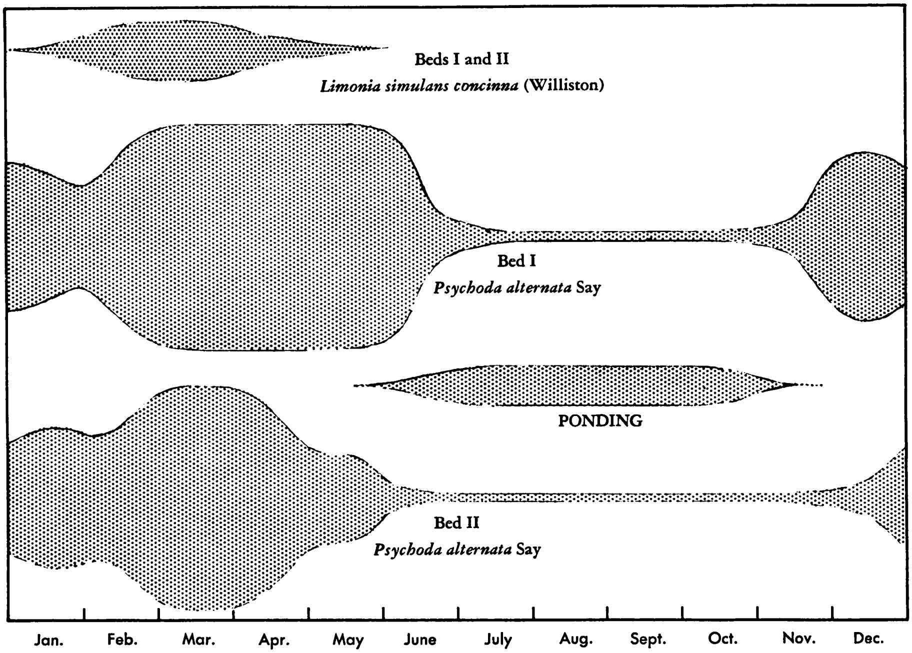


Fig. 9. Relative densities of fly populations observed at San Rafael Meadows, California, in 1953. Area labeled "ponding" indicates a period of flooding that prevented a high density of larvae from developing in filter beds.

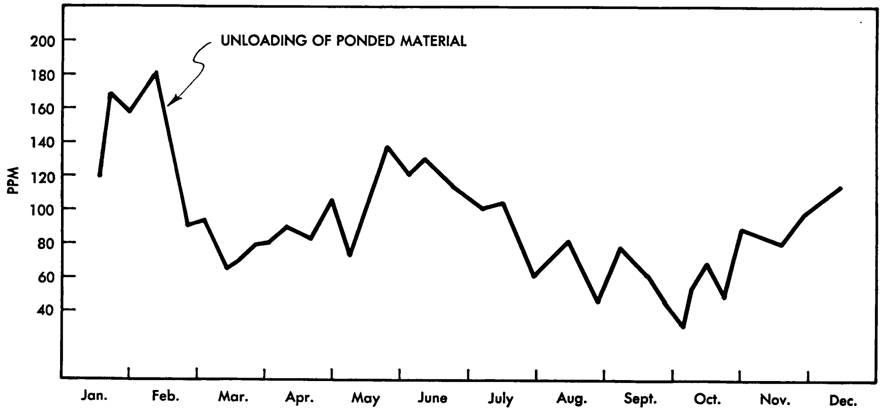


Fig. 10. Effluent suspended solids observed at San Rafael Meadows, California, in 1953. (Sampling at 1:00 p.m.)

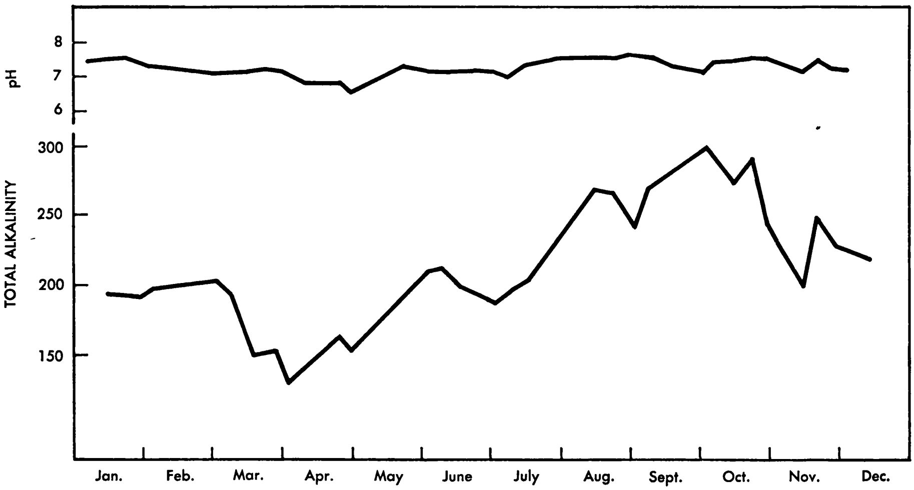


Fig. 11. Effluent pH and total alkalinity observed at San Rafael Meadows, California, in 1953. (Sampling at 1:00 p.m.)

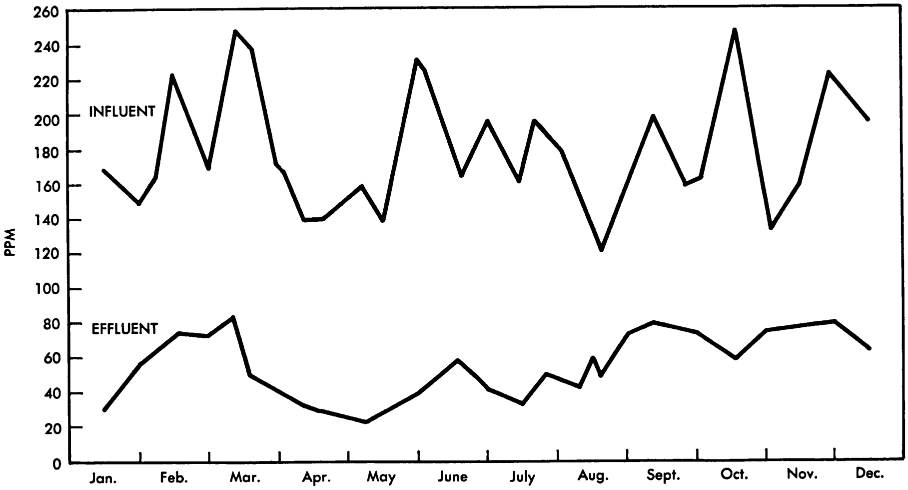


Fig. 12. Biochemical oxygen demand of influent and effluent observed at San Rafael Meadows, California, in 1953. (Sampling at 1:00 p.m.)

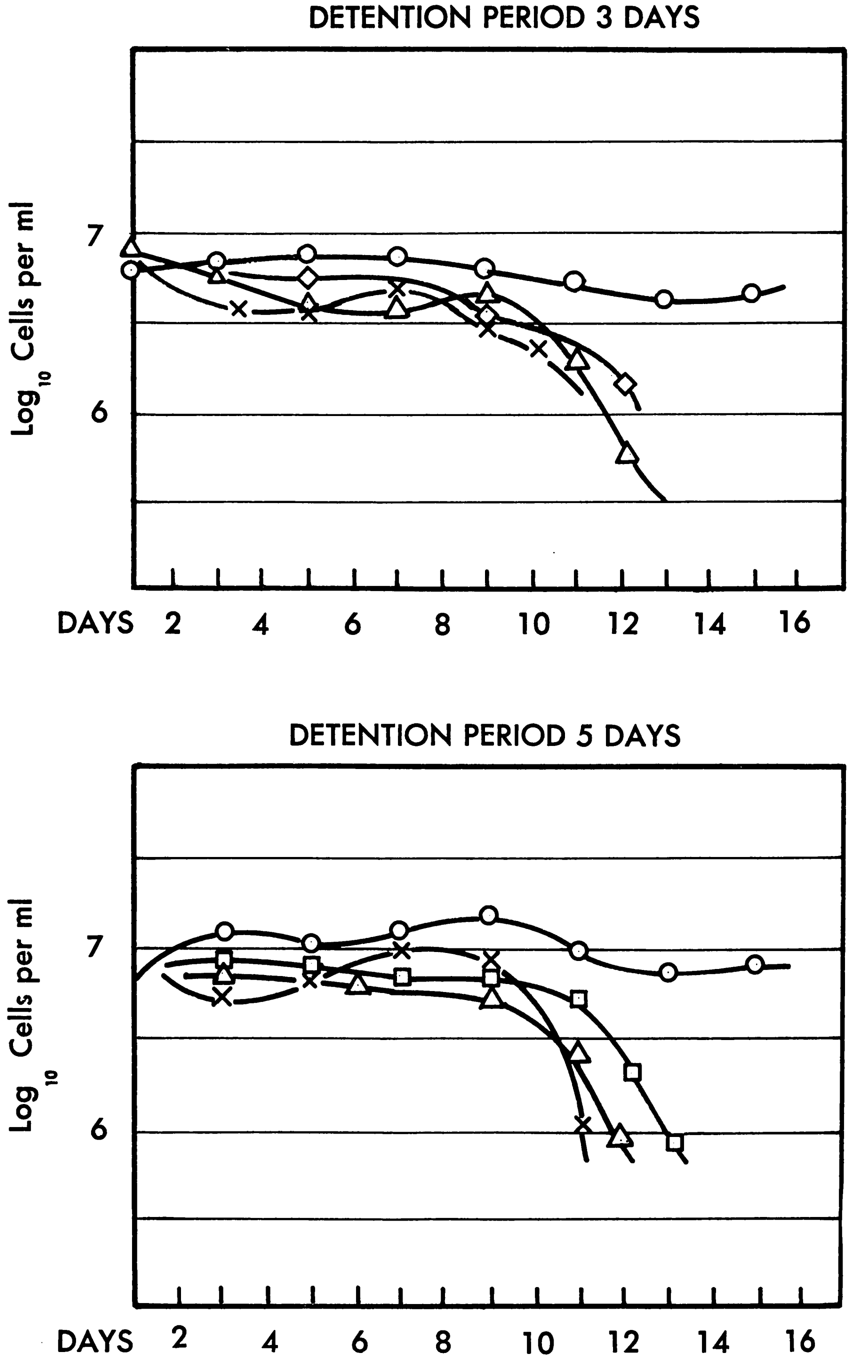
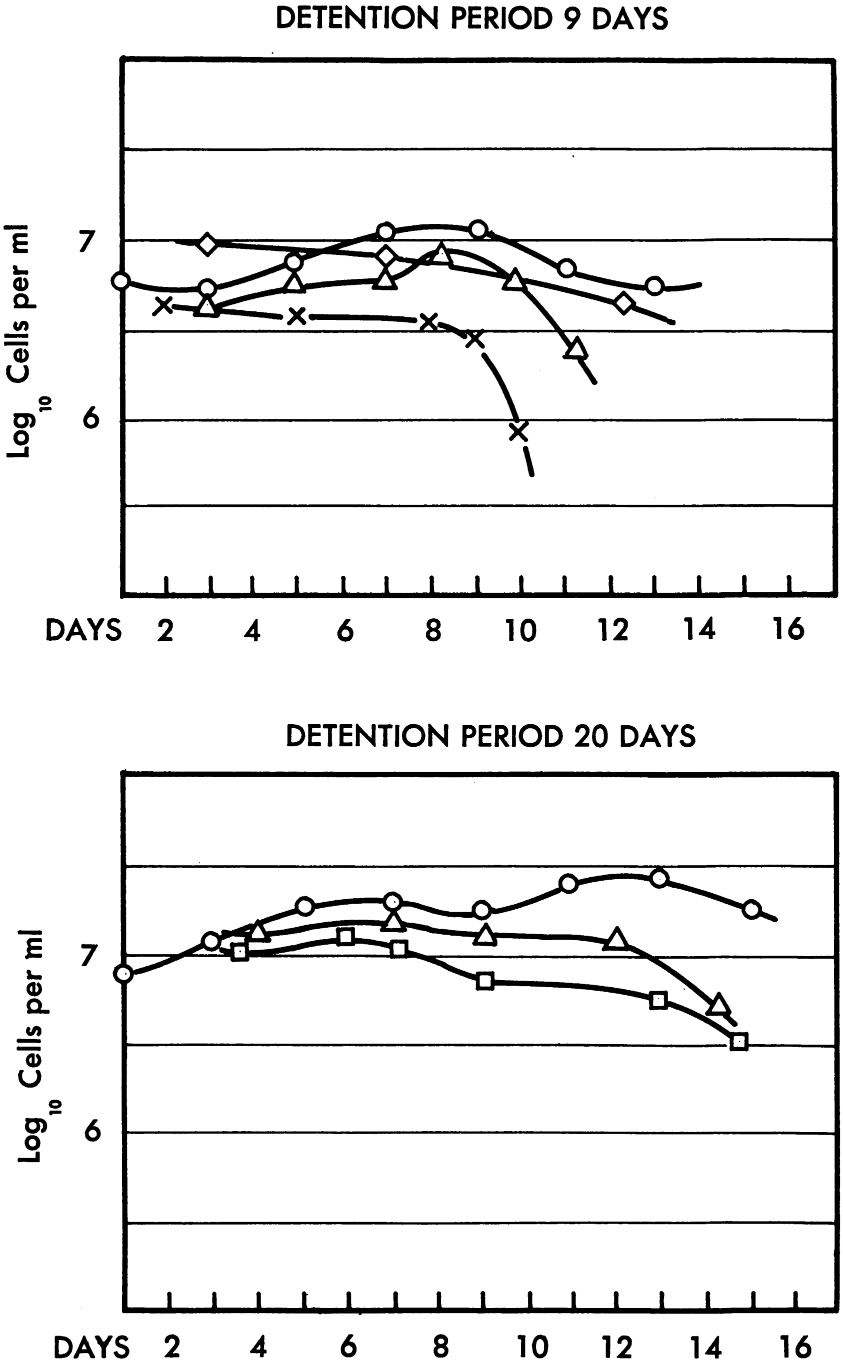


Fig. 13. Growth curves of algal cell populations observed in laboratory in continuous growth units.

