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Environmental Protection Technology Series

Tertiary Treatment with a Controlled Ecological System

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December 1973

TERTIARY TREATMENT

WITH A CONTROLLED ECOLOGICAL SYSTEM

by

Las Virgenes Municipal Water District
4232 Las Virgenes Road
Calabasas, California 91302

Project 16080 FBH
Program Element 1BB045

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OFFICE OF RESEARCH AND MONITORING

U. S. ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D. C. 20460

ABSTRACT

A two-stage pond system was constructed and operated as a process for polishing secondary sewage effluent. In the shallow first pond a luxuriant growth of algae was maintained. In the second stage a population of Daphnia pulex effectively removed the algae. Total volume of the system was 1,135 cubic meters. A program of chemical and biological monitoring was followed over a twelve-month period. Objectives were to demonstrate feasibility of the process for producing recreational-grade water, acquire operating data on a completely biological process, and determine its potential for nutrient removal. The system was operated with about 10 days' detention in each stage.

The Daphnia remained as the dominant zooplankton species in the second stage pond throughout the observation period, and during periods when their concentration was above 500 organisms/liter, were able to hold water transparence at Secchi disk readings around 2 meters. At such times COD reduction was above 40 percent across the system. Significant removal of nutrients occurred only during the months of July and August when nitrogen and phosphate reductions were 48 percent and 63 percent, respectively. Nutrient removal performance was hampered by occasional invasions of Daphnia or rotifers in the first stage pond, which decimated the algae population; such events were not successfully controlled and remain the principal obstacle to further development of the process.

This report was submitted in fulfillment of Project Number 16080 FBH by the Las Virgenes Municipal Water District under the (partial) sponsorship of the Environmental Protection Agency. Work was completed as of December 1971.

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ACKNOWLEDGMENTS

A Board of consultants consisting of Dr. Charles R. Hazel, Dr. John Klock, Dr. James L. Morgan, and Dr. George O. Schumann met three times during the project and provided valuable advice and assistance. Mr. Lloyd D. Hedenland, Sanitation Superintendent of the Las Virgenes Municipal Water District, acted as project field manager. Dr. Andrew L. Gram of Gram/Phillips Associates provided general direction, designed the pond system, and compiled the final report.

The organic carbon analyses were performed courtesy of the research and development laboratory of the Los Angeles County Sanitation Districts.

SECTION I

CONCLUSIONS

A two-stage pond process based on growth of algal cells and their subsequent ingestion by Daphnia can achieve effluent clarity together with substantial reductions in organic matter, nitrogen, and phosphate provided that active populations of both organisms are maintained.

In such a process, phosphate removal occurs mainly by precipitation at the elevated pH of the first-stage pond, while nitrogen is converted to solid organic forms by the algae and Daphnia and removed by sedimentation in the second-stage pond. Effective removal of both nitrogen and phosphorus occurs only during the warm-weather months.

Daphnia concentrations on the order of several hundred organisms per liter are easily capable of filtering virtually all algae from a pond of several days' detention.

The principal obstacle to reliable nutrient removal performance is the problem of preventing blooms of algal predators which occur in the first-stage pond.

SECTION II

RECOMMENDATIONS

The process studied herein is chiefly applicable to the polishing of effluent from small secondary treatment plants. Where high nutrient removal efficiencies are necessary, or where land is costly or unavailable, other methods must be used. In order to put the algae-Daphnia process into the plant designer's practical repertoire, there should be further investigation to develop design data. The principal questions relate to biological criteria; performance is closely associated with maximizing algae production and maintaining a vigorous Daphnia population. Increased reliability can only come through process modifications which successfully accommodate the various physiological requirements of these organisms. The next logical step would be to correlate the growth and feeding behavior of Daphnia with the composition of the treated waste water in which they are to perform.

On the basis of one year's operation of a particular pond configuration, the following tentative design guidelines are suggested for use in subtropical climates (pond temperature 10°C to 25°C seasonal range):

1. First-stage pond: Depth, 60 to 90 cm; Detention, 6 to 8 days

2. Second-stage pond: Depth, 2.5 meters minimum; Detention, 5 days

3. Provide overflow connection from first pond to second, and make horizontal distance between the two stages as great as practical.

4. Provide for dewatering and cleaning of second pond on an annual or more frequent schedule.

5. Divide first-stage pond into several compartments, with capability of operating either in series or in parallel.

SECTION III

INTRODUCTION

The Controlled Ecology demonstration project was based on a phenomenon observed in the system of effluent irrigation ponds at the Tapia Plant of Las Virgenes Municipal Water District. There, water which had acquired a heavy growth of algae during short storage in the initial pond was clarified in the downstream storage pond through the predatory action of a persistent Daphnia population. Five sets of analyses made between July and October, 1968, showed considerable reductions of phosphate and nitrate. In the demonstration project a similar system of smaller ponds was constructed and operated independently of the sewage treatment plant. Objectives were (a) to demonstrate technical and economic feasibility of producing recreational-grade water from secondary sewage effluent by means of a two-stage algae-crustacean process; (b) acquire operating data which could be used in formulating design criteria and interpreting water quality phenomena in artificial lakes; and (c) to determine the process's potential for removing nitrogen and phosphorus.

The basic processes involved consist of (1) the photosynthetic growth of green algae on inorganic carbon, nitrogen, and phosphorus present in the sewage effluent, and (2) the consumption of the algae by Daphnia. The two processes are carried out consecutively in separate ponds so that the production of algae can be maximized. There are several important side effects. Oxygen is produced in the first pond as a by-product of photosynthesis, and is consumed in the second by respiration of the Daphnia and other organisms. During rapid algae growth, bicarbonate is depleted, resulting in a rise in pH, which in turn promotes the removal of phosphate by precipitation, presumably as calcium phosphate. Finally, there is an accumulation of organic sediments on the bottoms of both ponds, made possible by several days' detention in each under quiescent conditions.

One promising feature of the system is the extent of clarification attainable. Removal of the algae by Daphnia is very effective, and costs are negligible in contrast to any other means of algae separation which has yet been studied. It is also possible to go one step further in the food chain and use fish to remove the Daphnia or regulate their population. However a comparison of food intake versus growth of both fish and Daphnia shows that the removal of nitrogen and phosphorus nutrients from the sewage effluent by incorporation in the body tissues of these organisms would be negligible. For example, Richman[1] measured enthalpy conversions in a system of Daphnia pulex feeding on the green alga Chlamydomonas reinhardi. He found that only 4 to 13 percent of the algal heat content reappears in the cell tissue of young Daphnia, and with adult organisms the conversion is less than 1.5 percent. Since algae and Daphnia have similar nitrogen and phosphorus compositions, as well as roughly the same heat value, the Daphnia could retain no more than about 10 percent of the N and P content of their algal diet. The same situation prevails during the transformation of Daphnia into fish tissue. Bennett[2] has summarized food conversion data for several warm water fish; the weight ratio of food fish produced to food consumed is generally less than 0.4, even when the fish are actively growing. Any substantial nutrient removal must therefore be due to other mechanisms, such as conversion to a solid form capable of sedimentation.

SECTION IV

PLANT DESCRIPTION

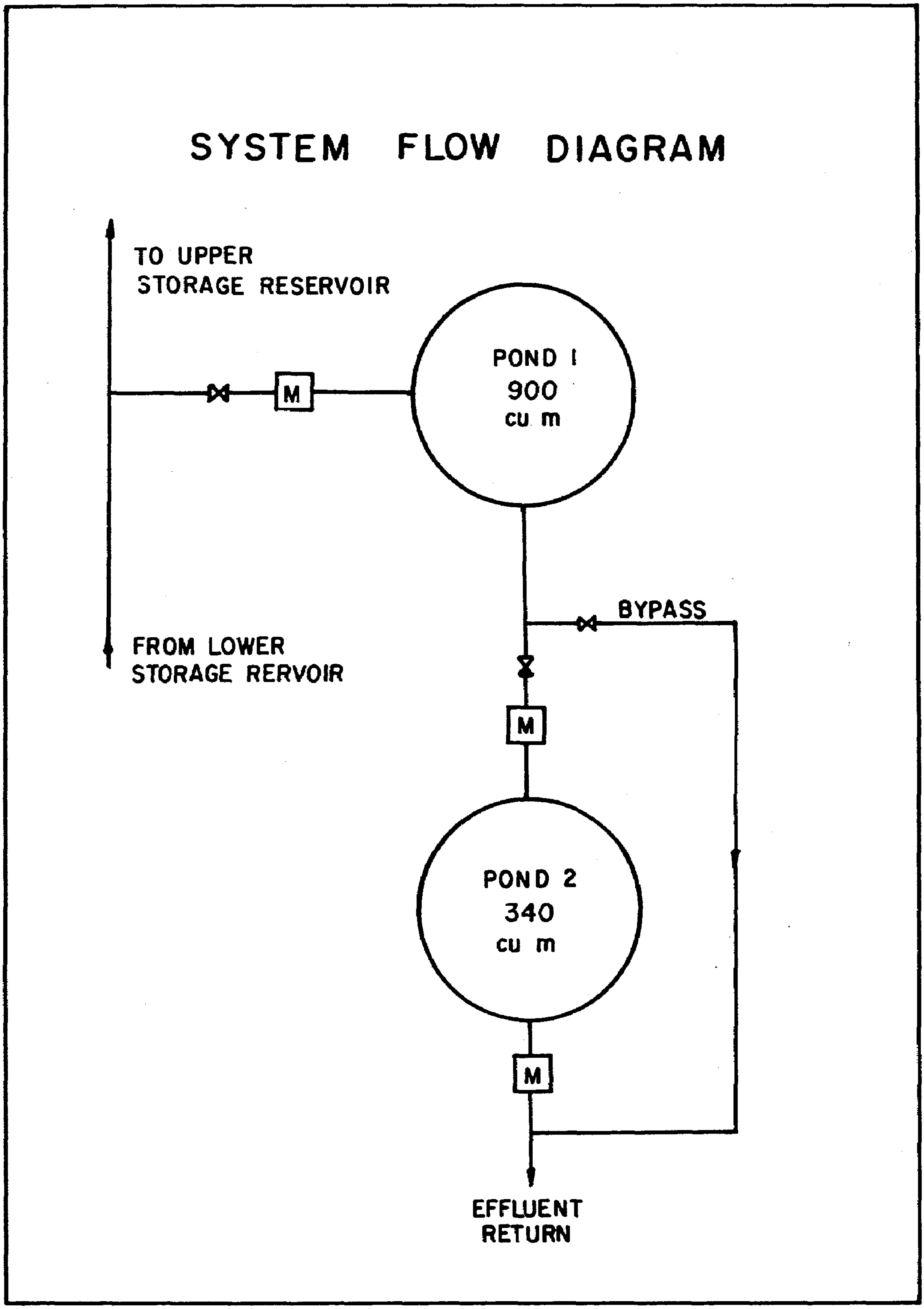
The project was located on the grounds of the District's Tapia Treatment Plant. The site is in Malibu Canyon about 6 kilometers inland from the Pacific Ocean. Terrain is rather steep and rugged, and the project ponds were constructed on small level areas which were fortuitously available. The climate is temperate and relatively mild, but the site is somewhat insulated from the ocean's influence by mountains. Daily air temperatures range from over 32 °C on hot summer days to below freezing on winter nights.

Scale of the project units was chosen on the basis of simulating natural environmental conditions at the lowest possible cost. Initially it was not known to what extent the success of the Daphnia population might depend on such things as illumination, available water depth, nature of pond bottom, etc. A relatively deep earth basin was therefore provided, with a ratio of surface to volume approximating that of the reservoir in which the Daphnia had been observed to thrive. Similarly, the algae pond was made large enough that sunlight illumination level and other growth-determining factors would be comparable to those prevailing in a full-scale plant.

A schematic diagram of the system is shown in Figure 1. The feed was activated sludge effluent taken from the Tapia Plant's spray irrigation system. Flow rates were adjusted manually and were totalized on 5-cm water meters at three points in the system. Part of the algae pond effluent could be diverted around the second pond to permit independent selection of detention periods.

Both ponds were circular in plan. Pond 1 was designed along the lines of a conventional oxidation pond. Gunite lining was provided to permit occasional thorough cleaning and to avoid entrainment of sediment. Water depth was 1.4 m and its volume about 900 cu m. Pond 2 was left unlined so that natural vegetation could develop, and its 3-meter depth provided a range of illumination levels. Two tiers of benches were left in the side slopes to accommodate sediment sample collectors. The volume of Pond 2 was 340 cu m.

FIGURE I


SYSTEM FLOW DIAGRAM
TO UPPER
STORAGE RESERVOIR
M
POND I
900
cu m
FROM LOWER
STORAGE RERVOIR
BYPASS
M
POND 2
340
cu m
M
EFFLUENT
RETURN

Inlets to both ponds consisted of a submerged 5-cm steel pipe discharging at one point near the periphery. In Pond 1 the inlet was 30 cm from the bottom and pointed in a circumferential direction. In Pond 2 the inlet pipe was 30 cm below the surface and pointed toward the center. The outlets of both ponds were concrete weir boxes located diametrically opposite the inlets. Figure 2 shows the general construction features.

When Pond 2 was first filled with water the leakage rate was about 1.3 liters per second. It gradually decreased over a period of several weeks to about 0.6 l/sec. In order to further reduce the loss rate a ton of bentonite was slurried into the pond and allowed to settle. Leakage thereafter remained at about 0.3 l/sec.

