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Stream Pollution

AQUATIC MACRO-INVERTEBRATE COMMUNITIES AS INDICATORS OF ORGANIC POLLUTION IN LYTLE CREEK

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Year-round field studies of the composition and ecology of stream communities associated with the purification of organic wastes in streams were initiated on Lytle Creek in October, 1949. This creek, which is located about 45 miles northeast of Cincinnati, Ohio, is a tributary of the Little Miami River. Its drainage basin comprises 27 sq. mi., a third of which is contained within the city limits of Wilmington, Ohio, a city of some 7,412 persons.

Lytle Creek is a permanent stream, approximately 11 miles long and has an average gradient of 25 ft. per mile. It is 3 to 35 ft. wide during low water stages and varies in depth from a few inches in the riffles to more than 6 ft. in a few pools. The principal natural source of water in the stream is surface drainage. This flow is augmented, however, at a point about 7.3 miles above the mouth, by the effluent from the Wilmington sewage treatment plant. Primary treatment including chemical precipitation was provided at this plant until 1954, when secondary treatment of the activated sludge type was installed. This improvement changed the stream conditions at that time. The plant treats an average of about 750,000 g.p.d. of sewage. The sewer system is of the combined type, and is overloaded during heavy rains. This necessitates bypassing a large part of the total flow directly into the creek. During low flow stage in summer and autumn, the sewage effluent comprises from 80 to 90 per cent of the total stream flow.

In the Lytle Creek investigations year-round studies have been conducted to determine seasonal variations in aquatic populations and environmental conditions. The environmental changes which occurred in the stream from October 1949 to June 1952 have been described by Gaufin and Tarzwell (1) (2), and the effects of sewage pollution on the fishes of the stream were considered by Katz and Gaufin (3). In this paper the composition and distribution of the invertebrate populations found in the various life (pollutional) zones of the stream at different seasons are considered in relation to the significant physical and chemical changes which have occurred. Further, an evaluation is made of the potential worth of these various invertebrate communities as indicators of the environmental conditions under which they exist.

Procedures

At the initiation of the Lytle Creek studies ten stations were selected for periodic sampling. These stations were designated by numbers which indicate their distance in miles from the stream mouth; namely, Stations, 0, 1, 2.8, 4.2, 5.2, 6.5, 7.2, 7.3 (sewage outfall), 7.6, and 8.7 (Figure 1). Monthly, or more frequently, samples were taken at these stations for the determination of dis-

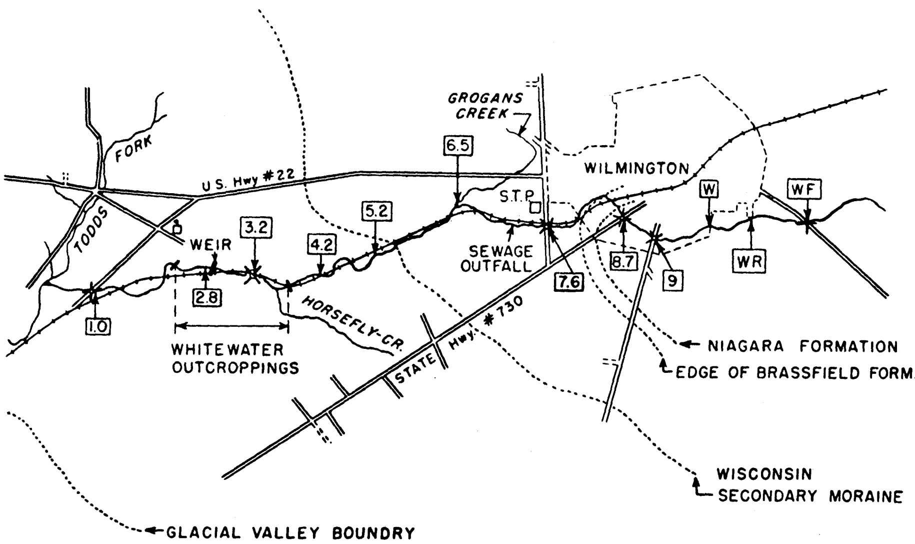

FIGURE 1.—Lytle Creek drainage basin.

solved oxygen, pH, CO_2, methyl orange and phenolphthalein alkalinity, and temperature. From July 1950 until the end of the study continuous measurements of stream flow were made by means of a sharp-crested, rectangular, contracted weir and a float-level recorder which were placed in the stream about 3 miles above its mouth.