Circles = no larvae; crosses = five larvae; triangles = ten larvae; squares = twenty larvae; diamonds = thirty larvae.

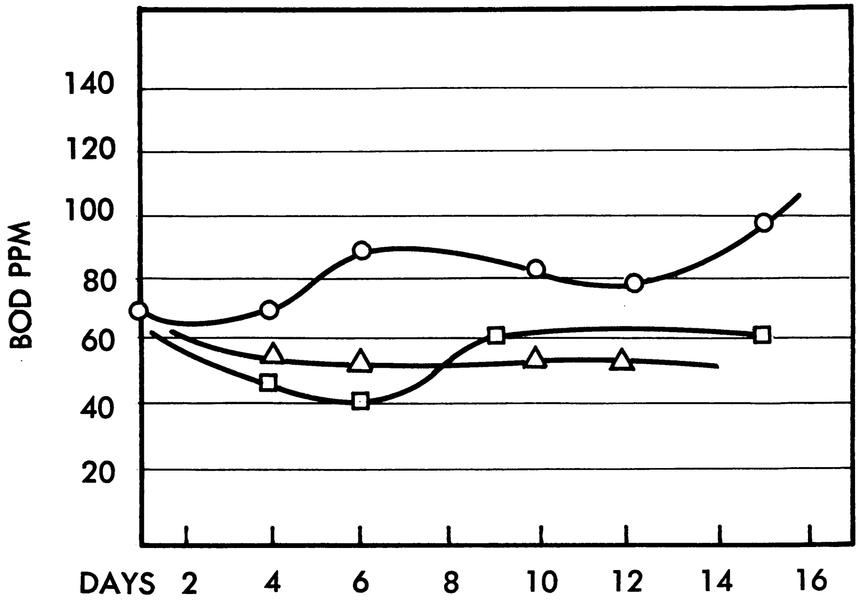

DETENTION PERIOD 9 DAYS

DETENTION PERIOD 20 DAYS

Fig. 14. Growth curves of algal cell populations observed in laboratory in continuous growth units.

Circles = no larvae; crosses = five larvae; triangles = ten larvae; squares = twenty larvae; diamonds = thirty larvae.

DETENTION PERIOD 3 DAYS



DETENTION PERIOD 5 DAYS

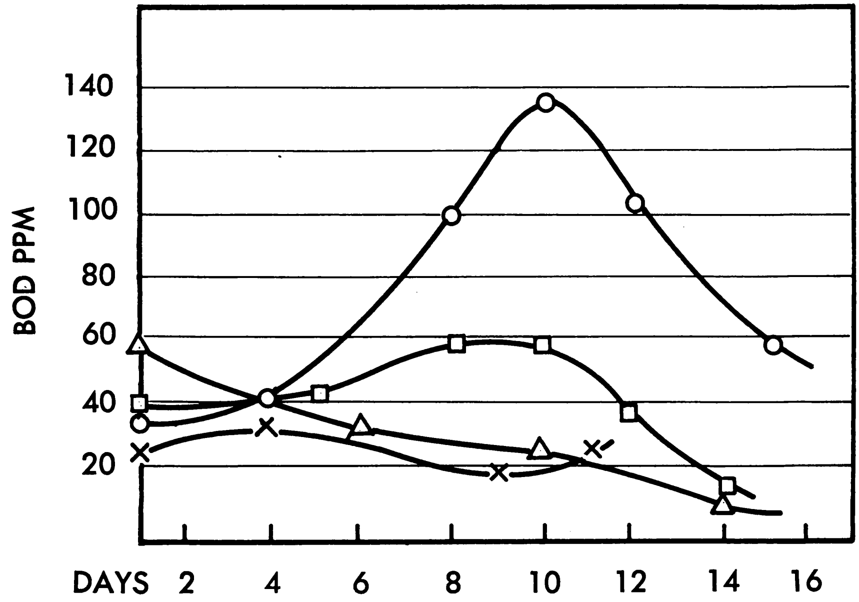


Fig. 15. Biochemical oxygen demand data obtained in laboratory from effluents of continuous growth units.

Circles = no larvae; crosses = five larvae; triangles = ten larvae; squares = twenty larvae; diamonds = thirty larvae.

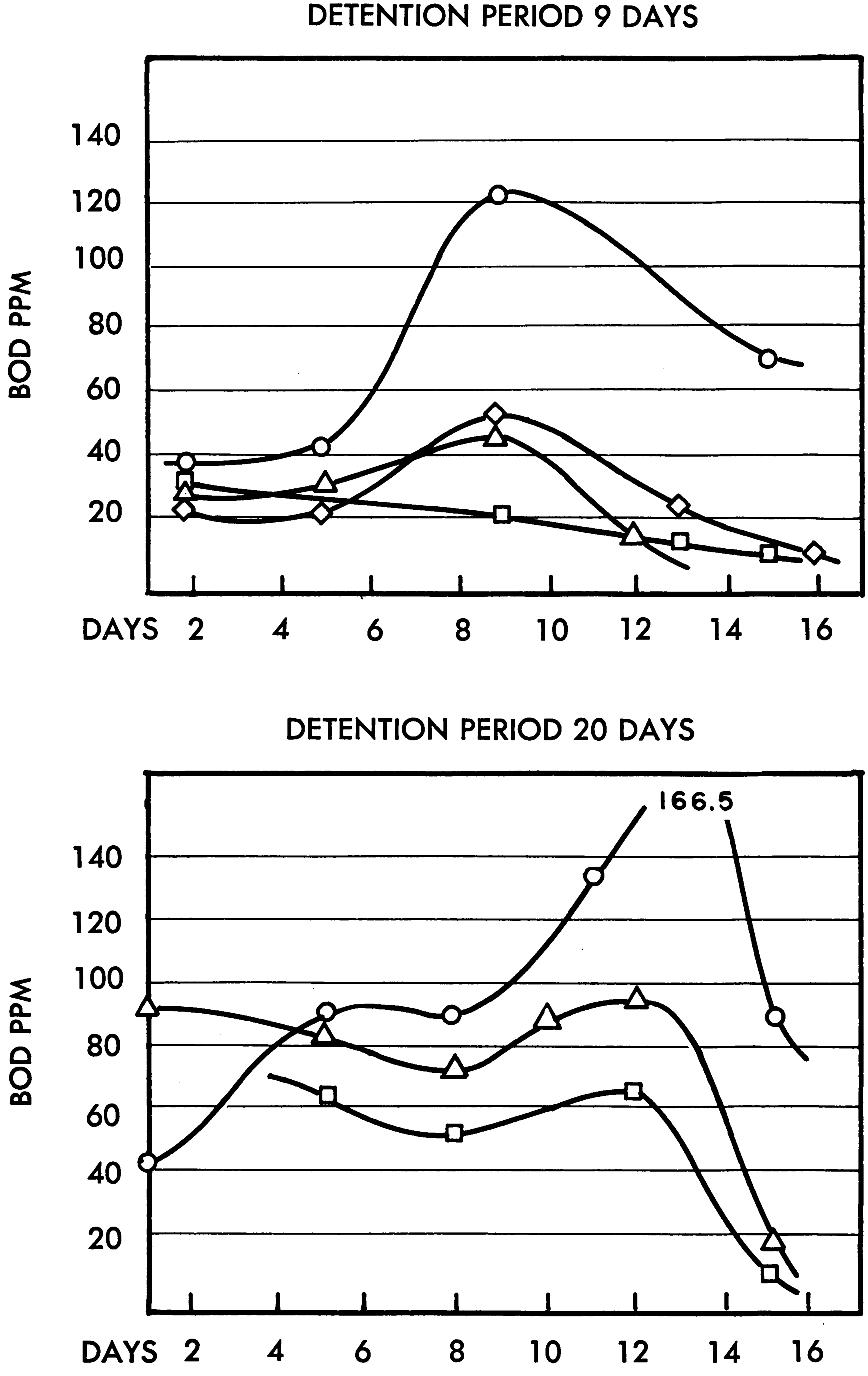
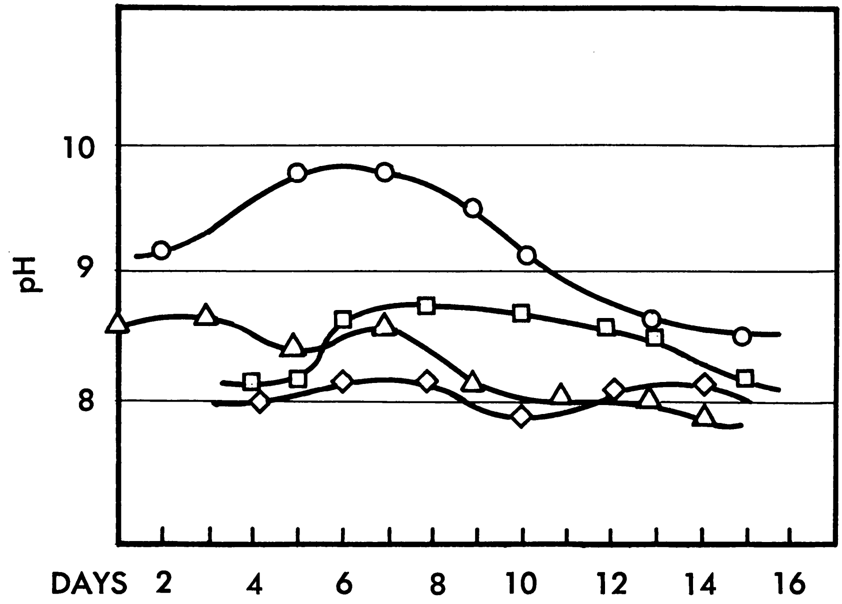


Fig. 16. Biochemical oxygen demand data obtained in laboratory from effluents of continuous growth units.

Circles = no larvae; crosses = five larvae; triangles = ten larvae; squares = twenty larvae; diamonds = thirty larvae.

DETENTION PERIOD 3 DAYS



DETENTION PERIOD 5 DAYS

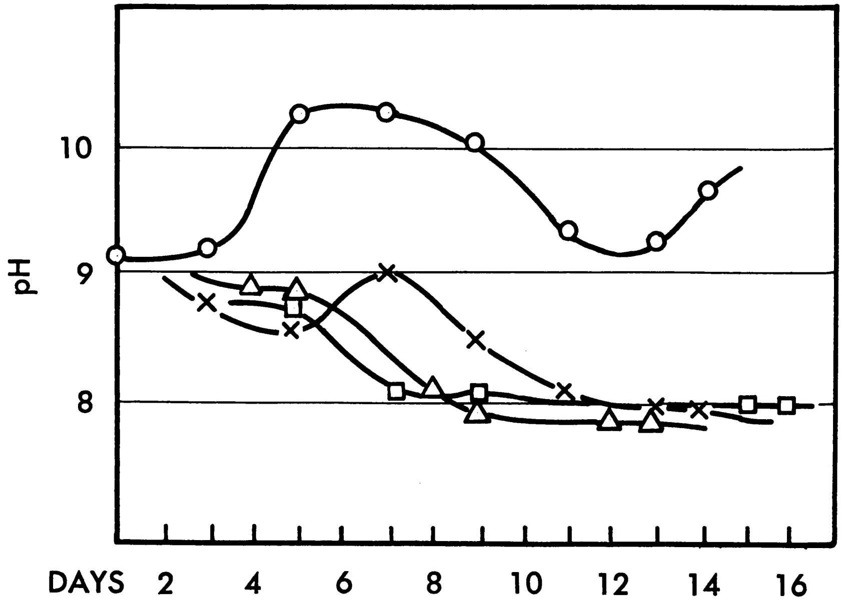
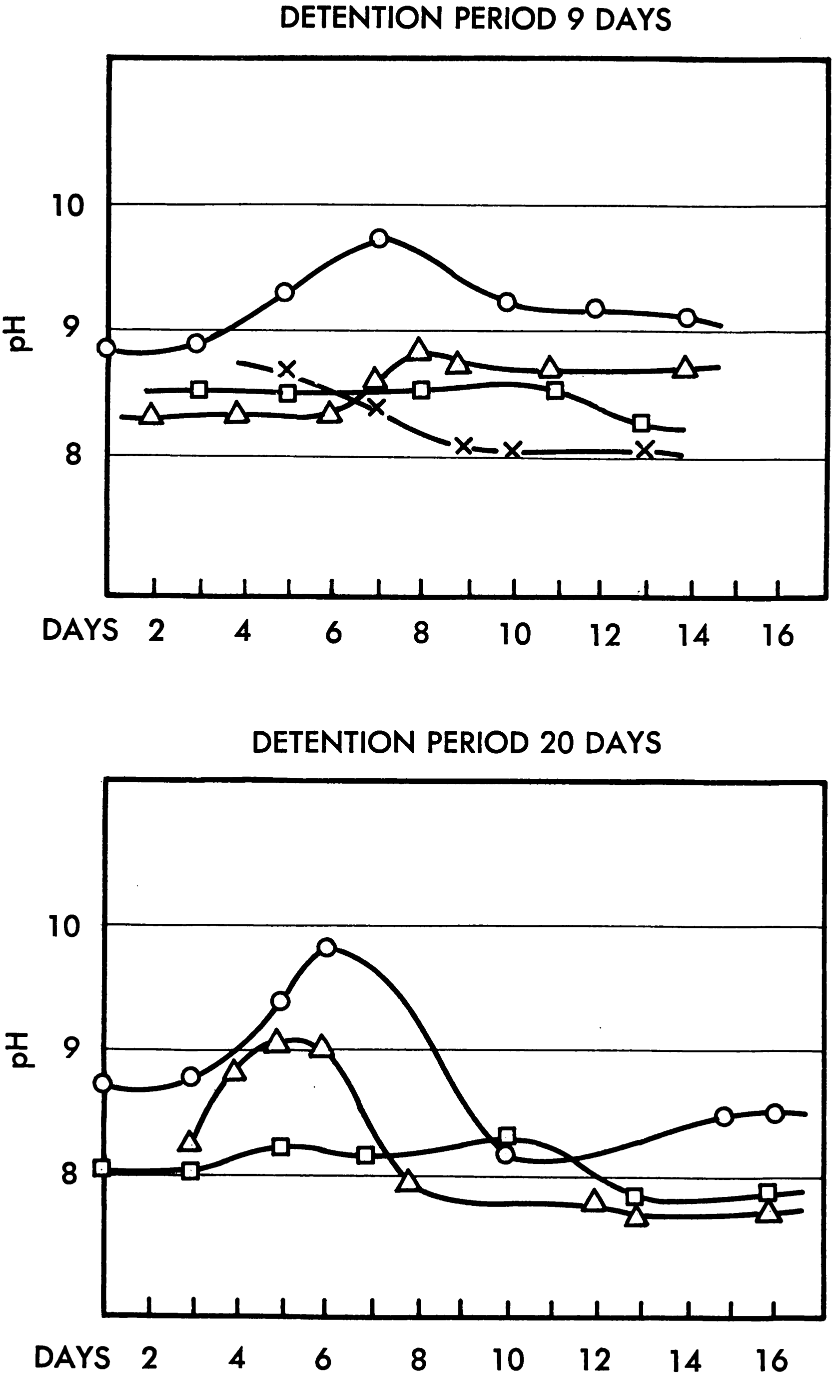