POND CONSTRUCTION DETAILS

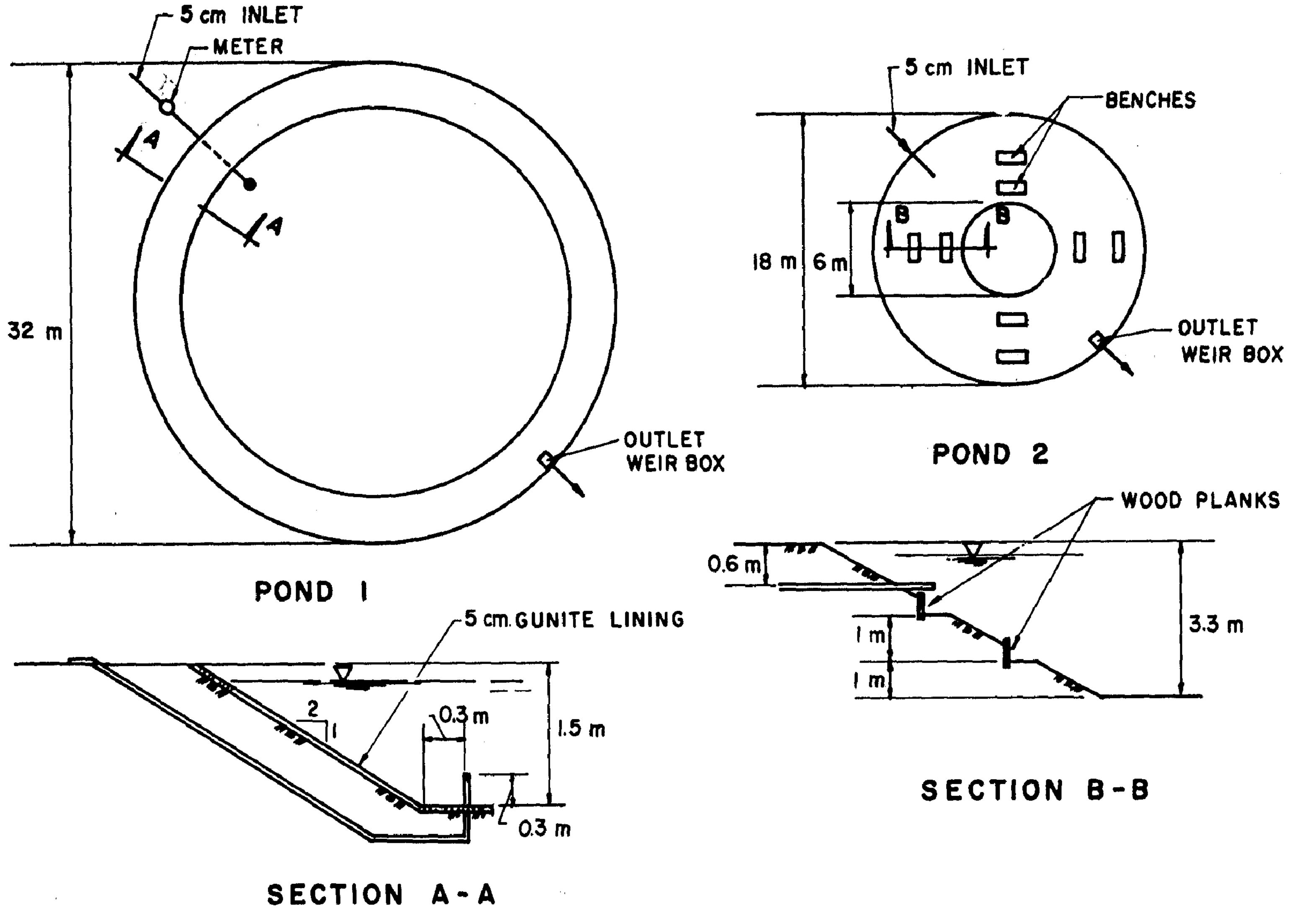


FIGURE 2

SECTION V

PROCEDURES

OPERATION

The liquid feed to the pond system consisted of activated sludge effluent of good quality. The District's sewage is almost entirely domestic and it arrives at the plant in fairly fresh condition. During the period of pond operation most of the plant's effluent flow was being disposed of by hillside spray irrigation. For this purpose the plant effluent was chlorinated and discharged to a storage pond of about two days' capacity. From there it was pumped to the spray system. A lateral connection to the spray system was the source of influent for the experimental ponds. At the upper end of the spray system is a second storage reservoir, which under normal operating conditions has a detention time of several days. It was in this upper reservoir that the persistent clarifying effect of *Daphnia* was observed to occur naturally.

At commencement of operations with the experimental system in August, 1970, Pond 1 was filled with water pumped from the lower storage reservoir in the normal manner. Pond 2 was filled from the upper storage reservoir to assure the presence of some *Daphnia* as seed. Within one week a dense bloom of algae, mostly *Chlorella*, had formed in both ponds. After one month of operation *Daphnia* appeared at high concentration, first in Pond 1 and a few days later in Pond 2.

The algae were decimated in both ponds. In Pond 2 the *Daphnia* were sustained temporarily by feeding water from the upper storage reservoir, which at that time had a fairly high algae count. In Pond 1 the *Daphnia* died out in several days; feeding with the normal influent was resumed, and a relatively stable algae population returned. From this point on, the system operated generally as intended with algae growth in Pond 1 and subsequent clarification in Pond 2.

Flow rates were initially set at 1.9 l/sec through both ponds. Beginning in January, 1971, when analyses were being made on a regular schedule, the flow through the system was reduced to 1.3 l/sec. Detention times were then approximately 8.3 days and 3.1 days in Ponds 1 and 2 respectively. In June the flow through Pond 1 was dropped to 0.9 l/sec to encourage more complete nutrient removals. The Pond 2 flow was also lowered at the same time for a different reason. The Daphnia population began to falter, and the flow was cut to about 0.4 l/sec to avoid losing them altogether. In September, 1971, the Pond 1 feed was brought back up to 1.9 l/sec as a measure to control Daphnia plagues.

A continuous temperature record was obtained of the liquid in both ponds, and of the air in the vicinity of Pond 1. Wooden shelters at each site housed spring-wound 7-day recorders. Temperature probes were suspended at mid-depth.

Collection of samples from Pond 1 for chemical and biological analysis was carried out by casting a tethered bottle from three points on the pond periphery and pooling the contents. In Pond 2 the Daphnia population tended to concentrate somewhat in different regions, so a series of five samples was taken along a pond diameter. An aluminum boat was used as a platform, and it was maneuvered by pulling along a fixed cable suspended above the pond surface. Vertical "core" samples were taken by lowering a length of 2-cm PVC pipe through the liquid depth, stoppering the upper end, and removing carefully.

Sediment samplers were placed on the earth benches which had been provided for them in Pond 2. These consisted of shallow open-top wooden boxes, one foot square, partially filled with earth in simulation of the natural bottom. Sets of the samplers were removed at intervals during the study, and portions of the accumulated sediment were analyzed for organic nitrogen and total phosphate. The earth bedding tended to interfere with subsequent selection of a representative aliquot of bottom area, and it was difficult to avoid washing of the open samplers when hoisting them to the surface. In May, 1971, a different type of sediment collector was installed and proved to be more suitable. They were short lengths of 5-cm PVC pipe, capped at the bottom and attached to a steel stake which would hold them on the pond bottom in a vertical position.

DAPHNIA SEPARATION

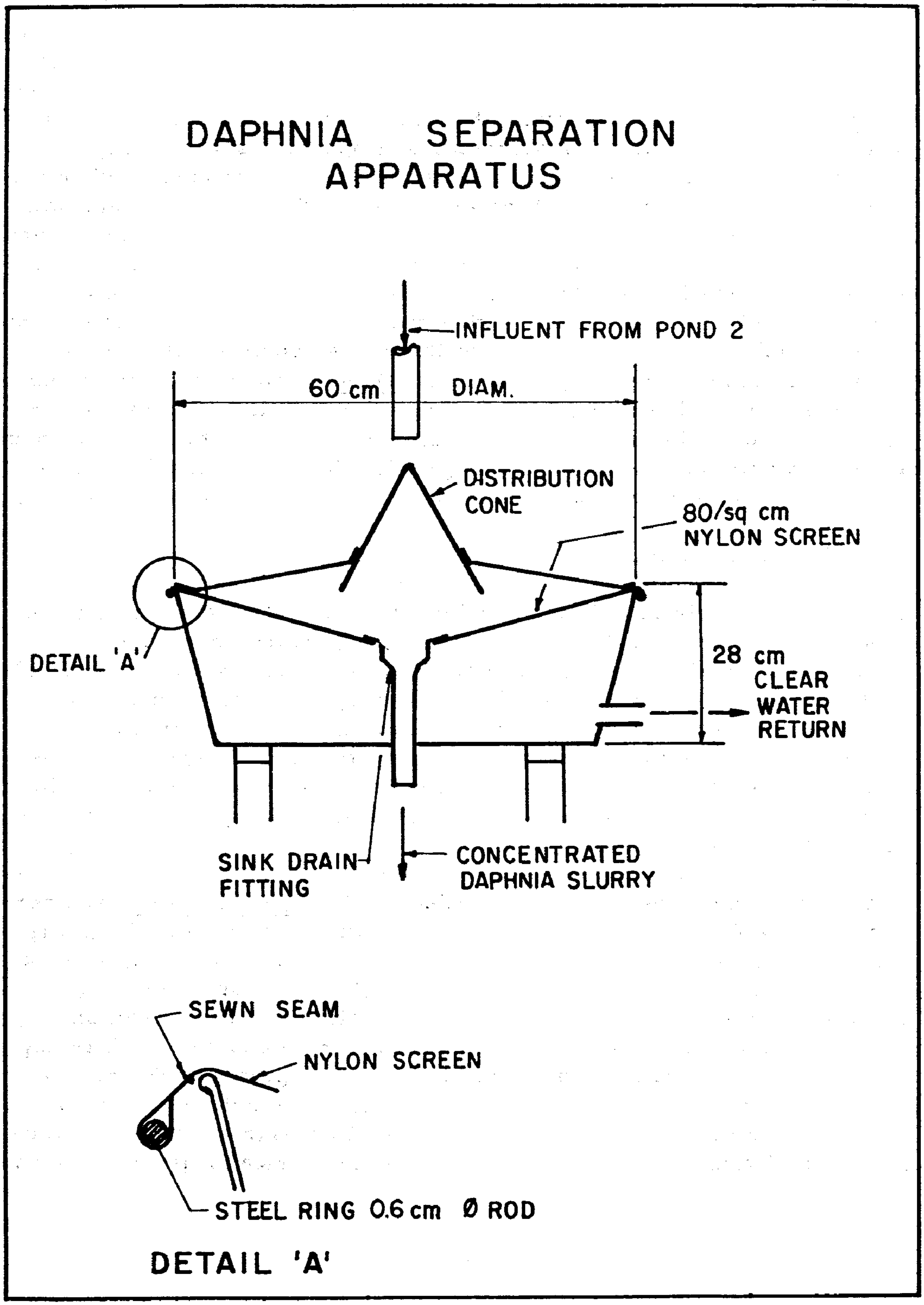
There were periods of operation in which the Daphnia population in Pond 2 increased to a level much greater than was necessary to consume all of the algae being delivered. Also, as described subsequently, there were several instances of Daphnia becoming established in Pond 1 where their presence was undesired. In both situations it would have been of some benefit to have available a means of removing Daphnia without interfering with the liquid flow. A device to accomplish this was designed, constructed, and operated successfully, although it was never used as a regular process component. Figure 3 shows the general features of the apparatus. Water from Pond 2 was pumped over a 60° sheet metal cone onto a funnel-shaped nylon screen having 80 filaments per sq cm. Most of the water passed through the screen and flowed back into the pond. Daphnia accumulated on the screen and sloughed gradually with a small flow of water to the center of the funnel. The central 23 cm of the screen could easily handle 6 1/sec of feed, concentrating an initial Daphnia concentration of several hundred per liter 20 fold. Passage through the pump mutilated most of the organisms. If there should be any reason to preserve them alive, it would be necessary to use gravity flow or some other relatively non-violent means of transport.

ANALYTICAL WORK

The group of analytical determinations carried out on a regular basis was selected partly to demonstrate process performance, but also to provide information on process mechanisms. Table I gives a schedule of the various measurements. "Daily" analyses were normally performed every day except weekends and holidays. In addition to the regular determinations, organic carbon in the influent and two ponds was estimated several times with a Beckman analyzer. Also, Kjeldahl nitrogen and total phosphate were run on bottom samples taken from the ponds.

Analytical techniques were in accordance with Standard Methods wherever applicable. Transparence in Pond 2 was measured by lowering a 20-cm Secchi disk to the depth of disappearance. Turbidity was expressed as percent transmittance of white light through a one-cm sample thickness. All colorimetric determinations were read on a Lumetron colorimeter. Suspended solids were measured by filtering a 25-ml sample through a tared 0.45-micron Millipore disk, drying at 103° C, and weighing. The EDTA titration method was used for calcium, brucine method for nitrate, direct nesslerization for ammonia, and Kjeldahl nitrogens were read colorimetrically after distillation.

FIGURE 3


DAPHNIA SEPARATION APPARATUS
INFLUENT FROM POND 2
60 cm DIAM.
DISTRIBUTION CONE
80/sq cm NYLON SCREEN
DETAIL 'A'
28 cm
CLEAR WATER RETURN
SINK DRAIN FITTING
CONCENTRATED DAPHNIA SLURRY
SEWN SEAM
NYLON SCREEN
STEEL RING 0.6 cm Ø ROD
DETAIL 'A'

"Filtered" determinations were made on the filtrate generated during the suspended solids procedure.

Algae counts and identification were carried out under a microscope at 400X magnification using a haemacytometer cell. *Daphnia* concentrations were determined by manually removing the specimens found in a 100-ml aliquot of sample using an eye dropper.

Table 1. PROCESS DETERMINATIONS

Measurement	Location	Frequency
Physical		
Flow	I, 1, 2	Daily
Temperature	Air, 1, 2	Continuous
Transparence	2	Daily
Suspended Solids	I, 1, 2	Daily
Turbidity	I, 1, 2	Daily
Color	2	Daily
Chemical		
pH	I, 1, 2	2/day
Alkalinity	I, 1, 2	2/day
COD, filtered and unfiltered	I, 1, 2	1/week
Calcium	I, 1, 2	1/week
Kjeldahl nitrogen, filtered and unfiltered	I, 1, 2	1/week
Ammonia nitrogen	I, 1, 2	1/week
Nitrate nitrogen	I, 1, 2	1/week
Orthophosphate, filtered and unfiltered	I, 1, 2	1/week
Dissolved oxygen	1, 2	2/day
Biological		
Algae count and species	1	Daily
Daphnia count	1, 2	Daily

Figure 4. Pond 1

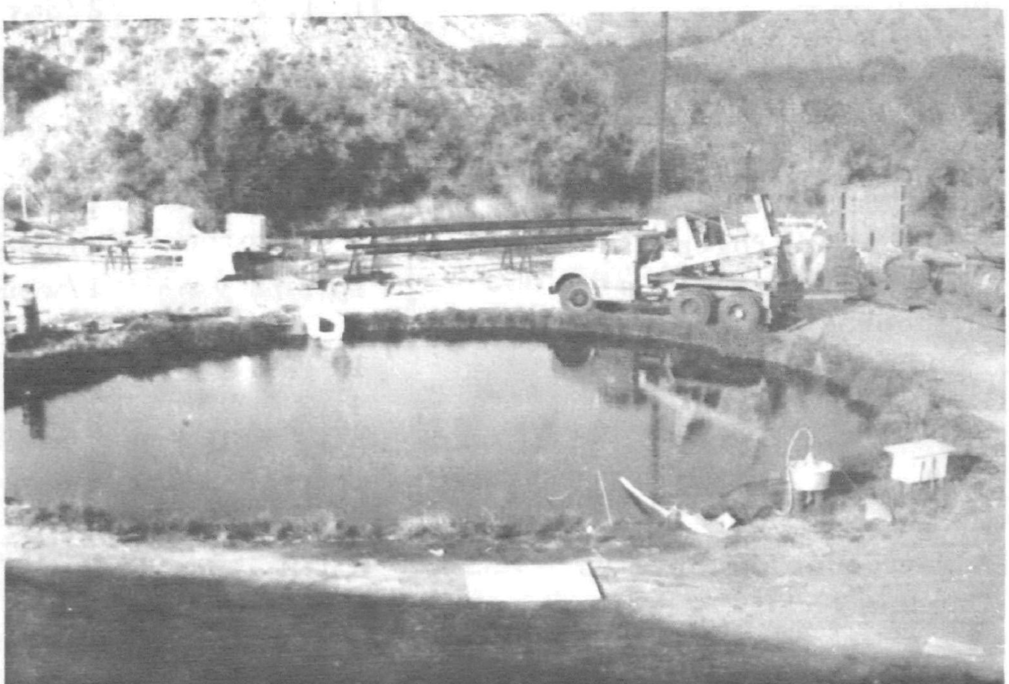


Figure 5. Pond 2

SECTION VI

RESULTS

GENERAL PERFORMANCE

Figures 7 through 21 provide a chronological record of system conditions over a 14-month period. On most of the charts parallel records are given for influent, Pond 1, and Pond 2. The bar-type plots show the times and spacing of samples, as well as the pattern of change. Table 2 contains the average results by months of several performance parameters.