Daily and seasonal physical-chemical variations were determined by taking during each season hourly samples at each of the stations over 24-hr. periods. During the first year of the study, five intensive 24-hr. sampling runs were carried out through the concerted efforts of biologists, bacteriologists, and chemists of the Center staff. In these studies samples of the sewage effluent were taken at each hour throughout a 24-hr. period. This selected 24-hr. discharge was followed downstream and sampled successively at each station over a 24-hr. period. Rate of flow was determined by the use of fluorescein dye placed in the stream just prior to the study. Dissolved oxygen and temperature determinations were made at each station every 2 hr. and samples were collected for bacteriological and chemical studies. Determinations of B.O.D.; oxygen consumed; pH; total alkalinity; chlorides; total and soluble phosphates; and ammonia, organic, nitrite, and nitrate nitrogen were made on 24-hr. composited samples from each of the stations. Determinations of coliform organisms and enterococcus densities were also made.

During the second year of the study nine additional 24-hr. sampling runs were conducted. Hourly studies were made at each station during 24-hr. periods for the determination of air and water temperatures, dissolved oxygen content, and pH.

From January 1950 through December 1951, quantitative bottom samples were taken at monthly intervals from pools, runs, and riffles at each station. A Surber square-foot sampler was used in the riffles and runs, whereas a Peterson or Ekman dredge was used in the pools. Marginal samples were taken by means of a special sampler formerly used by the junior author (4).

During 1952 the quantitative samples were supplemented by large qualitative collections taken from all sections of the stream by means of a Needham handscreen sampler or a Ward's sieve net. These collections were made to detect the environmental changes which occurred from season to season along the entire stream, rather than at fixed points.

Results

Physical-Chemical Data

The Lytle Creek studies revealed that during the summer months, when flows are low, septic, recovery, and clean water zones are distinct. Further, the major satisfaction of the biochemical oxygen demand, most of which was contributed by the effluent from the primary sewage treatment plant, took place in a relatively short section below the sewage outfall. From May to November each year variations in dissolved oxygen and pH were at a maximum, and the most severe conditions of oxygen depletion occurred. During the winter months higher flows and lower temperatures resulted in the pollutional zones changing their location and extent. During the period from December to April, natural purification proceeded at a slower rate, sewage fungus grew farther downstream, and dissolved oxygen was abundant throughout the stream.

Maximum and minimum flow rates and temperatures are given in Table I. In general, the summer and autumn months were periods of low flow and high temperatures, whereas during the winter and spring months the reverse condition prevailed.

The intensive sampling runs which were carried out in December 1949, and March, July, August, and September 1950, revealed considerable variation in the average B.O.D. values for the sewage effluent and the stream water below the outfall. The amount of dilution of the sewage, time of flow, and water temperature greatly influenced the rate of stream purification and oxygen depletion during the different seasons of the year. During each of the intensive sampling runs conducted, the peak sewage flow occurred between noon and midnight. The 5-day B.O.D. of the stream just below the treatment plant varied from a low of 6.8 p.p.m. at 8:00 AM during the March run to a high of 193 p.p.m. at 6:00 PM during the August run. The former low B.O.D. resulted from the dilution of the small night-time sewage flow by the greater stream flow occurring at the time of the study.