Fig. 17. Variations in pH observed in laboratory in continuous growth units.
Circles = no larvae; crosses = five larvae; triangles = ten larvae; squares = twenty larvae; diamonds = thirty larvae.



DETENTION PERIOD 9 DAYS

DETENTION PERIOD 20 DAYS

Fig. 18. Variations in pH observed in laboratory in continuous growth units.

Circles = no larvae; crosses = five larvae; triangles = ten larvae; squares = twenty larvae; diamonds = thirty larvae.

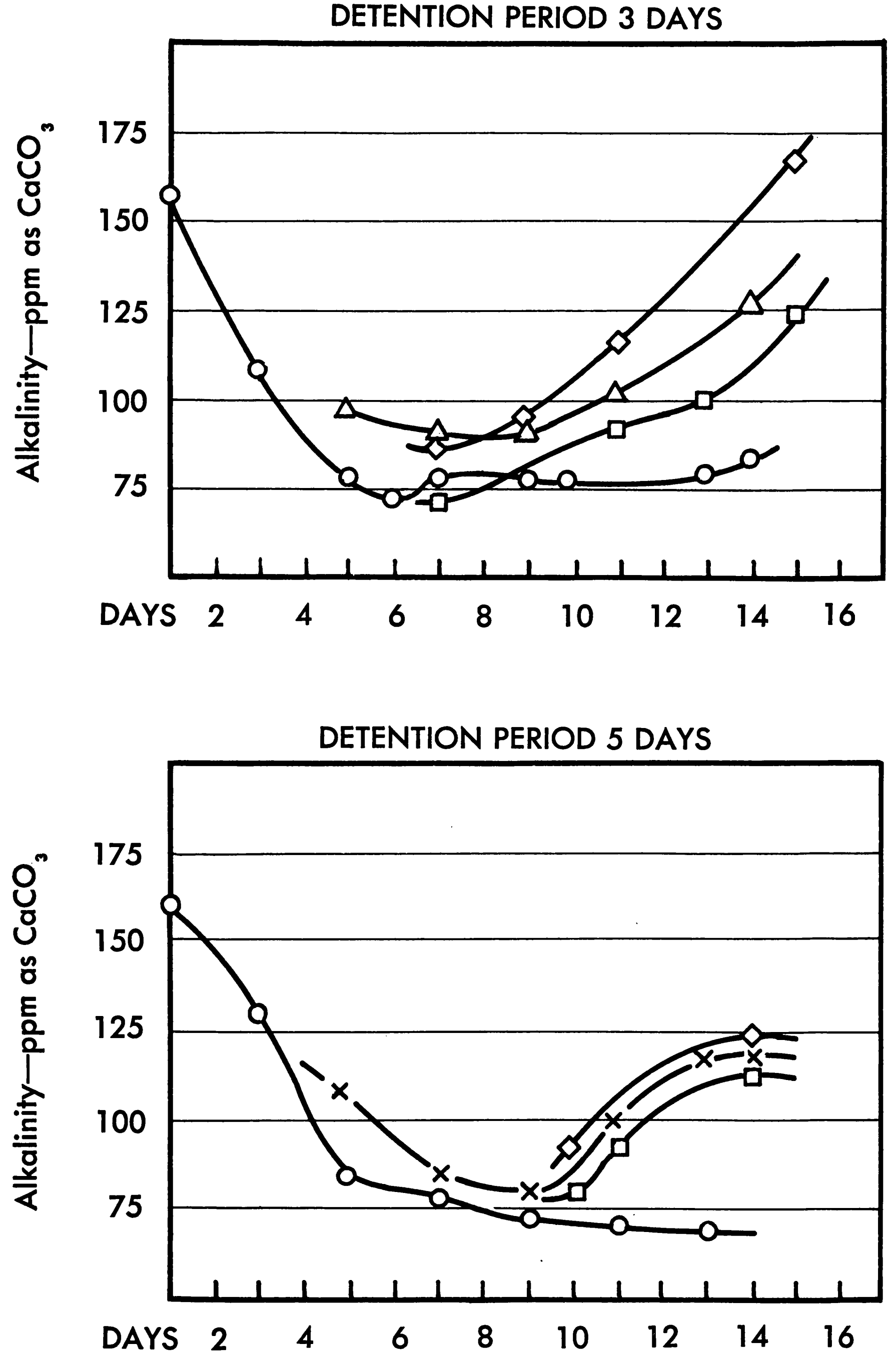
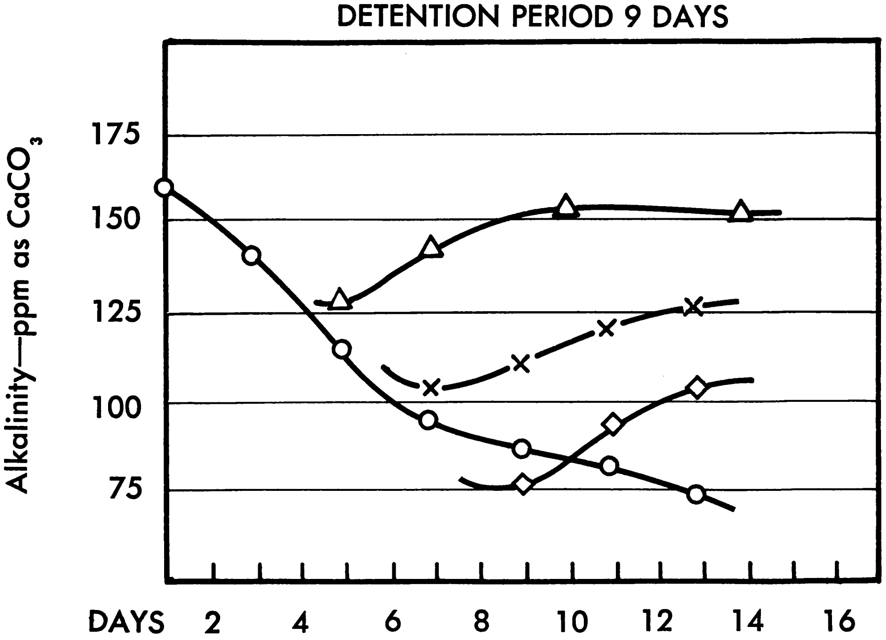


Fig. 19. Variations in total alkalinity observed in laboratory in continuous growth units.

Circles = no larvae; crosses = five larvae; triangles = ten larvae; squares = twenty larvae; diamonds = thirty larvae.



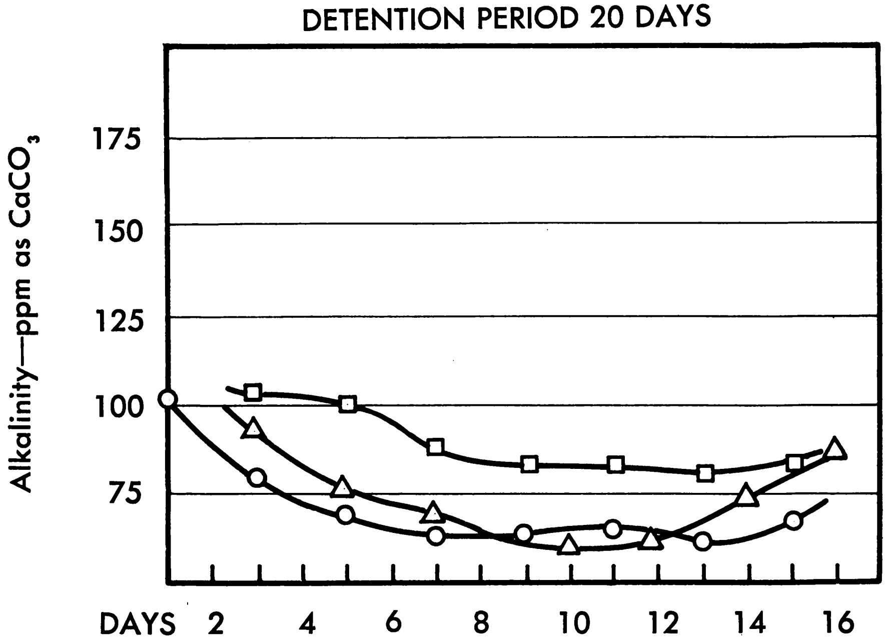


Fig. 20. Variations in total alkalinity observed in laboratory in continuous growth units.

Circles = no larvae; crosses = five larvae; triangles = ten larvae; squares = twenty larvae; diamonds = thirty larvae.

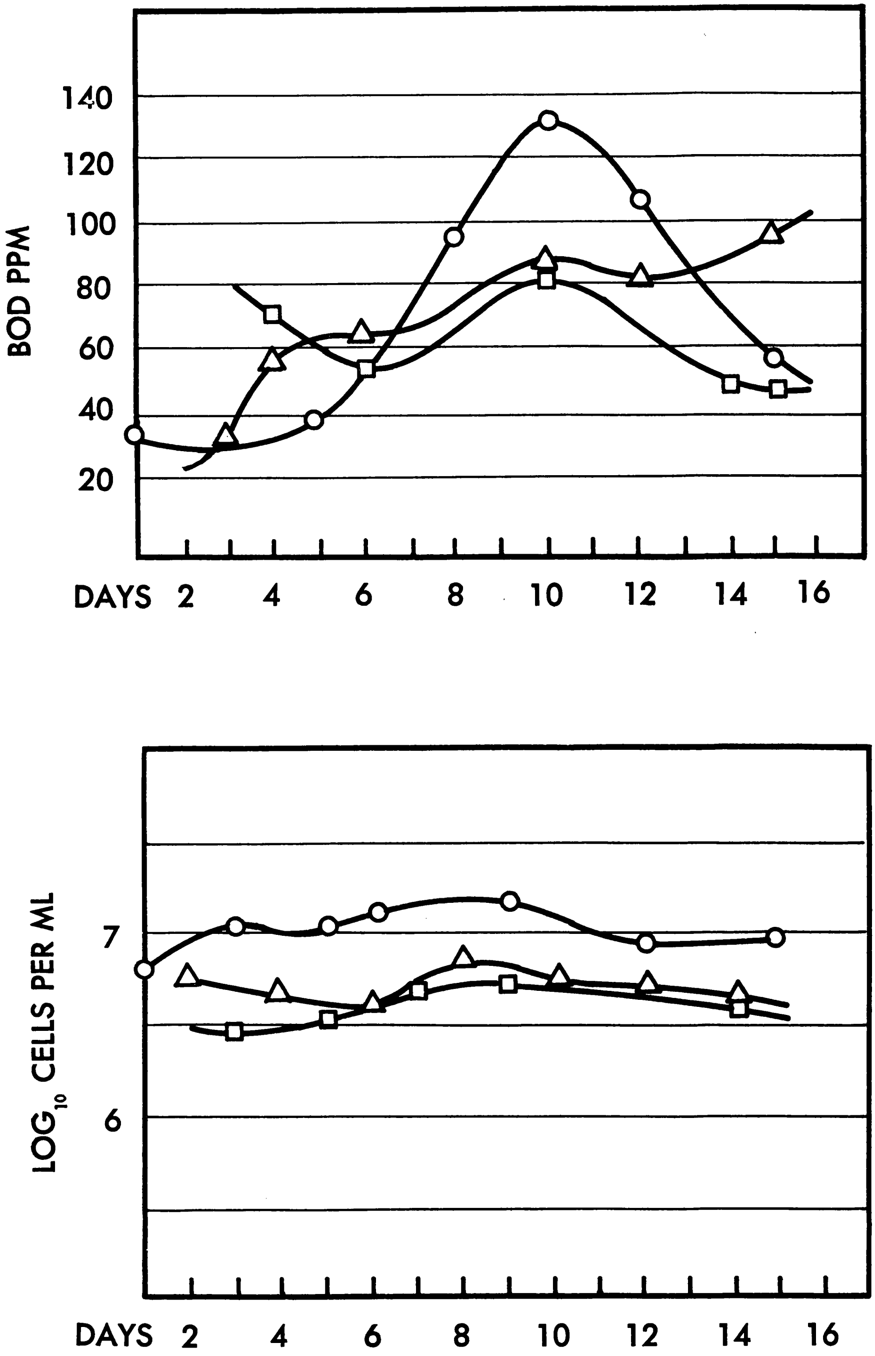


Fig. 21. Data obtained from hydrophilid beetle cultures in laboratory, using continuous growth units.
Circles = no beetles; triangles = five beetles; squares = ten beetles.