The seasonal temperature progression in the two ponds is shown on Figure 7. Values ranged from a low of about 5° C in early January to 28° in early August. Daily fluctuations are not shown on the chart, but the peak-to-peak span was about 5° in Pond 1 and about 1.5° in Pond 2 (during clear weather). Flow data are represented on Figure 8 in the form of a smoothed hydraulic throughput rate. Plotted values are the reciprocal of the mean residence time of the current pond contents. The computation was made under the assumptions of complete mixing and of steady delivery of flow between daily readings; the residence time at the end of a given day is given by the formula $T_n = V/Q_n + (T_{n-1} - V/Q_n)\exp(-Q_n/V)$ where V is pond volume and Q_n the volume of influent during day "n". The spikes in the Pond 1 graph represent flushing events which were instituted to suppress incursions of Daphnia and rotifers, as discussed later.

Transparence and clarity of the Pond 2 effluent are shown on Figure 9. Secchi disk readings were from 2.0 to 2.5 m all through the spring months. The decline during the subsequent summer corresponds exactly to a reduction in the Daphnia population. Comparison of influent and effluent light transmittance in the spring suggests a reduction in colloidal matter of about 50 percent (log 0.93 versus log 0.86). During the summer Pond 2 was slightly more turbid than the influent because of incomplete algae removal. Turbidity in Pond 1 was not measured; at most times it was far greater than that of the influent because of its algae content. The suspended solids record, Figure 10, illustrates the growth of algal cells in Pond 1 and their subsequent removal in

Table 2. SUMMARY DATA

	Temp. °C	Trans-mittance %	Susp. Solids mg/l	COD mg/l	pH	Diss. Oxygen mg/l
Influent						
Feb		84.5	23	76	7.0	
Mar		86.0	23	67	7.0	
Apr		88.7	15	71	7.1	
May		85.3	17	43	7.0	
June		87.5	14	32	7.1	
July		88.1	31	43	7.2	
Aug		86.0	18	43	7.2	
Sept		83.7	21	69	7.1	
Oct		82.2	21	51	7.2	
Pond 1						
Feb	10.4		31	95	7.9	20.0
Mar	10.9		42	100	8.2	19.5
Apr	16.6		48	91	8.2	18.2
May	19.4		21	48	8.0	12.0
June	22.5		31	73	8.4	22.8
July	24.7		66	122	9.2	18.6
Aug	25.0		73	89	9.2	20.0
Sept	22.2		49	111	8.3	13.4
Oct	16.1		48	88	8.2	21.9
Pond 2						
Feb	12.7	93.7	8	69	7.4	1.0
Mar	14.9	94.1	12	68	7.4	0.8
Apr	18.1	93.4	11	56	7.7	4.6
May	20.6	90.9	16	34	7.8	2.5
June	22.2	81.5	30	35	7.9	7.0
July	25.2	78.7	35	60	8.7	5.6
Aug	25.8	74.3	42	58	9.0	12.4
Sept	23.6	79.6	32	80	8.2	5.3
Oct	20.1	80.1	16	38	7.6	1.8

Pond 2. Algae production during the summer months of July and August was almost twice as great as it was throughout the spring.

The COD values (Figure 11) also reflect the transformation of inorganic carbon to organic form in Pond 1, and its removal in Pond 2. Overall COD reductions were substantial during the spring period of high Daphnia activity, but were small or negative during the summer. Organic carbon analyses made during the spring are given in Table 3. The samples were composited from once-daily grabs over the periods indicated. Results are quite parallel to the COD's.

Table 3. TOTAL ORGANIC CARBON DETERMINATIONS
Values in mg/l C

Period	Influent	Pond 1	Pond 2
2/24 - 3/12	15.8	25.2	11.6
3/15 - 3/31	17.2	35.6	15.0
4/1 - 4/19	14.2	18.4	12.4
4/20 - 4/30	12.0	19.8	10.2

NUTRIENT REMOVAL

Nutrient Removals achieved by the system are shown in Table 4, which displays monthly average nitrogen and phosphate concentrations, and on Figures 12 through 16. The removals given in Table 4 are the differences between plant influent and effluent concentrations. Significance levels are based on Student's "t" distribution using the statistic $t=(\bar{x}_1 - \bar{x}_2)/(s\sqrt{1/n_1 + 1/n_2})$. Here $\bar{x}_1$ and $\bar{x}_2$ are the measured average concentrations for a given month, n_1 and n_2 are the numbers of samples contributing to the averages, and $s = \sqrt{[(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2] / (n_1 + n_2 - 2)}$, where s_1 and s_2 are the sample standard deviations. The number of degrees of freedom is $n_1 + n_2 - 2$. The notation N.S. means that the removal was not significant at the 10 percent level.

Performance in this respect was mediocre throughout the cold weather months, but in May there was significant reduction in both nutrients. Best performance was in July and August, when the overall removals were 48% for nitrogen and 63% for phosphate. It may be noted that most of the nitrogen removal occurs in Pond 2. In Pond 1, the nitrogen is incorporated into the algal cells, and in Pond 2 the algae are ingested by Daphnia and other higher organisms and converted to settleable solids. On the other hand, the bulk of the phosphate is removed in the first pond, evidently through precipitation as the result of the high pH environment generated by the algae.

Table 4. MONTHLY AVERAGE NUTRIENT CONCENTRATIONS

Total Nitrogen, mg/l

	Influent	Pond 1	Pond 2	Removal	Significance Level, %
February	15.2	15.8	13.5	1.7	1.5
March	16.1	14.3	11.6	3.5	0.1
April	13.4	12.2	9.8	3.6	1.0
May	12.0	11.3	8.6	3.4	0.2
June	12.8	11.1	11.4	1.4	---
July	15.3	14.0	7.9	7.4	0.1
August	13.6	14.4	6.9	6.7	0.1
September	18.4	15.6	10.3	8.1	0.1
October	18.6	17.8	11.2	7.4	5.5

Total Phosphate, mg/l

	Influent	Pond 1	Pond 2	Removal	Significance Level, %
February	28.4	27.0	27.3	1.4	N.S.
March	28.9	27.5	27.6	1.4	9.3
April	27.2	25.5	24.8	1.7	N.S.
May	27.7	21.4	16.6	6.3	0.3
June	29.0	26.1	25.8	2.9	N.S.
July	29.5	13.5	10.5	17.0	0.1
August	29.3	17.7	10.9	11.6	0.1
September	34.8	23.1	18.2	11.7	8.5
October	24.4	23.7	23.9	0.7	N.S.

Balances on the amounts of nutrients passing through Ponds 1 and 2 are given in Tables 5 and 6. There was very little net change in nitrate nitrogen in either pond, but in Pond 1 part of the ammonia nitrogen was converted to organic, which was then removed in Pond 2. A similar conversion of soluble phosphate to insoluble phosphate occurred in Pond 1, although here it is probable that most of the insoluble phosphate formed was inorganic. Algal cells contain only about two percent phosphate, compared to around nine percent nitrogen. At any rate, during July and August more than half of the phosphate entering Pond 1 remained there.

Collection devices had been placed on the bottom of both ponds for the purpose of acquiring samples of deposited settleable material. Kjeldahl nitrogen accumulated at the rate of about 4.5 kg per month in Pond 1, and at about 5.5 kg per month in Pond 2, during the summer period (May through August). These figures agree reasonably well with the nitrogen removals shown in Table 5. Phosphate accumulated on the bottom on Pond 2 at about one pound per month, again in agreement with the liquid measurements. However the Pond 1 bottom samples contained little or no phosphate above background, suggesting that the removed phosphate may have been deposited on the concrete pond lining in crystaline form.

On September 24, 1971, a phosphate tracer was injected into Pond 1 for the purpose of observing the distribution process. The tracer was 40 millicuries of phosphorus-32 prepared by neutron activation of ammonium diacid phosphate. Samples of the liquid and bottom sediment in Pond 1 were taken for two weeks, as well as liquid and Daphnia samples from Pond 2. Filtered and unfiltered portions were later dried on planchets and counted using a thin-window Geiger system. The results are shown graphically in Figure 6. There was very rapid initial disappearance of filtrable phosphate; within two days some 65 percent had been either precipitated or converted to suspended solids. Tracer removed from the liquid reached a peak of 45 percent in five days and then slowly began to reappear, perhaps by exchanging with non-radioactive phosphate. Samples of bottom sediment showed the same level of radioactivity as the interior liquid, again suggesting precipitation rather than sedimentation as the dominant mechanism. The peak in the fraction converted to suspended form may be only apparent since the concentrations were changing rapidly at that time, and the mass balance calculation hence subject to inaccuracy. In any case, transformations in the pond were essentially complete in five days. From then on the only changes which occurred were the washout of remaining suspended and filtrable tracer, and the gradual return of tracer previously removed from the liquid.

Table 5. NITROGEN BALANCE
(Figures in kg N)

Pond 1

	Input				Output					
	Org N	NH_3-N	NO_3-N	Total	Org N	NH_3-N	NO_3-N	Total	Decrease	%
Feb	10.8	50.5	13.1	70.6	18.0	43.1	12.0	73.1	-2.5	-3.5
Mar	6.2	32.1	16.1	54.4	12.7	18.6	17.0	48.3	6.1	11.3
Apr	6.0	42.0	20.5	68.6	11.9	36.1	14.2	62.2	6.4	9.3
May	2.5	56.2	24.6	83.3	12.0	47.4	19.0	78.4	4.9	5.9
June	8.9	21.4	12.9	43.2	14.1	10.4	12.8	37.3	5.9	13.6
July	9.8	44.2	1.7	55.6	22.0	26.9	1.8	50.7	4.9	8.7
Aug	3.8	24.1	1.2	29.0	18.0	12.0	0.8	30.6	-1.6	-5.6
Sept	16.0	69.2	1.2	86.4	22.9	47.8	1.7	72.4	14.0	16.2
Oct	10.5	45.7	0.7	56.9	16.2	37.0	1.2	54.4	2.5	4.3

Pond 2

	Org N	NH_3-N	NO_3-N	Total	Org N	NH_3-N	NO_3-N	Total	Decrease	%
Feb	11.7	27.8	8.7	48.2	2.8	29.6	8.9	41.4	6.8	14.2
Mar	10.4	19.1	15.7	45.2	3.1	20.8	13.0	37.0	8.2	18.2
Apr	8.8	24.8	9.4	43.0	3.3	21.1	10.0	34.4	8.6	19.9
May	3.9	7.4	8.8	20.1	2.0	8.8	6.8	17.7	2.4	12.2
June	0.4	8.7	5.4	14.5	1.4	7.3	4.3	13.0	1.5	10.3
July	11.1	2.7	1.0	14.8	3.9	3.4	1.0	8.4	6.4	43.3
Aug	11.2	2.2	0.3	13.7	3.5	2.4	0.2	6.1	7.6	55.5
Sept	6.2	10.4	0.3	16.9	2.5	7.7	0.1	10.3	6.5	38.7
Oct	6.0	14.2	0.5	20.7	4.1	12.1	0.5	16.6	4.1	19.7

Table 6. PHOSPHATE BALANCE
(Figures in kg PO_4)

Pond 1

	Input			Output				
	Sol	Insol	Total	Sol	Insol	Total	Decrease	%
Feb	117.4	14.2	131.6	119.0	6.1	125.1	6.4	4.9
Mar	91.6	5.9	97.5	86.2	6.6	92.8	4.7	4.8
Apr	133.7	5.2	138.9	120.0	14.6	130.6	8.3	6.0
May	182.1	10.2	192.3	143.8	5.2	149.0	43.3	22.5
June	88.5	9.2	97.7	77.5	10.2	87.7	10.0	10.2
July	100.3	6.4	106.7	10.2	42.1	52.3	54.4	60.0
Aug	60.6	1.8	62.4	25.4	12.5	37.9	24.5	39.4
Sept	155.6	8.2	163.8	103.0	5.6	108.6	55.2	33.7
Oct	71.3	3.2	74.5	70.3	4.4	74.7	-0.2	0.0

Pond 2

	Sol	Insol	Total	Sol	Insol	Total	Decrease	%
Feb	77.9	5.0	82.9	80.2	3.4	83.6	-0.7	-0.1
Mar	82.0	6.2	88.2	86.7	1.5	87.6	0.6	0.1
Apr	65.4	18.2	83.6	85.6	0.4	86.0	-2.4	-2.9
May	16.8	8.7	25.5	28.1	1.7	29.8	-4.3	-17.1
June	25.8	4.6	30.4	29.6	1.0	30.6	-0.2	-0.6
July	3.4	8.9	12.3	9.5	1.7	11.2	1.1	9.2
Aug	7.3	5.9	13.2	6.7	2.2	8.9	4.3	32.3
Sept	23.8	1.3	25.1	19.0	0.4	19.4	5.7	22.8
Oct	26.0	1.6	27.6	27.5	3.4	30.9	-3.3	-11.8

