# THE PLANKTON POPULATIONS OF AN OIL REFINERY EFFLUENT PONDING SYSTEM


By

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THE PLANKTON POPULATIONS OF AN OIL REFINERY

EFFLUENT PONDING SYSTEM

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# CHAPTER I

## INTRODUCTION

Many methods have been used to treat industrial effluents. One of the promising methods of treatment is ponding. Ponding as a method of stabilization of effluents is not new (Caldwell, 1946); however, it is relatively new as a means of improving oil refinery effluents. Common practice utilizes one or a few ponds. An improved method appears to be to construct a series of ponds so that fresh effluent enters the first pond and continues through the series, remaining in each pond at least one day (Irwin, 1958).

A serial pond system incorporates features of both flowing and standing water. Longitudinal ecological succession occurs in flowing water. Standing water develops plankton faster and produces larger populations than flowing water, other factors being equal (Welch, 1952). Organisms in natural waters have the ability to reduce the toxicity or unfavorable conditions occurring with introduced pollutants. The combination of advantageous features in a pond series favors the "self-purification" process.

A pond system has been operating at an oil refinery in Kansas since 1958. A study of the kinds of plankters, including bacteria, their numbers and successional stages was begun in June 1958, and extended through May 1959. These data are compared with more recent work conducted on the same pond system and other comparable systems.

# CHAPTER II

## DESCRIPTION OF PONDS

The ponds were surrounded by dikes so that only direct precipitation and effluent from the refinery could enter. Ponds were numbered in sequence from 1 through 12 (Figure 1). Effluent flowed from Ponds 1, 2, 4, 5 and 9 through submerged pipes. Effluent flowed from Ponds 6, 7, 8, 10 and 11 through broad openings left by incomplete dikes. From Pond 3, effluent ran over a concrete flume for about 60 feet (55.4 meters); then was pumped about 100 yards (92.3 meters) through a 4 inch (10 centimeters) pipe and sprayed into Pond 4. The final effluent from Pond 12 flowed over a weir into a nearby river.

There was considerable variation in size and depth among the ponds. Ponds 1 and 2 covered 1/3 acre (.13 hectares) each and were 14 feet (13 meters) deep. Pond 3 covered 5 acres (2.02 hectares) and had depths to 13 feet (12 meters). The remaining ponds ranged from 1/2 to 2-3/4 acres (.92 hectares) in size and were about five feet (4.6 meters) deep.

Twelve collection stations were established. Station 1 was at the base of a cooling tower just before the effluent entered Pond 1. The other eleven collecting stations represented each pond, except Pond 1, which was not sampled due to a heavy layer of oil on the water.

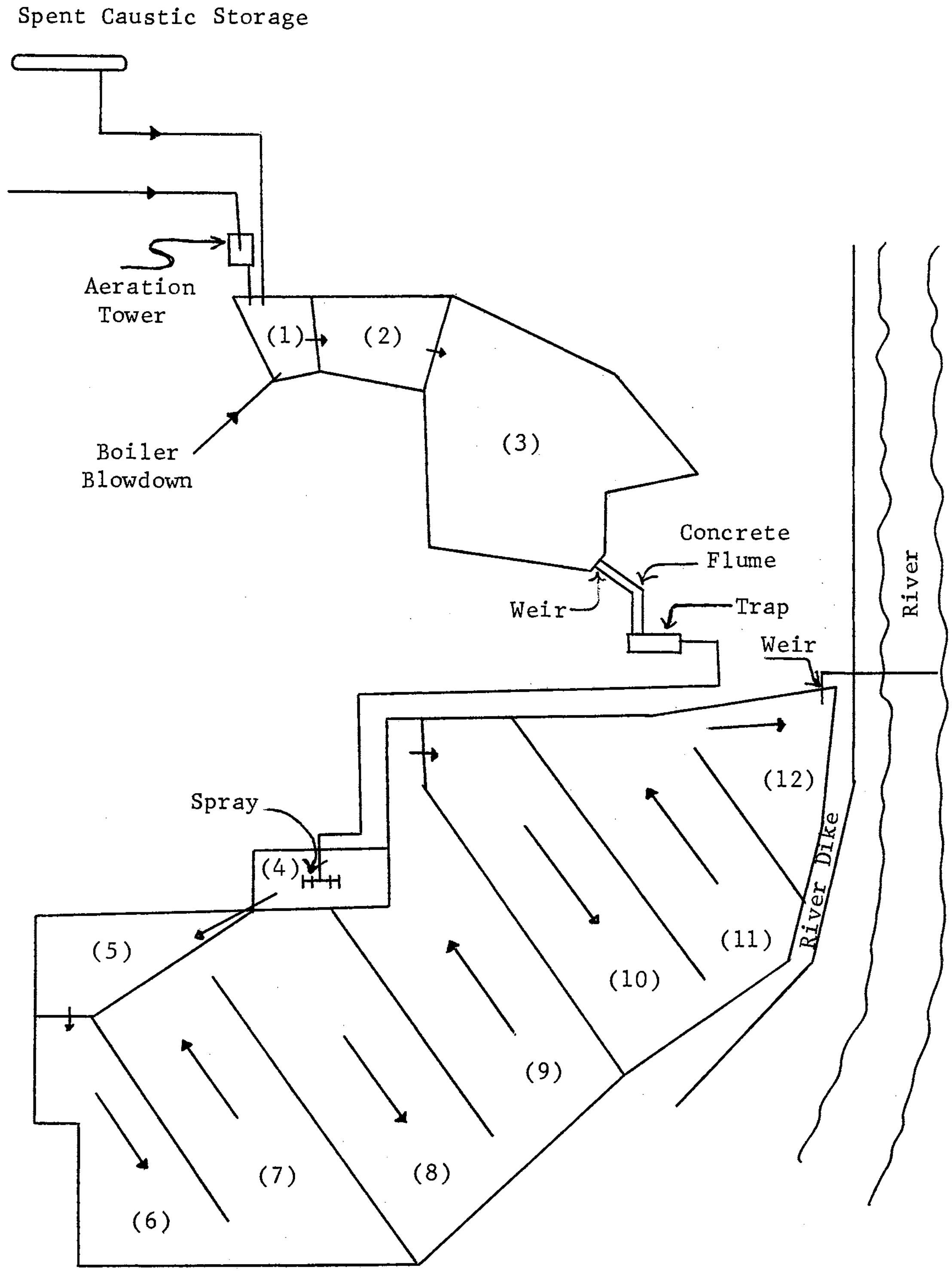


Figure 1. Diagram of the Ponding System
(Pond numbers in parentheses)

## CHAPTER III

## LITERATURE AND HISTORY

Ponding of industrial wastes has been practiced for many years, the main purposes being storage, controlled discharge, and sedimentation to remove solids. In the petroleum industry, ponds have been used mainly for the separation of oil from brine, with the latter discharged during periods of high stream flow (Pearse et al., 1948). More recently, ponds have been utilized for oil refinery waste treatment.

The role of algae in the stabilization of organic effluents has been discussed by many workers. The green algae assist in the clarification and purification of sewage (Renn, 1954, Abbott, 1950). Phytoplankton plays a major role in the reduction of nutrients in waste water through assimilation and eventual deposition in the bottom sediments (Mackenthun and McNall, 1959). Reduction of nutrients occurs when midges mature and leave the pond system. Sewage lagoons are effective regardless of the algal crop present, although the algae assist in keeping the ponds aerobic (Allen, 1955). Only a small proportion of the nutrients are converted into algae. Algae may do no more than pay their own way in terms of oxygen production and consumption, due to the heavy organic load caused by the death and decay of algal cells (Dorris, Patterson and Copeland, 1963).

Algae and bacteria, in the proper commensal relationship, can stabilize organic wastes (Hermann and Gloyna, 1957). Algae are dependent to an unknown degree on various aquatic bacteria (Neel and Hopkins, 1956). Under laboratory conditions, Chlorella does not oxidize organic matter in sewage, but bacteria do (Allen, 1955). Rod- and coccus-shaped bacteria occur in cooling tower oxidation systems (Elkin, Mohler and Kumnick, 1956). The bacteria were heterotrophic, depending on organic compounds for food. Bacteria may be largely responsible for the breakdown of organic matter (Silva and Papenfuss, 1953).

The ponding system on which the present study was conducted has received considerable attention since 1958. Dorris, Gould, and Jenkins (1958) discussed the various refinery processes, giving the toxic components produced in each. A progressive decrease in toxic components occurred in the pond series, although phenol and ammonia were not removed as completely during the winter as were other components. Ammonia nitrogen was reduced 80 - 90%, phenols and sulfides more than 90%, and pH and alkalinity were reduced in less than 35 days or about half the maximum holding time in the ponds (Dorris, Patterson, and Copeland, 1963). Chemical oxygen demand was reduced slightly more than 50% in about five weeks, and biochemical oxygen demand was reduced 80% in 25 days under both summer and winter conditions. Fish did not die within 48 hours when placed in 100% undiluted final effluent (Jenkins, 1964, Refinery A). Copeland (1963) compared the photosynthetic productivity, community respiration, chlorophyll concentration, and suspended organic matter between this ponding system and another. The present pond series was used in a discussion of the basis of ponding systems (Irwin, 1958).

In a study of population dynamics of herbivorous insects in refinery holding ponds (Tubb, 1963), insect populations were correlated with effluent quality. Emergence of adult insects removed a significant amount of energy from the ponding system. The structure of littoral insect communities in refinery effluent ponds varied with the season and stage of stabilization (Ewing, 1964).

## CHAPTER IV

## METHODS AND MATERIALS

Effluent flowed out of each pond continuously and was replaced at the entrance by effluent in an earlier stage of stabilization. It was assumed there might be differences in plankton from one end to the other, and to get a representative sample, effluent was taken from each of the four sides of the pond and mixed together. Samples were dipped from the upper layer of water about eight feet from shore. Formalin preservative was added to the sample to make a 5% concentration.

Phytoplankton and zooplankton were identified to genus. Dr. G. W. Prescott identified *Chlorococcum humicola*, *Synechocystis Pevalekii* Erceg., and *Chlamydomonas Ehrenbergii*. A Spencer compound microscope equipped with a 20x ocular and a 16 mm objective was used for counting organisms in combination with a Whipple Micrometer and a Sedgewick-Rafter counting chamber. Individual organisms were counted in ten ocular micrometer fields selected at random. Each algal cell was considered as a separate organism, with the exception of *Anacystis* and *Scenedesmus*, in which each colony was counted as a single unit.

The volume of plankton was computed by using measurements of each genus, as given by standard references, in formulas for shapes which most nearly approximated that of each plankter.

In grouping months into seasons, summer included June through August; fall, September through November; winter, December through February; and spring, March through May.

For counting bacteria, the same microscope was used but with a 4 mm objective and a Petroff-Housser counting chamber with special cover slip. Identification was as rod- or coccus-shape. Crystal violet stain was used to make the bacteria more visible. Bacteria in twenty of the smaller grid squares were counted.