The satisfaction of the 5-day B.O.D. as the water moved downstream during four of the 24-hr. studies is shown in Figure 2. The average 5-day B.O.D. at the different stations is shown in Figure 3. Eighty per cent of the B.O.D. was satisfied in the first three miles of stream below the sewage outfall in all except the March run. On that occasion low water temperature (average 40° F.), which decreased bacterial activity, and the much greater runoff, which reduced the time of flow,

TABLE I.—Extreme Physical and Chemical Variations, Lytle Creek 1949–52

Item	Maximum			Minimum		
	Sta.	Value	Sample	Sta.	Value	Sample
Flow (c.f.s.)	2.8	100+	Mar. 1951	2.8	1.0	Aug. 1951
Time of flow (days)	—[1]	7 to 8	Aug. 1950	—[1]	1½	Mar. 1950
Water temp. (°F.)	2.8	91	July 1952	2.8	32	Jan. 1951
B.O.D., 5-day (p.p.m.)	7.2	193.0	Aug. 1950	7.2	6.8	Mar. 1950
Diss. oxygen (p.p.m.)	5.2	19.4	May 1951	5.2	0.0	Aug. 1951
pH	2.8	9.1	May 1951	7.2	6.9	Aug. 1951
Tot. alk., as $CaCO_3$ (p.p.m.)	4.2	318	Dec. 1949	1.0	169	Dec. 1949
Tot. phosphate, as PO_4 (p.p.m.)	6.5	26.2	Dec. 1949	7.6	0.55	Aug. 1950
TKN-N (p.p.m.)	6.5	38.0	Dec. 1949	7.6	0.04	Mar. 1950

[1] Sewage plant to mouth.

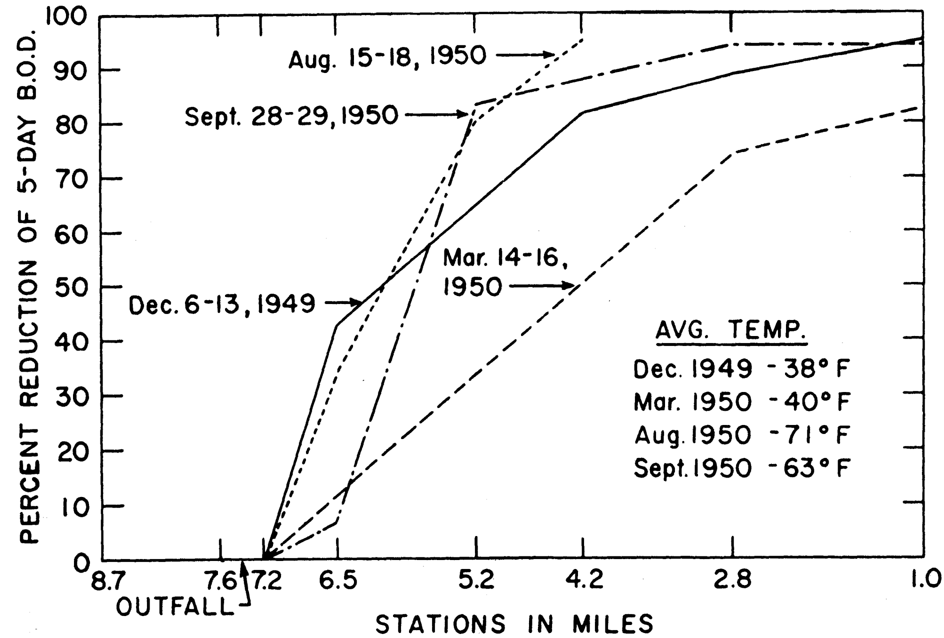

FIGURE 2.—Rate of satisfaction of B.O.D. in Lytle Creek, 1949–50.

resulted in the water flowing 5.5 miles before a similar B.O.D. reduction was obtained. During the March run a stream section moved four miles in 20 hr. whereas during July, September, and December, 36 to 48 hr. were required for travel of only two miles. Time of flow was, therefore, about four times as long in the latter studies as it was in March.

The nutrient content of the water just below the sewer outfall varied considerably depending upon the time of day, the season, the amount of dilution, the temperature, and other factors. In general, however, the analyses of water samples revealed that nitrogen in its various forms and the total phosphates, which were present in only small quantities in the stream above the sewage outfall, were contributed in large amounts by the sewage, and underwent a gradual but marked reduction as the water moved downstream. For example, from August 15 to 18, 1950, during a period of low flow, 24-hr. composite water samples taken at a point 0.2 miles above the outfall contained 1.40 p.p.m. total phosphates expressed as PO_4 ions, and 1.11 p.p.m. total Kjeldahl nitrogen; those taken 0.1 mile below the outfall contained 12.0 and 28.7 p.p.m., respectively. Water samples taken from the stream at a point about three miles below the outfall contained 8.0 p.p.m. total phosphates and 18.6 p.p.m. total nitrogen. A heavy rain prevented the completion of this study, but water samples taken from near the mouth of the stream during similar periods of low flow yielded phosphate and nitrogen values similar to those found in the stream above the sewage treatment plant, indicating a return to normal stream conditions.