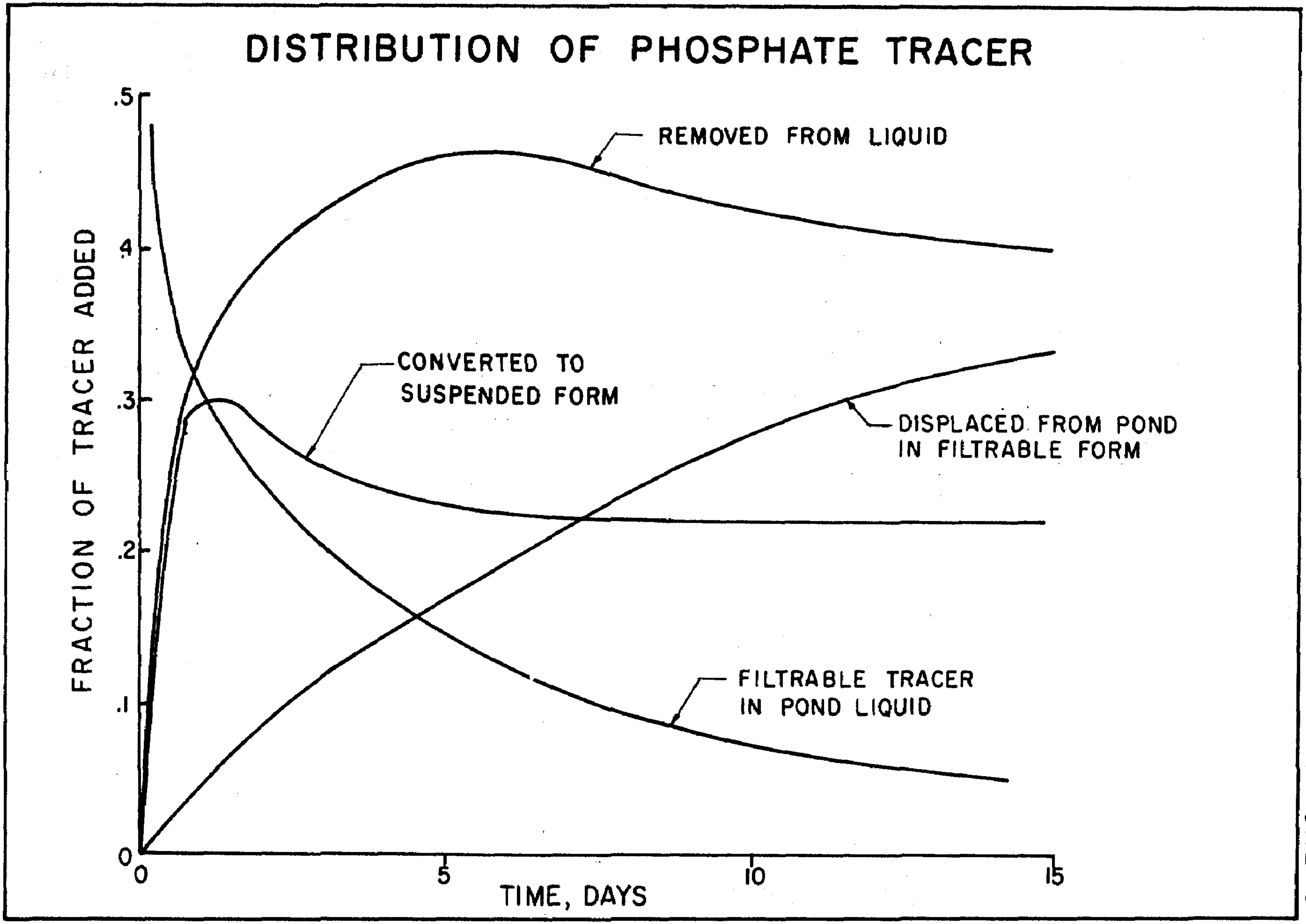

DISTRIBUTION OF PHOSPHATE TRACER
FRACTION OF TRACER ADDED
.5
.4
.3
.2
.1
0
0
5
10
15
TIME, DAYS
REMOVED FROM LIQUID
CONVERTED TO
SUSPENDED FORM
DISPLACED FROM POND
IN FILTRABLE FORM
FILTRABLE TRACER
IN POND LIQUID

FIGURE 6

Populations of Chlorella and Daphnia throughout the year are shown in Figures 17 and 18. Small numbers of other algae were occasionally observed, but Chlorella strongly predominated and cell counts of this genus were therefore used as a measure of algal population. Although the cell concentrations during February and March were similar to those reached in July, it should be noted that the average cell size was much smaller in the earlier period, and the biomass concentration was much greater in summer. The suspended solids determination provides a more significant measure of algal substance.

Like Chlorella among the Pond 1 algae, Daphnia pulex was the sole dominant invertebrate organism of Pond 2. Various water bugs occurred, including some fairly large types, but none in appreciable mass concentrations compared to the Daphnia. No other cladoceran species were encountered. The low temperature reached in Pond 2 never caused complete cessation of asexual reproduction, and they remained at high concentrations in the face of a hydraulic detention time of five days (pond volume divided by overflow). The actual wash-out rate of the organisms could be somewhat less because of rheotaxis; the Daphnia tended to swim "upstream" in the vicinity of the outlet weir. Throughout the winter-spring season the Daphnia produced winter eggs (ephippia). These tended to float and frequently collected on the leeward side of Pond 2 as a granular scum. The period of ephippia production was approximately November through April.

The potential clarification effect of Daphnia can be estimated from the filtration rates which have been measured by others. A conservatively low value is 5 ml/day for an average organism. At this rate 100 organisms per liter would be sifting their medium once every two days. Since Pond 2's Daphnia concentrations were frequently above 1000 organisms per liter it is not surprising that they were able virtually to eliminate the algae at such times.

With the onset of summer the Daphnia population in Pond 2 began to fall off unaccountably. The cause is not known; possibilities include warm water temperatures (above 22° C), activity of some unrecognized predator, and some toxic factor. Ryther[3] has found definite experimental evidence that senescent Chlorella cells contain a substance which inhibits growth and reproduction of Daphnia magna feeding upon them, while rapidly growing algae exhibit no such antagonistic effect. At any rate, with the Daphnia concentration less than 100 per liter, as it was most of the time from June to October, removal of algae was not complete.

Biological upsets of the algae population in Pond 1 occurred in almost periodic fashion at approximately two-month intervals. The first time, in September, 1970, Daphnia invaded the pond and slaughtered most of the algae. After recovery a school of Gambusia was introduced, and it appeared to be holding the Daphnia under control, since the fish were observed occasionally in small concentrations for six months. Minor raids on the algae happened in December and February, but were readily controlled by temporarily increasing the flow. However, a serious Daphnia pulse came on suddenly about the first of April. The algae were nearly wiped out, but normal conditions were restored in about a week by maintaining a high flow rate. Another invasion began about May 15. This time flushing for several days brought little change, and the pond was drained. After refilling, the algae population was quickly reestablished, but it built up rather slowly during June, and finally reached a peak level around the middle of July. The next upset was a bloom of rotifers which appeared suddenly at the end of July. Flushing for two days brought the rotifers under control. Algae recovery was slow; at the end of August their population was about half of the July peak when a second rotifer bloom again cut them down. The deterioration in Pond 1 phosphate removal during times of low algae concentration can be seen in Figure 16.

Intensity of photosynthesis which occurred in Pond 1 is indicated by the pH and alkalinity data of Figures 19 and 20, and by dissolved oxygen concentrations given in Table 2. The most sensitive measure is pH. Influent pH was rather steady throughout the year at about 7.2. In the pond, however, the removal of carbon dioxide by photosynthesis resulted in higher pH values at nearly all times, with peaks of 10 being reached under the intense light conditions of summer days. Lapses in photosynthetic activity caused by loss of the algae population are clearly apparent in the pH record. In Pond 2 the pH was normally about 7.4, the carbon dioxide having been restored by the respiration of higher organisms, principally Daphnia. The drop in population of these creatures which occurred with the onset of summer allowed the pH to remain elevated during this period. The alkalinity record is also affected by photosynthesis, though less dramatically. The removal of carbon dioxide itself does not alter alkalinity, but the incorporation of ammonia nitrogen into algal protein and the precipitation of carbonates and phosphates at raised pH both consume alkalinity at nearly stoichiometric ratios. Alkalinity reductions in Pond 1 coincided with periods of strong pH rise. In Pond 2 the alkalinity loss persisted although the pH had been brought back down by new carbon dioxide.

Dissolved oxygen remained high in Pond 1 during the entire period of operation. Peak daytime values of over 40 mg/l were experienced when the algae were actively growing. Influent oxygen demand was relatively low and as a result the nightly drop in D. O. due to respiration was not pronounced. Typical early morning D. O. readings were around 5 mg/l lower than the afternoon highs, meaning that Pond 1 was supersaturated most of the time. In the winter and spring months the D. O. levels in Pond 2 were quite low, mostly because of Daphnia respiration. The oxygen consumption rate of Daphnia is on the order of 0.25 mg/day/mg dry weight[1]. At 1000 organisms per liter and average organism weight of 0.02 mg, respiration would amount to 5 mg/l/day. Exertion of BOD in the pond liquid and bottom sediments would also reduce dissolved oxygen.

Later, when the Daphnia population had fallen off, there were wide daily fluctuations in the dissolved oxygen on Pond 2. High influent D. O. and some photosynthetic activity produced supersaturation in the daytime, while respiration (of bottom sediments and a varied pelagic population) lowered the oxygen during the night down to a few milligrams per liter. Lack of oxygen by itself was probably not the reason for disappearance of the Daphnia, since they thrived at considerably lower D. O. levels earlier in the year.

SECTION VII

DESIGN IMPLICATIONS

From a nutrient removal standpoint, optimization of the process consists in achieving both maximum algae growth and complete algae entrapment. The inorganic carbon in the feed should be photosynthesized to an extent which raises the pH above 10, since only then will there be extensive precipitation of phosphate. Uptake of nitrogen is also proportional to algae production. To accomplish relatively complete conversion the hydraulic throughput must be controlled so as to pace the growth rate. Basically this means giving each volume of liquid its due quota of solar radiation. During portions of July and August when the pH of Pond 1 was being driven above 10, the hydraulic feed rate was about 11 cm/day. It may be inferred that this application rate represents an approximate upper limit for good carbon conversion in the summertime. The corresponding algae production was about 11 gm/sq m/day. To obtain equal results in the winter, when temperatures and light intensity are both lower, would require a considerable reduction in surface loading.

In the algae stage it is desirable to maintain as shallow a depth as possible, consistent with total absorption of impinging light. For a given pond surface area, shallower depth is associated with shorter residence time; short average generation time for the algae is desirable for maintaining a vigorous culture and minimizing loss of synthesized tissue by endogenous respiration. Since the phosphate removal process is substantially complete in a few days, it would seem possible to reduce the Pond 1 depth from 1.4 m used to 1.0 m or less with some advantage.

Under favorable environmental conditions Daphnia begin to produce broods of young at an age of less than two weeks. Each brood contains around a dozen new organisms, so that the effective generation time is only one or two days. As far as maintenance of the Daphnia population is concerned, the second-stage detention time could be very short, but it is also necessary to attain complete algae removal. The Daphnia can easily consume their own weight in algae each day. Using this as a loading criterion, the detention time should be at least equal to the weight ratio of algae feed concentration to Daphnia pond concentration. A parallel criterion is that the pond detention time should be several times the turnover time for Daphnia filtration. Under normal circumstances the feed ratio criterion would control. With an algae concentration of 50 mg/l and a Daphnia population consisting of 500 organisms per liter with 0.02 mg average weight, the detention time should be at least five days.

Depth in the Daphnia stage does not seem to be critical. Economics of construction and maintenance suggest using a fairly deep pond, on the order of 5 meters. One feature of probable importance is provision for seasonal cleaning. When the Daphnia are filtering effectively, most of the organic nitrogen received by the pond as algae remains as bottom sediment. Removal once a year, by draining and scraping, for example, would prevent the eventual resuspension of the deposited nutrients.

Predator incursions in the algae stage are a major operating problem. Control of these higher organisms by mechanical screening may be feasible, but such measures would destroy the system's principal virtue of simplicity and low cost. Another means of coping with predator plagues would be to divide the algae pond into multiple compartments, so that not all of the algal biomass would be simultaneously exposed. Upon the appearance of Daphnia in one compartment, it would be drained and refilled with culture from an adjacent unit. To reduce opportunity for seeding of the algae stage with Daphnia, it would be helpful to separate the two pond stages physically. A free overflow weir at the outlets of all algae units will prevent entry of Daphnia which might have worked their way upstream.

SECTION VIII

REFERENCES

1. Richman, S., The Transformation of Energy by Daphnia pulex. Ecol. Monographs. 28:273, 1958.

2. Bennett, G. W., Management of Lakes and Ponds. New York, Van Nostrand Reinhold, 1971. 365 p.

3. Ryther, J. H., Inhibitory Effects of Phytoplankton upon the Feeding of Daphnia magna with Reference to Growth, Reproduction and Survival. Ecology. 35:522, 1954.

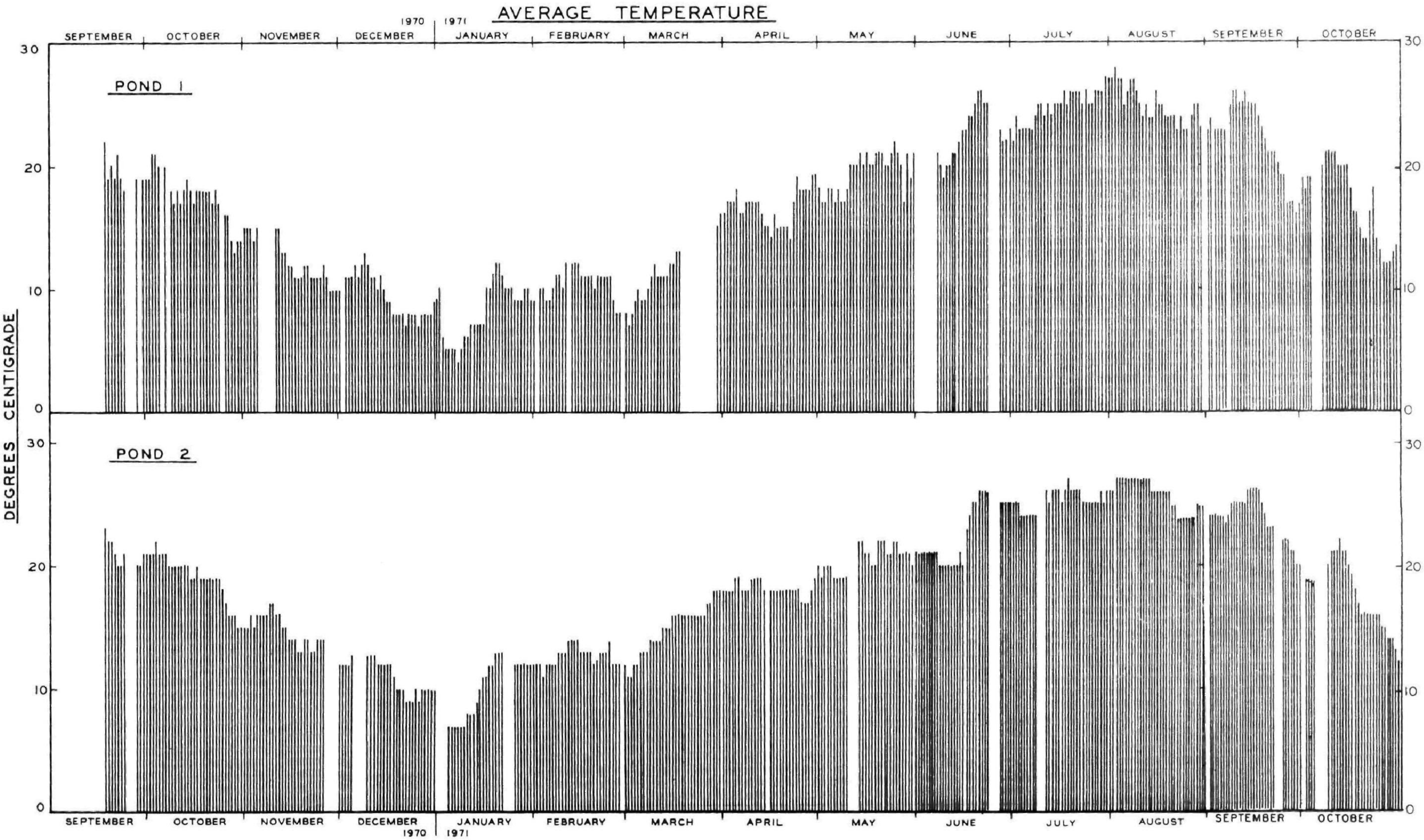

AVERAGE TEMPERATURE
1970 | 1971
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
POND 1
POND 2
DEGREES CENTIGRADE
0
10
20
30

