Comparative biochemical studies and counts of suspended algae and protozoa in a small Ohio stream give evidence that the effluent from a small sewage treatment plant characteristically causes an increase in certain green flagellates (Euglenophyceae) and the disappearance of the yellow brown flagellates (Chrysophyceae).

Stream Enrichment and Microbiota

By JAMES B. LACKEY, Ph.D.

IT has been well demonstrated that one ultimate effect of sewage or of treated sewage effluents is the fertilization of the receiving stream or body of water (*1–7*). This generalization, however, is seldom based on actual counts of species and of their numbers in the receiving waters. It is usually based on a study of a few kinds of organisms or of a broad classification, such as green flagellates. In the few instances in which the actual numbers and kinds of species occurring below points of waste or sewage admission have been studied (*1, 4, 8, 9*), no companion studies have been undertaken on nearby and somewhat similar waters as controls.

To provide more specific information on the fertilizing effects of a treated sewage effluent, an ecologic study of the suspended microbiota in Lytle Creek, a small stream in southwestern Ohio which receives such an effluent, was begun in the summer of 1944. In this study, the qualitative and quantitative distributions of the suspended algae and protozoa at points selected to reflect the effects of the effluent were determined. For comparison with Lytle Creek, similar studies were made of Cowan Creek, an unfertilized stream in the same area, and of the Santa Fe River, a larger unfertilized stream in Florida. The findings of these studies are presented in the following pages.

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Lytle Creek has been the scene of three earlier reports. Gaufin and Tarzwell (*10*) have described the known invertebrates (exclusive of protozoa); Cooke (*11*) has considered the ecology of the fungi; and Katz and Gaufin (*12*) have discussed the fish. These papers, together with the present one, give an account of the majority of the living organisms in Lytle Creek and provide what is perhaps the most nearly complete story of one stream.

The Ohio Streams

Lytle Creek is about 11 miles long. In the summer of 1944, when it was sampled for this study, it had a flow of about 1 cubic foot per second except after showers. The stream drained a small rural farming section and received the effluent from the sewage treatment plant for Wilmington, Ohio, a town of about 6,000 population (1940 census). The sewage effluent was the only pollution entering the stream. The stream showed a typical oxygen depletion just below the treatment plant outfall, with recovery before it entered Todd Fork about

7 miles away. Typical dissolved oxygen ranges, pH values, and temperatures in Lytle Creek are given in the paper by Gaufin and Tarzwell (*10*) or the one by Cooke (*11*). Although the work reported in this paper antedates theirs, conditions were probably very similar.

Cowan Creek is located in an adjoining watershed and is similar in length and flow. However, its watershed, which is arable, pasture, or wooded land, is sparsely inhabited, and the stream receives no visible pollution. According to samples from one point, biochemical oxygen demand (BOD), the dissolved oxygen, and the nitrate content corresponded roughly to station V on Lytle Creek.

Sampling Procedures

Samples from Lytle Creek were taken at five stations, which had been set up for a study conducted by the Public Health Service. Their locations are shown in figure 1. Station I, at mile 8.7 above the mouth of the creek, was within the city limits of Wilmington. Station II, at mile 7.2, was a short distance below the outfall of the sewage treatment plant. Station III was at mile 5.2, where there was little visible evidence of the sewage effluent, and stations IV and V were at miles 3.2 and 1.0, respectively, where the stream presented a practically normal appearance. Samples from Cowan Creek were taken at only one point, which corresponded in mileage from the mouth to Lytle Creek V.

These two streams were sampled approximately every 2 weeks beginning June 19 and ending August 15. The samples, totaling 33, were brought to the stream pollution investigation station at the Environmental Health Center (now the Robert A. Taft Sanitary Engineering Center), Public Health Service, and there analyzed for kinds and numbers of organisms and certain biochemical data. The sampling period covered the time of low flow and high temperature, when populations of algae and protozoa are normally at their highest. Practically every organism found had been recorded previously from other Ohio River Basin streams.

Results

The BOD values for Lytle and Cowan Creeks are shown in figure 2. The BOD figure of about 4 p.p.m. for Cowan Creek is close to the average for unpolluted creeks of this area that have been sampled. It is not surprising that the BOD value was so high at station II on Lytle Creek, since the dilution of the treatment plant effluent was not large. Perhaps the most sur-

Figure 1. Location of sampling stations in Lytle Creek.