# CHAPTER V

# STRUCTURE OF THE PLANKTON COMMUNITIES

## Incidence of Zooplankton

The zooplankton consisted entirely of protozoa, which were found irregularly during May through November in Ponds 8 through 12. Zooplankters were present in other ponds at other times, but their numbers were too low to make significant counts.

Summer populations contained more protozoan genera than did fall (Table I). The genera *Holophrya*, *Vorticella*, *Sphaerophrya*, and an unidentified hypotrich were present in summer, but only the hypotrich and *Gromia* were found in fall samples. *Gromia* was collected primarily during November, but was also present during June in very small numbers. Minter (1964) found a greater variety of zooplankton in another oil refinery pond series, including ciliate protozoa, omnivorous and carnivorous rotifers, and one species each of Cladocera and Copepoda.

## Incidence of Phytoplankton

Twenty-seven genera of algae, representing four phyla (Table II), were found during the study, but only eight were present in significant numbers. The eight genera were *Chlamydomonas*, *Chlorogonium*, *Chlorococcum*, *Schroederia*, *Scenedesmus*, *Synechocystis*, *Anacystis*, and *Oscillatoria*.

TABLE I

ZOOPLANKTON POPULATIONS
(Estimated numbers/liter x $10^3$)

| | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|
| JUNE | | | | | |
| *Holophrya* | | | 1,100 | 350 | |
| *Hypotricha* | | | 350 | 350 | 350 |
| *Sphaerophrya* | | 1,450 | | | |
| Unidentified | | 4,400 | | | 350 |
| JULY | | | | | |
| *Holophrya* | 759 | | | | |
| *Vorticella* | | | 350 | | |
| *Hypotricha* | | | 350 | | |
| Unidentified | | | | 700 | |
| AUGUST | | | | | |
| Unidentified | | | 350 | 350 | |
| SEPTEMBER | | | | | |
| *Hypotricha* | 350 | 700 | | | |
| Unidentified | | | | 350 | 700 |
| OCTOBER | | | | | |
| Unidentified | 350 | 350 | | | |
| NOVEMBER | | | | | |
| *Gromia* | | | 700 | 2,550 | 1,450 |
| MAY | | | | | |
| Unidentified | | | 750 | 1,100 | 1,100 |

TABLE II

GENERA OF PHYTOPLANKTON

| Chlorophyta | Myxophyta |
|---|---|
| *Chlamydomonas* | *Synechocystis* |
| *Chlorogonium* | *Gloeocapsa* |
| *Pandorina* | *Anacystis* |
| *Eudorina* | *Oscillatoria* |
| *Gloeocystis* | *Spirulina* |
| *Chlorococcum* | *Anabaena* |
| *Golenkinia* | |
| *Micractinium* | |
| *Schroederia* | Euglenophyta |
| *Sorastrum* | *Euglena* |
| *Pediastrum* | |
| *Ankistrodesmus* | |
| *Closteriopsis* | Chrysophyta |
| *Tetradesmus* | *Rhizochrysis* |
| *Scenedesmus* | *Asterionella* |
| *Actinastrum* | |
| *Closterium* | |
| *Cosmarium* | |

Maximal numbers of algae were found in early summer. Peak numbers increased from Pond 2 through Pond 8, and decreased sharply in Pond 9 and through the end of the pond series (Figure 2). Monthly peak numbers ranged from 1,713 to 4,891 x $10^6$ cells/liter (Table III). The first ponds contained an apparently unialgal population of *Synechocystis* in large numbers. After Pond 8, *Synechocystis* numbers dropped markedly and a variety of algae appeared, including *Oscillatoria* and *Scenedesmus*.

An early summer pulse may have developed just prior to collection of the June sample since algal cells generally were in the final stages of cell division. Each unit was therefore counted as two separate organisms. The existence of a population expanding under optimal conditions is indicated.

Another plankton pulse developed from November through January. Peak numbers ranged from 254 to 1,370.9 x $10^6$ cells per liter (Figure 2). Peak numbers decreased sharply from Pond 2 through Pond 4, after which a progressive and relatively regular increase in numbers occurred through Pond 10, where numbers exceeded those of the first ponds. Ponds 11 and 12 had successively smaller numbers of plankton.

Green algae were predominant in winter. The most numerous species was *Chlorococcum humicola*. *Schroederia*, *Scenedesmus*, *Chlamydomonas* and *Gloeocystis* were present in significant numbers. Other genera occurred in small numbers sporadically. *Euglena* was the only euglenophyte found, and it appeared in November and April only.

In April, a successive decrease in peak numbers occurred through Pond 8, but peak numbers in the last three ponds were much higher (Table III). Numbers during May decreased continuously through the pond series.

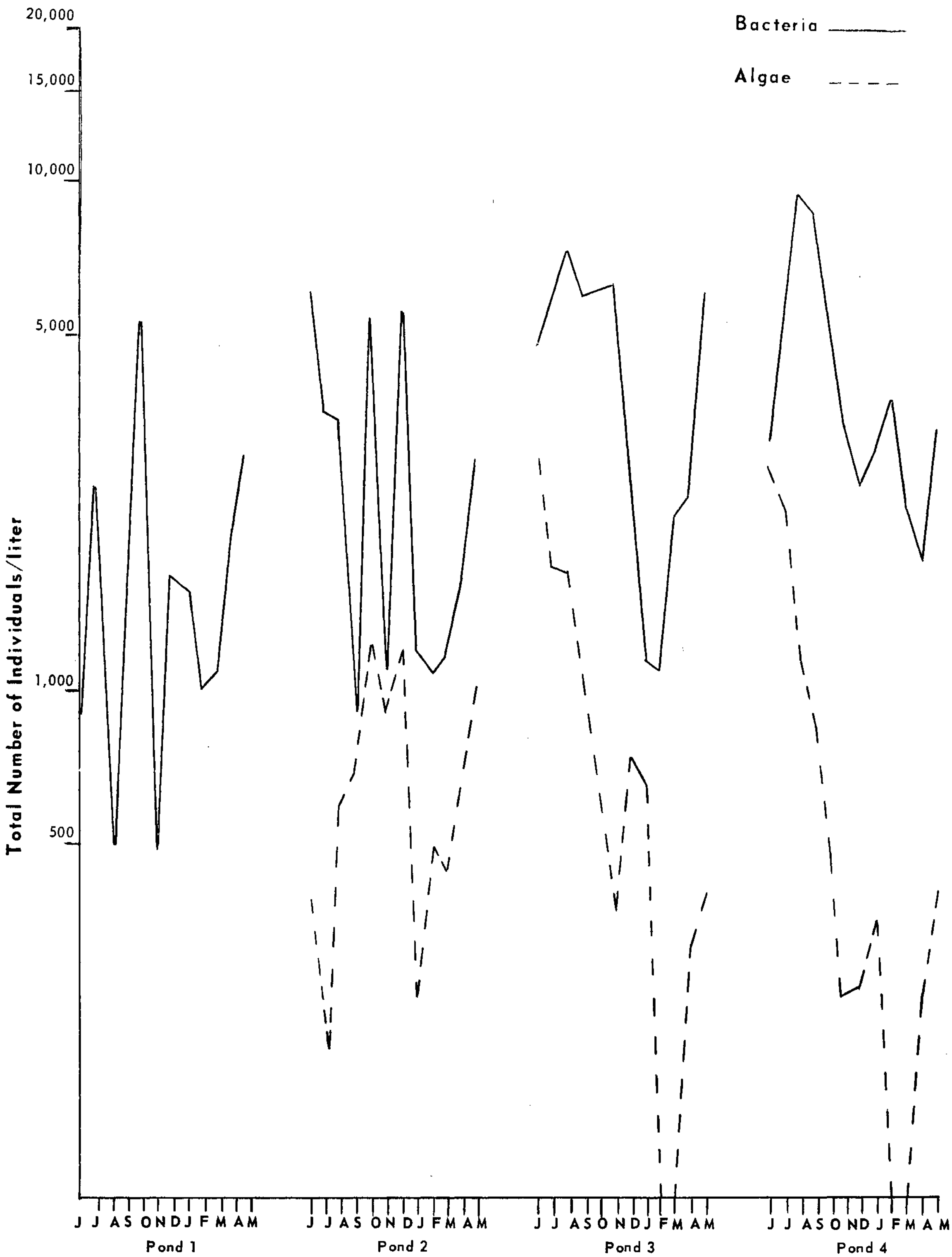


Figure 2. Comparison of Total Numbers of Algae and Bacteria. All Numbers Are x$10^6$.

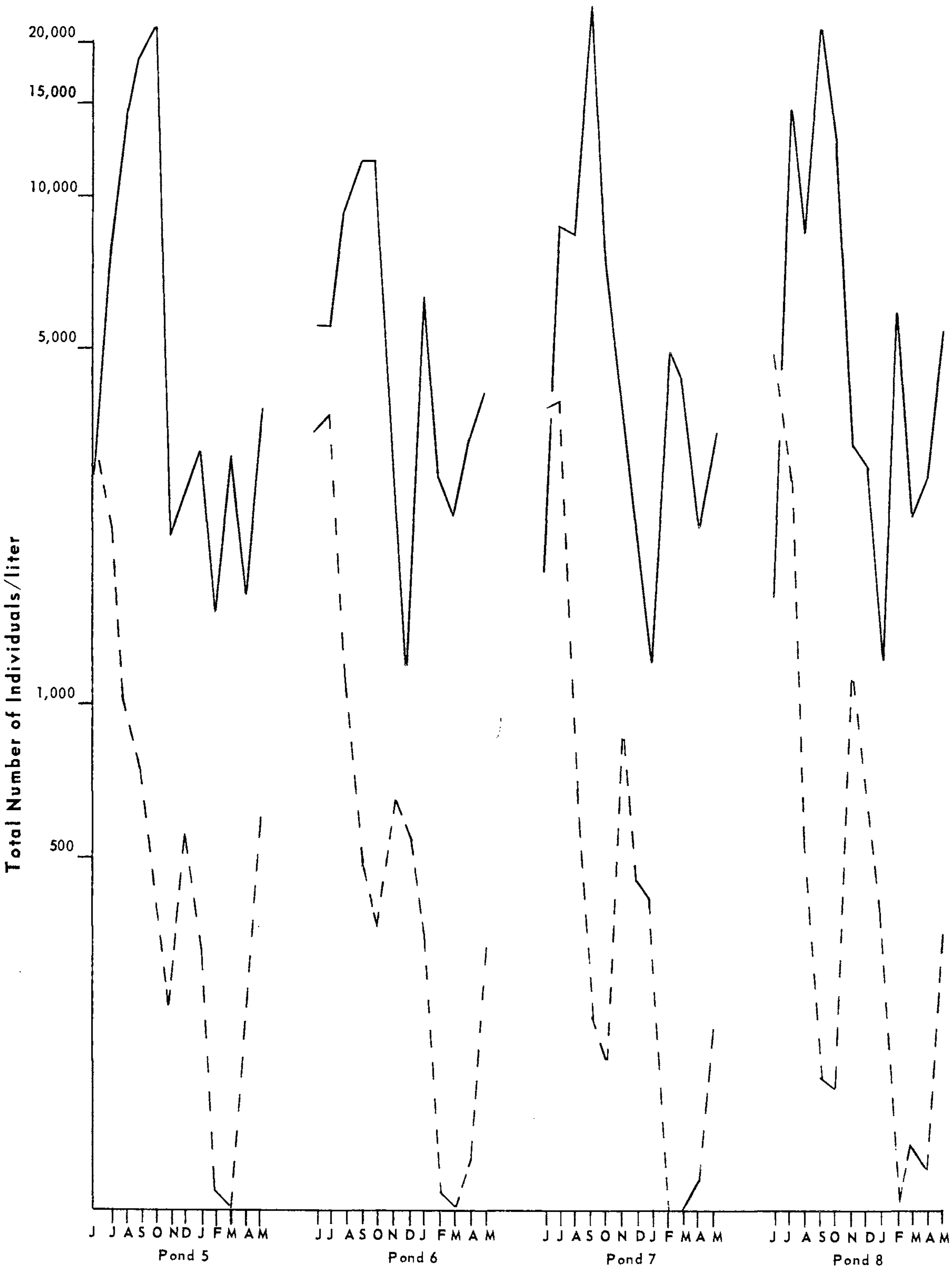


Figure 2. (Continued)