Figure 7

HYDRAULIC TURN-OVER RATE

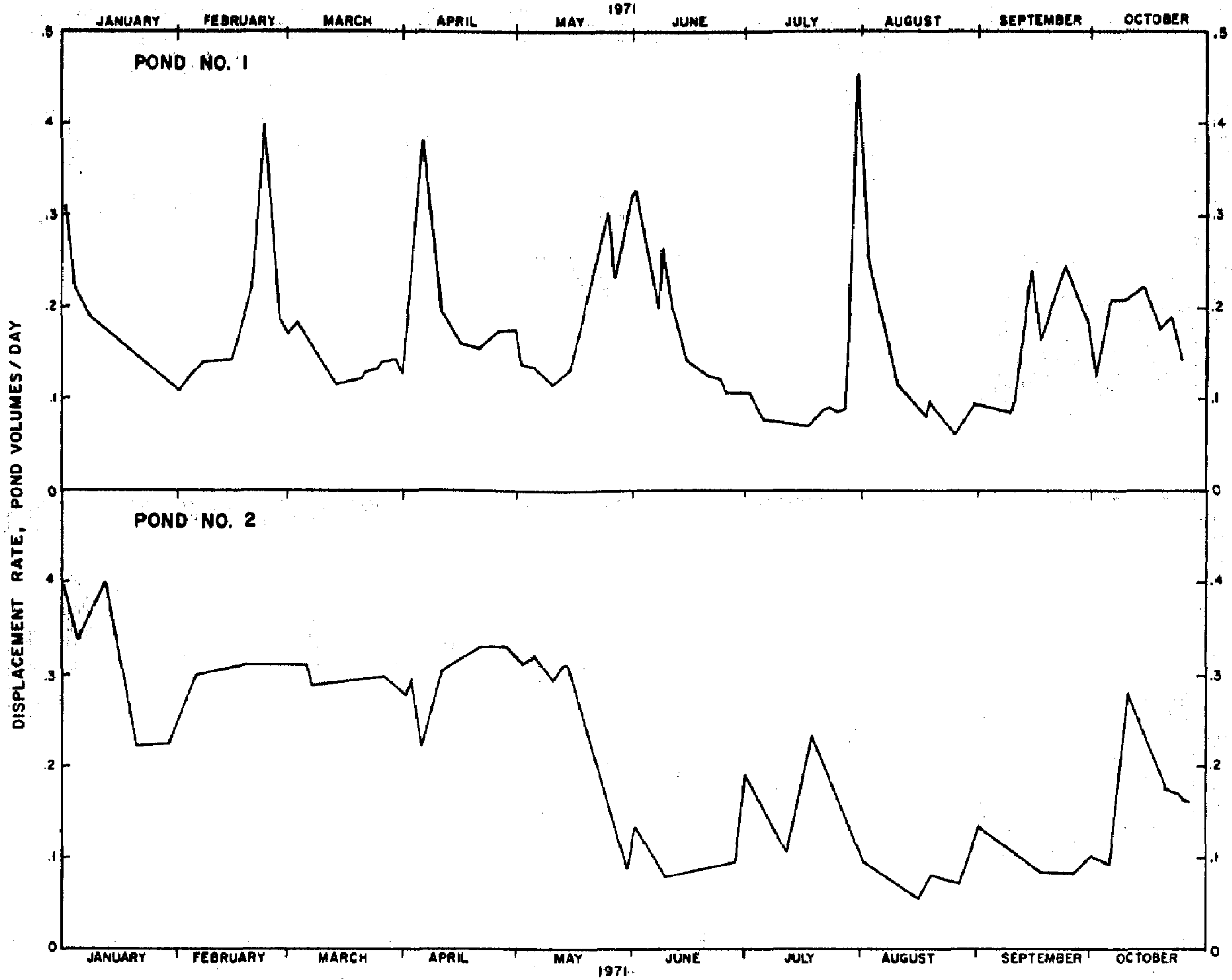
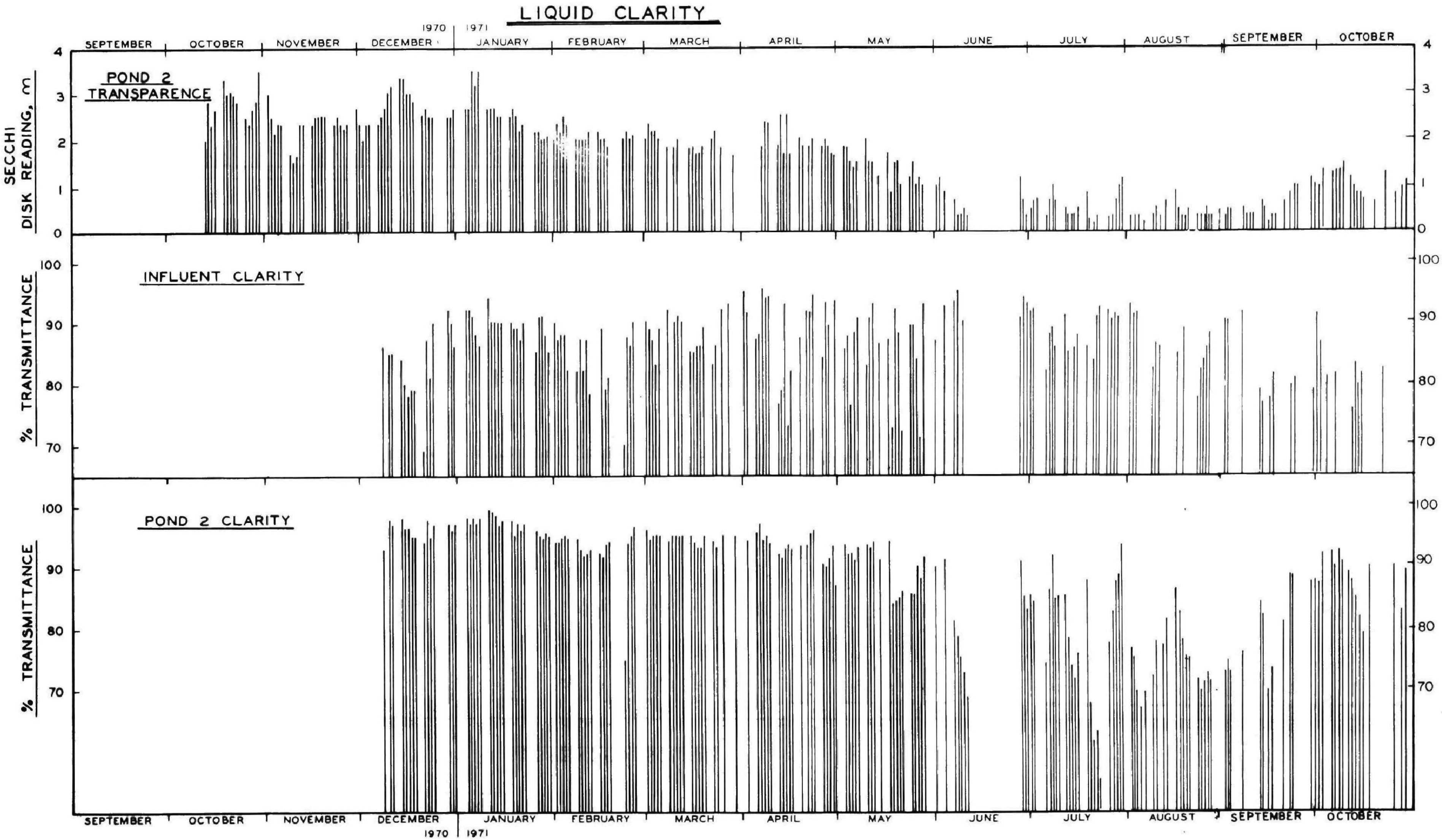


Figure 8



LIQUID CLARITY
POND 2 TRANSPARENCE
SECCHI DISK READING, m
INFLUENT CLARITY
% TRANSMITTANCE
POND 2 CLARITY
% TRANSMITTANCE
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
1970
1971

Figure 9

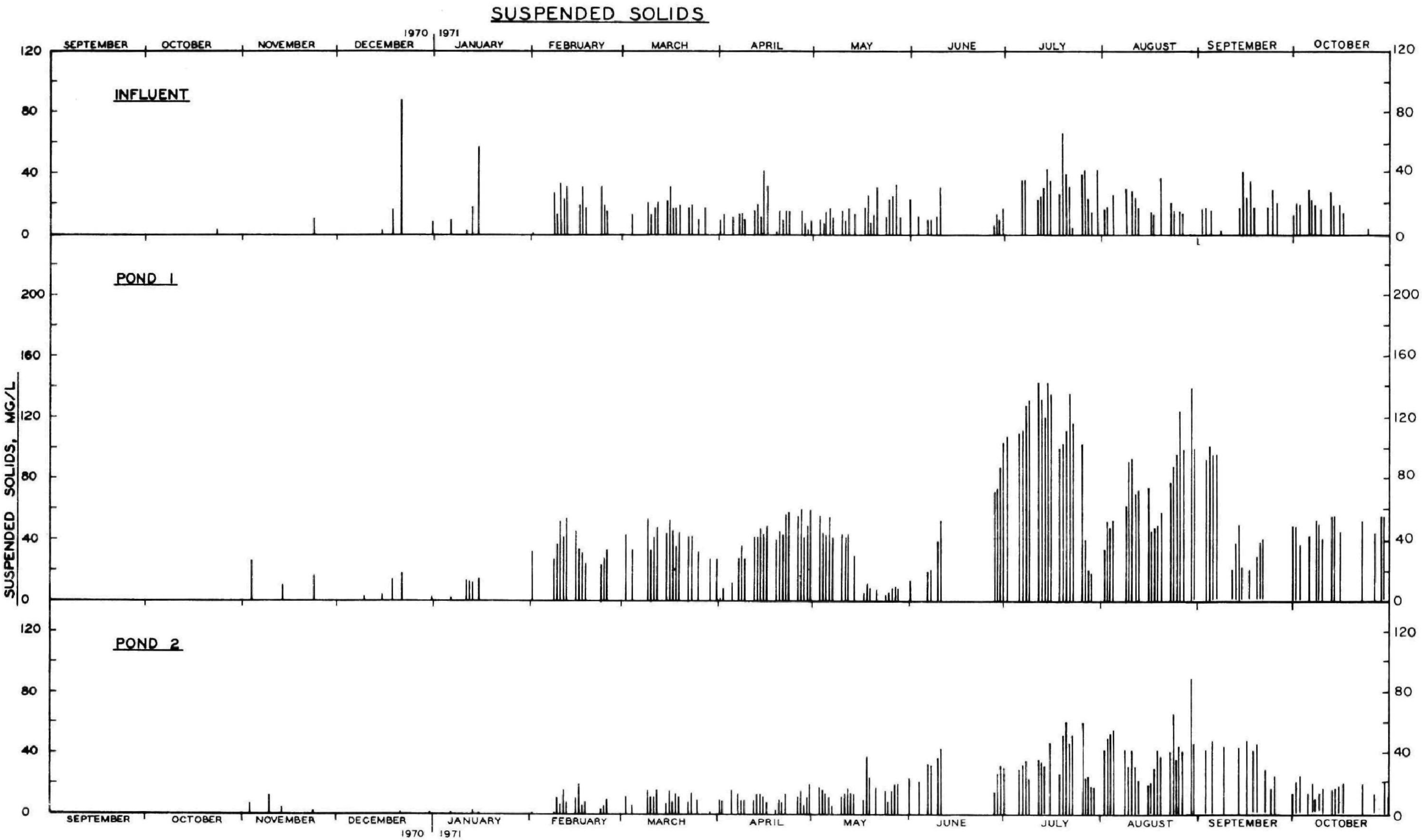

SUSPENDED SOLIDS
1970 1971
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
INFLUENT
POND 1
POND 2
SUSPENDED SOLIDS, MG/L
0
40
80
120
160
200