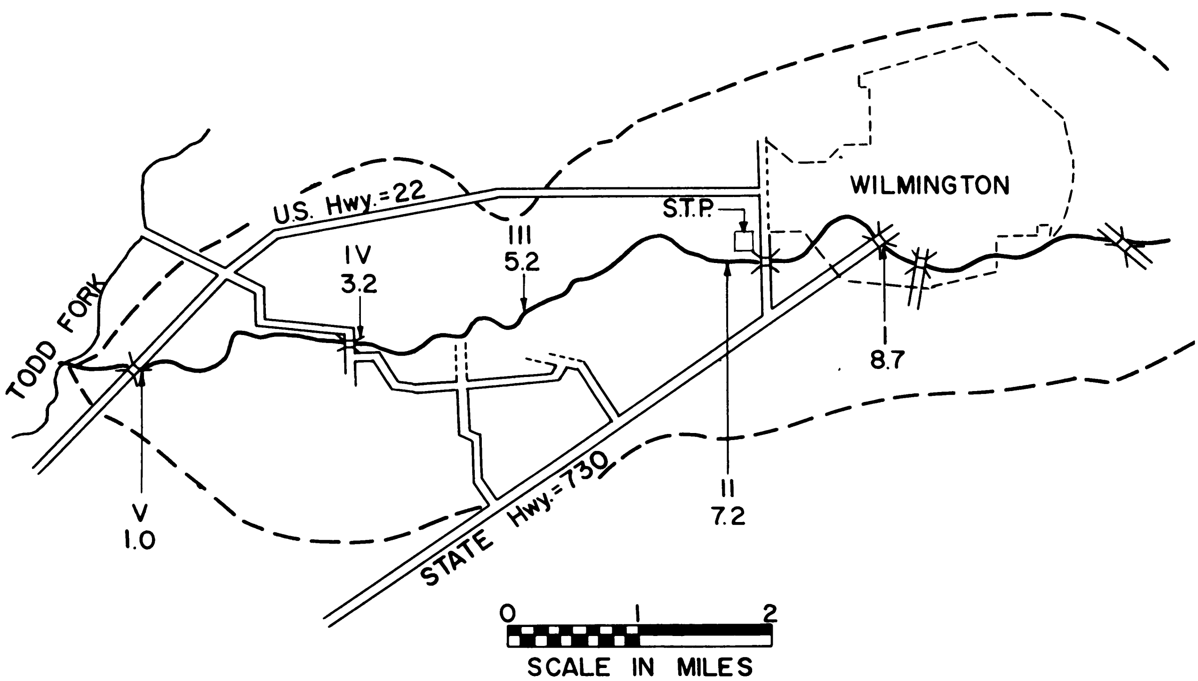
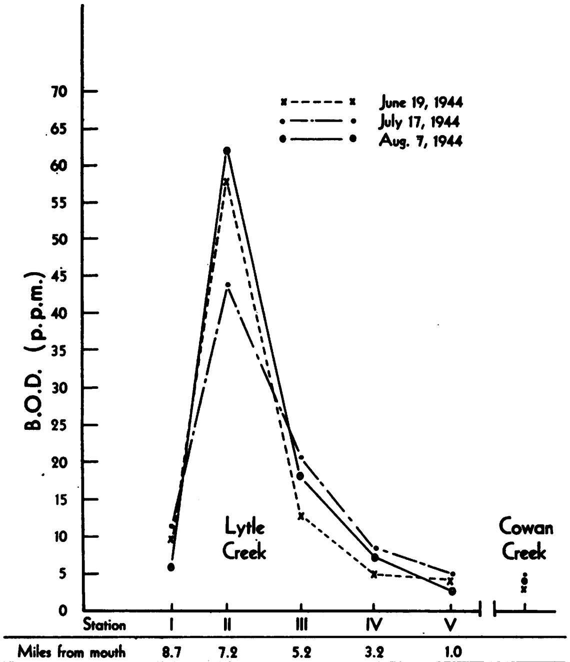


Figure 2. Range in biochemical oxygen demand (BOD) in Lytle and Cowan Creeks, summer of 1944.



prising thing about the BOD values is the rapid decrease at the downstream stations.

The BOD values accord well with the values for nitrates and nitrites, shown in table 1. All three values, decreasing rapidly downstream, argue for large numbers of organisms, both saprophytic and holophytic (or saprozoic and holozoic), moving downstream.

A total of 167 species or genera of algae and protozoa were found in the 27 samples from Lytle Creek. In the 6 samples from the single Cowan Creek station, 92 species or genera were found. Table 2 lists all the species and gives the number of occurrences of each, by station.

Station I on Lytle Creek, the clean water station, had a total of 101 species, which occurred 195 times in the samples analyzed. These should have provided an excellent seeding for the downstream stations, if they were able to pass through the short zone of oxygen depletion, or possibly toxic zone, just below the treatment plant outfall. Forty-three of these species were found at station II. All of the Chrysophyceae and most of the Cryptophyceae, the Volvocales, and the diatoms (Bacillarieae) were killed in the zone of pollution, but the remaining groups, which recovered only slightly in species, recovered surprisingly in numbers of organisms.

The number of species found at station I in Lytle Creek was never again equaled. The only group showing a downstream increase in species was the green Euglenophyceae. Station V, the lowest on Lytle Creek, showed 20 fewer species than the comparable Cowan Creek station, which had 9 fewer species than the uppermost Lytle Creek station. Eleven of the twelve species of Chrysophyceae found in Cowan Creek were found not at all in Lytle Creek after station I: Evidently this group will not stand recent sewage pollution. Even some of the blue-green algae disappeared. Other groups adversely affected at stations II, III, and IV were the Cryptophyceae, the Dinoflagellata, the Volvocales, the Chlorophyceae (which die rather slowly in polluted water), and the diatoms.