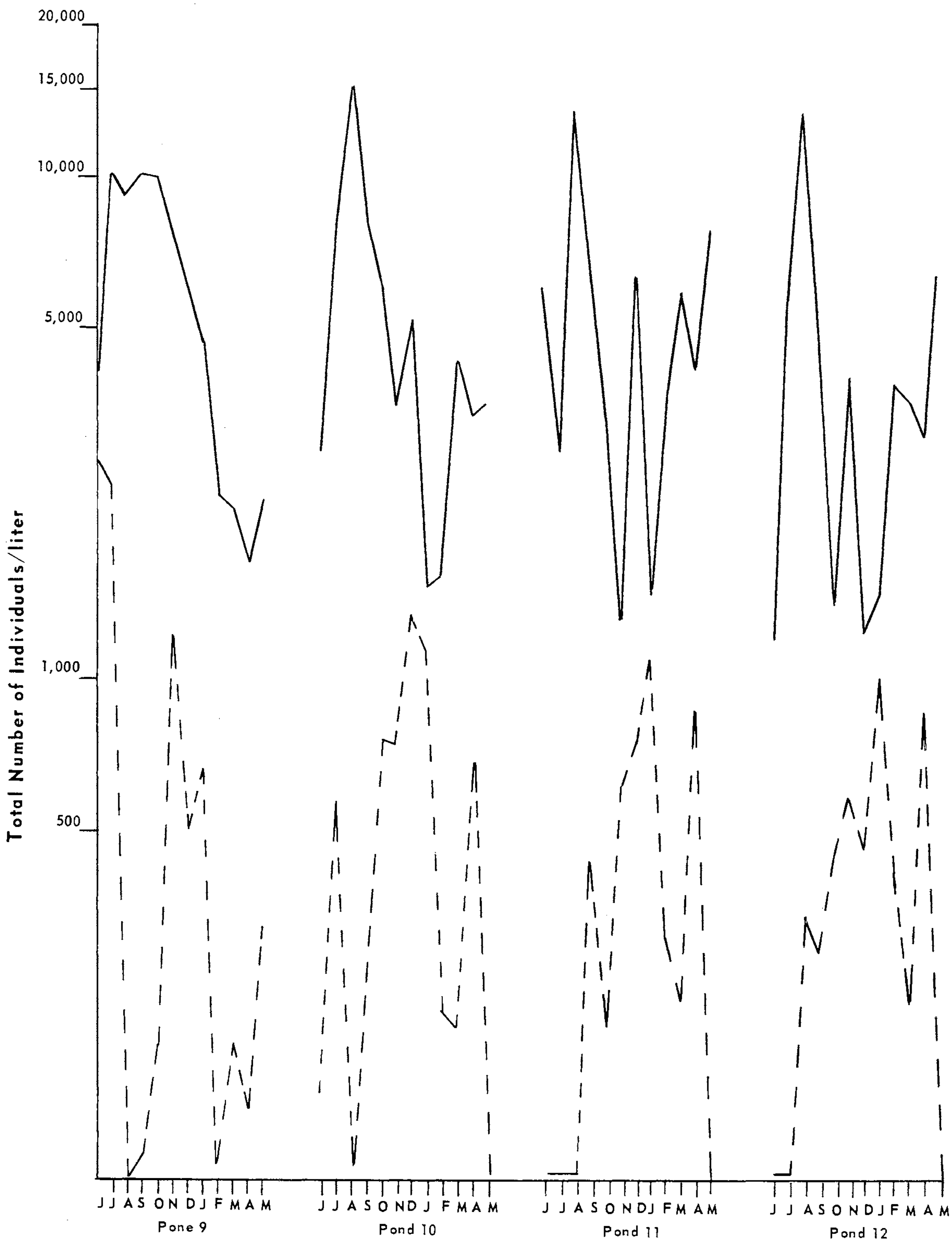


Figure 2. (Continued)

TABLE III

PHYTOPLANKTON POPULATIONS
(Estimated numbers/liter x $10^6$)

| GENERA | PONDS 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| JUNE TOTAL | 390 | 2,915 | 2,808 | 3,432 | 3,377 | 3,776 | 4,891 | 2,751 | 147 | 61 | 72 |
| *Synechocystis* | 390 | 2,915 | 2,806 | 3,432 | 3,375 | 3,776 | 4,872 | 2,685 | 126 | 11 | 32 |
| *Chlorococcum* | | | | | | | | | 10 | 18 | 11 |
| *Oscillatoria* | | | | | 2 | | 2 | 40 | 10 | 15 | 20 |
| Other | | | 1 | | | | 16 | 32 | 18 | 17 | 8 |
| JULY TOTAL | 195 | 1,742 | 2,320 | 2,042 | 3,695 | 3,932 | 2,770 | 2,435 | 641 | 78 | 62 |
| *Synechocystis* | 195 | 1,742 | 2,316 | 2,042 | 3,690 | 3,928 | 2,770 | 2,419 | 622 | 77 | 25 |
| *Oscillatoria* | | | | | 3 | 4 | | 12 | 5 | | 7 |
| *Scenedesmus* | | | | | | | | | 4 | 3 | 9 |
| Other | | 1 | 4 | | 2 | | | 14 | 9 | 18 | 21 |
| AUGUST TOTAL | 613 | 1,713 | 1,180 | 1,072 | 1,127 | 727 | 526 | 91 | 93 | 66 | 345 |
| *Synechocystis* | 613 | 1,713 | 1,180 | 1,072 | 1,112 | 719 | 499 | 60 | 59 | 47 | 310 |
| *Oscillatoria* | | | | | 2 | | 5 | 17 | 10 | 11 | 5 |
| Other | | | | | 13 | 8 | 22 | 15 | 24 | 8 | 30 |
| SEPTEMBER TOTAL | 718 | 970 | 863 | 787 | 477 | 244 | 184 | 114 | 200 | 421 | 283 |
| *Synechocystis* | 718 | 647 | 511 | 279 | 154 | 51 | 12 | 3 | 191 | 420 | 278 |
| *Flagellate* | | 322 | 352 | 508 | | | 6 | 2 | | | |
| *Schroederia* | | | | | 28 | | 5 | 1 | | | |
| *Scenedesmus* | | | | | 48 | 84 | 45 | 7 | 1 | | 1 |
| *Oscillatoria* | | | | | 6 | | 5 | 11 | 1 | 1 | 1 |
| *Chlorococcum* | | | | | 152 | | 17 | 4 | | | |
| Other | | | | | 88 | 9 | 94 | 86 | 8 | 1 | 3 |

TABLE III (Continued)

| GENERA | PONDS 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| OCTOBER TOTAL | 1,284 | 676 | 479 | 470 | 354 | 190 | 173 | 189 | 757 | 197 | 444 |
| Synechocystis | 1,284 | 671 | 472 | 465 | 204 | 67 | 70 | 58 | 725 | 177 | 424 |
| Schroederia | | | 3 | 3 | 23 | 13 | 15 | 23 | 3 | 1 | 2 |
| Gloeocystis | | | | | 40 | 53 | 22 | | | | |
| Scenedesmus | | | | | 26 | 26 | 31 | 50 | 6 | 6 | 13 |
| Other | | 5 | 4 | 2 | 80 | 31 | 36 | 59 | 24 | 13 | 5 |
| | | | | | | | | | | | |
| NOVEMBER TOTAL | 908 | 363 | 254 | 255 | 670 | 872 | 1,154 | 1,279 | 732 | 599 | 593 |
| Chlorococcum | 908 | 346 | 240 | 240 | 498 | 644 | 816 | 883 | 520 | 476 | 467 |
| Schroederia | | 8 | 12 | 10 | 95 | 115 | 171 | 197 | 54 | 28 | 20 |
| Scenedesmus | | 3 | | | 55 | 104 | 132 | 160 | 70 | 40 | 21 |
| Chlamydomonas | | 4 | 2 | 5 | 5 | | 12 | 8 | 5 | 3 | 2 |
| Gloeocystis | | | | | 12 | | 15 | 17 | 19 | 4 | 6 |
| Other | | 2 | | | 5 | 8 | 8 | 14 | 54 | 48 | 76 |
| | | | | | | | | | | | |
| DECEMBER TOTAL | 1,251 | 735 | 268 | 560 | 531 | 456 | 649 | 499 | 1,371 | 748 | 461 |
| Chlorococcum | 1,251 | 732 | 262 | 548 | 520 | 445 | 636 | 478 | 1,229 | 688 | 425 |
| Schroederia | | | | | | 6 | 8 | 7 | 57 | 24 | 14 |
| Chlamydomonas | | | 7 | 9 | 11 | 5 | 5 | 13 | 47 | 19 | 14 |
| Scenedesmus | | | | | | | | | 32 | 10 | 4 |
| Other | | 3 | | 3 | | | | | 6 | 6 | 4 |
| | | | | | | | | | | | |
| JANUARY TOTAL | 252 | 667 | 364 | 354 | 328 | 419 | 387 | 679 | 1,134 | 1,146 | 1,023 |
| Chlorococcum | | 661 | 361 | 351 | 327 | 414 | 387 | 675 | 1,105 | 1,112 | 986 |
| Schroederia | | 6 | 3 | 1 | 1 | 4 | | 4 | 11 | 16 | 20 |
| Chlamydomonas | | | 1 | 2 | | | | | 18 | 16 | 18 |
| Other | | | 1 | 2 | | | | | | 2 | |