Figure 10

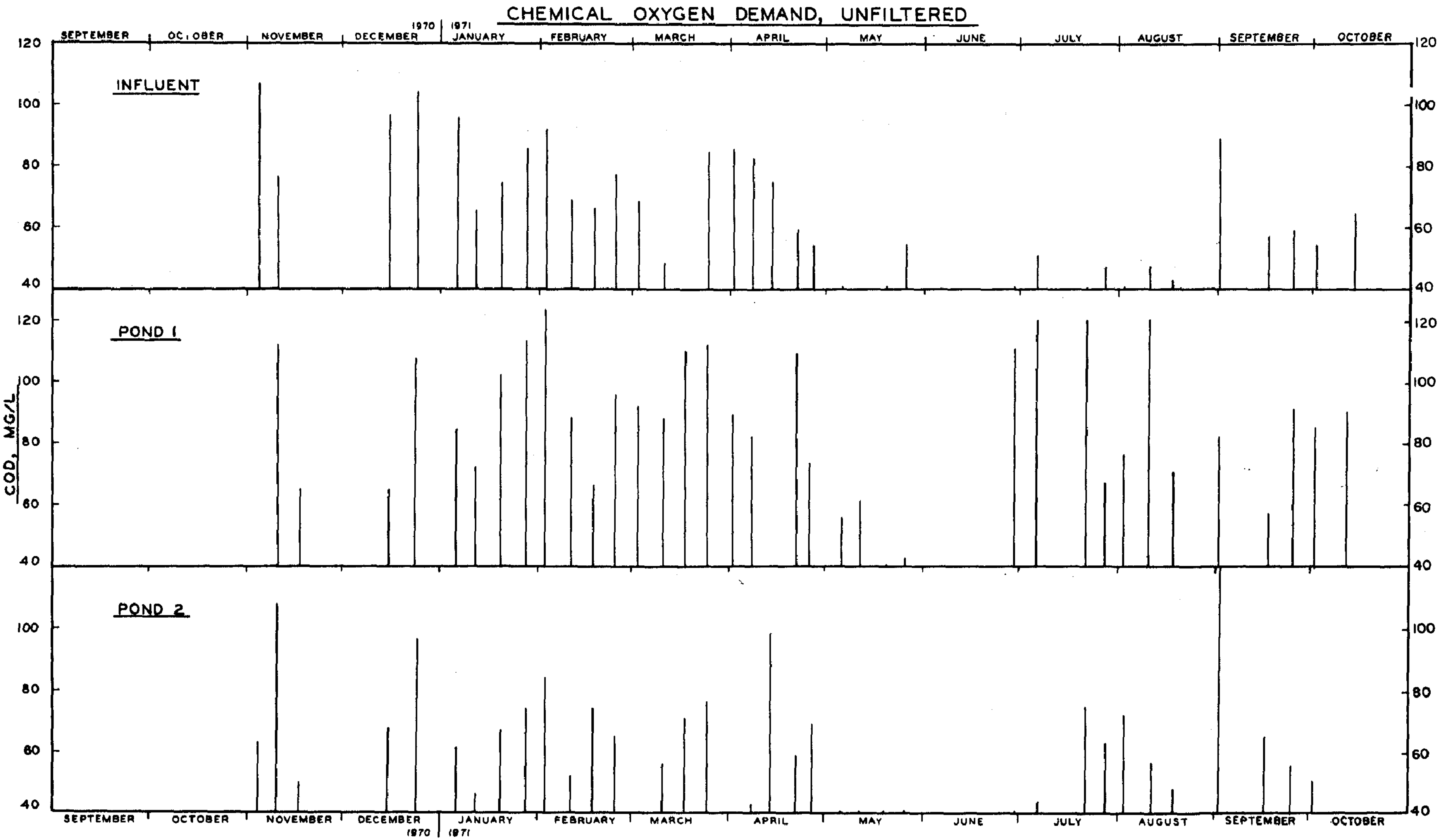

CHEMICAL OXYGEN DEMAND, UNFILTERED
INFLUENT
POND 1
POND 2
COD, MG/L
1970
1971
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
120
100
80
60
40

Figure 11

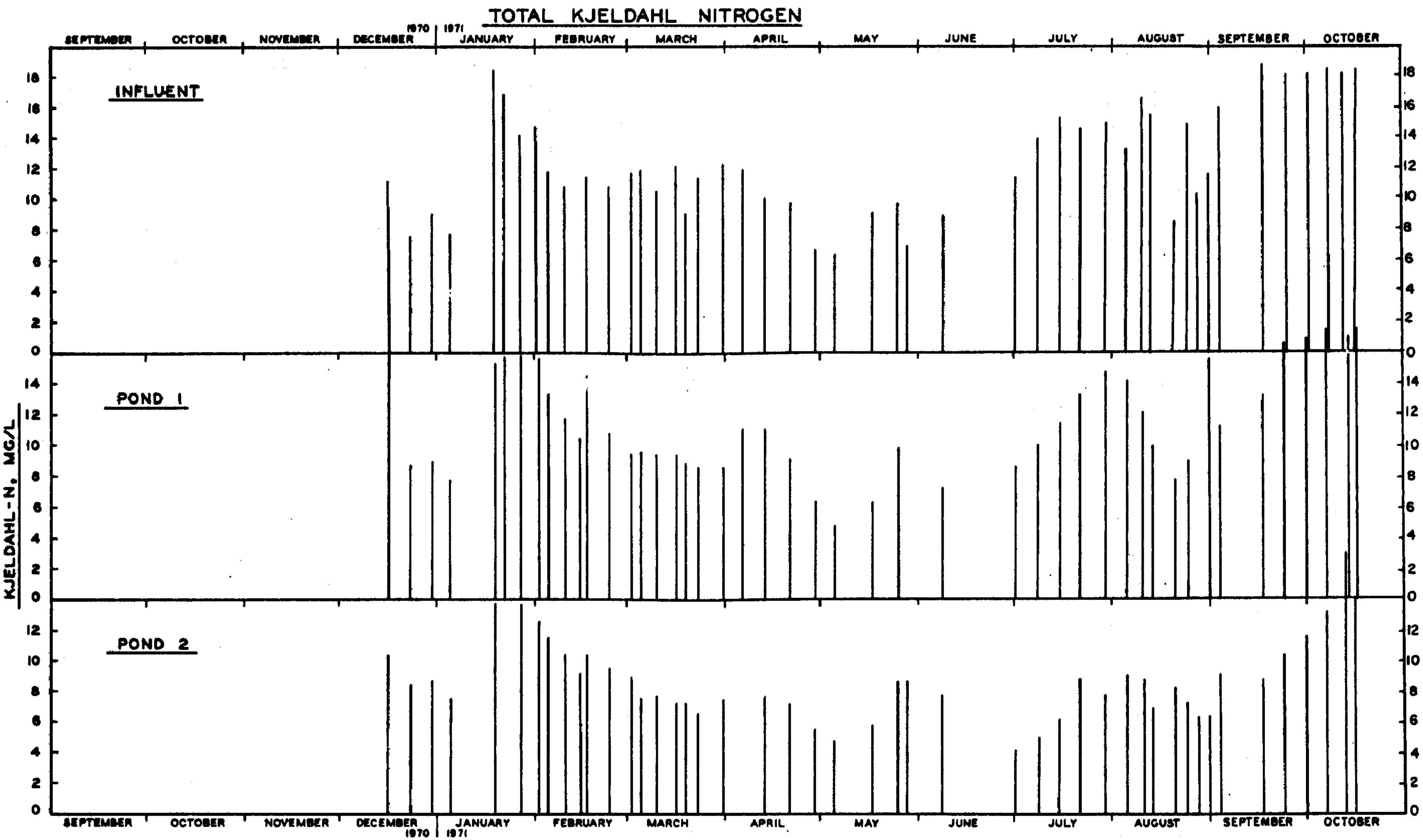

TOTAL KJELDAHL NITROGEN
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
1970
1971
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
INFLUENT
POND 1
POND 2
KJELDAHL-N, MG/L

Figure 12

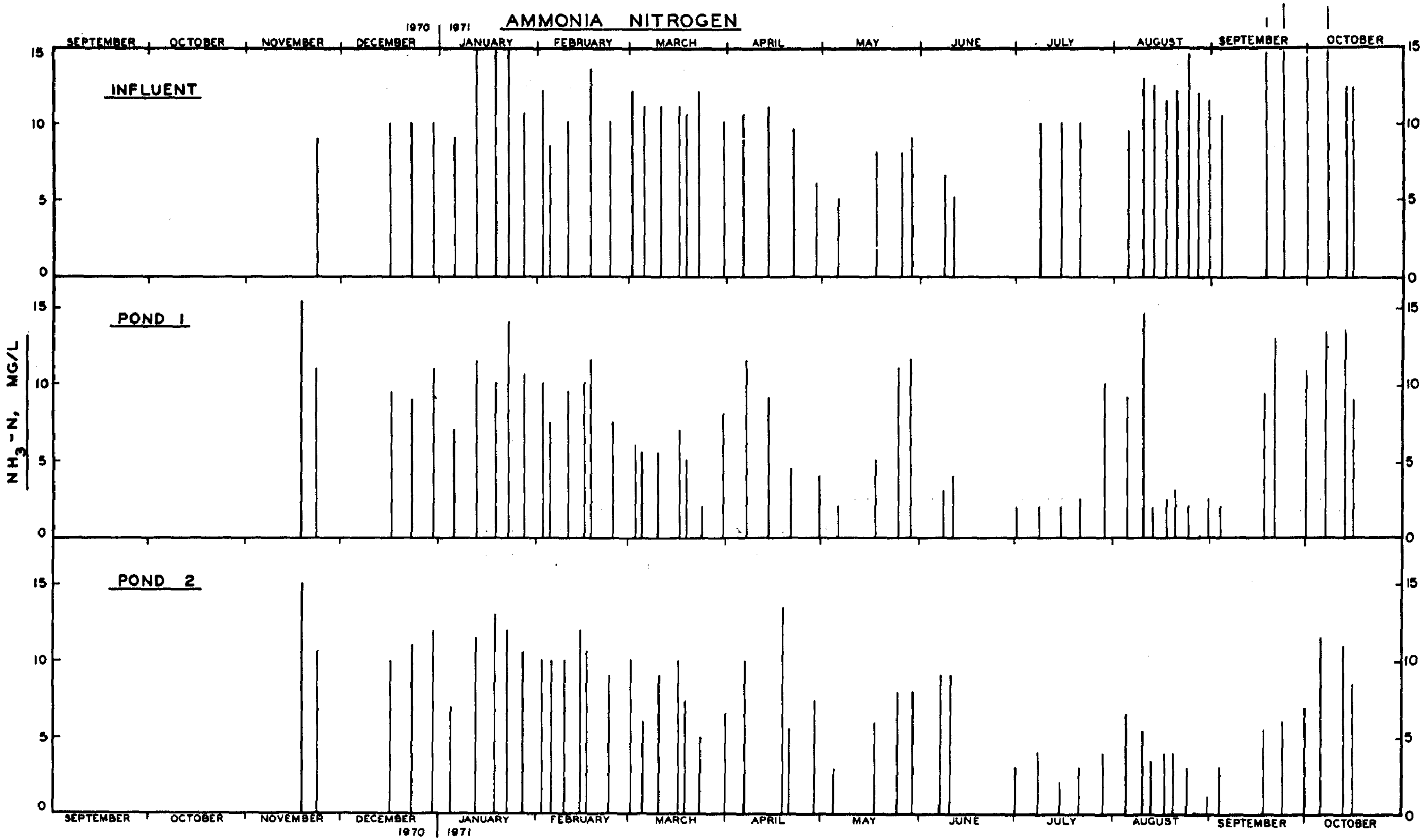

AMMONIA NITROGEN
1970 | 1971
SEPTEMBER OCTOBER NOVEMBER DECEMBER JANUARY FEBRUARY MARCH APRIL MAY JUNE JULY AUGUST SEPTEMBER OCTOBER
INFLUENT
POND 1
POND 2
NH3-N, MG/L
0
5
10
15

Figure 13

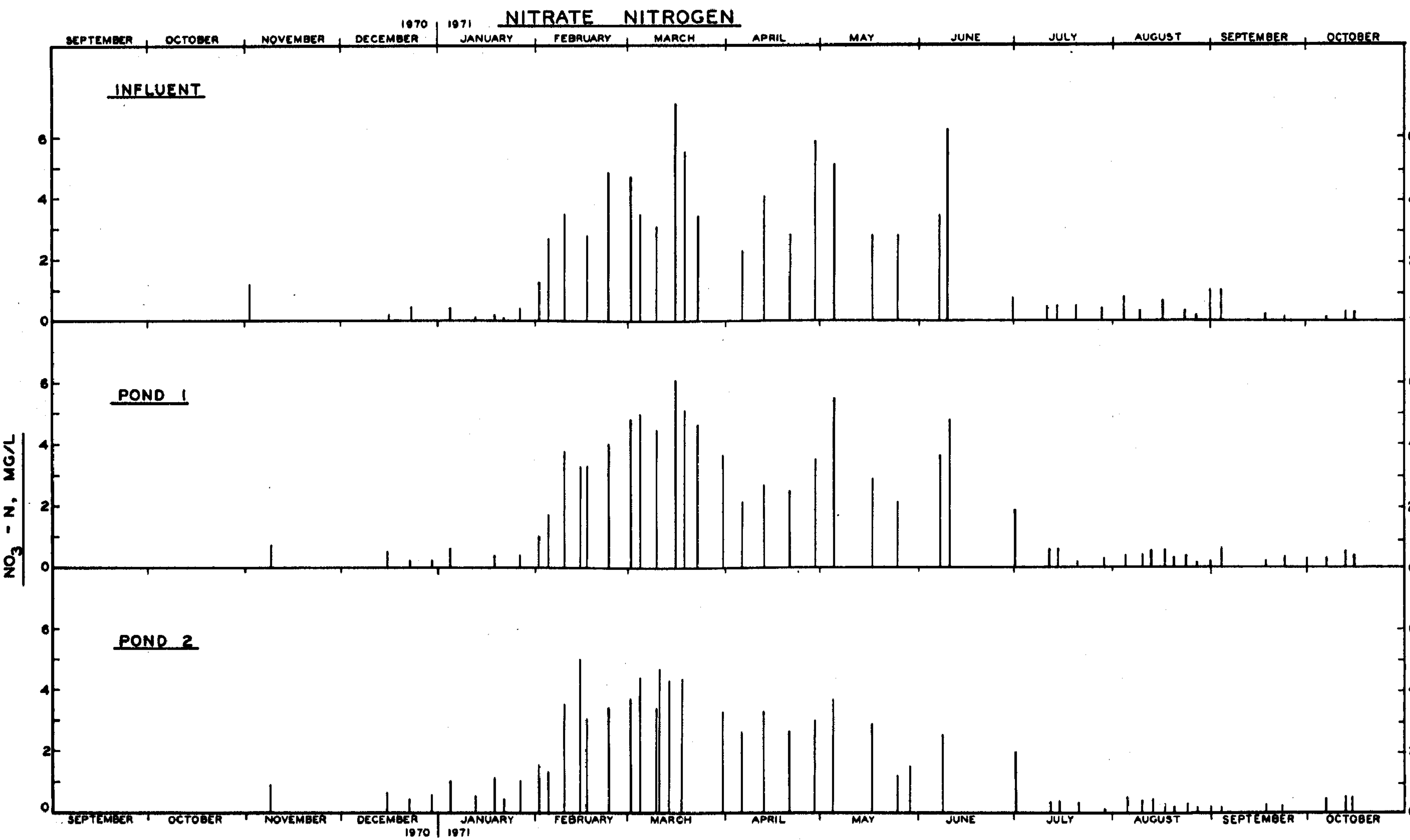

NITRATE NITROGEN
1970 | 1971
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
INFLUENT
POND 1
POND 2
NO_3 - N, MG/L
0
2
4
6