Determinations of pH and alkalinity of water samples taken during the various 24-hr. sampling runs revealed that these environmental factors were at all times favorable to the production of a varied fauna in any section of the stream. The maximum and minimum pH and total alkalinity values recorded from the stream during the study are listed in Table I. The pH of the sewage effluent was neutral or slightly acid

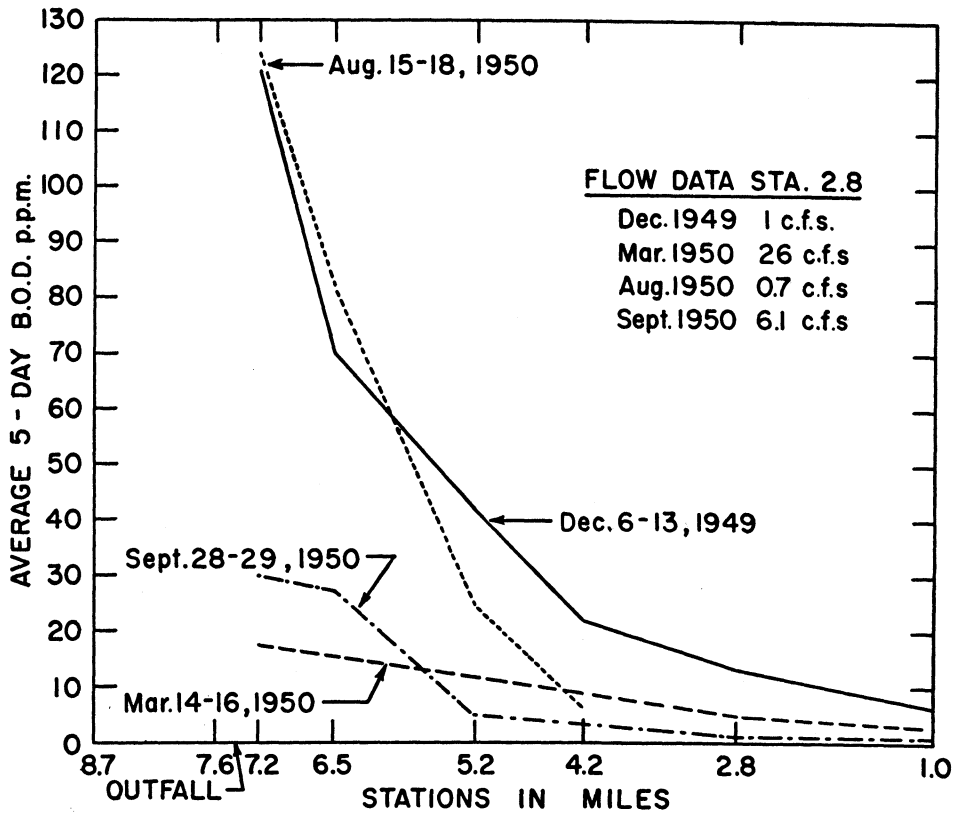

FIGURE 3.—Range in B.O.D., Lytle Creek, 1949–50.

during dry periods, but ranged from 7.0 to 8.5 during periods of heavy precipitation. The pH values increased progressively downstream from the sewage outfall. (Table II). Methyl orange alkalinity values, recorded as p.p.m. $CaCO_3$ equivalent, were in excess of 168 p.p.m. at all times.

Lakes and streams normally exhibit a noticeable diurnal variation in their dissolved oxygen content (5) (6) (7) (8) (9) (10) (11) (12). In streams polluted with domestic sewage this variation is usually more marked in certain sections due to dense algal growths and the large oxygen demand of the organic material. In Lytle Creek diurnal variations due to these factors were most marked in the recovery zone.