TABLE III (Continued)

| GENERA | PONDS 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FEBRUARY TOTAL | 514 | 85 | 84 | 109 | 109 | 92 | 81 | 77 | 212 | 300 | 413 |
| Chlorococcum | | | | | 108 | 92 | 79 | 73 | 130 | 147 | 255 |
| Chlamydomonas | | | | | | | 1 | 3 | 78 | 146 | 152 |
| Other | | | | | 1 | | 1 | 1 | 6 | 6 | 6 |
| MARCH TOTAL | 444 | 75 | 64 | 43 | 88 | 97 | 135 | 191 | 200 | 218 | 230 |
| Chlorococcum | 444 | 72 | 62 | 36 | 25 | 14 | 28 | 65 | 143 | 166 | 196 |
| Schroederia | | 1 | 1 | 3 | 55 | 63 | 48 | 39 | 6 | 6 | 6 |
| Chlamydomonas | | | | | | | | | | | |
| Flagellate | | 2 | 1 | 4 | 8 | 19 | 54 | 77 | 40 | 24 | 16 |
| Other | | | | | | | | | | | |
| APRIL TOTAL | 719 | 321 | 254 | 202 | 128 | 115 | 118 | 136 | 709 | 972 | 927 |
| Chlorococcum | 719 | 321 | 237 | 142 | 100 | 81 | 78 | 80 | 565 | 830 | 789 |
| Schroederia | | | | | | | | 1 | 2 | | 3 |
| Chlamydomonas | | | 17 | 61 | 28 | 19 | 25 | 28 | 47 | 87 | 66 |
| Scenedesmus | | | | | | | | | | 30 | 51 |
| Other | | | | | | 14 | 15 | 28 | 96 | 4 | 18 |
| MAY TOTAL | 1,005 | 418 | 414 | 598 | 333 | 232 | 340 | 324 | 79 | 62 | 49 |
| Chlorococcum | 1,005 | 417 | 252 | 407 | 212 | 145 | 192 | 171 | 45 | 32 | 25 |
| Schroederia | | | | | | | | | | 1 | |
| Chlamydomonas | | 271 | 162 | 179 | 121 | 81 | 143 | 39 | 15 | 15 | 11 |
| Micractinium | | | | | | | | 100 | 18 | 10 | 13 |
| Other | | | | 13 | | 5 | 5 | 14 | 1 | 5 | |

Fluctuations in numbers occurred with approximately parallel increase and decrease in numbers in all algal genera.

## Phytoplankton Biomass

Algal volume was low during June through August, and was relatively uniform throughout the pond series (Figure 3). Ponds 8 and 9 had almost twice the biomass of any other pond in June. Genera of larger individuals first occurred in Ponds 8 and 9 (Table IV). Ponds 10 through 12 were lower in volume due to a large decrease in numbers of *Synechocystis*.

Maximum volumes of algae occurred in September through November, with greatest volumes in Ponds 6 through 9 (Figure 3). Most genera increased in volume through Pond 9, and decreased sharply in Ponds 10 through 12. *Anacystis* composed much of the total volume in Ponds 10 through 12 in November. A trend to larger volumes in the last of the pond series is indicated (Figure 3). By November, the dominant alga had changed from *Synechocystis* to *Chlorococcum*, which remained dominant through April.

The largest volumes of algae during December through April were generally at the two ends of the pond series. The early peak volume was mostly *Chlorococcum*. The second peak resulted from an increase in almost all genera. Volume was relatively large in December, decreased through February, then increased through March to April (Figure 3). Generic variety was relatively constant through this period with fluctuations in volume caused by different numerical ratios among the genera present.

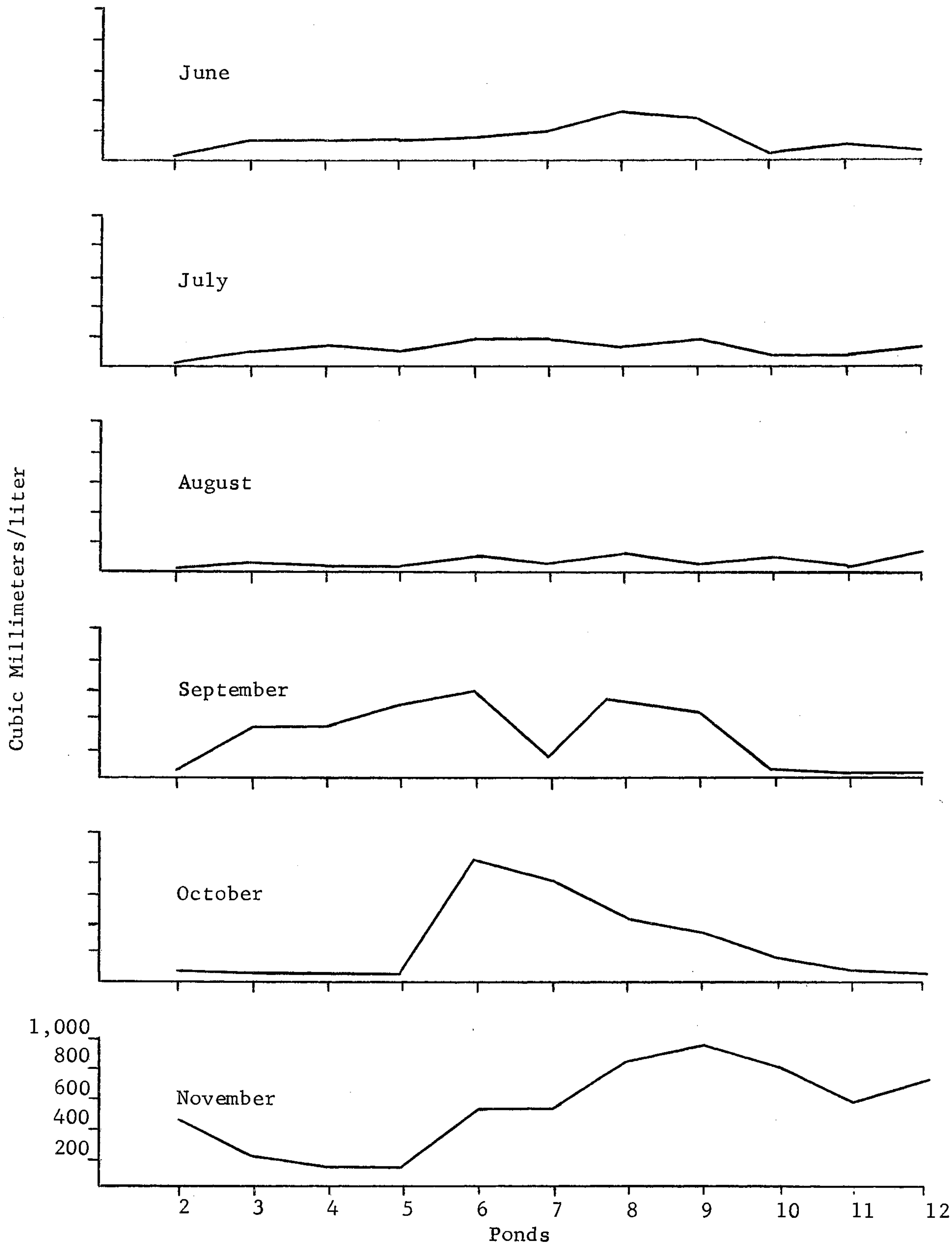


Figure 3. Volume of Algae by Month

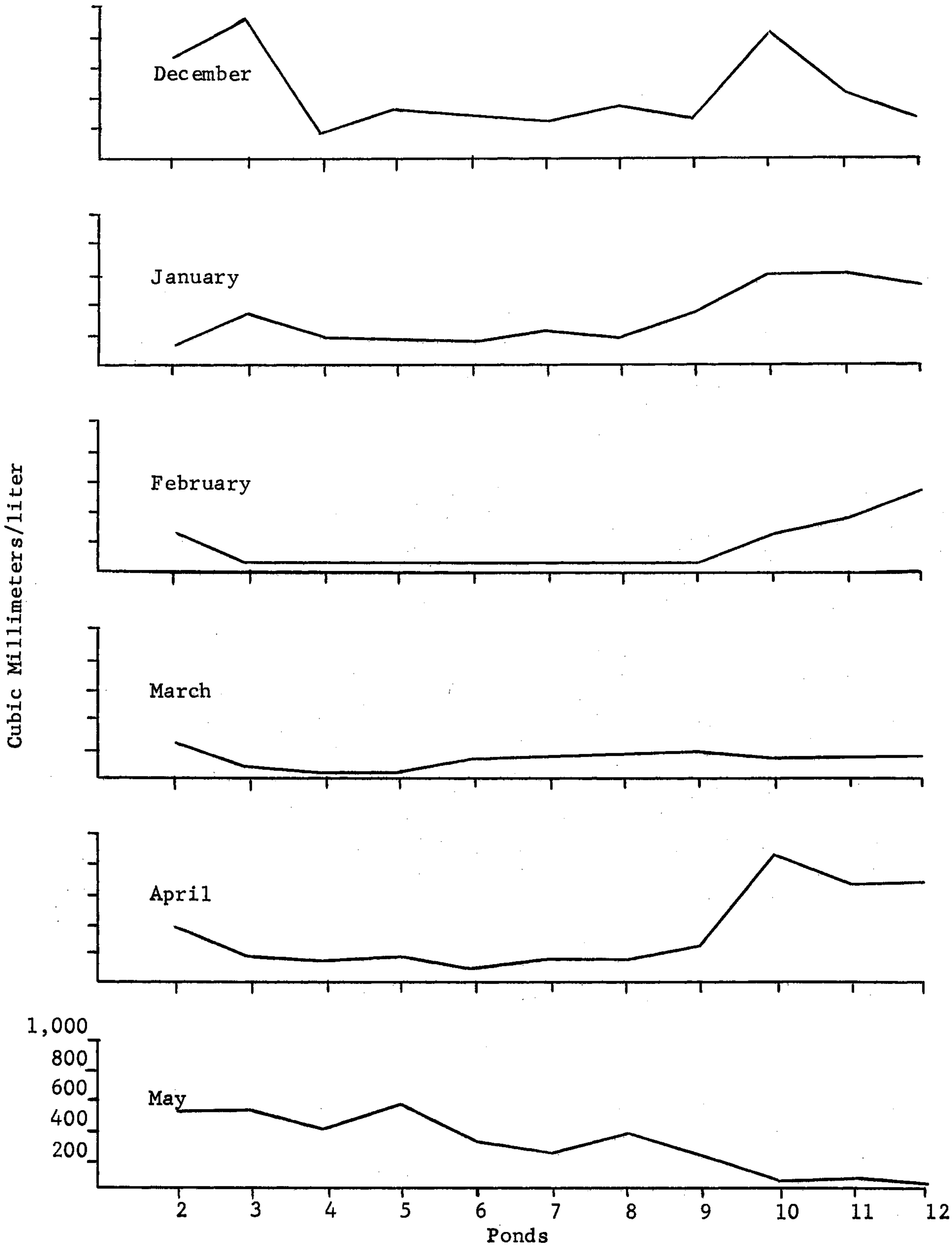


Figure 3. (Continued)