Figure 14

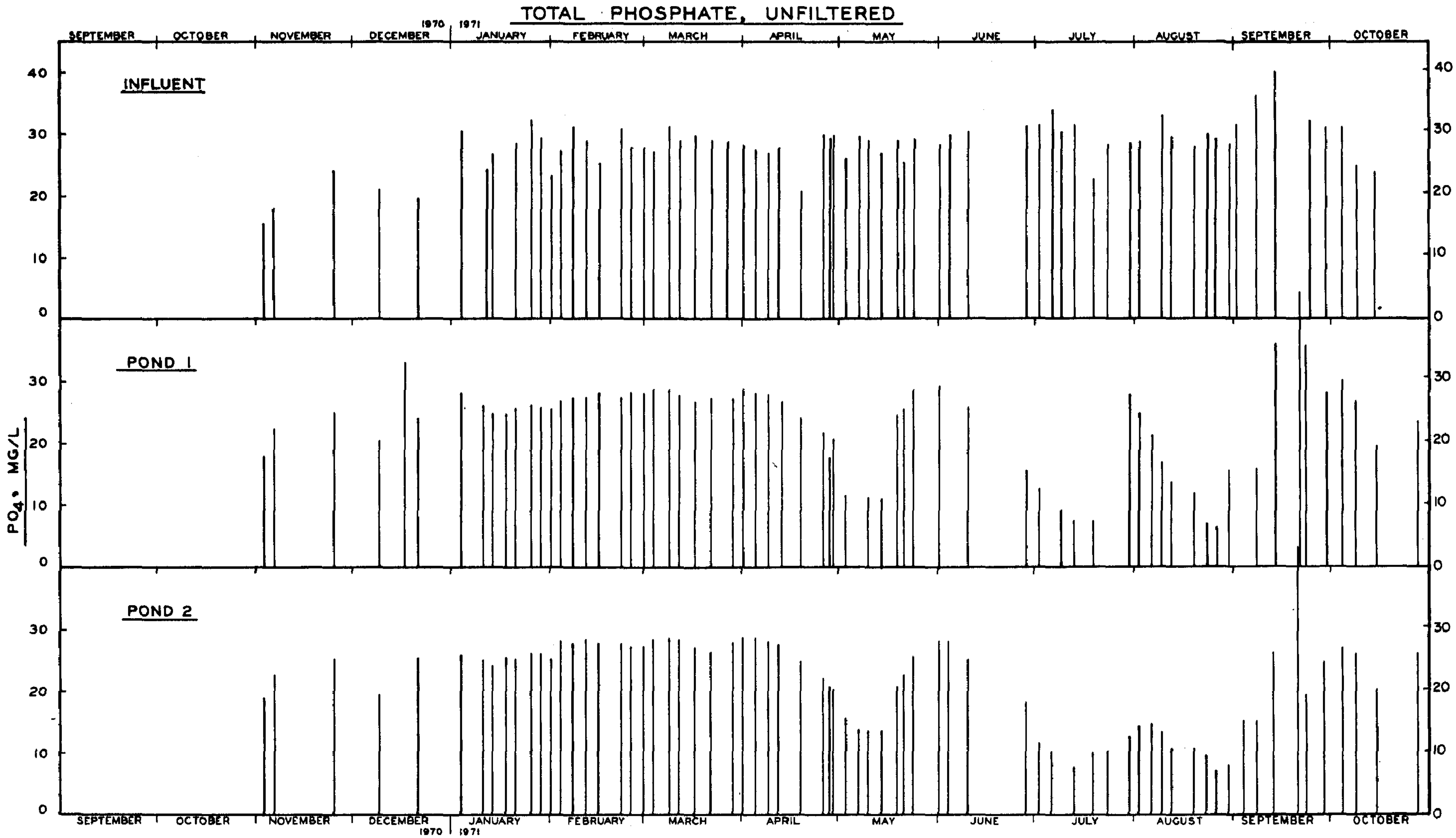
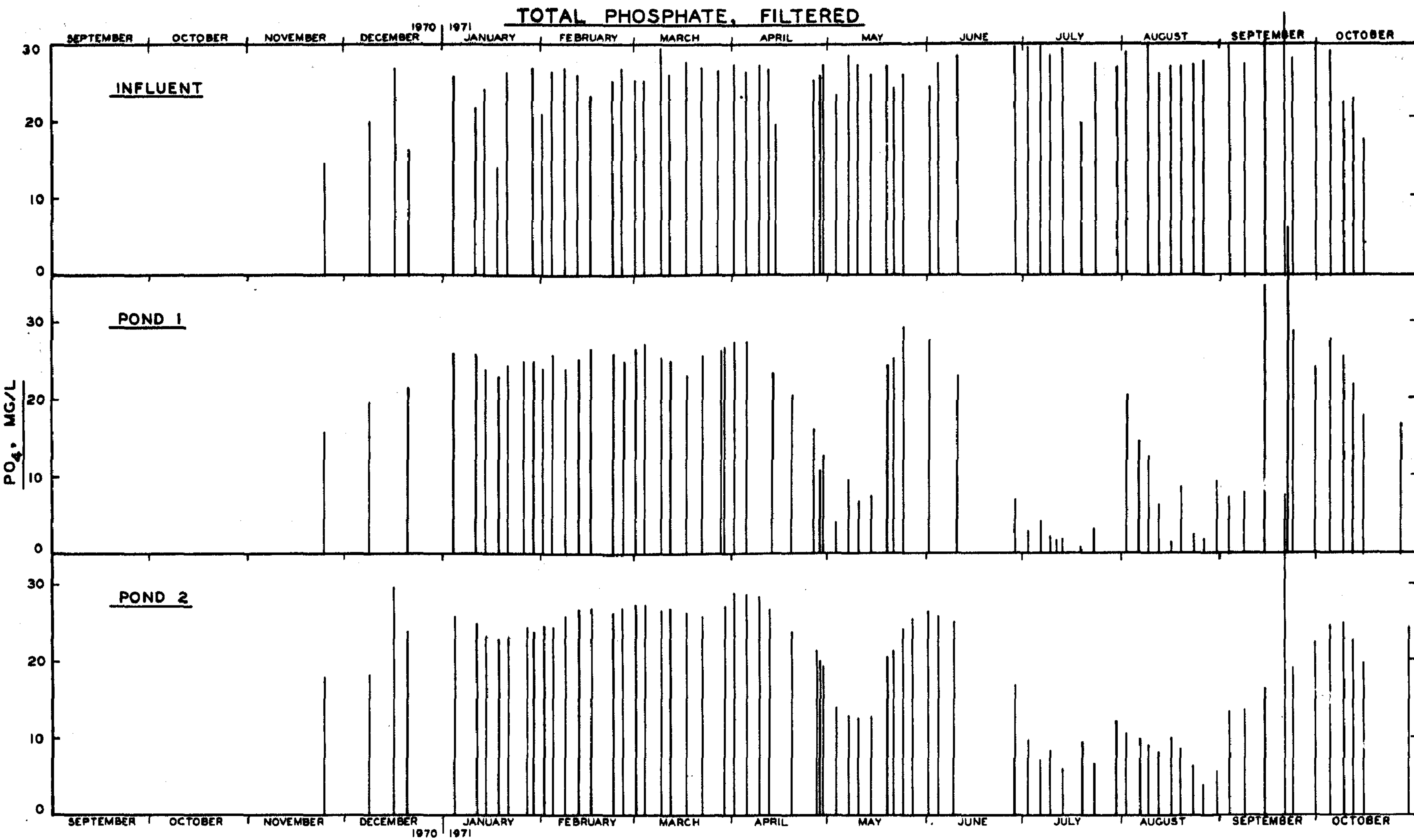

TOTAL PHOSPHATE, UNFILTERED
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
1970
1971
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
INFLUENT
POND 1
POND 2
PO_4, MG/L
0
10
20
30
40

Figure 15



TOTAL PHOSPHATE, FILTERED
INFLUENT
POND 1
POND 2
PO4, MG/L
0
10
20
30
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
1970
1971
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

Figure 16

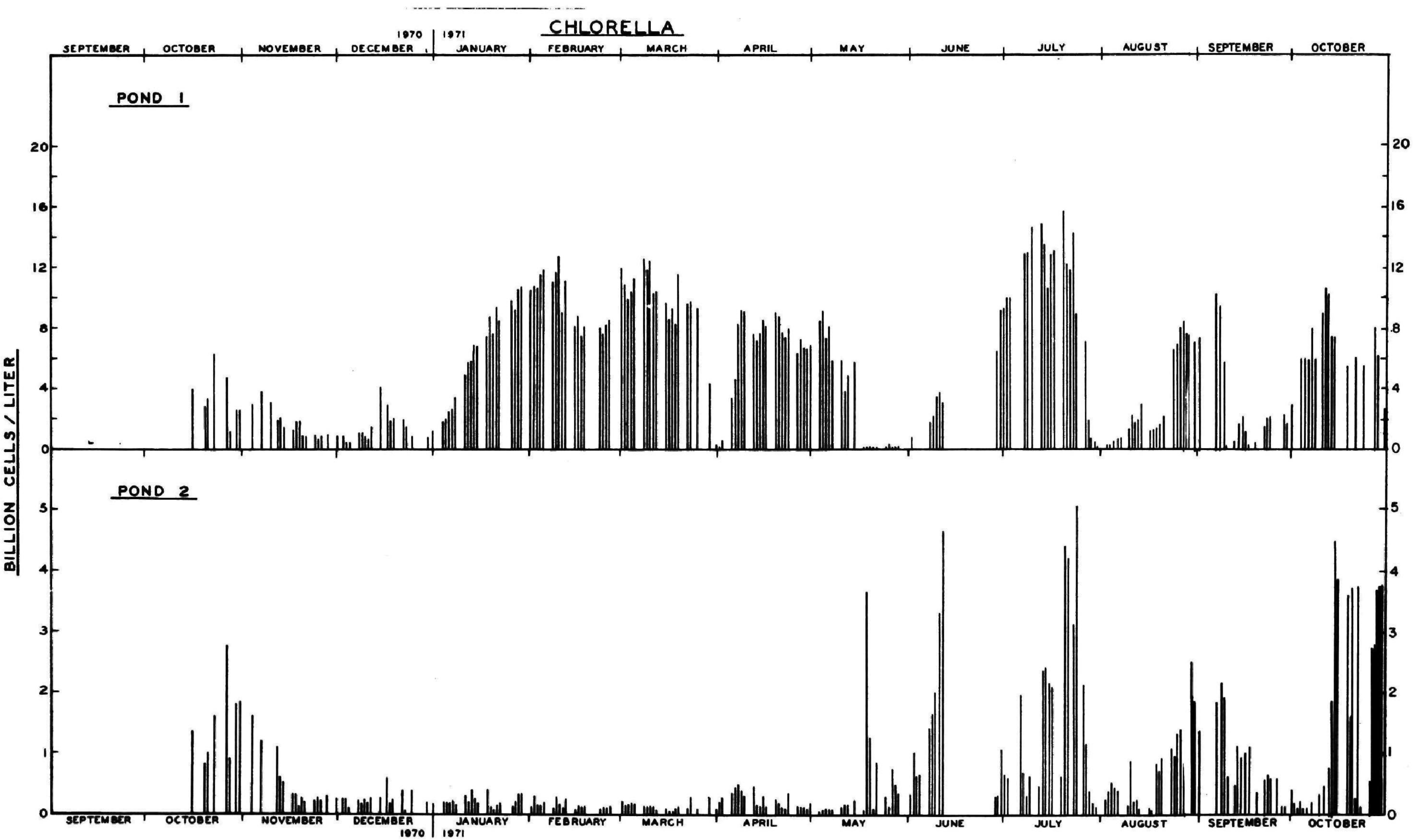

CHLORELLA
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
1970
1971
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
POND 1
POND 2
BILLION CELLS / LITER
0
4
8
12
16
20
1
2
3
4
5

Figure 17

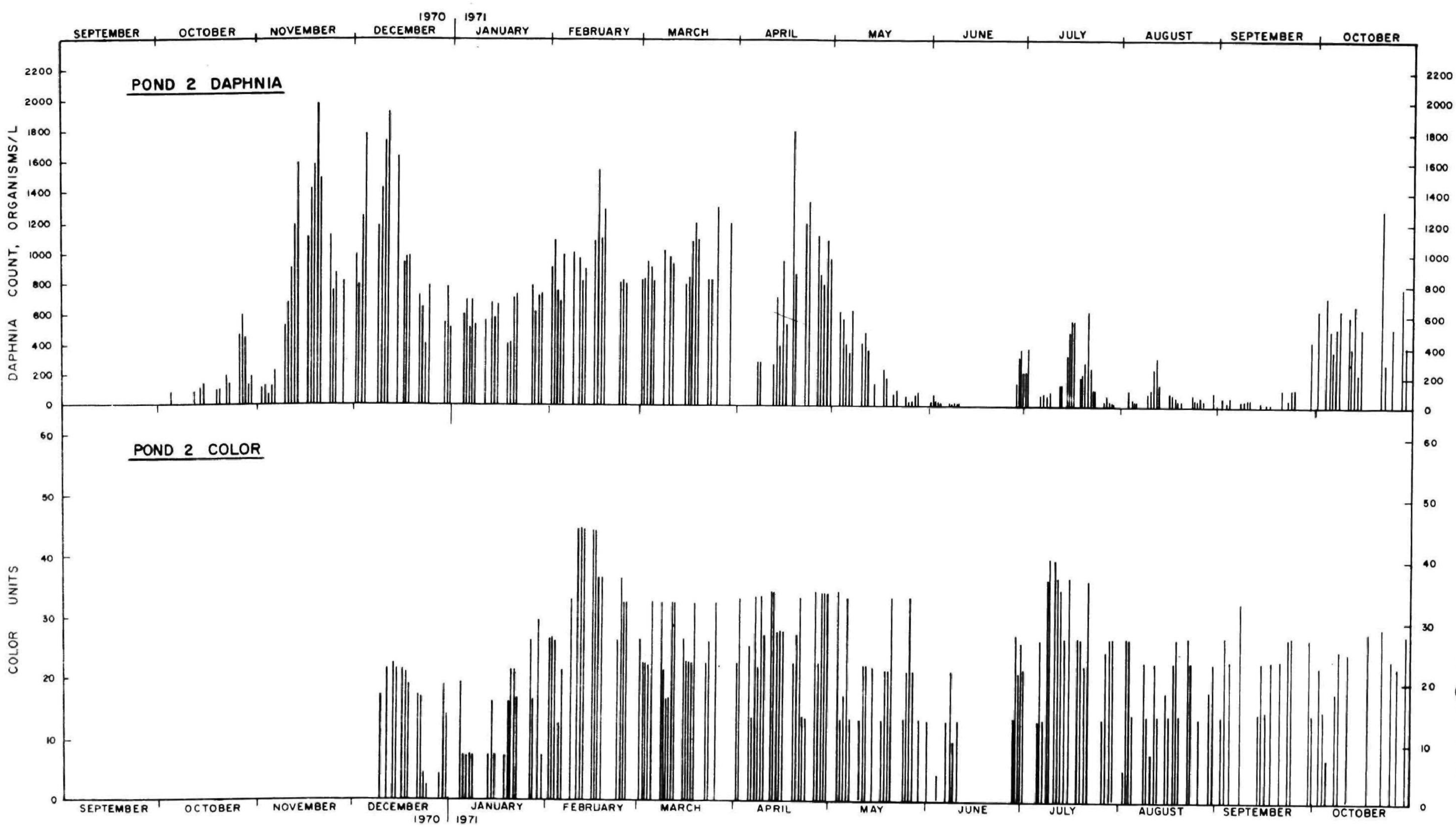

POND 2 DAPHNIA
DAPHNIA COUNT, ORGANISMS/L
POND 2 COLOR
COLOR UNITS
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
1970
1971
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