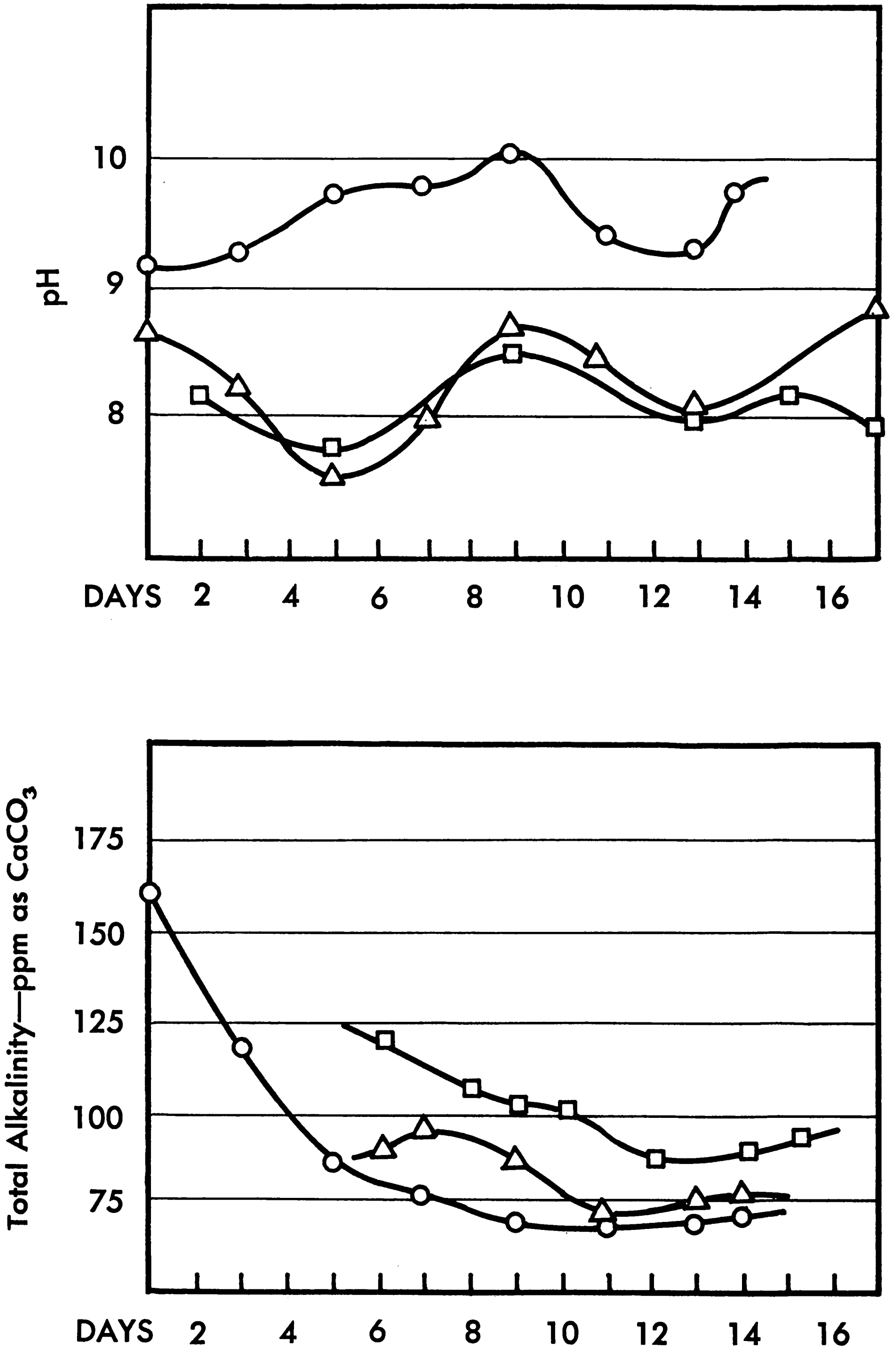
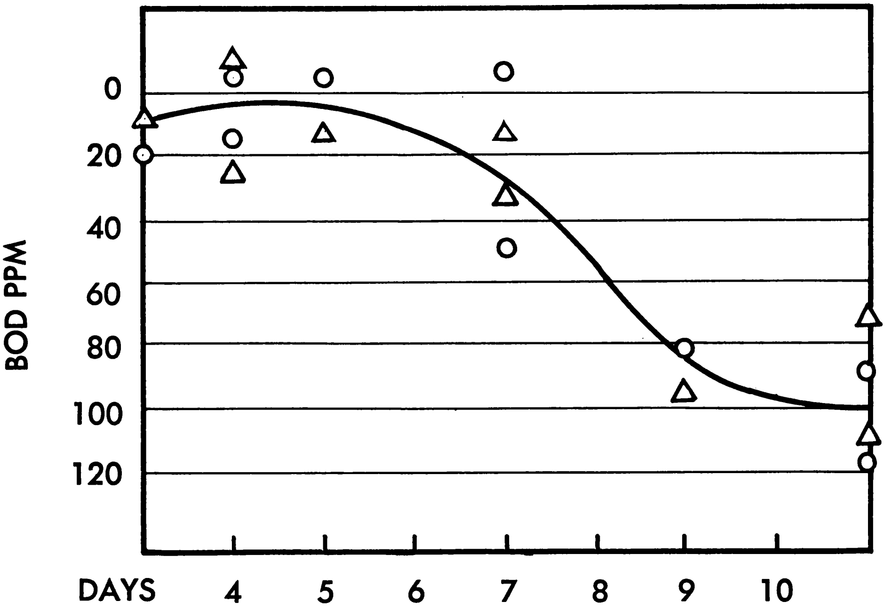


Fig. 22. Data obtained from hydrophilid beetle cultures in laboratory, using continuous growth units.
Circles = no beetles; triangles = five beetles; squares = ten beetles.



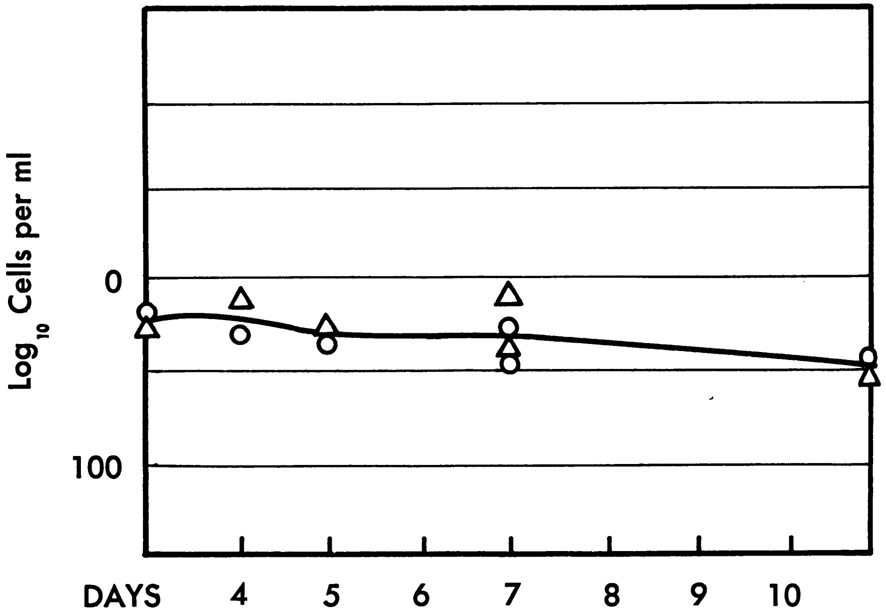


Fig. 23. Data obtained in laboratory from two series of batch-culture tests, each using ten tendipedid larvae.
Triangles = first series; circles = second series.

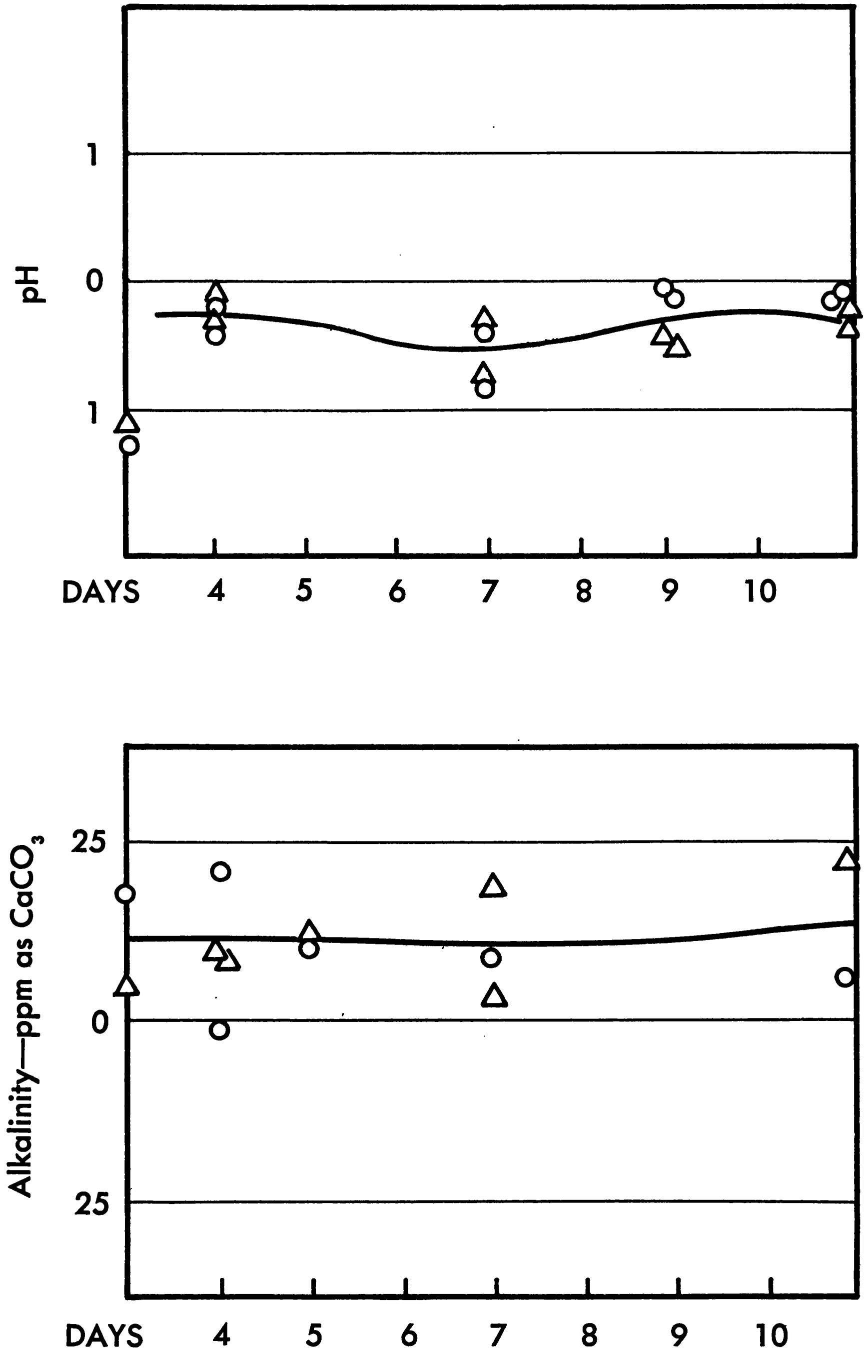


Fig. 24. Data obtained in laboratory from two series of batch-culture tests, each using ten tendipedid larvae.
Triangles = first series; circles = second series.

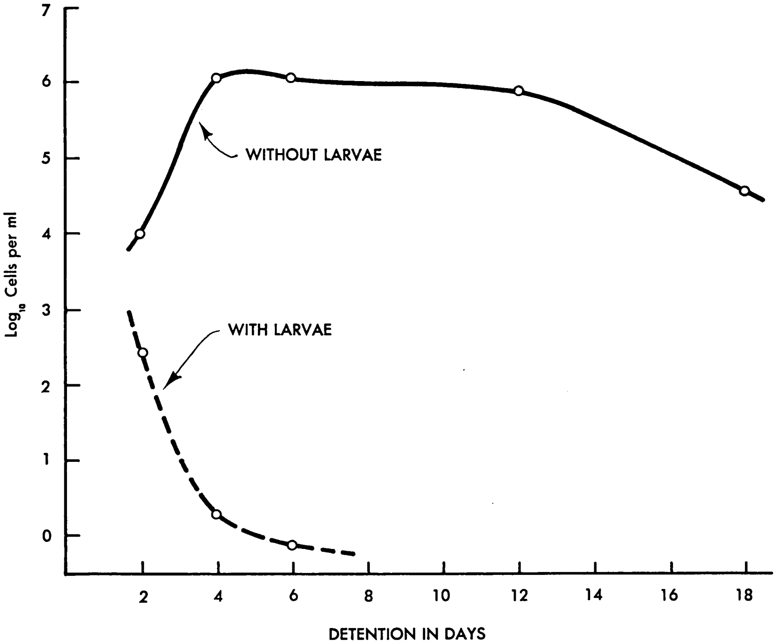


Fig. 25. Growth curves of the alga *Scenedesmus obliquus* (Turp.) Kutz, versus detention periods, observed in laboratory in growth-unit cultures.