TABLE IV

VOLUME OF PHYTOPLANKTON

($mm^3$/liter)

| GENERA | PONDS 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| JUNE TOTAL | 19 | 139 | 143 | 164 | 161 | 180 | 312 | 294 | 53 | 98 | 50 |
| *Synechocystis* | 19 | 139 | 134 | 164 | 161 | 180 | 232 | 128 | 6 | | 2 |
| *Chlorococcum* | | | | | | | | | 6 | 9 | 6 |
| *Oscillatoria* | | | | | | | | 4 | 1 | 2 | 2 |
| Other | | | 9 | | | | 80 | 162 | 40 | 86 | 40 |
| JULY TOTAL | 9 | 87 | 128 | 105 | 176 | 188 | 132 | 188 | 83 | 94 | 114 |
| *Synechocystis* | 9 | 83 | 110 | 97 | 176 | 187 | 132 | 115 | 30 | 4 | 1 |
| *Oscillatoria* | | | | | | | | 1 | | | 1 |
| *Scenedesmus* | | | | | | | | | 4 | 3 | 7 |
| Other | 4 | 18 | 8 | | | | | 72 | 48 | 88 | 105 |
| AUGUST TOTAL | 29 | 82 | 56 | 51 | 117 | 73 | 134 | 79 | 125 | 41 | 163 |
| *Synechocystis* | 29 | 82 | 56 | 51 | 53 | 34 | 24 | 3 | 3 | 2 | 15 |
| *Oscillatoria* | | | | | | | 1 | 2 | 1 | 1 | |
| Other | | | | | 64 | 38 | 110 | 74 | 121 | 38 | 148 |
| SEPTEMBER TOTAL | 34 | 337 | 359 | 496 | 592 | 135 | 537 | 444 | 49 | 23 | 28 |
| *Synechocystis* | 34 | 31 | 24 | 13 | 7 | 2 | 1 | | 9 | 20 | 13 |
| *Flagellate* | | 306 | 335 | 483 | | | 6 | 2 | | | |
| *Schroederia* | | | | | 14 | | 2 | | | | |
| *Scenedesmus* | | | | | 51 | 88 | 48 | 8 | 1 | | |
| *Oscillatoria* | | | | | 1 | | | 1 | | | |
| *Chlorococcum* | | | | | 80 | | 9 | 2 | | | |
| Other | | | | | 440 | 44 | 472 | 431 | 39 | 3 | 14 |

TABLE IV (Continued)

| GENERA | PONDS 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| OCTOBER TOTAL | 61 | 56 | 43 | 34 | 823 | 681 | 422 | 360 | 160 | 79 | 60 |
| <u>Synechocystis</u> | 61 | 32 | 22 | 22 | 10 | 3 | 3 | 3 | 35 | 8 | 20 |
| <u>Schroederia</u> | | | 2 | 2 | 11 | 6 | 7 | 11 | 1 | | 1 |
| <u>Gloeocystis</u> | | | | | 372 | 489 | 201 | | | | |
| <u>Scenedesmus</u> | | | | | 27 | 27 | 32 | 52 | 6 | 6 | 14 |
| Other | | 24 | 18 | 10 | 402 | 155 | 179 | 294 | 118 | 64 | 26 |
| | | | | | | | | | | | |
| NOVEMBER TOTAL | 476 | 206 | 136 | 140 | 513 | 545 | 851 | 968 | 826 | 590 | 718 |
| <u>Chlorococcum</u> | 476 | 181 | 126 | 126 | 261 | 338 | 427 | 463 | 273 | 249 | 245 |
| <u>Schroederia</u> | | 4 | 6 | 5 | 47 | 57 | 84 | 98 | 26 | 14 | 10 |
| <u>Scenedesmus</u> | | 3 | | | 57 | 109 | 139 | 168 | 74 | 42 | 22 |
| <u>Chlamydomonas</u> | | 7 | 3 | 8 | 8 | | 21 | 14 | 10 | 6 | 4 |
| <u>Gloeocystis</u> | | | | | 114 | | 140 | 157 | 174 | 40 | 57 |
| Other | | 11 | | | 26 | 42 | 38 | 70 | 270 | 239 | 379 |
| | | | | | | | | | | | |
| DECEMBER TOTAL | 656 | 921 | 150 | 317 | 292 | 244 | 345 | 277 | 819 | 446 | 281 |
| <u>Chlorococcum</u> | 656 | 908 | 137 | 287 | 273 | 233 | 333 | 250 | 644 | 360 | 222 |
| <u>Schroederia</u> | | | | | | 3 | 4 | 4 | 28 | 12 | 7 |
| <u>Chlamydomonas</u> | | | 12 | 15 | 19 | 8 | 8 | 23 | 84 | 34 | 25 |
| <u>Scenedesmus</u> | | | | | | | | | 34 | 11 | 5 |
| Other | | 13 | 2 | 14 | | | | | 29 | 29 | 22 |
| | | | | | | | | | | | |
| JANUARY TOTAL | 132 | 349 | 192 | 187 | 172 | 220 | 204 | 356 | 616 | 628 | 558 |
| <u>Chlorococcum</u> | 132 | 346 | 189 | 184 | 171 | 217 | 203 | 354 | 579 | 582 | 517 |
| <u>Schroederia</u> | | 3 | 1 | | | 2 | | 2 | 5 | 8 | 10 |
| <u>Chlamydomonas</u> | | | 1 | 3 | | | 1 | | 31 | 29 | 31 |
| Other | | | | | | | | | | 9 | |

TABLE IV (Continued)

| GENERA PONDS | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FEBRUARY TOTAL | 270 | 45 | 44 | 57 | 62 | 50 | 46 | 49 | 236 | 364 | 558 |
| Chlorococcum | 270 | 45 | 44 | 57 | 56 | 48 | 42 | 38 | 68 | 77 | 133 |
| Chlamydomonas | | | | | | | 1 | 6 | 140 | 260 | 271 |
| Other | | | | | 6 | 2 | 4 | 6 | 29 | 28 | 29 |
| MARCH TOTAL | 232 | 80 | 35 | 29 | 119 | 138 | 155 | 182 | 128 | 131 | 134 |
| Chlorococcum | 232 | 39 | 32 | 18 | 13 | 7 | 15 | 34 | 75 | 87 | 102 |
| Schroederia | | 36 | | | | | 2 | 6 | 6 | 11 | 6 |
| Chlamydomonas | | 1 | 2 | 6 | 98 | 112 | 86 | 69 | 10 | 10 | 10 |
| Flagellate | | 2 | 1 | 4 | 7 | 18 | 51 | 73 | 38 | 23 | 15 |
| Other | | 2 | | | | | | | | | |
| APRIL TOTAL | 377 | 169 | 154 | 182 | 103 | 148 | 160 | 232 | 859 | 644 | 676 |
| Chlorococcum | 376 | 168 | 124 | 74 | 52 | 43 | 41 | 42 | 296 | 435 | 413 |
| Schroederia | | | | | | | 1 | 1 | 1 | | 1 |
| Chlamydomonas | | 1 | 30 | 108 | 51 | 34 | 44 | 49 | 84 | 155 | 118 |
| Scenedesmus | | | | | | | | | | 32 | 53 |
| Other | | | | | | 71 | 75 | 140 | 478 | 22 | 90 |
| MAY TOTAL | 527 | 560 | 420 | 594 | 327 | 245 | 381 | 236 | 57 | 69 | 34 |
| Chlorococcum | 527 | 77 | 132 | 213 | 111 | 76 | 101 | 90 | 24 | 17 | 13 |
| Schroederia | | | | | | | | | | | |
| Chlamydomonas | | 483 | 288 | 317 | 216 | 144 | 254 | 69 | 28 | 26 | 19 |
| Micractinium | | | | | | | | 6 | 1 | 1 | 1 |
| Other | | | | 64 | | 25 | 26 | 70 | 5 | 26 | 2 |

The algal volume in May differed from all others in that the volume was high in the first ponds and decreased through Pond 12. The catalytic cracker of the refinery was not in use from April 19 through May 7 and absence of this toxic effluent may have affected algal volume in Ponds 2 through 6. The amount of biomass in Ponds 10 through 12 resembled that of the previous summer; however, different algae were present. A large bloom of Chlamydomonas occurred in Ponds 3 through 8 in May. Although this alga is relatively small, it is much larger than most other genera found during the study.

Marked differences in variety, numbers and volumes of genera often occurred between Ponds 9 and 10 (Table III). These ponds are separated by a complete dike, with effluent passing through a submerged pipe. Complete dikes may contribute to better effluent stabilization than incomplete dikes.

Relationships between seasons and toxicity of early ponds on the rate of stabilization may be indicated in Figure 4. Peak algal volumes are found early in the pond series during summer, but peaks occur successively later in the pond series through the winter months. Slower and less complete stabilization may have occurred during winter months, or algae may have been unable to utilize nutrients in the early ponds during the winter.

## Species-Diversity

To determine the species-diversity index, the sum of individual cells in each separate microscopic field was plotted against the number of species in the field on semi-logarithmic graph paper (Yount, 1956).