However, the downstream decrease in number of species was compensated for by the increase in number of organisms. If 500 organisms of one species per milliliter is accepted as a bloom value (*13*), there were from 2 to 6 blooms at the downstream stations as compared with one bloom at station I and no blooms at the Cowan Creek station:

Blooms in Lytle Creek

	I	II	III	IV	V
Chromatium sp	----	------	----	648	-------
Cryptomonas erosa	888	------	----	------	512; 1,100; 15,040
Navicula spp	----	800; 1,120	660; 680; 568; 800	2,640	512
Trachelomonas urceolata	----	------	----	------	640
Euglena sp	----	784; 504	----	------	5,760

Table 1. Nitrate and nitrite nitrogen at six sampling points in Lytle and Cowan Creeks, July 24, 1944

Station	Nitrate nitrogen (p.p.m.)	Nitrite nitrogen (p.p.m.)
Lytle Creek:		
I	0.10	0.01
II	.44	.02
III	.80	.08
IV	.08	trace
V	.08	trace
Cowan Creek	.04	trace

Table 2. Micro-organisms found in Lytle and Cowan Creeks in the summer of 1944: number of occurrences of each species at each station sampled

Genus and species	Lytle Creek					Cowan Creek
	I	II	III	IV	V	
SCHIZOMYCETES						
Beggiatoa alba	1	---	---	4	---	---
Blastocaulis sp	---	---	---	---	1	---
Chromatium sp	---	---	1	3	---	---
Sphaerotilus natans	---	1	---	1	---	---
Spirillum spp	1	---	---	1	2	---
Spirochaeta sp	1	---	1	---	---	---
MYXOPHYCEAE						
Aphanocapsa sp	---	1	---	---	---	---
Chroococcus turgidus	2	---	---	---	---	---
Lyngbya sp	1	---	1	2	1	---
Merismopedia elegans	2	2	---	---	---	1
Merismopedia glauca	2	---	---	---	1	---
Oscillatoria spp	3	3	5	4	3	3
Phormidium sp	2	---	---	---	---	---
CRYPTOPHYCEAE						
Chilomonas paramecium	1	---	---	---	1	---
Chroomonas spp	3	1	2	---	2	4
Cryptomonas ovata	---	---	---	---	1	1
Cryptomonas spp. (including *erosa*)	6	5	3	1	5	6
Cyathomonas truncata	2	---	---	---	---	1
Rhodomonas lacustris	2	---	2	---	---	3
CHRYSOPHYCEAE						
Chromulina globosa	1	---	---	---	---	3
Chromulina ovalis	2	---	---	---	---	3
Chromulina pascheri	---	---	---	---	---	1
Chrysapsis sagene	---	---	---	---	---	2
Chrysococcus asper	---	---	---	---	---	2
Chrysococcus ovalis	---	---	---	---	---	2
Chrysococcus rufescens	---	---	---	---	---	2
Chrysococcus spirale	---	---	---	---	---	2
Dinobryon sertularia	---	---	---	---	---	3
Mallomonas tonsurata	---	1	---	---	---	1
Mallomonas sp	---	---	---	---	---	1
Rhizochrysis scherffeli	---	---	---	---	---	1
DINOFLAGELLATA						
Gymnodinium gracilis	1	---	---	---	---	2
Gymnodinium sp	---	---	---	---	2	---
Peridinium tabulatum	---	---	---	---	---	1
VOLVOCALES						
Brachiomonas sp	---	---	---	1	---	---
Carteria elongata	1	---	---	---	---	---
Cephalomonas granulata	3	---	---	---	1	1
Chlamydomonas spp	6	6	5	5	4	5
Chlorogonium minimum	---	---	---	---	---	1
Gonium pectorale	---	---	---	---	---	1
Heteromastix angulosa	2	2	1	1	1	1
Lobomonas rostrata	---	---	---	---	---	1
Pandorina morum	---	---	---	---	---	1
Pedinomonas rotunda	1	---	---	---	---	---
Phacotus angulosa	---	---	1	---	---	4
Phacotus lenticularis	4	---	---	1	1	1
Pyramidomonas inconstans	2	---	---	1	1	3
Scherffelia phacus	---	---	---	---	1	---
Spermatozopsis exultans	---	---	---	---	---	4
Spondylomorum quaternarium	---	1	---	4	---	---
Thoracomonas sp	---	---	---	---	---	2
VOLVOCALES—Continued						
Collodictyon triciliatum	2	4	3	---	2	1
Polytoma uvella	---	1	---	4	---	---
BACILLARIEAE						
Achnanthes coarctata	4	---	1	---	---	---
Cocconeis placentula	3	3	1	1	1	---
Cyclotella meneghiniana	6	5	2	1	1	3
Cymbella sp	3	---	---	---	1	2
Diploneis sp	---	---	1	---	---	---
Eunotia sp	2	1	---	---	---	2
Fragilaria crotonensis	---	---	---	---	---	1
Fragilaria sp	1	---	---	1	---	1
Gomphonema olivaceum	---	1	2	---	---	1
Gyrosigma sp	1	1	---	---	---	5
Melosira granulata	1	---	---	---	---	---
Melosira varians	---	---	---	---	---	1
Navicula spp	6	6	5	3	5	6
Nitzschia closterium	1	---	---	2	1	2
Nitzschia sigmoidea	1	---	---	---	1	1
Pinnularia sp	1	---	2	1	2	---
Rhizosolenia eriensis	---	---	---	---	---	1
Rhoicosphenia sp	---	1	---	---	---	---
Surirella sp	---	1	---	1	---	2
Synedra acus	---	---	---	---	---	2
Synedra biceps	1	---	---	---	---	1
Synedra ulna	1	1	2	---	2	3
Synedra sp	2	1	2	2	---	3
EUGLENOPHYCEAE (green)						
Cryptoglena pigra	---	1	1	1	1	---
Euglena acus	3	6	4	1	3	3
Euglena agilis	---	1	1	1	1	1
Euglena anabaena	1	1	1	1	---	---
Euglena deses	---	3	5	3	1	1
Euglena ehrenbergii	---	3	---	---	1	---
Euglena fusca	1	1	---	1	---	---
Euglena gracilis	---	---	1	1	2	---
Euglena granulata	1	3	1	2	1	---
Euglena oxyuris	1	4	3	---	2	---
Euglena pisciformis	2	4	4	3	4	1
Euglena polymorpha	2	5	3	3	3	1
Euglena quartana	---	---	---	4	---	---
Euglena sanguinea	---	1	1	---	---	---
Euglena sciotensis	1	2	1	1	1	2
Euglena spirogyra	---	---	1	---	1	1
Euglena tripteris	---	1	---	---	---	---
Euglena viridis	4	6	2	4	3	1
Euglena sp	4	6	5	4	5	2
Lepocinclis marssoni	1	4	2	1	1	2
Lepocinclis ovum	2	5	3	1	3	3
Lepocinclis steinii	---	1	---	---	---	---
Lepocinclis texta	---	3	2	---	---	---
Phacus anacoleus	---	2	2	1	1	---
Phacus brevicauda	---	3	1	---	---	---
Phacus longicauda	---	---	---	---	1	---
Phacus pleuronectes	---	2	3	3	---	1
Phacus pyrum	3	3	3	2	1	---
Phacus stokesi	---	3	2	---	---	---
Phacus suecica	---	1	---	---	---	1
Phacus triqueter	---	2	2	1	---	---
Phacus sp	---	---	1	---	---	---
Trachelomonas crebea	---	---	---	---	---	2
Trachelomonas hispida	---	1	---	---	---	1