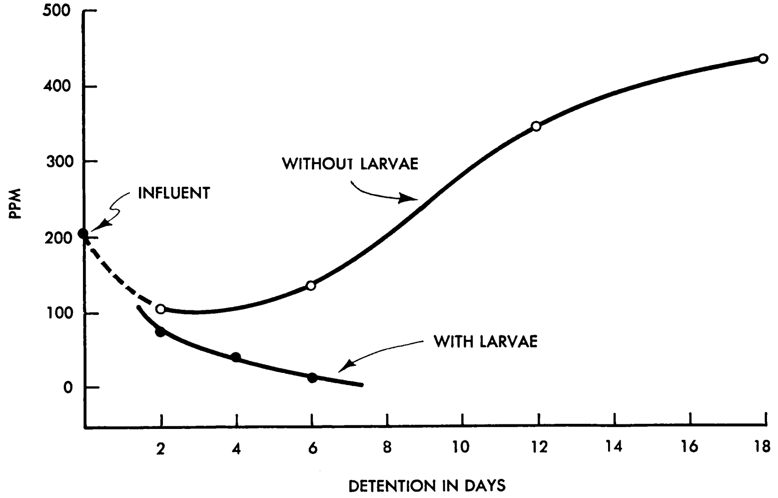


Fig. 26. Supernatant biochemical oxygen demand of effluent observed in laboratory in growth-unit cultures.

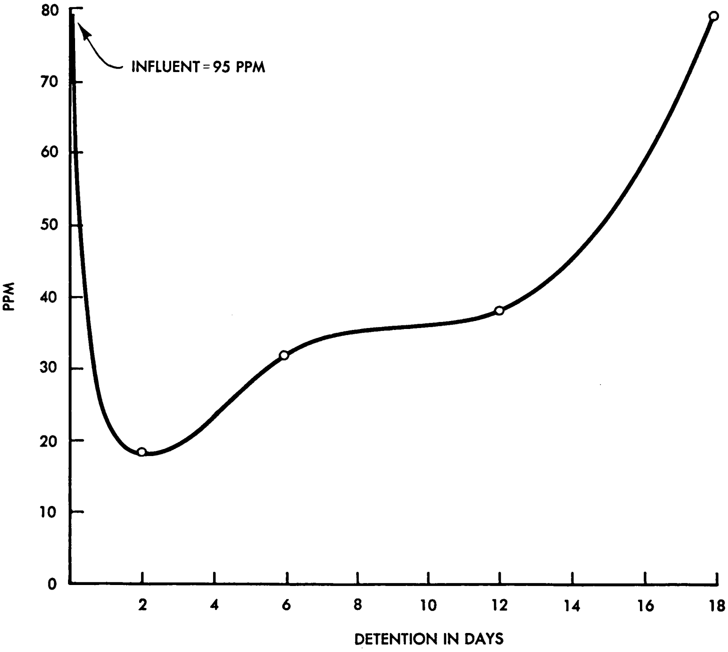


Fig. 27. Carbohydrate (expressed in ppm) in supernatant, without larvae, observed in laboratory in growth-unit cultures.

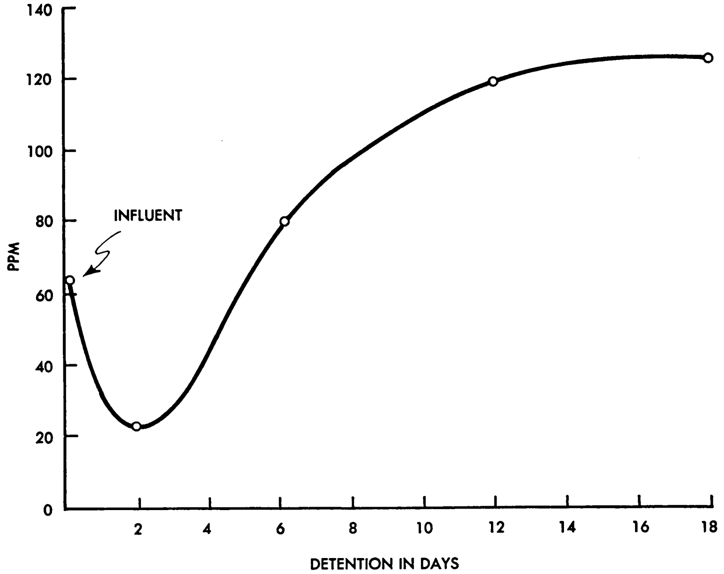


Fig. 28. Fat (expressed in ppm) in supernatant, without larvae, observed in laboratory in growth-unit cultures.

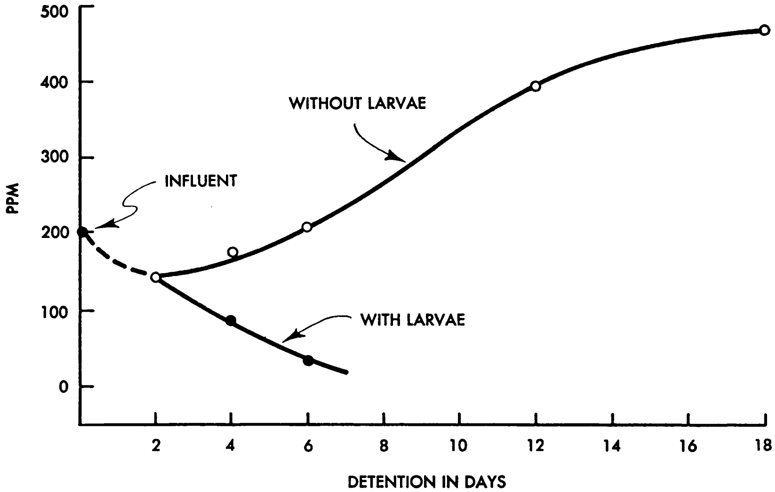


Fig. 29. Total biochemical oxygen demand of effluent observed in laboratory in growth-unit cultures.

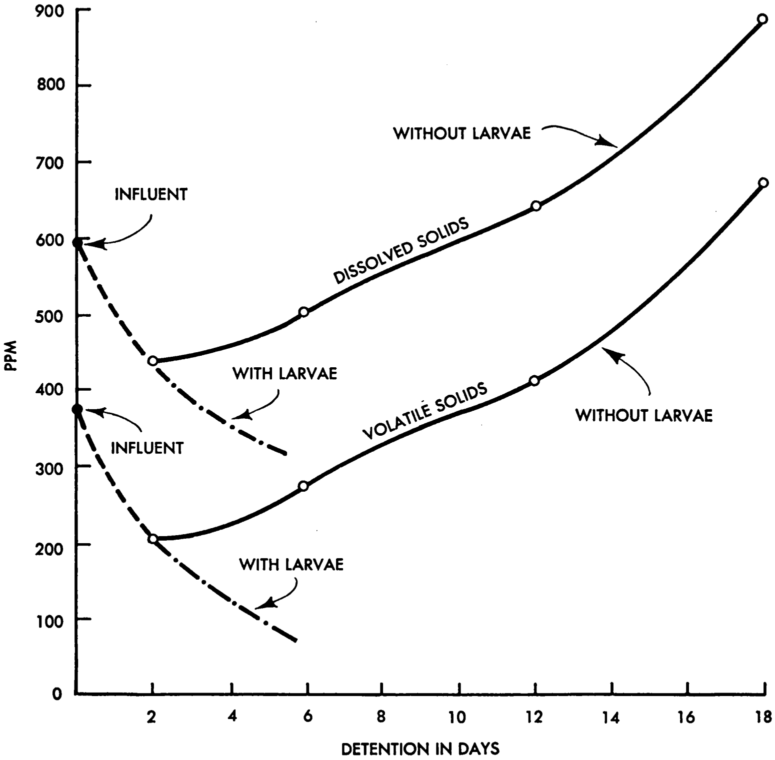


Fig. 30. Dissolved and volatile solids in supernatant observed in laboratory in growth-unit cultures.

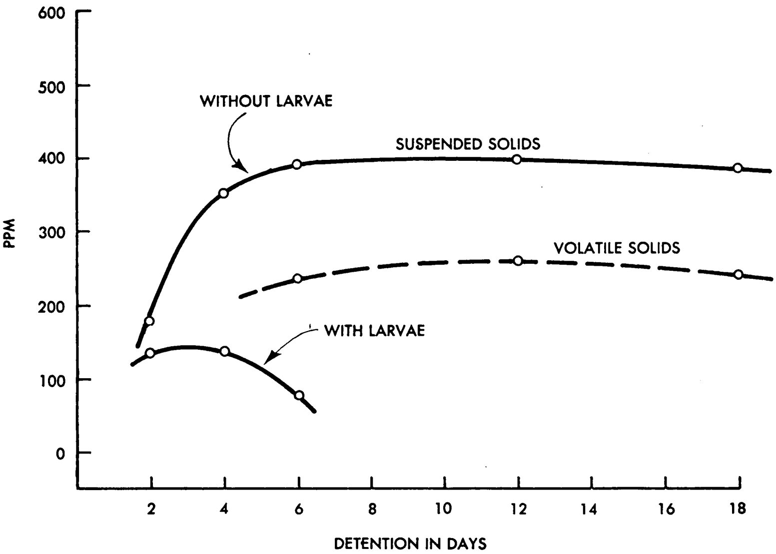


Fig. 31. Total and volatile suspended solids observed in laboratory in growth-unit cultures.

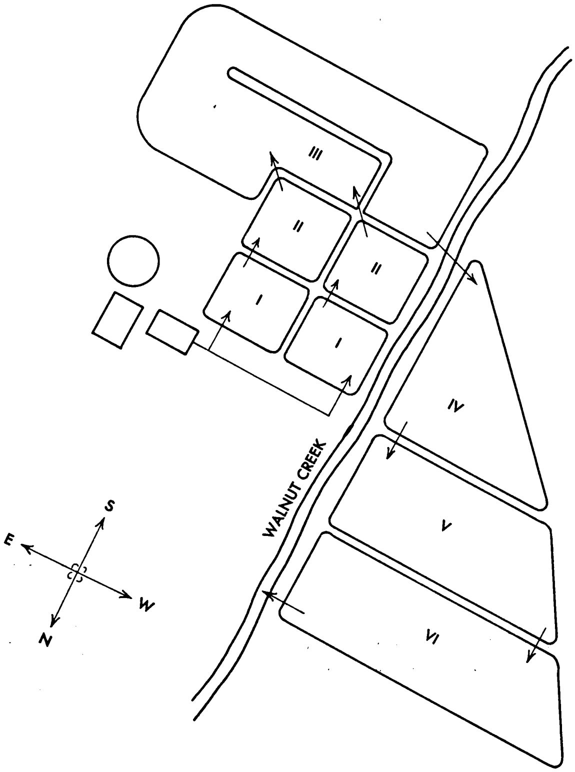


Fig. 32. Oxidation ponds at Concord, California. (For comparison, each unit of pond I measures 200 x 230 feet.)

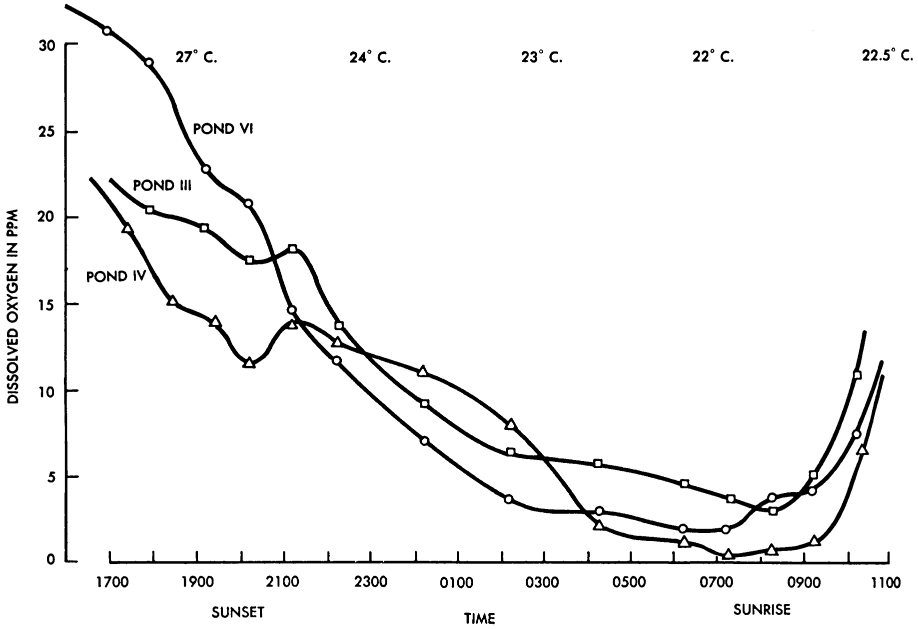


Fig. 33. Fluctuations in photosynthetic oxygenation observed in oxidation ponds III, IV, and VI at Concord, California, September 26–27, 1952.