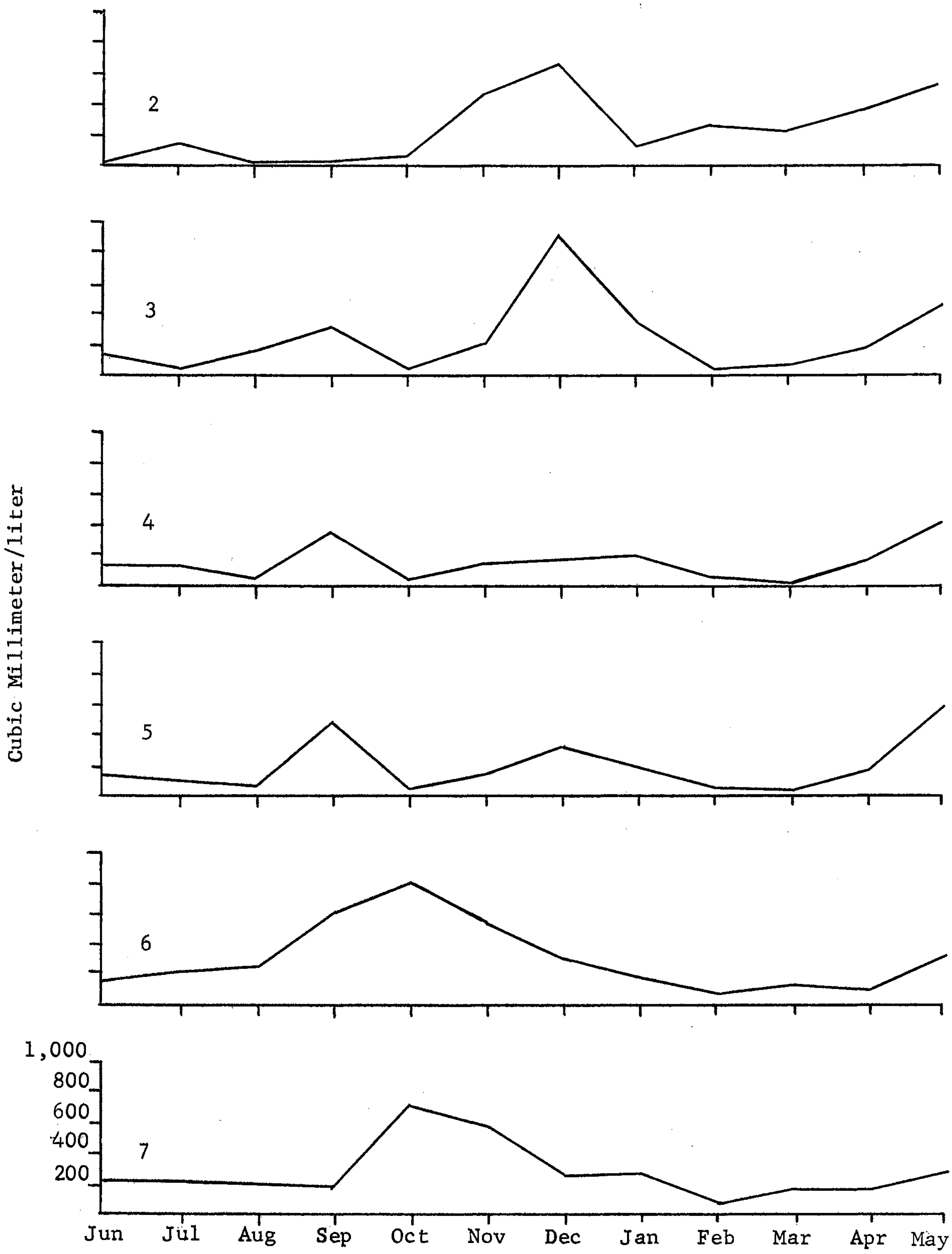


Figure 4. Volume of Algae by Pond

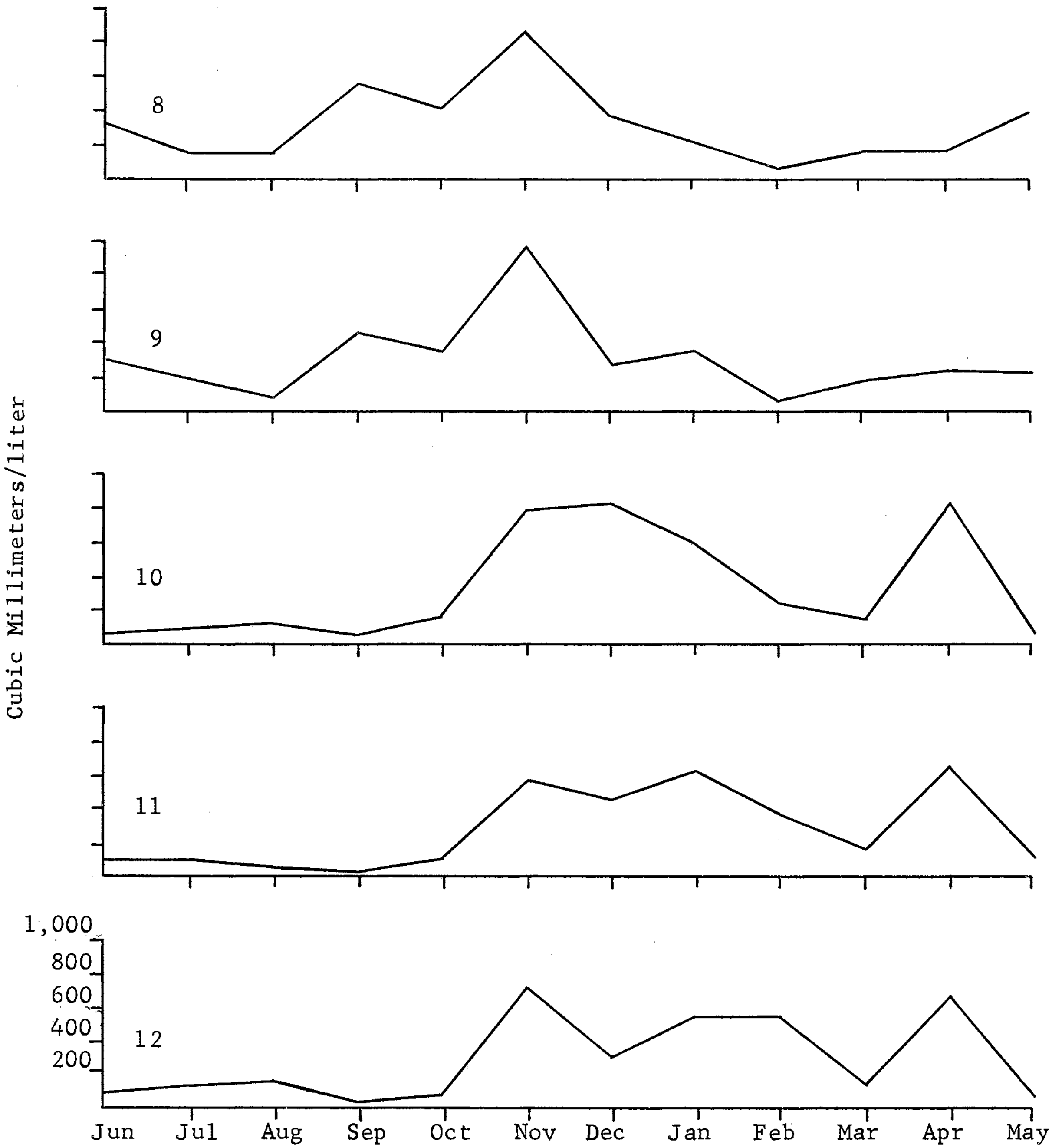


Figure 4. (Continued)

Each field almost always contained more than 10 individuals and often more than 100 individuals, but the entire number of cells in the 10 fields counted often did not total 1,000. Exact determination of 10 fold increases was not possible. Instead, the species-diversity index was computed by dividing the number of cumulative species by a fraction of 3 cycles. Thus, the index number of the June sample for Pond 10 was 2.4 cycles, since only 400 total individuals were counted from that sample (Table V).

A diversity index allows evaluation of the degree of organization of the community. A higher diversity may indicate a more highly organized system and a more stabilized effluent.

The highest diversities were found during the summer months (Table V). Diversity increased through the series, ranging from 0.3 to 5.0 species per cycle. Minter (1964) also found more diversity during the optimal environment of summer months.

Plankton succession is dependent, among other things, upon depletion of nutrients (Margalef, 1958). As nutrients are depleted, species more adapted to lower amounts of nutrients will replace those requiring highly concentrated nutrients. High nutrient content usually results in high numbers of a few species, but a low nutrient content usually results in many species in low numbers. In June, succession from large numbers of a few species in the early ponds to a variety of species in smaller numbers occurred in the later ponds (Figure 5). Ponds 7 through 9 were the most diverse during October (Figure 6). The total number of cells was lower than in Ponds 10 through 12.

Winter and spring diversities may indicate a slower stabilization of the effluent (Table V). Although the highest diversity was found in

TABLE V

PLANKTON SPECIES-DIVERSITY
(Species per Cycle)

| POND | SUMMER | | | FALL | | | WINTER | | | SPRING | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May |
| 2 | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.4 | 0.3 | 0.4 | 0.3 | 0.4 |
| 3 | 0.3 | 0.7 | 0.3 | 0.7 | 1.0 | 2.3 | 0.7 | 0.7 | 0.4 | 1.8 | 0.7 | 0.7 |
| 4 | 0.7 | 0.7 | 0.3 | 0.7 | 1.4 | 1.1 | 1.1 | 1.0 | 0.4 | 1.4 | 1.2 | 0.7 |
| 5 | 0.3 | 0.3 | 0.3 | 0.7 | 1.4 | 1.1 | 1.0 | 1.0 | 0.4 | 1.4 | 0.8 | 1.4 |
| 6 | 0.7 | 0.3 | 1.3 | 3.7 | 2.4 | 2.0 | 0.7 | 1.0 | 0.9 | 1.8 | 0.8 | 0.7 |
| 7 | 0.7 | 0.3 | 1.7 | 4.8 | 3.6 | 2.0 | 1.0 | 1.0 | 0.9 | 1.7 | 1.3 | 1.5 |
| 8 | 1.0 | 0.3 | 2.6 | 4.8 | 3.6 | 2.3 | 1.0 | 0.7 | 1.8 | 1.7 | 1.3 | 2.4 |
| 9 | 1.3 | 1.0 | 2.3 | 4.8 | 3.2 | 2.0 | 1.0 | 1.0 | 1.4 | 1.6 | 1.7 | 2.5 |
| 10 | 2.4 | 1.7 | 4.1 | 3.5 | 1.7 | 3.0 | 1.7 | 1.0 | 1.2 | 1.6 | 1.4 | 2.7 |
| 11 | 4.5 | 2.6 | 2.7 | 1.7 | 2.4 | 3.0 | 1.7 | 1.0 | 1.4 | 1.5 | 2.3 | 3.3 |
| 12 | 5.0 | 4.3 | 3.4 | 2.7 | 2.0 | 3.3 | 1.7 | 1.0 | 1.3 | 1.5 | 1.7 | 2.5 |