Table 2. Micro-organisms found in Lytle and Cowan Creeks in the summer of 1944: number of occurrences of each species at each station sampled—Continued

Genus and species	Lytle Creek					Cowan Creek
	I	II	III	IV	V	
EUGLENOPHYCEAE—Con. (green)						
Trachelomonas stokesi	---	---	1	1	2	---
Trachelomonas teres	1	---	---	---	---	3
Trachelomonas urceolata	1	5	1	1	5	2
Trachelomonas volvocina	2	1	1	1	3	3
EUGLENOPHYCEAE (colorless)						
Anisonema ovale	1	---	---	---	1	---
Astasia klebsii	---	1	---	3	1	---
Copromonas subtilis	---	---	---	1	---	---
Distigma proteus	1	---	---	---	---	---
Entosiphon sulcatum	2	---	---	---	---	---
Menoidium incurvum	1	1	1	2	---	---
Metanema sp	1	---	---	---	---	---
Notosolenus apocamptus	---	---	1	---	---	---
Peranema trichophorum	1	1	1	---	---	1
Petalomonas angusta	---	---	---	---	1	---
Petalomonas carinata	---	---	---	---	1	---
Sphenomonas quadrangularis	---	3	4	1	---	---
CHLOROPHYCEAE						
Actinastrum gracillmum	---	---	---	---	---	2
Ankistrodesmus falcatus	---	3	---	---	---	2
Ankistrodesmus convolutus	---	---	---	---	1	---
Ankistrodesmus mirabile	4	4	1	---	2	4
Ankistrodesmus tumidus	2	2	1	---	1	---
Chlorella spp	---	1	2	---	---	---
Closterium sp	1	---	1	---	---	---
Coelastrum microporum	2	---	---	---	2	1
Coelastrum reticulatum	1	---	---	---	---	1
Cosmarium sp	4	2	---	---	---	1
Desmatractum sp	---	---	---	---	---	1
Kirchneriella lunaris	2	---	1	---	---	---
Lagerheimia chodati	1	---	---	---	---	1
Micractinium pusillum	---	1	---	---	---	---
Oocystis lacustris	2	1	1	---	1	---
Pediastrum duplex	1	---	---	---	---	---
Pediastrum boryanum	3	---	1	---	---	---
Pediastrum tetras	3	---	---	---	---	---
Schizochlamys gelatinosa	1	---	---	---	---	---
Schroederia setigera	---	1	---	---	---	---
Selenastrum gracile	---	1	---	---	---	---
Scenedesmus spp	4	3	---	---	2	3
Tetradesmus wisconsinensis	1	---	---	---	---	---
Tatraedron minutum	1	---	---	---	---	1
Tatraedron muticum	---	---	---	---	1	---
Tetrallantos lagerheimii	1	---	---	---	---	---
Treubaria triappendiculata	---	---	---	---	---	1
Westella botryoides	1	1	---	---	1	---
Unidentified green cells	---	---	1	---	---	---
CILIATA						
Balanitozoon agilis	---	---	---	---	1	---
Chilodonella cucullulus	2	---	---	1	1	---
Cinetochilum margaritaceum	3	---	---	---	---	---
Coleps hirtus	---	2	2	1	---	---
Colpidium colpoda	---	1	---	---	---	---
Cyclidium glaucoma	2	3	3	1	1	2
Cyclidium spp	---	1	---	---	1	---
Glaucoma pyriformis	---	1	---	---	---	---
Halteria grandinella	---	1	---	---	2	---
Holophrya viridis	---	---	---	1	---	---
Lembadion bullinum	---	---	---	---	---	1
Lionotus fasciola	1	---	---	---	---	---
Microthorax sulcatus	3	---	---	---	---	---
Pleuronema chrysalis	---	---	---	---	1	---
Strobilidium sp	1	---	---	---	2	2
Trachelocerca phoenicopterus	1	---	---	---	---	---
Uronema marina	---	---	---	1	---	---
Urotricha farcta	2	3	1	1	2	2
Vorticella spp	1	---	2	1	---	---
RHIZOPODA						
Actinophrys sol	1	---	---	---	---	---
Amoeba vespertilio	---	1	---	---	---	---
Amoebulae	---	---	---	---	1	---
Hartmanella hyalina	---	---	---	3	---	---
Microgromia sp	---	---	1	---	---	---
Nuclearia dilicatula	2	---	---	---	---	---
Rhapidiophrys elegans	1	---	---	1	---	---
Rhapidiophrys pallida	2	---	---	1	---	1
Vahlkampfia albida	---	1	---	---	---	---
Vahlkampfia limax	---	---	---	---	1	---
Vampyrella sp	1	---	---	---	---	---
MASTIGOPHORA						