Figure 18

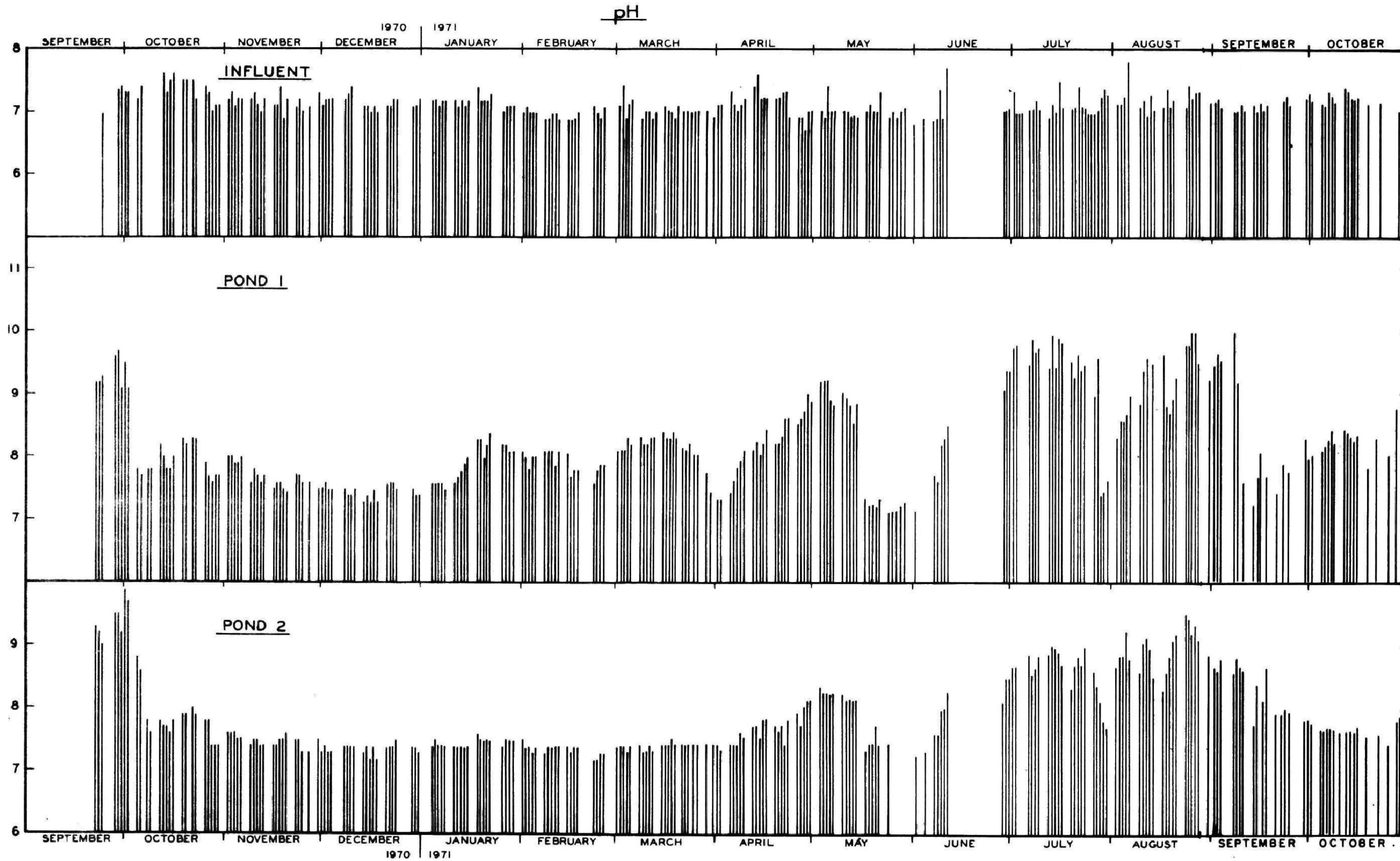

pH
1970
1971
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
INFLUENT
POND 1
POND 2
8
7
6
11
10
9
8
7
9
8
7
6

Figure 19

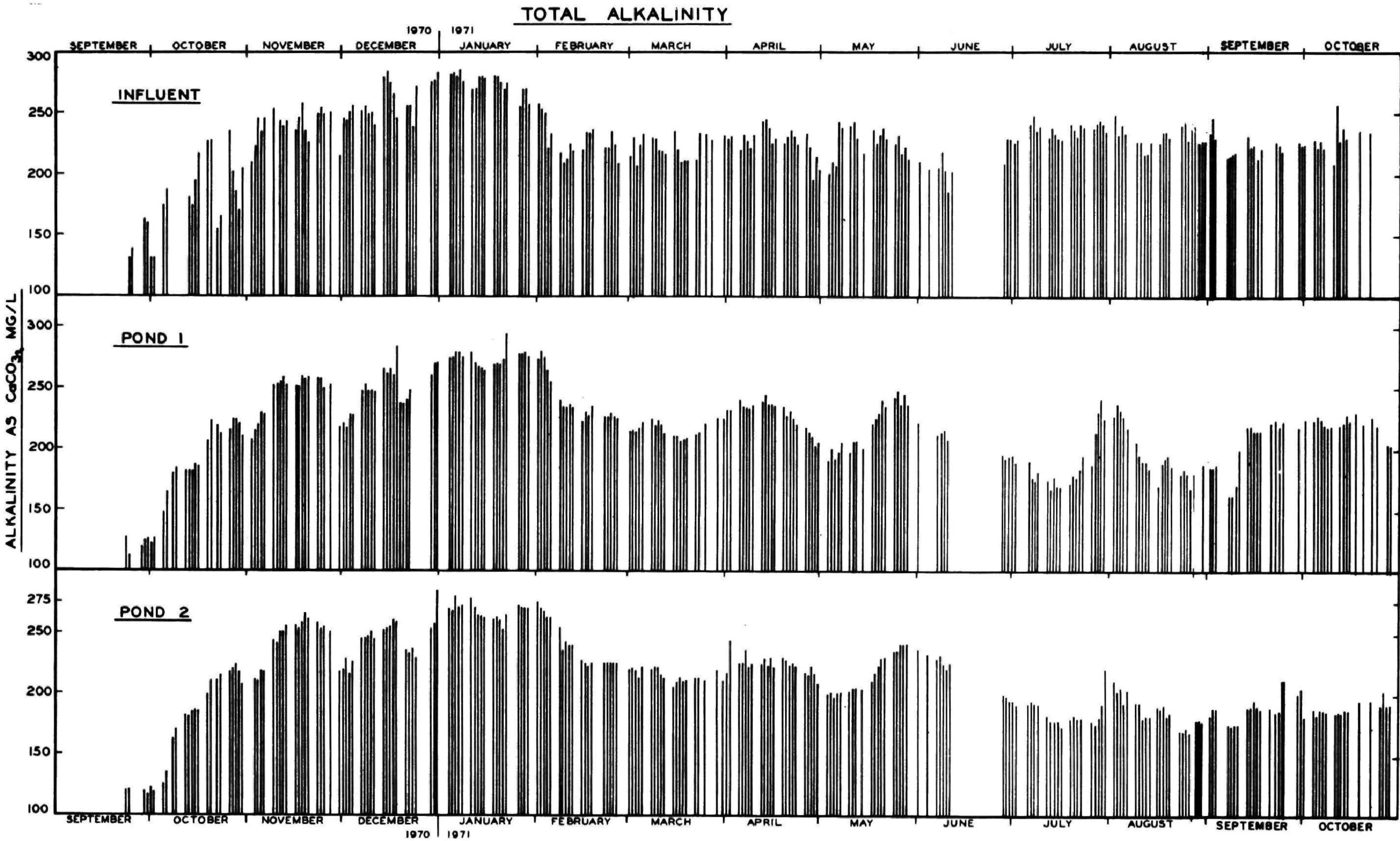

TOTAL ALKALINITY
1970
1971
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
INFLUENT
POND 1
POND 2
ALKALINITY AS $CaCO_3$ MG/L
300
250
200
150
100
275

Figure 20

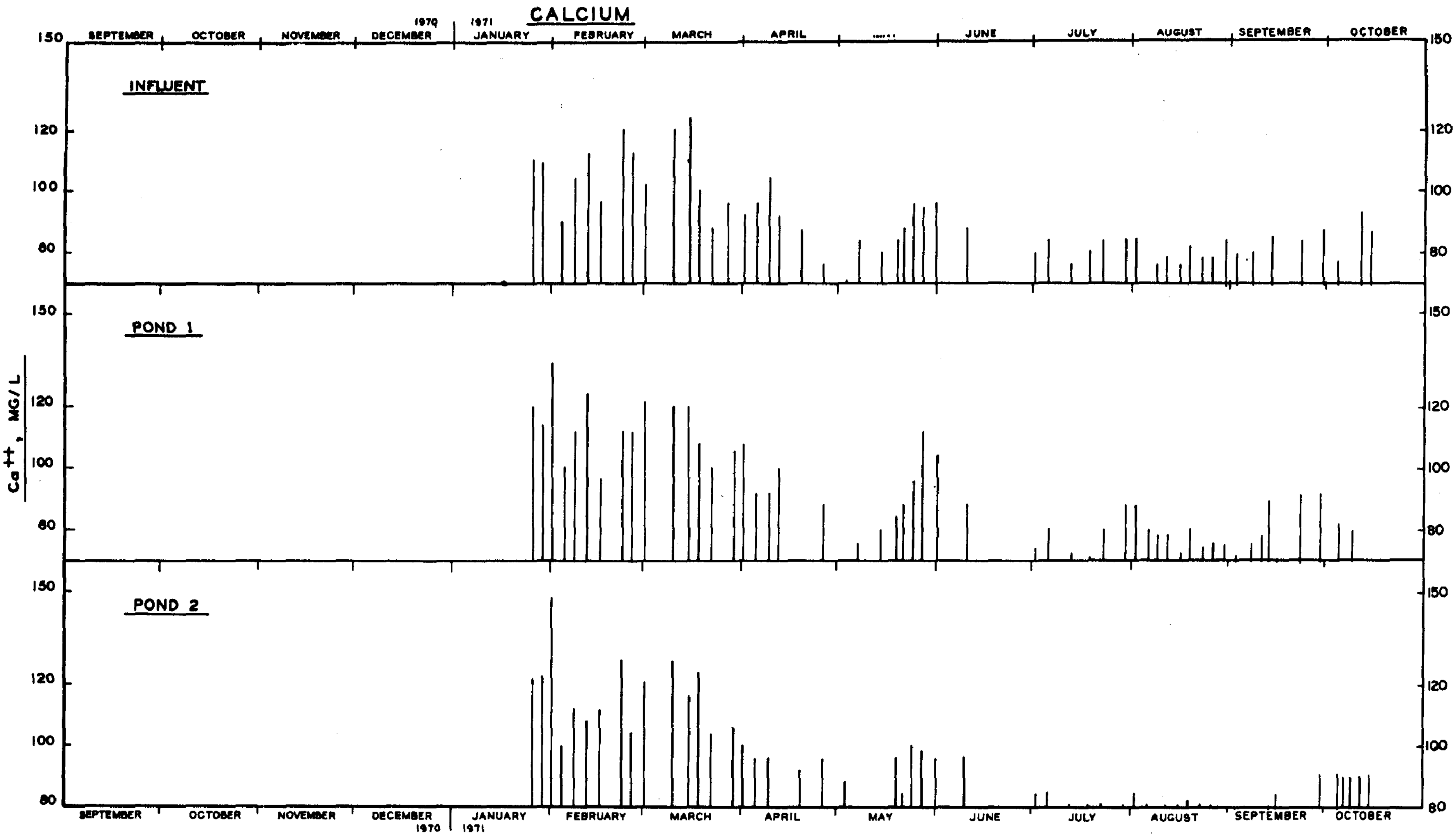

CALCIUM
1970
1971
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
INFLUENT
POND 1
POND 2
Ca++, MG/L
150
120
100
80

Figure 21

SELECTED WATER RESOURCES ABSTRACTS

INPUT TRANSACTION FORM

1. *Report No.*

2.

3. *Accession No.*

W

4. *Title* Tertiary Treatment with a Controlled Ecological System

5. *Report Date*

6.

8. *Performing Organization Report No.*

7. *Author(s)* Gram, Andrew L.

10. *Project No.*

9. *Organization* Gram/Phillips Associates, Inc. under contract to Las Virgenes Municipal Water District

11. *Contract/Grant No.* 16080 FBH

13. *Type of Report and Period Covered*

12. *Sponsoring Organization* Environmental Protection Agency

15. *Supplementary Notes*

Environmental Protection Agency report number, EPA-660/2-73-022, December 1973.

16. *Abstract* A two-stage pond system was operated as a process for polishing secondary sewage effluent. The shallow first stage was an oxidation pond in which a heavy growth of algae was permitted to develop. In the second stage a population of Daphnia pulex consumed the algae. Detention times were about 10 days in each stage. Chemical and biological monitoring were carried out over a year's period to determine feasibilityof using the process to produce recreational-grade water and reduce algae growth potential.

While the Daphnia remained as the dominant zooplankton species in the second pond throughout the observation period, their concentration varied between 100 and 1,500 organisms/liter. Excellent water clarity was obtained when the Daphnia were above 500 organisms/liter, and at such times the over-all COD reduction was greater than 40 percent. Significant removal of nutrients occurred only during the months of July and August, when N and P reductions were 48 percent and 63 percent respectively. Performance was hampered by occasional invasions of Daphnia or rotifers in the first-stage pond, which decimated the algae. Such events were not successfully controlled and remain the principal obstacle to further process development.

17a. *Descriptors* Waste water treatment, Water reclamation, Aquatic biology

17b. *Identifiers* Biological processes, Oxidation ponds, Zooplankton

17c. *COWRR Field & Group*

18. *Availability*

19. *Security Class. (Report)*

20. *Security Class. (Page)*

21. *No. of Pages*

22. *Price*

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