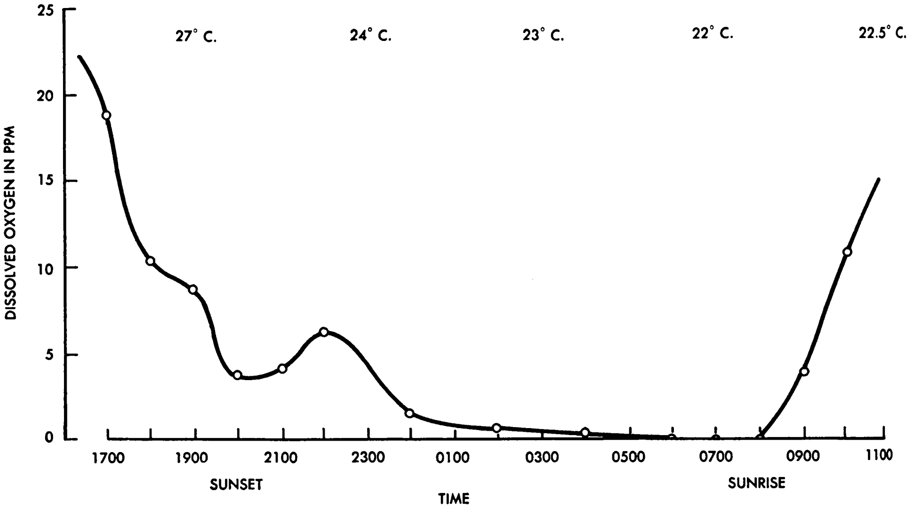


Fig. 34. Fluctuations in dissolved oxygen observed at edge of oxidation pond V at Concord, California, September 26–27, 1952.

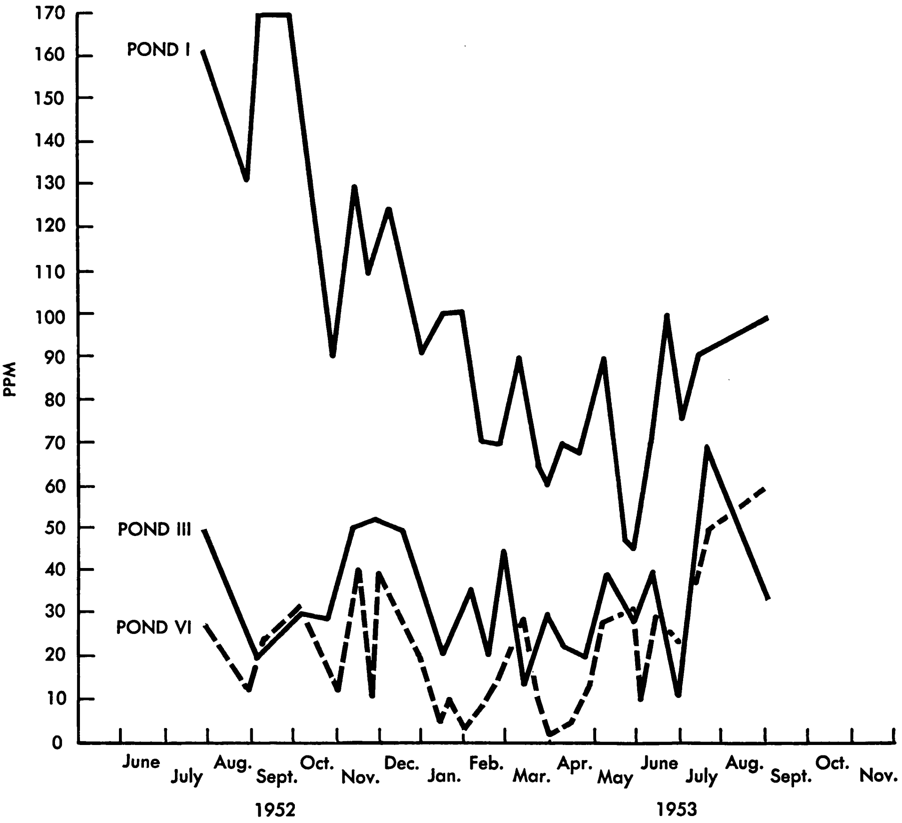


Fig. 35. Biochemical oxygen demand data obtained in oxidation ponds at Concord, California, in 1952 and 1953.

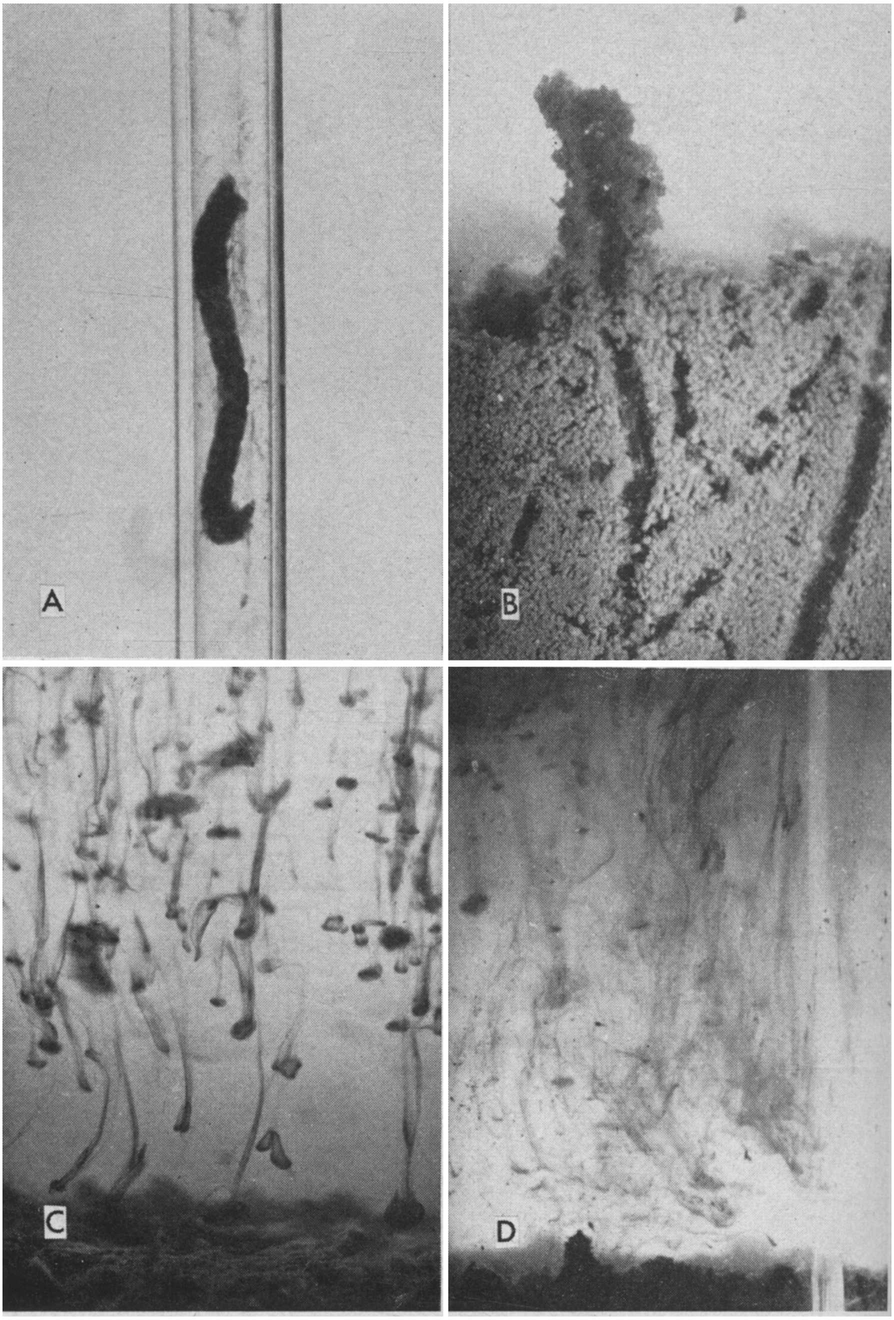


Fig. 36. *A,* larva of *Glyptotendipes barbipes* (Staeger) in glass tube; *B,* larval tubes in substrate of oxidation pond; *C,* ink droplets in water without larvae; *D,* turbulence created by larval action.

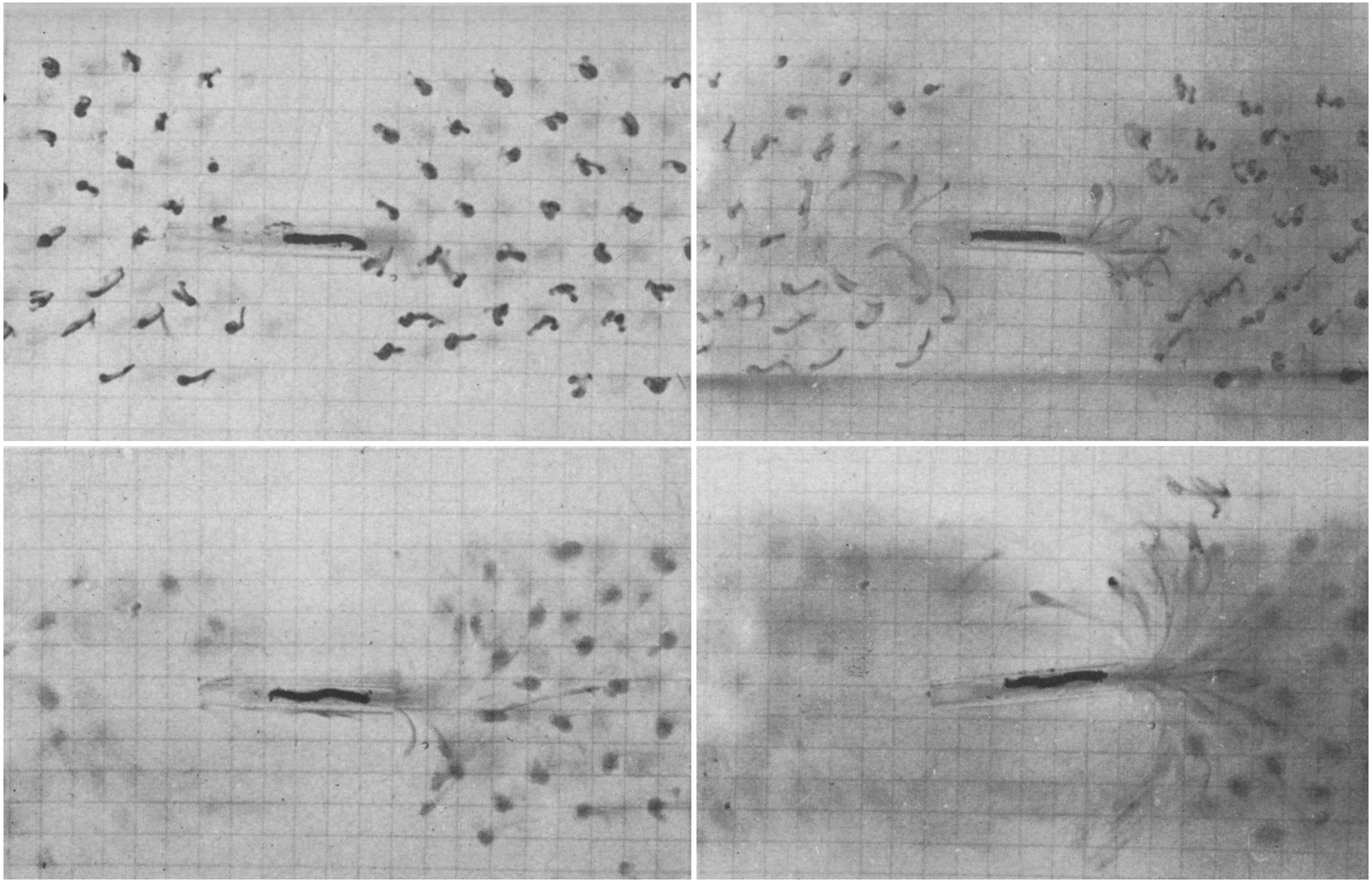


Fig. 37. Turbulance created by a larva of *Glyptotendipes barbipes* (Staeger). Time, five minutes. Back grid, half-cm squares.