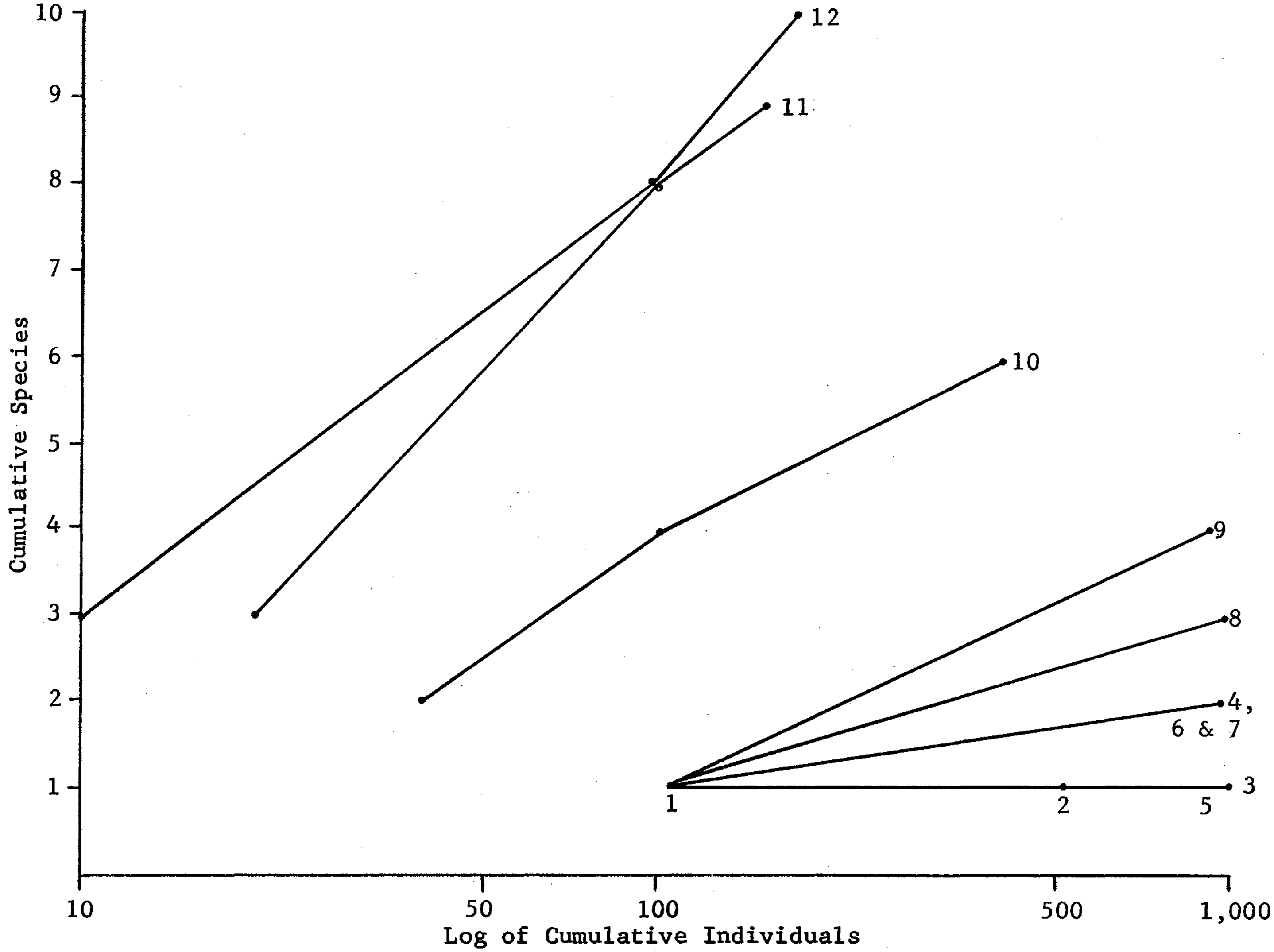


Figure 5. Species-Diversity Curves Comparing Ponds During June
(Pond numbers are at the end of each curve)

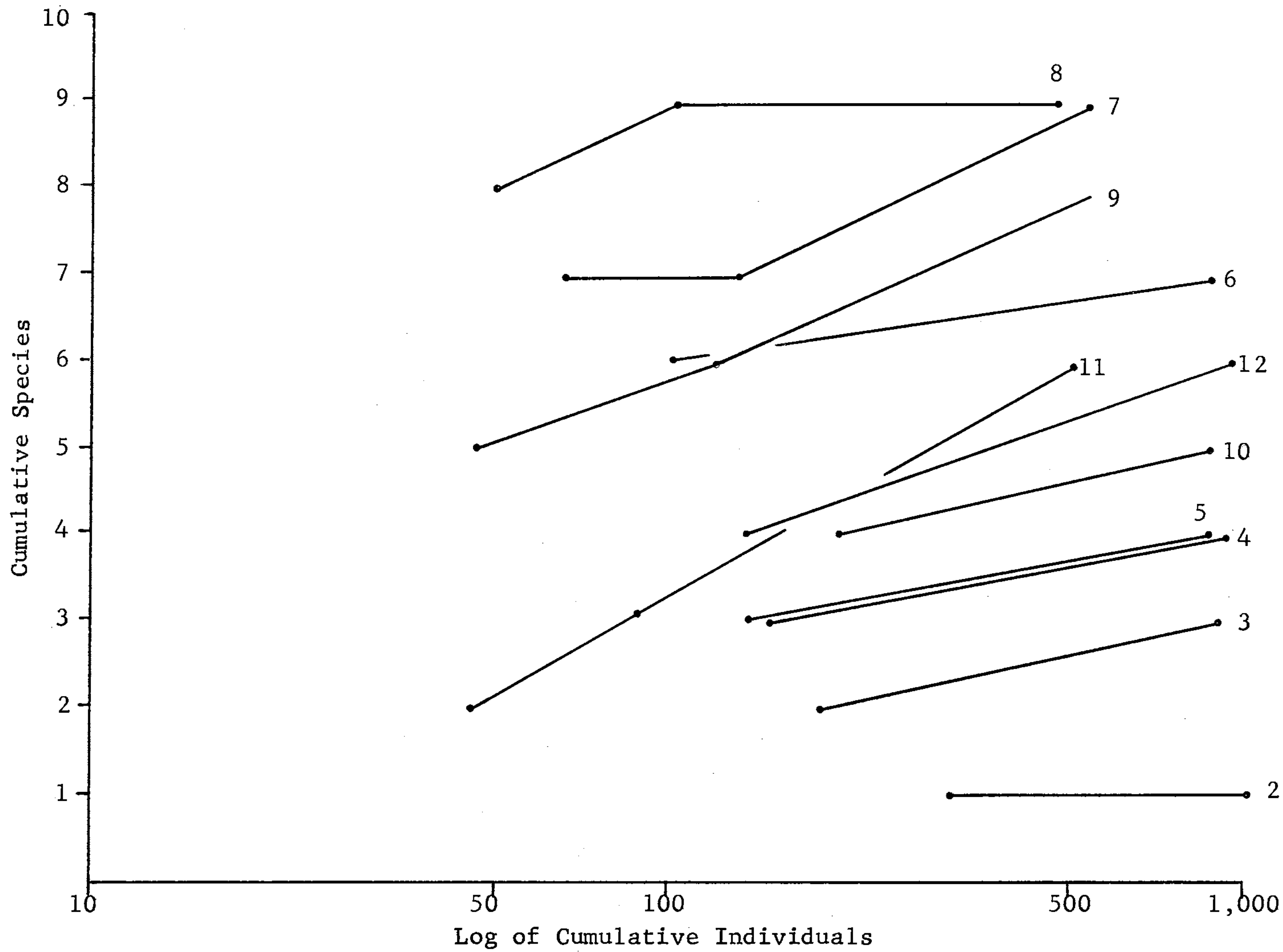


Figure 6. Species-Diversity Curves Comparing Ponds During October
(Pond numbers are at the end of each curve)

the last ponds of the series as during summer, the diversity was not as great. Minter (1964) found equivalent results which he attributed to higher toxicity and lessened phenol reduction during winter. Chlorococcum composed most of the plankton population, other genera appearing regularly but in small numbers (Table III), which may indicate a high productivity throughout the series (Margalef, 1958).

Diversity was much lower than Minter found, even though effluent holding time was much longer. Generally, Minter found 4 to 8 species per cycle, but only 1 to 5 were found in the present study. Longer holding time should have provided more time for stabilization of the effluent resulting in a more diverse plankton community; however, the effluents studied by Minter had extensive pre-treatment before ponding.

Diversity in Pond 12 in June was much lower than in Silver Springs (Yount, 1956), in a laboratory stream microcosm steady state (Odum and Hoskin, 1957), or in a study of oil refinery holding ponds (Minter, 1964) (Figure 6). Pond 12 was only slightly above the successional stage described by Odum and Hoskin (1957).

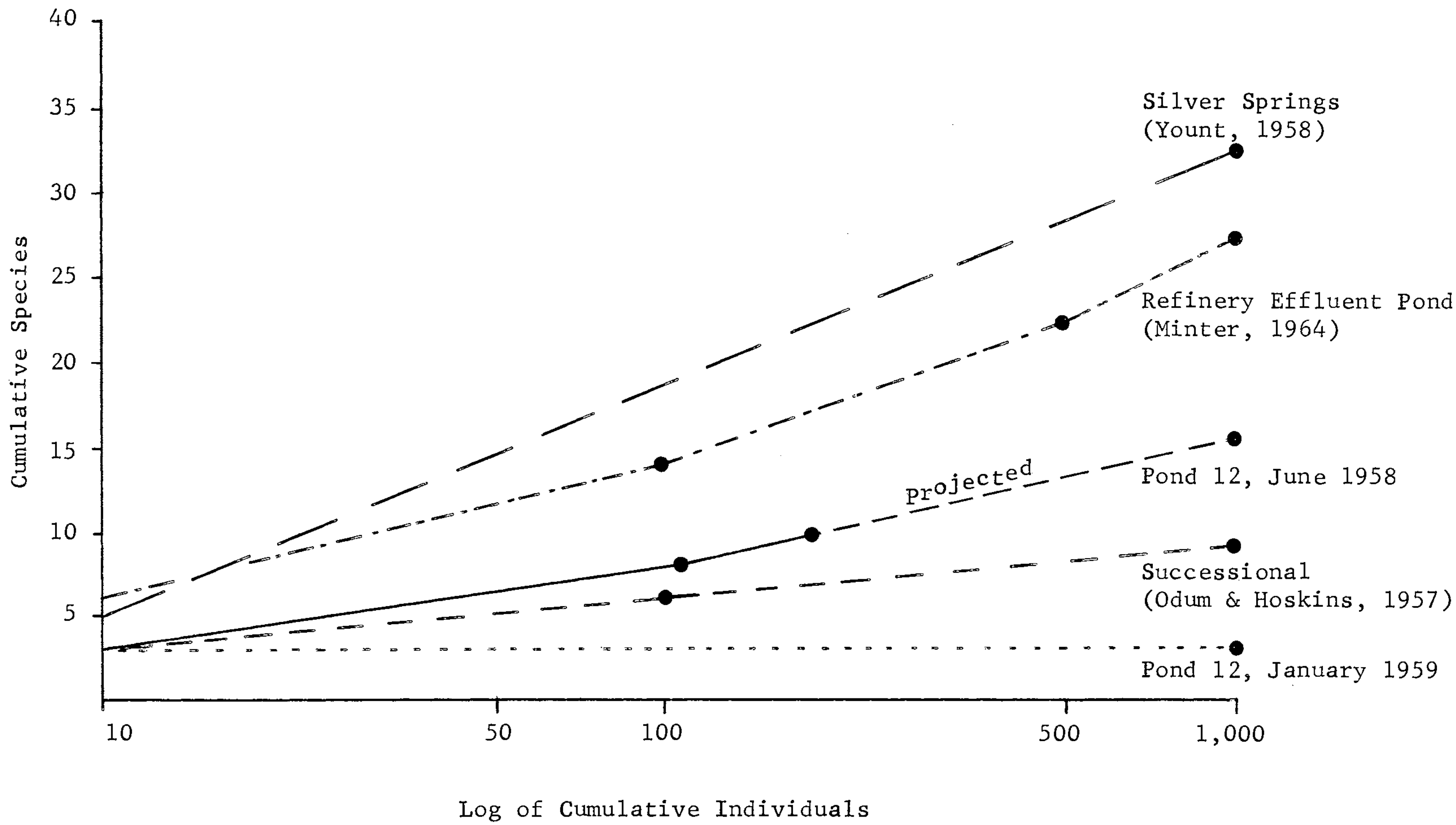


Figure 7. Selected Species-Diversity Curves Compared With Other Plankton Communities

## CHAPTER VI

## STRUCTURE OF THE BACTERIAL COMMUNITY

Rod-shaped bacteria generally were two to four times more numerous than coccus-shaped ones at all times of the year (Table VI). On rare occasions the cocci exceeded the rods in numbers. Numbers of each tended to increase or decrease together.