During the summer months, the dissolved oxygen content in the four-mile section below the sewage treatment plant ranged from supersaturation to virtual depletion. The dissolved oxygen content at any time depended on the oxidizable material, temperature, sunlight, stream flow, and turbidity, and varied considerably with the seasons, showing the widest variation during periods of high temperature and low flow. Daily maximum and minimum dissolved oxygen values recorded during four representative sampling runs are shown graphically in Figures 4 and 5. Seasonal variations in the dissolved oxygen content throughout the stream have been discussed in detail by the authors in previous papers (1) (2).

Biological Data

Bacteriological studies of Lytle Creek were conducted during the first year of the project. Two indicators of sewage pollution were investigated; namely,

TABLE II.—pH Data,[1] Lytle Creek Surveys

Station	Mar. 14–15, 1950	July 11–13, 1950	Aug. 15–18, 1950	Sept. 28–29, 1950	Feb. 25–26, 1952
8.7	—	—	7.8	—	8.1
7.6	8.3	8.1	8.1	7.9	8.3
Effluent	7.7	7.1	6.8	7.2	—
7.2	8.4	7.2	6.9	7.3	8.1
6.5	—	7.4	7.2	7.5	7.8
5.2	—	7.7	7.9	7.7	7.9
4.2	—	—	7.9	—	8.1
2.8	8.2	—	—	7.9	8.1
1.0	8.3	—	—	7.9	8.2

[1] All samples taken at 12:00 Noon.

coliforms and enterococci. Coliform densities were determined by fermentation tube procedures described in the 9th edition of "Standard Methods for the Examination of Water and Sewage," while enterococcus densities were determined by the Winter and Sandholzer method. "Total" bacterial estimates were based on the "five-tube most probable number" method, using growth (turbid *versus* clear) end points in lactose broth. One set of tubes was incubated at 37° C. and a duplicate set at 20° C.

Sampling runs of 24-hr. duration conducted in December 1949, and March, July, and August 1950, revealed that Lytle Creek above the sewage outfall contained relatively few coliforms and enterococci. Approximately 99 per cent of the coliforms in the stream below the outfall were attributed to the sewage effluent. The two indicator groups showed a rapid death rate in the stream section between the sewage outfall and Station 2.8, approximately four miles downstream. In December and March, "total" bacterial densities, as indicated by samples incubated at temperatures of 37° C. and 20° C., showed a progressive decrease below the outfall. In marked contrast, summer samples incubated for 48 hr. at 20° C. showed nearly consistent in-

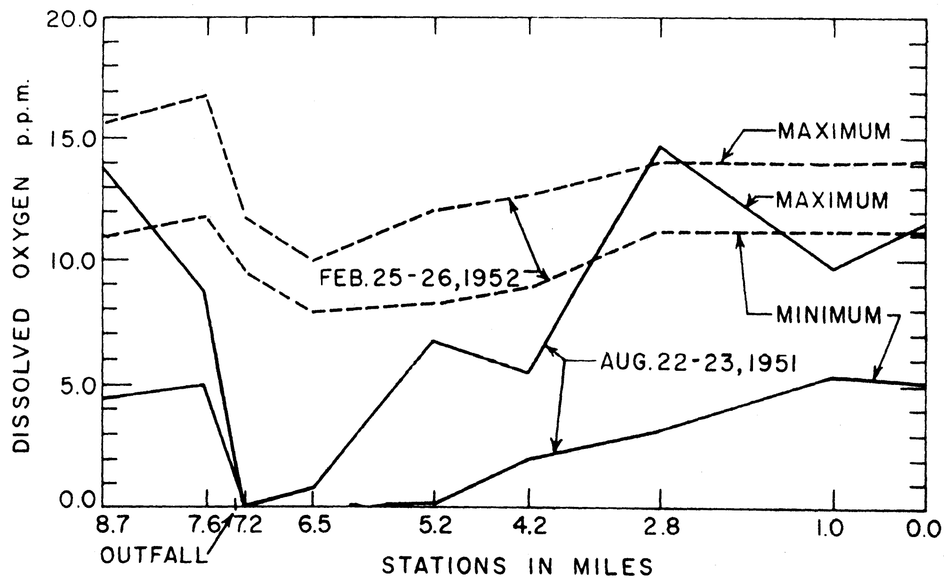

FIGURE 4.—Range in dissolved oxygen, Lytle Creek.