Bodo caudatus	---	1	---	2	---	---
Bodo pulcher [1]	1	2	2	1	1	---
Dinomonas vorax	---	---	---	---	---	2
Oicomonas socialis	1	---	---	---	---	1
Oicomonas termo	2	1	2	1	2	1
Phyllomitus amylophagus	---	1	---	---	---	---
Physomonas vestita	1	1	---	---	---	1
Pleuromonas jaculans	2	---	---	---	---	---
Pteridomonas pulex	---	---	---	---	---	1
Spiromonas angusta	1	---	---	---	---	---
Unidentified colorless flagellates	4	2	2	2	2	3
Total number of species or genera	101	82	68	63	72	92

[1] Provisional name only.

The numbers of blooms at the downstream stations in comparison with the numbers at station I and in Cowan Creek are one evidence of enrichment. Further evidence is afforded by a comparison of the total number of organisms at each station. The numbers of organisms at stations II through V were much greater than the numbers at station I and in Cowan Creek, as shown in table 3. It should be noted, too, that only 5 samples were analyzed for each of the lower three stations, as compared with 6 for each of the others.

Actually, the fertilization of the downstream waters is apparent, on the basis of a marked increase in the number of organisms, for only a few groups. The blue-green algae were up sharply at station III, but they declined thereafter. The Cryptophyceae first dropped sharply, then rose to high numbers at station V. This pattern is a common occurrence for the Cryptophyceae. They apparently are favorably influenced by recent fertilization, but they seem to avoid high BOD values. The same is true of the small colorless flagellates, whose behavior in a stream seems to differ from their behavior in a sewage treatment plant. This difference, however, may be a sampling fault, since most of these organisms occur on or near the bottom.

Some of the data in table 3 are very difficult to explain. For example, the number of ciliates dropped steadily until station V, where there was suddenly a fourfold increase. This increase, however, was due almost entirely to *Balanitozoon agilis* and *Urotricha farcta*, two related ciliates whose food is largely unknown. These might have been feeding on some small bacteria that develop late in the cycle of organic degradation. Just how far we are from being able to foretell, or account for, the presence of a given organism in a stream is emphasized in a recent paper by Wuhrmann (*14*). He showed an inability to produce a given biota in effluents similar as to BOD, oxygen consumed, nitrate content, and so on. He concluded that there were still unknown organic substances present that determine the nature of the biota.

The Euglenophyceae, however, clearly demonstrate the effects of stream enrichment. They were the largest group in number of species at each station, but they were low in number of organisms at the Cowan Creek station and Lytle Creek I. At Lytle Creek V they were more abundant than any other group, except for the single bloom of *Cryptomonas erosa* that occurred there. The Euglenophyceae showed substantial increases at stations II, III, IV, and V, and they were the most abundant group at station II. At station III, only diatoms and small green cells (*Chlorella*) outnumbered them; at station IV, only diatoms.

Use of the whole group of Euglenophyceae as indicators of pollution or of recent pollution has been questioned (*14*).