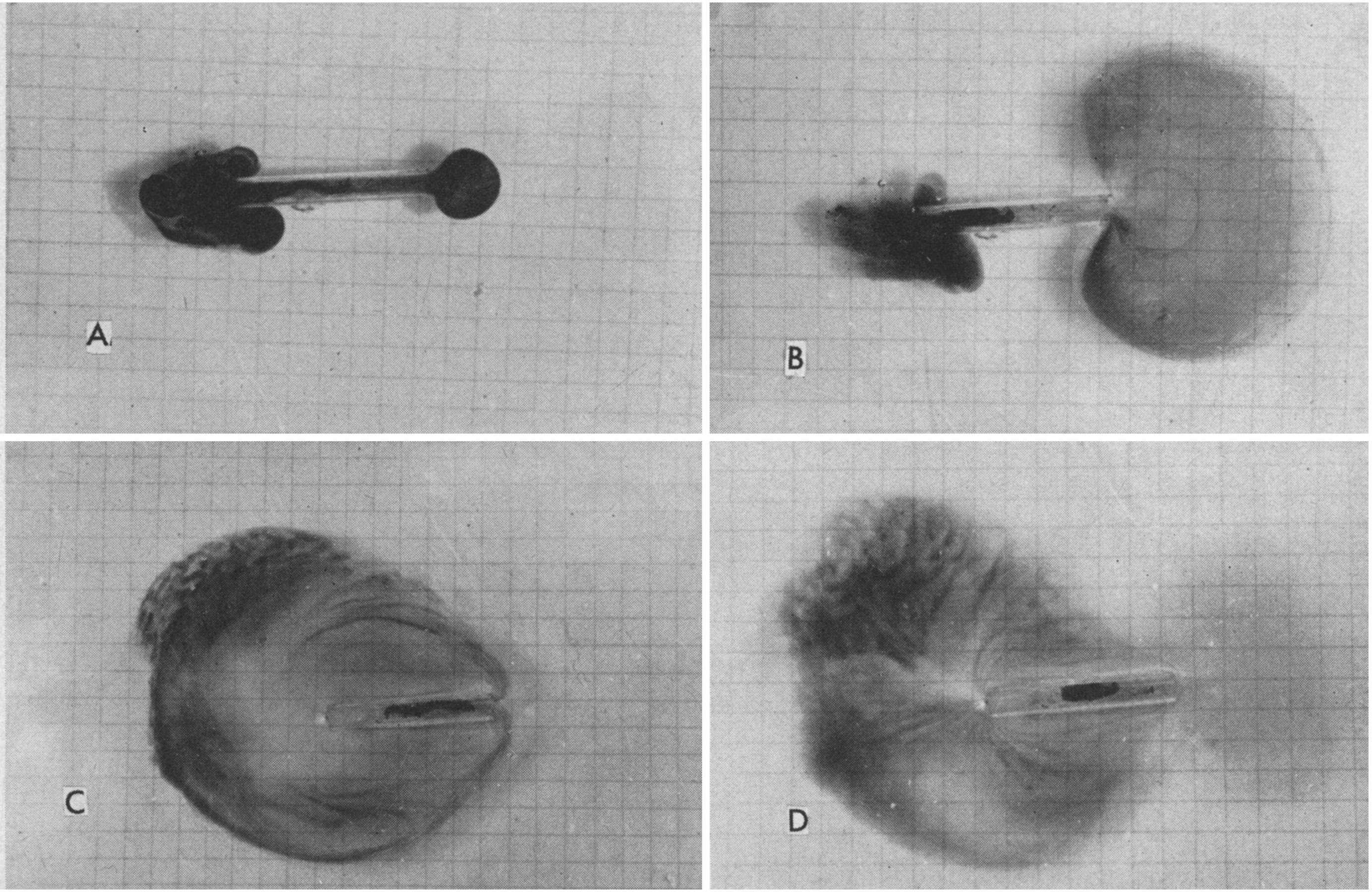


Fig. 38. *A* and *B*, larva of *Glyptotendipes barbipes* (Staeger) pumping ink droplet throught glass tube (time, five minutes); *C*, currents created by larva; *D*, effect of larval reversal of undulation. Back grid, half-cm squares.

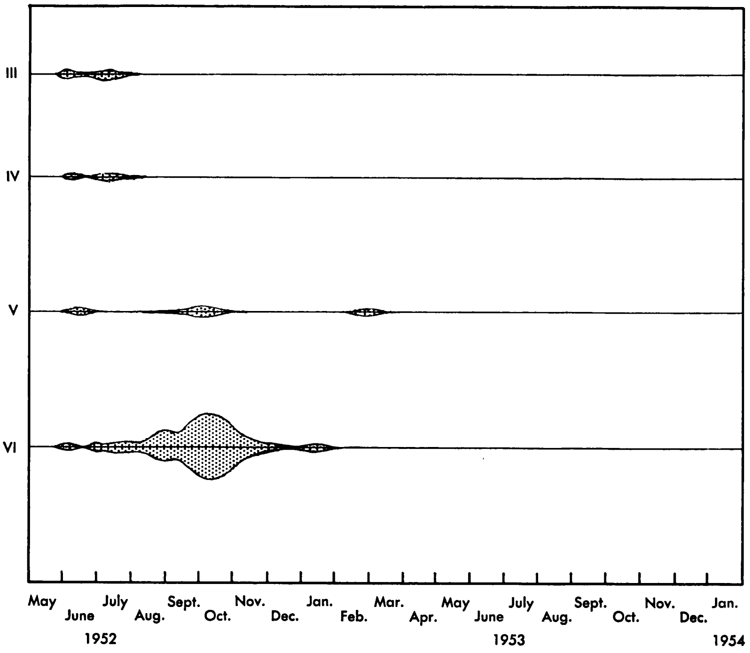


Fig. 39. Relative abundance of *Notonecta unifasciata* Hungerford in oxidation ponds III–VI at Concord, California, in 1952 and 1953.

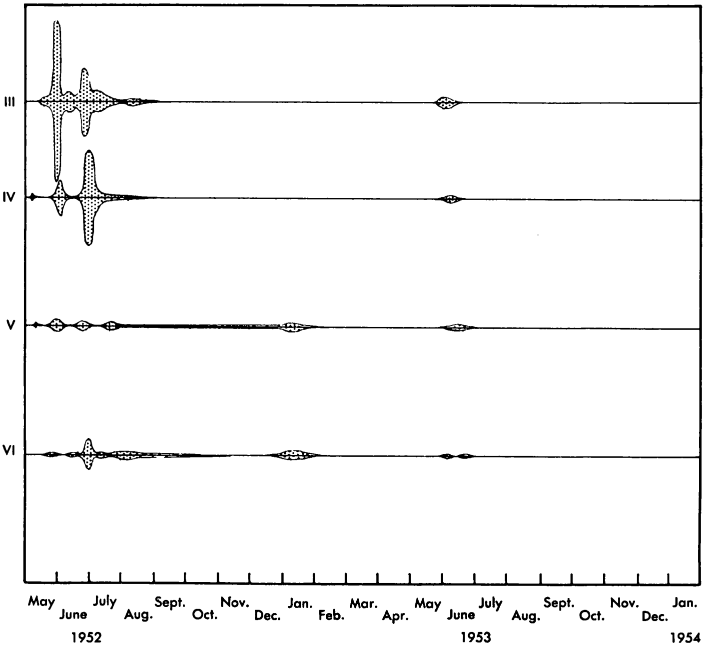


Fig. 40. Relative abundance of *Hesperocorixa laevigata* (Uhler) in oxidation ponds III–VI at Concord, California, in 1952 and 1953.

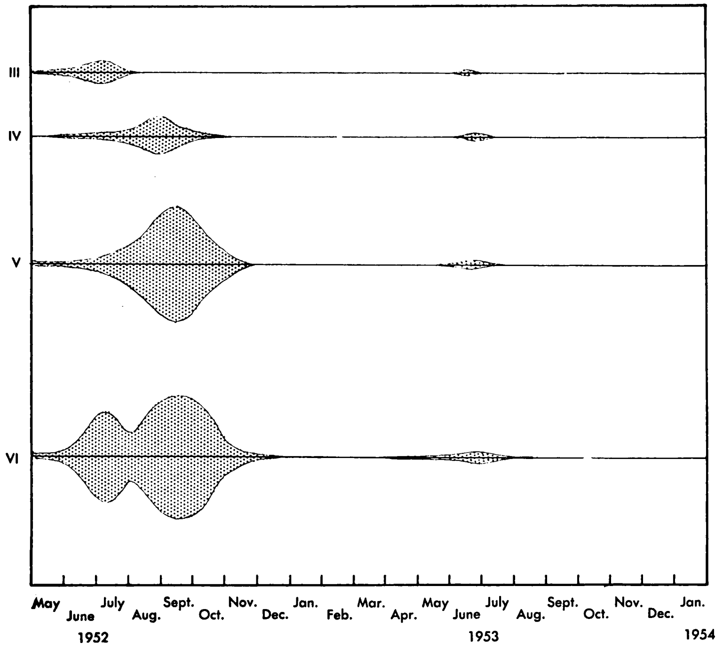


Fig. 41. Relative abundance of *Corisella inscripta* (Uhler) in oxidation ponds III–VI at Concord, California, in 1952 and 1953.

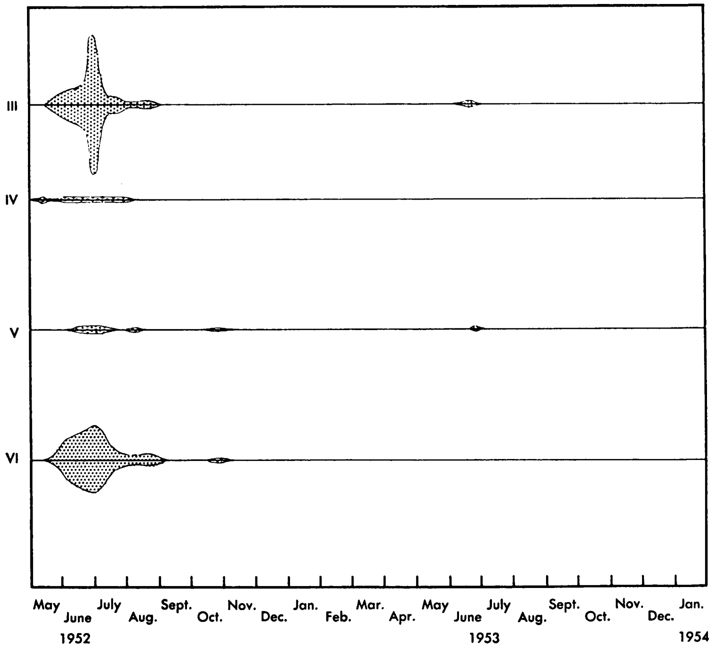


Fig. 42. Relative abundance of *Corisella decolor* (Uhler) in oxidation ponds III–VI at Concord, California, in 1952 and 1953.

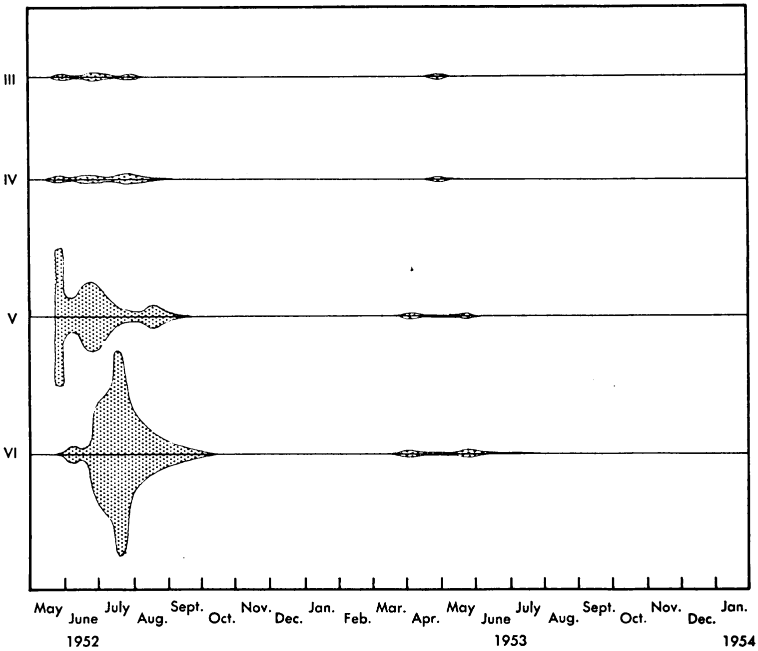


Fig. 43. Relative abundance of *Laccophilus decipiens* LeConte in oxidation ponds III–VI at Concord, California, in 1952 and 1953.

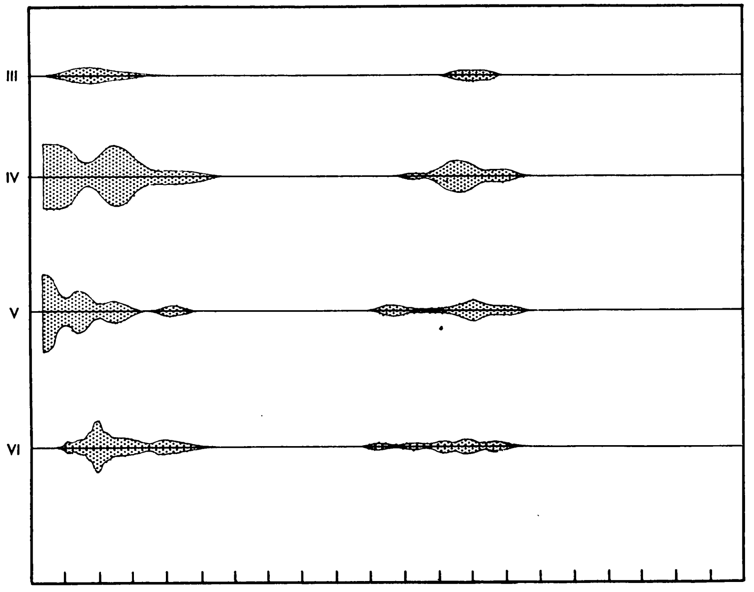


Fig. 44. Relative abundance of *Rantus anisonychus* (Crotch) in oxidation ponds III–VI at Concord, California, in 1952 and 1953.