Total numbers for the year ranged from a high of 236 x $10^8$/liter to a low of 5 x $10^8$/liter (Table VI). Peak numbers of bacteria in summer and fall were two to five times the peak numbers in winter and spring (Figure 2). Peak numbers of bacteria during summer were progressively greater through Pond 7, then lower through Pond 12. Numbers of bacteria during colder months fluctuated more than during warmer months.

During summer and fall in Ponds 3 through 9, numbers of algae decreased but bacteria increased (Figure 2). Algal and bacterial numbers in Ponds 10 through 12 generally increased and decreased together. Pond 2 differed from the others, probably because of higher toxicity and a generally lower stage of stabilization. The numbers of algae and bacteria increased or decreased similarly in all ponds during winter and spring. Changes in algal numbers were usually followed by changes in numbers of bacteria.

The process of waste stabilization probably depends on bacterial decomposition of organic compounds found in effluents. Dorris, et al.,

TABLE VI

BACTERIAL POPULATIONS
(Estimated numbers/liter x $10^8$)

| | JUN. | JUL. | AUG. | SEP. | OCT. | NOV. | DEC. | JAN. | FEB. | MAR. | APR. | MAY | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| STA. 1 | | | | | | | | | | | | | |
| Rod | 6 | 10 | 1 | 8 | 40 | 2 | 13 | 6 | 1 | 5 | 4 | 23 | 119 |
| Coccus | 3 | 16 | 4 | 10 | 14 | 3 | 4 | 10 | 9 | 6 | 17 | 6 | 102 |
| Total | 9 | 26 | 5 | 18 | 54 | 5 | 17 | 16 | 10 | 11 | 21 | 9 | 221 |
| STA. 2 | | | | | | | | | | | | | |
| Rod | 44 | 30 | 27 | 7 | 38 | 7 | 32 | 6 | 5 | 7 | 16 | 14 | 233 |
| Coccus | 18 | 5 | 7 | 2 | 16 | 4 | 25 | 6 | 6 | 5 | 1 | 15 | 110 |
| Total | 62 | 35 | 34 | 9 | 54 | 11 | 57 | 12 | 11 | 12 | 17 | 29 | 343 |
| STA. 3 | | | | | | | | | | | | | |
| Rod | 31 | 52 | 59 | 43 | 49 | 47 | 18 | 6 | 8 | 14 | 18 | 45 | 390 |
| Coccus | 16 | 8 | 14 | 16 | 12 | 16 | 6 | 7 | 4 | 10 | 11 | 16 | 136 |
| Total | 47 | 60 | 73 | 59 | 61 | 63 | 24 | 13 | 12 | 24 | 29 | 61 | 526 |
| STA. 4 | | | | | | | | | | | | | |
| Rod | 24 | 48 | 75 | 58 | 42 | 23 | 24 | 16 | 31 | 16 | 14 | 22 | 393 |
| Coccus | 7 | 6 | 20 | 28 | 11 | 11 | 1 | 14 | 7 | 7 | 4 | 11 | 127 |
| Total | 31 | 54 | 95 | 86 | 53 | 34 | 25 | 30 | 38 | 23 | 18 | 33 | 520 |
| STA. 5 | | | | | | | | | | | | | |
| Rod | 20 | 64 | 130 | 109 | 151 | 11 | 20 | 22 | 14 | 22 | 12 | 31 | 606 |
| Coccus | 10 | 12 | 19 | 70 | 68 | 10 | 6 | 11 | 1 | 10 | 4 | 8 | 229 |
| Total | 30 | 76 | 149 | 179 | 219 | 21 | 26 | 33 | 15 | 32 | 16 | 39 | 835 |

TABLE VI (Continued)

| | JUN. | JUL. | AUG. | SEP. | OCT. | NOV. | DEC. | JAN. | FEB. | MAR. | APR. | MAY | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| STA. 6 | | | | | | | | | | | | | |
| Rod | 27 | 52 | 66 | 75 | 102 | 30 | 15 | 16 | 23 | 23 | 21 | 30 | 480 |
| Coccus | 28 | 4 | 26 | 41 | 17 | 12 | 3 | 50 | 5 | 0 | 12 | 12 | 210 |
| Total | 55 | 56 | 92 | 116 | 119 | 42 | 18 | 66 | 28 | 23 | 33 | 42 | 690 |
| STA. 7 | | | | | | | | | | | | | |
| Rod | 13 | 30 | 63 | 84 | 62 | 40 | 19 | 5 | 39 | 25 | 18 | 19 | 407 |
| Coccus | 5 | 56 | 20 | 152 | 14 | 2 | 3 | 7 | 12 | 18 | 4 | 15 | 308 |
| Total | 18 | 86 | 83 | 236 | 76 | 42 | 22 | 12 | 51 | 43 | 22 | 34 | 715 |
| STA. 8 | | | | | | | | | | | | | |
| Rod | 4 | 86 | 62 | 108 | 101 | 28 | 19 | 7 | 47 | 15 | 24 | 39 | 540 |
| Coccus | 12 | 62 | 20 | 107 | 21 | 4 | 10 | 5 | 12 | 8 | 4 | 18 | 283 |
| Total | 16 | 148 | 82 | 215 | 122 | 32 | 29 | 12 | 59 | 23 | 28 | 57 | 823 |
| STA. 9 | | | | | | | | | | | | | |
| Rod | 28 | 91 | 54 | 78 | 80 | 73 | 53 | 14 | 21 | 11 | 12 | 15 | 530 |
| Coccus | 8 | 9 | 38 | 40 | 27 | 4 | 8 | 33 | 2 | 11 | 5 | 8 | 193 |
| Total | 36 | 100 | 92 | 118 | 107 | 77 | 61 | 47 | 23 | 22 | 17 | 23 | 723 |
| STA. 10 | | | | | | | | | | | | | |
| Rod | 24 | 40 | 109 | 48 | 48 | 32 | 39 | 10 | 12 | 30 | 30 | 28 | 450 |
| Coccus | 4 | 38 | 41 | 32 | 12 | 2 | 15 | 5 | 4 | 13 | 3 | 7 | 176 |
| Total | 28 | 78 | 150 | 80 | 60 | 34 | 54 | 15 | 16 | 43 | 33 | 35 | 626 |

TABLE VI (Continued)

| | JUN. | JUL. | AUG. | SEP. | OCT. | NOV. | DEC. | JAN. | FEB. | MAR. | APR. | MAY | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| STA. 11 | | | | | | | | | | | | | |
| Rod | 44 | 16 | 116 | 56 | 17 | 13 | 50 | 8 | 29 | 31 | 32 | 67 | 479 |
| Coccus | 18 | 12 | 20 | 8 | 14 | 0 | 12 | 6 | 8 | 30 | 10 | 11 | 149 |
| Total | 62 | 28 | 136 | 64 | 31 | 13 | 62 | 14 | 37 | 61 | 42 | 78 | 628 |
| STA. 12 | | | | | | | | | | | | | |
| Rod | 8 | 12 | 90 | 23 | 10 | 30 | 12 | 8 | 34 | 22 | 25 | 47 | 321 |
| Coccus | 4 | 44 | 45 | 28 | 4 | 10 | 0 | 7 | 5 | 14 | 5 | 17 | 183 |
| Total | 12 | 56 | 135 | 51 | 14 | 40 | 12 | 15 | 39 | 36 | 30 | 64 | 504 |

(1963) stated that stabilization appears to be as effective under winter conditions as it is under summer conditions, as measured by reduction of biochemical oxygen demand. However, the greater number of bacteria present during the warm months (Figure 2) could indicate a faster and more complete stabilization of the effluent from June through October. Copeland (1963) theorized that dead cells from the large algal population of summer produced a heavy organic load on the system. Therefore, the decomposition of dead algae by bacteria may have caused the increase in bacteria in early fall.

The timing of an effluent release to a stream is very important (Silva and Papenfuss, 1953). A pond series should contain effluents long enough for the toxicity to decrease. Also, the effluent release should not place a heavy organic load on the receiving stream. Thus, as shown by algal and bacterial numbers (Figure 2) and algal volumes (Figure 4), the effluent was probably held as long as necessary during summer months. But in winter, algal volume and numbers were still increasing in the last ponds, indicating that a large amount of organic matter was still being produced and the effluent should have been held longer in the pond series (Copeland, 1963), or the efficiency of the series during winter should be increased.

## CHAPTER VII

## SUMMARY

1. The structure of the planktonic and bacterial populations of an oil refinery ponding system was determined. Numbers of zooplankton, numbers and biomass of phytoplankton, plankton diversity and numbers of bacteria were analyzed and compared with related studies to yield information on the process of stabilization of oil refinery effluents in ponds.

2. Zooplankton was restricted to summer and fall months and to Ponds 8 through 12. Zooplankton was restricted to five genera of the Phylum Protozoa.

3. Eight of twenty-seven genera of algae were present in significant numbers. Dominant genera were *Synechocystis*, *Chlorococcum*, and *Chlamydomonas*.

4. Maximal numbers of algae were found during early summer, with a peak number of $4,890.5 \times 10^6$ cells/liter. Maximal numbers of cells/liter were $1,284.1 \times 10^6$ during fall, $1,370.9 \times 10^6$ during winter, and $1,005.9 \times 10^6$ during spring. During summer, the last ponds of the series contained low numbers and a variety of genera. In winter and early spring, numbers of algae were lower, and the last ponds of the series contained the largest numbers.

5. Maximal volumes of algae were 312 $mm^3$/liter during summer, 968 $mm^3$/liter during fall, 921 $mm^3$/liter during winter, and 859 $mm^3$/liter

during spring. The maximal volume was found during fall due to the large size of individuals of the algal genera present. Summer and fall peak volumes occurred in Ponds 6 through 9. The peak volumes during winter and spring were in Ponds 10 through 12. A faster rate of stabilization during the summer is indicated.

6. Species-diversity generally increased from pond to pond. Pond 12 was the most diverse, except during the fall when Ponds 7 through 9 were most diverse. Diversity was much lower than in several other plankton communities. The pond with the greatest diversity was considered to be the most stabilized.

7. Numbers of bacteria ranged from $5 \times 10^8$ to $236 \times 10^8$ cells/liter. Rods were more numerous than cocci. Peak numbers of bacteria during summer and fall were 2 to 5 times those of winter and spring. The high numbers of bacteria during summer may have been partially due to the presence of dead algal cells and the additional bacteria required for their decomposition.

8. The structure of plankton and bacterial populations indicate that a high degree of stabilization occurred during summer and fall, but that stabilization was less complete during the winter.

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