In the present study, the genera *Cryptoglena*, *Euglena*, *Lepocinclis*, and *Phacus* were found to be well represented in the enriched or recently polluted water; and many of the species not only tolerated the condition, they multiplied in it. Most of the species of these four genera that

Table 3. Total number of organisms[1] in all samples by station

Group	Lytle Creek I (6 S)	Lytle Creek II (6 S)	Lytle Creek III (5 S)	Lytle Creek IV (5 S)	Lytle Creek V (5 S)	Cowan Creek (6 S)
Schizomycetes	92	224	4	1, 337	7	0
Myxophyceae	174	148	263	162	108	30
Chrysophyceae	16	0	0	0	1	1, 742
Cryptophyceae	1, 029	441	31	168	16, 778	434
Bacillarieae	1, 370	3, 217	3, 329	2, 690	797	1, 198
Volvocales	817	860	668	733	607	561
Euglenophyceae (green)	306	3, 633	1, 436	2, 617	10, 539	161
Euglenophyceae (colorless)	10	16	41	16	17	11
Chlorophyceae	664	723	7, 086	0	5, 253	212
Ciliata	46	35	10	9	194	20
Rhizopoda	20	6	1	23	17	3
Mastigophora	764	140	124	197	7, 830	122
Total	4, 925	9, 443	11, 845	8, 071	42, 287	4, 494

S=Samples.

[1] An organism in this paper usually means a single cell. Exceptions include filaments whose cells are distinguished with difficulty (such as *Beggiatoa* and *Lyngbya*) and some colonies (such as *Aphanocapsa*, *Spondylomorum*, and *Coelastrum*).

Table 4. Total number of Trachelomonas organisms in all samples, by station

Species	Lytle Creek					Cowan Creek
	I	II	III	IV	V	
Trachelomonas crebea	0	0	0	0	0	7
Trachelomonas hispida	0	2	0	0	0	1
Trachelomonas stokesii	0	0	1	1	96	0
Trachelomonas teres	1	0	0	0	0	36
Trachelomonas urceolata	2	364	8	32	1,092	54
Trachelomonas volvocina	36	2	8	8	648	12
Total	39	368	17	41	1,836	110

were found occurred at or below station II in Lytle Creek, and most of the occurrences of these genera were in the polluted or recovery areas.

The genus *Trachelomonas* offers a different story. It was represented by only 6 of its many species, and only 1 of these 6, *urceolata*, increased markedly in the area of pollution, as shown in table 4. This is in decided contrast to the genus *Euglena*, which was represented by 18 species. Table 5 shows the behavior of the nine most common of these. All achieved substantial to large increases at stations II, III, and IV. All except *Euglena quartana*, which is a saprophyte, were present either in Cowan Creek or Lytle Creek I but in very small numbers.

Actually, then, the occurrence of many of the Euglenophyceae was favored by existing or recent sewage pollution, and there were a few species, such as *Euglena acus*, *E. agilis*, *E. pisciformis*, *E. polymorpha*, *E. gracilis*, and *E. quartana*, *Lepocinclis ovum*, and *Trachelomonas urceolata*, which showed heavy increases as a result of such pollution. These same species may bloom for other reasons, of course.

A few other organisms, such as *Oicomonas termo* and *Chlorella* spp., certain chlamydomonads, and naviculoid diatoms, behaved in the same manner. On the whole, however, it is easier to list the organisms that died as a result of the pollution. Here special emphasis would be on the Chrysophyceae or the Chlorophyceae. Perhaps analysis of a much larger number of samples would show some additional species to be favorably influenced by the pollution.

One species not identified in samples from other Ohio Valley streams was found in this study. This was *Cephalomonas granulosa*, one of the Volvocales, which is apparently rare. It occurred in Cowan Creek once, at Lytle Creek I three times, and at Lytle Creek V once. No significance can be attached to these occurrences, although there were 216 organisms per milliliter in Lytle Creek I in one sample.

Comparison With a Florida Stream

It may be argued that the numbers of organisms in Lytle Creek are not unusual and therefore do not support the idea that heavy growths follow enrichment. Cowan Creek, which was used as a control, was fairly similar to Lytle Creek chemically and biologically. For a comparison with a stream having different characteristics, the Santa Fe River in north central Florida was selected.

No data on BOD, nitrates, nitrites, or phosphorus for the Santa Fe River are available. However, it received virtually no sewage or industrial pollution and probably little agricultural drainage. The Santa Fe differs from Ohio Valley streams in that it is a brown-water (tannic and perhaps humic acid) stream with a pH tending toward acidity.

The Santa Fe River and two small lakes that contribute to the headwaters of the river were routinely sampled in 1953–54. A total of 81 samples from six points in the river and one point in each of the lakes were analyzed for kinds and numbers of organisms. In these 81 samples, 332 species or genera of algae and protozoa were found. Roughly, this is two times as many species in three times as many samples as were found in Lytle Creek. The groups of organisms found in the Santa Fe system, by station, are shown in table 6. It is evident from this table and from table 2 that routine sampling of any body of water of fair size will reveal a large variety of algae and protozoa, unless there is some special restrictive reason such as extreme pollution.

At one river station, Mikeville, cattle used the small slough-like branch of the river proper, and this water was at times polluted. The pollution was evidently mild, however. Forty-three of the forty-seven observed species of Euglenophyceae occurred at this station, but none of them ever attained bloom proportions.