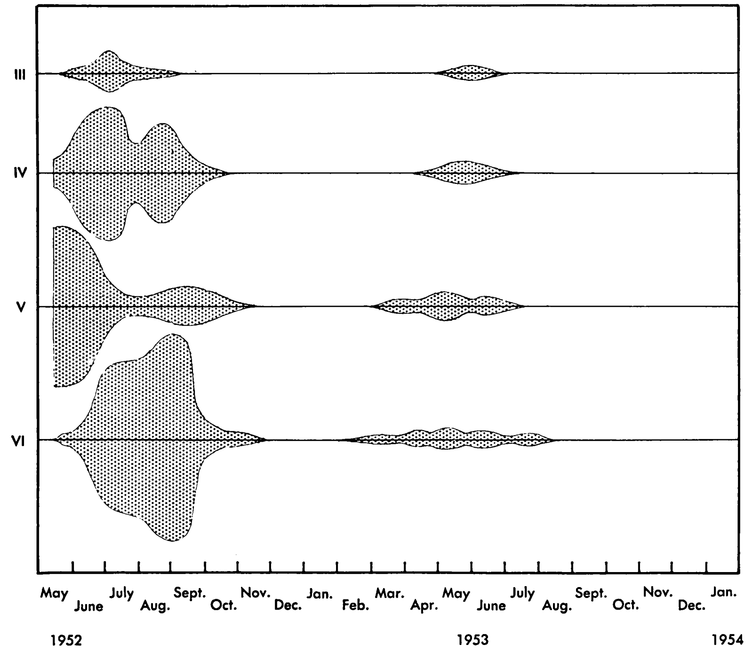


Fig. 45. Relative abundance of *Tropisternus californicus* (LeConte) in oxidation ponds III–VI at Concord, California, in 1952 and 1953.

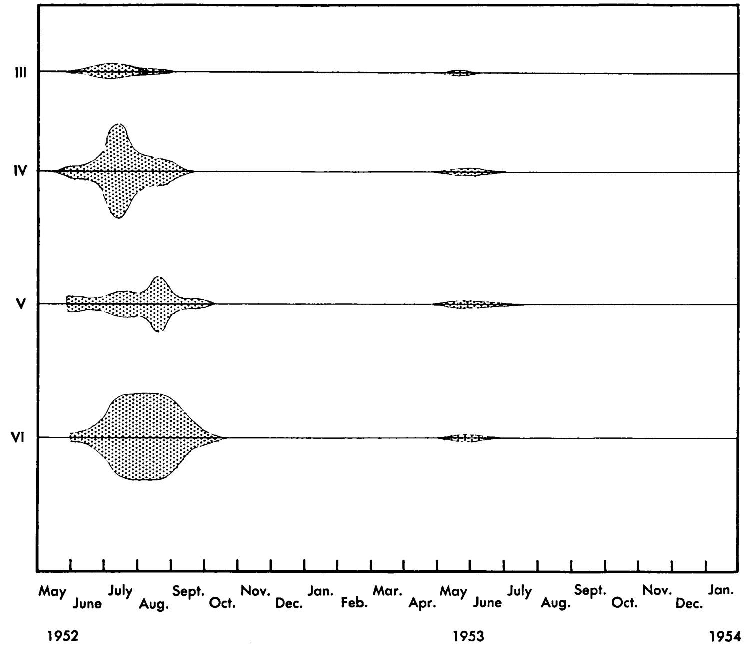


Fig. 46. Relative abundance of *Tropisternus lateralis* (Fabricius) in oxidation ponds III–VI at Concord, California, in 1952 and 1953

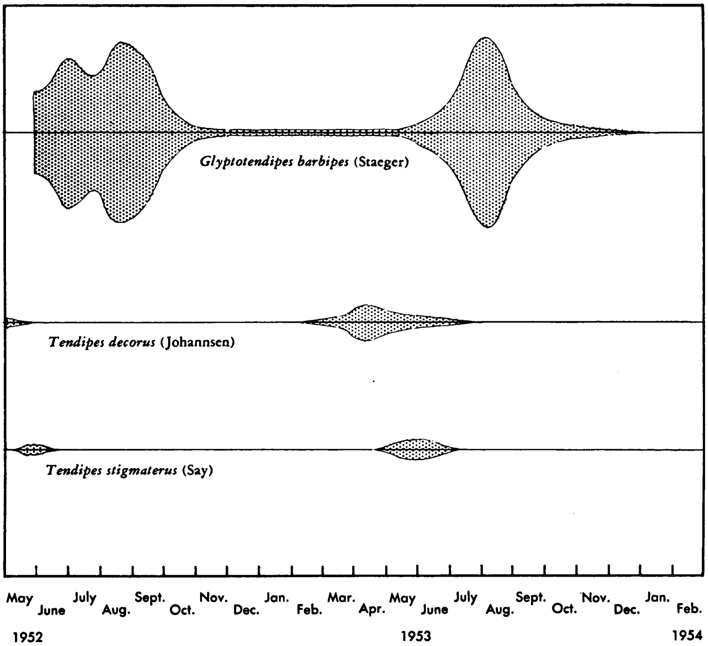


Fig. 47. Relative abundance of three species of tendipedid larvae in oxidation pond III at Concord, California, in 1952 and 1953.

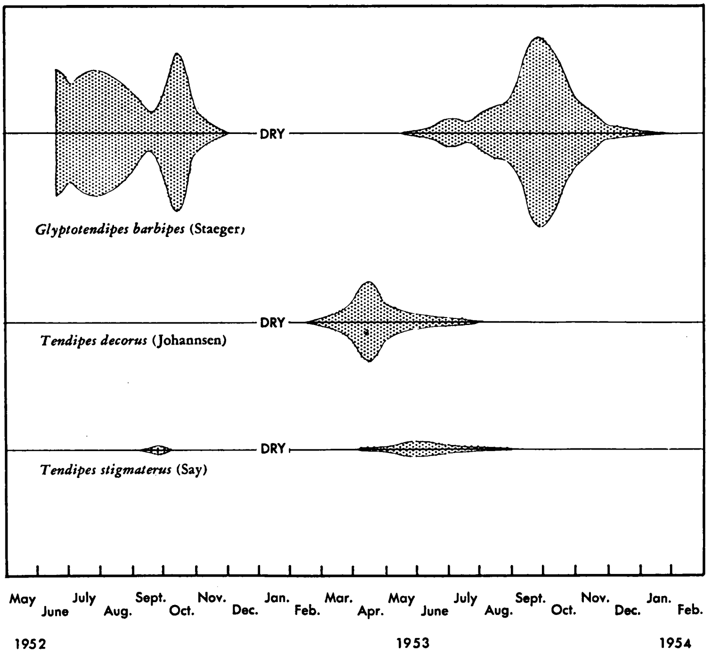


Fig. 48. Relative abundance of three species of tendipedid larvae in oxidation pond IV at Concord, California, in 1952 and 1953.

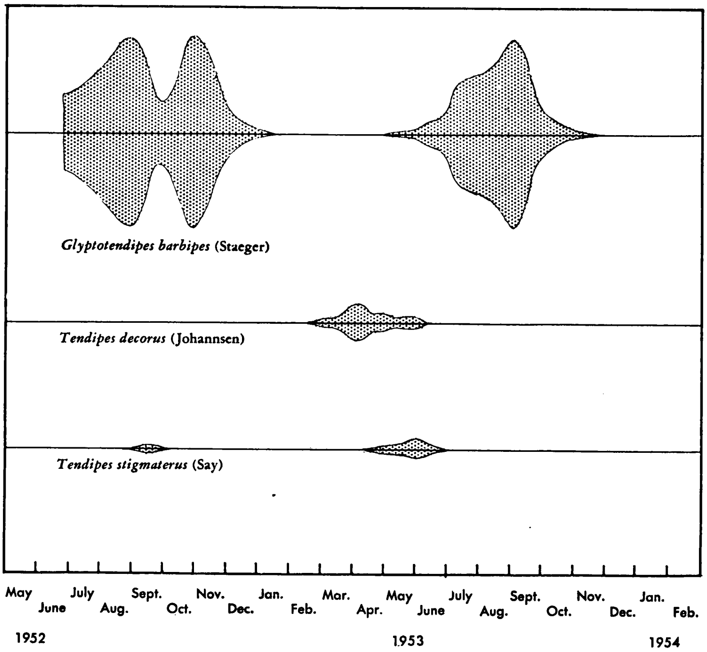


Fig. 49. Relative abundance of three species of tendipedid larvae in oxidation pond V at Concord, California, in 1952 and 1953.

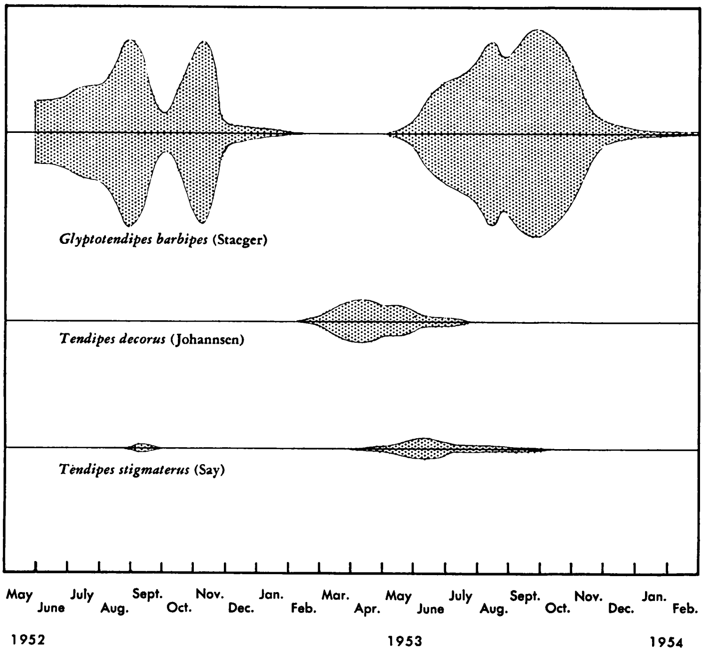


Fig. 50. Relative abundance of three species of tendipedid larvae in oxidation pond VI at Concord, California, in 1952 and 1953.

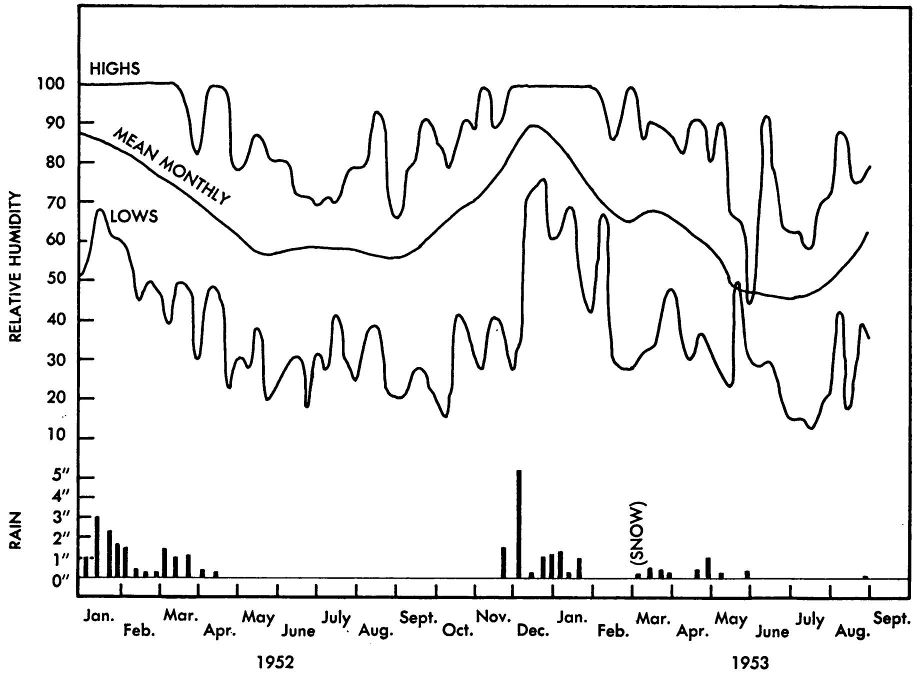


Fig. 51. Relative humidity and rainfall recorded at Concord, California, in 1952 and 1953.

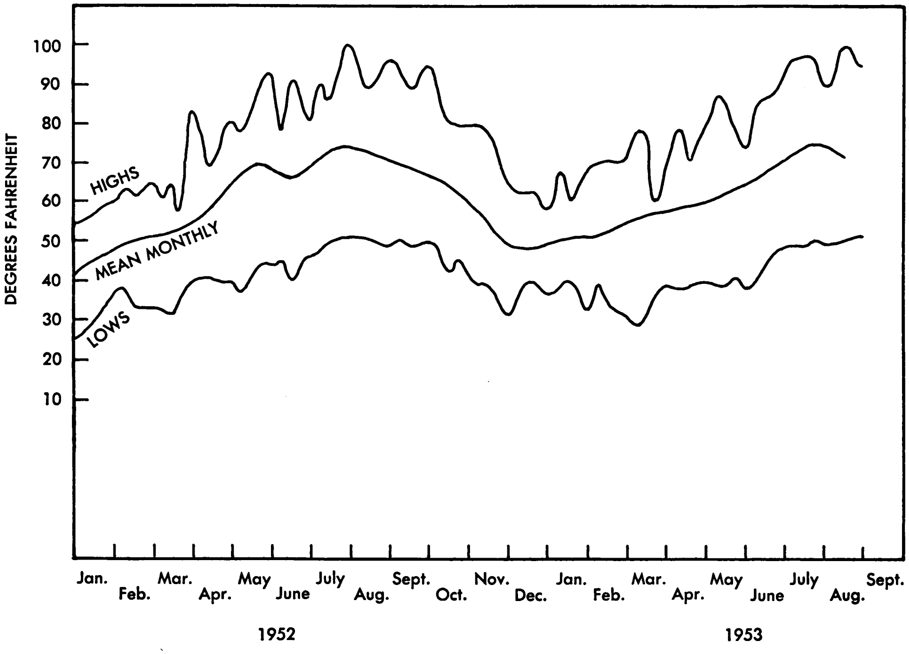


Fig. 52. Temperatures recorded at Concord, California, in 1952 and 1953.

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