There were only three blooms in the river during the time it was studied. All three were at Mikeville: one of a species of *Gymnodinium*, one of *Ankistrodesmus falcatus*, and one of the minute green *Chlorella.* Hampton Lake had three blooms, and Sante Fe Lake had eight. Of the latter, four were late summer blooms of blue-green algae, quite in keeping with lake behavior. The bloom organisms did not enter the river to any extent because the very small amount of water draining from the lakes passes through marshy, grass-grown channels.

The Santa Fe, then, is a largely unpolluted stream that is rich in kinds of algae and pro-

Table 5. Total number of certain Euglena organisms in all samples, by station

Species	Lytle Creek					Cowan Creek
	I	II	III	IV	V	
Euglena acus	29	445	307	520	648	13
Euglena agilis	0	24	4	1	64	1
Euglena deses	0	4	22	13	8	1
Euglena pisciformis	82	60	461	279	85	1
Euglena polymorpha	24	102	40	145	532	1
Euglena quartana	0	0	0	709	0	0
Euglena sciotensis	2	42	2	36	16	3
Euglena viridis	14	260	5	256	297	1
Other species (mostly *Euglena gracilis*)	25	1, 904	115	620	6, 530	25
Total	176	2, 841	1, 356	2, 559	8, 180	46

Table 6. Number of species in principal groups of algae and protozoa occurring in 81 samples from the Santa Fe River system, Fla., 1953–54

Group	Santa Fe Lake (12 S)	Hampton Lake (5 S)	Waldo (12 S)	Worthington Springs (10 S)	Mikeville (9 S)	Oleno (13 S)	High Springs (11 S)	Bell (9 S)
Schizomycetes					1	1		
Myxophyceae	11	6	4	4	13	6	5	6
Chlorophyceae:								
Volvocales	3	3	1	3	13	6	3	7
Other	30	25	7	10	53	13	4	13
Xanthophyceae	1	2	1	0	5	1	0	1
Chrysophyceae	6	4	5	5	5	4	3	1
Cryptophyceae	3	2	5	3	5	2	3	3
Dinoflagellata	7	9	1	5	8	5	2	3
Euglenophyceae	4	1	0	12	43	15	12	4
Bacillarieae	16	7	11	22	16	25	21	21
Mastigophora	4	2	6	1	13	3	7	2
Rhizopoda	3	1	7	7	17	3	5	5
Ciliata	17	3	8	7	17	6	8	6
Total species	105	65	56	79	219	90	73	72

S=samples.

tozoa, but poor in numbers of organisms. It contained many organisms common to Lytle Creek, and the increase in kind and number of organisms at the Mikeville station indicates that it might well exhibit blooming if well fertilized. These observations strengthen the idea that plentiful enrichment of a stream causes a great increase in organisms, and also that the kind of bloom is a function of the type of enrichment. At Mikeville, where the water was muddied by cattle and polluted by their droppings, a sharp and heavy increase in Euglenophyceae occurred. This fact and the Lytle Creek study both indicate that some Euglenophyceae increase as a result of fecal pollution.

Bloom Potentials

A plentiful supply of the proper nutrients is certainly essential for blooming. That the nature of the nutrient material determines both the strength and nature of blooming has been indicated by a number of observations. In the laboratory at the University of Florida, for example, commercial fertilizer has been repeatedly added to concrete tanks that are filled with water from a small brook. The brook is spring-fed but it contains a varied plankton, including Euglenophyceae. These tanks develop heavy blooms—of small Chlorophyceae. A few *Euglena*, *Phacus*, and *Lepocinclis* organisms occur in the bloom, but their numbers are always small. As another example, never in any pond I have observed has the addition of commercial fertilizer produced a bloom of *Euglena sanguinea;* but when similar ponds are invaded by cattle this organism frequently blooms heavily.

In addition to the proper nutrients, the proper seed must be present. Few protozoa or algae, however rare they may be, seem likely to be absent in most environments. There are broad limits—acid water vs. hard; low salinity vs. high—for certain species or groups. *Gonyostomum semen* is practically never found in hard water, and *Gymnodinium brevis* has never been recorded from any part of the world other than the Gulf of Mexico off the Florida coast and Trinidad, B. W. I. (*16*). Instances such as these are rare, however, and probably would be greatly decreased by additional and much more critical observation in many parts of the world. In almost every instance in which a single group of organisms has been studied extensively in a particular geographic area, investigators have found most of the known species of that group (within their broad ecologic limits). A recent example of this is the study by Decloitre (*17*) of the thecate rhizopods in French Equatorial Africa. He found most of the known species. He recognized climate as a barrier for some species, but also stated: "The intertropical zone is little known as a whole; it is very probable that a certain number of these species will be found, sooner or later, in this climatic zone and will be recognized as ubiquitous."

It is unwise to state that a micro-organism species—that is, the seed—is absent from a given environment. One reason is that the environment may not have been adequately sampled; another is that the sample may not have been completely analyzed. It is difficult to examine completely even a single drop of water in a sample. Many workers make one examination, then set the slide aside in a moist chamber to reexamine later. The question inevitably arises as to what size sample must be analyzed to yield a given species. Or otherwise stated, what is the chance of finding a given species in a random sample?

Fisher, Corbett, and Williams (*18*) consider that the majority of species are rare, only a few being common. Therefore, the species in a biological group would not be equally abundant, even though an environment might be sampled a number of times under uniform conditions. This is equivalent to stating that conditions in the habitat sampled were optimal or nearly so for the "common" species, but only within the range of tolerance for those that occurred less frequently. I do not recall ever having seen *Trachelomonas reticulata* but once, despite having examined thousands of samples of foul water, presumably its preferred habitat. This one occasion was a sample from a tree hole, and it contained a dense population of this species.

No answer has been evolved thus far as to approximating the maximum probability of finding a given species. It may reside in knowing the preferred habitat for the species, then sampling as near to it as possible. The range of tolerance should be known, too. That some organisms exhibit a wide range is shown by the studies of Lytle Creek and the Santa Fe River. Lytle Creek is a hard-water stream, well fertilized, in a temperate zone. The Santa Fe is a larger, soft-water stream, with little fertilization and probably much tannic and humic acid, located in a subtropical environment. Yet these streams had 99 species in common. In addition, many of the species occurring in only one of these streams have been found in habitats near the other stream. More complete analyses, or perhaps more numerous ones, would probably reduce still further the species found in only one or the other area. The question, then, is what peculiar conditions give rise to an abundance of a given species.

Such considerations as these emphasize the importance to the ecologist of careful chemical and physical studies of a habitat. Perhaps we may yet be able to say with certainty that, since an environment presents certain characteristics, we can expect to find certain species there.

Summary and Conclusions

In comparative studies of three streams in the United States, quantitative and qualitative determinations of the suspended algae and protozoa provided specific evidence of the fertilizing effects of a treated sewage effluent on some species of these organisms.

In Lytle Creek, a small stream in southwestern Ohio which receives effluent from a primary sewage treatment plant, a total of 167 species were found. Certain species of Euglenophyceae were exceptionally abundant at points below the plant outfall. Chlorophyceae and Chrysophyceae were adversely affected by the effluent.

Of 92 species of microbiota in Cowan Creek, a similar but unfertilized stream in the same area, only Chrysophyceae and diatoms (Bacillarieae) were abundant.

In the Santa Fe River, a larger unfertilized stream in Florida, 332 species were found, but none of them occurred in large numbers.

Species common to both the Ohio and Florida waters totaled 99, indicating a wide environmental tolerance for these species.

The more intensively a given environment is sampled, the greater is the possibility of finding a given species therein, provided the environment falls within its range of tolerance. The environmental ranges of many micro-organisms are wide enough for the organisms to be termed ubiquitous. But such organisms may reproduce rapidly enough to form blooms only within a narrow range the critical factor, or combination of factors, of which rarely occurs. Recent fecal pollution appears to be one such factor for certain species of Euglenophyceae. The same environment appears to be limiting for some species of Chrysophyceae.

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A list of the micro-organisms found in the Santa Fe River may be obtained from the author.

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Air Pollution Research

Five Federal agencies have been awarded contracts for community air pollution research in the Public Health Service air pollution program, for use during fiscal year 1956.

The Weather Bureau, Department of Commerce, allocated $196,000, is studying the dilution and dispersal of contaminants in the atmosphere. The assignment includes devising ways of surveying problem areas, evaluating existing weather data to determine air pollution potentialities, and predicting weather conditions that may intensify air pollution.

The National Bureau of Standards, Department of Commerce, has been allocated $98,000 for developing methods of analyzing and identifying various gaseous contaminants of the atmosphere. This bureau is exploring ways of collecting and treating condensable pollutants and of analyzing concentrated samples of the atmosphere. It is also studying reactions among gases and other chemicals in the air.

The Bureau of Mines, Department of the Interior, also allocated $98,000, is investigating causes of inadequate incineration of combustible wastes and means of improving incineration. It will study sulfur dioxide removal processes and evaluate elements from internal combustion in engines which may contribute to air pollution. It will also sample a limited selection of stack effluents.

Additional agreements with other Federal agencies include one with the Library of Congress for the preparation of a continuing annotated air pollution bibliography and one with the Department of Agriculture for the assignment of a plant physiologist to the program. The physiologist will initiate investigation of the use of plants as air pollution indicators and assist in coordinating the air pollution activities of the Service and the Department of Agriculture.

Contracts for research to be conducted by non-Federal agencies were consummated with the following: Baylor University for a preliminary study to determine the feasibility of using tissue enzymes to evaluate the toxicity of air pollutants ($34,000); the University of Nebraska for a study to determine the feasibility of using tissue culture to evaluate the toxicity of air pollutants ($31,000); the State College of Washington for the development of an automatic air pollution sampling and recording instrument ($17,590); and the Franklin Institute for a study of the feasibility of collecting and storing air samples by compressing atmospheric air and storing it in suitable containers for subsequent chemical or physical analysis of vapor-phase contaminants ($16,064). Other contracts for research are being negotiated.