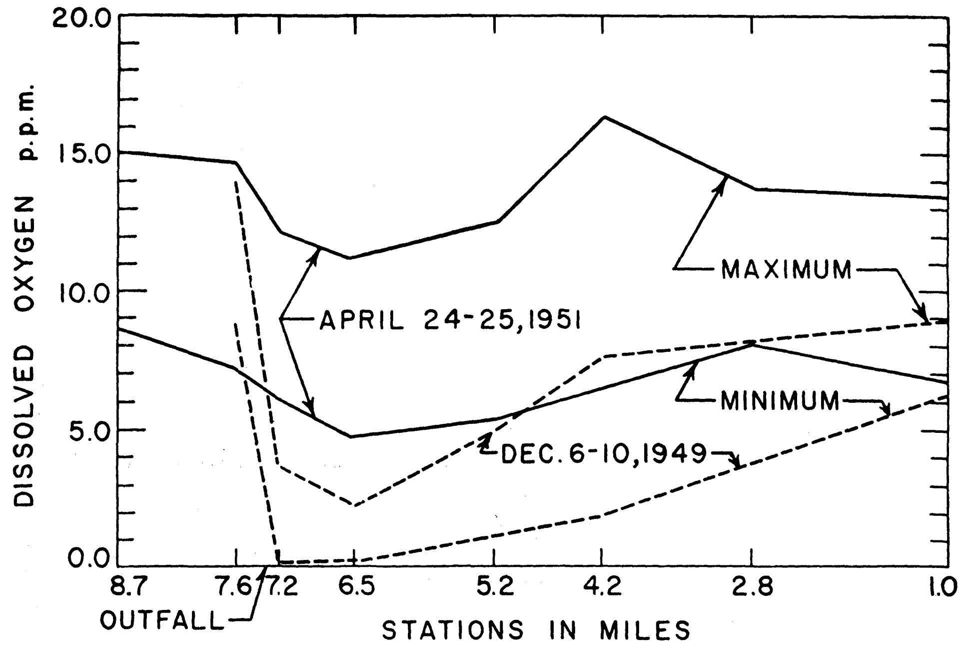

FIGURE 5.—Range in dissolved oxygen, Lytle Creek.

creases in concentration with distance from the sewer outfall to Station 6.5 in July, and to Station 5.2 in August. Tests for coliforms and "total" bacterial growth on the bottoms of the riffles and pools showed consistently higher concentrations of organisms in the riffles than in the pools.

Studies on the microbenthic biota of the creek were also confined to the first year of the project. Detailed analyses of such biota were made in connection with the sampling runs conducted in December 1949, and March, July, and August 1950. Bottom growths from rocks or from paving bricks placed in riffles and mud samples from pools were analyzed, using the Lackey drop method (13) and high power (323 ×) of the compound microscope. Counts of organisms were made on an areal basis. Not only rotifers, crustaceans, and protozoans, such as rhizopods, and ciliates, but also visible bacteria such as zoogloea and *Sphaerotilus natans,* and the blue-green algae and diatoms were counted.

In conducting these studies of the microbenthos, pools and riffles were considered separately. Only the most significant of the trends as revealed by the December 1949 run are considered in this paper. During that month, under low flow conditions, pollution-tolerant blue-green algae, largely *Oscillatoria* spp., increased greatly in the pools below the sewage treatment plant in response to the increased fertility, reaching a peak at Station 6.5 and rapidly decreasing to the point of scarcity at Station 4.2. Because of limited sunlight, heavy sedimentation, and low oxygen supply on the bottoms of pools in the zone of active decomposition, the diatom population was low above Station 4.2; but below this point their numbers increased to over 110 million per square foot. A large population of diatoms such as *Navicula* spp., *Nitzschia* sp., and *Gomphonema* spp., was characteristic of most of the lower part of the stream. Flagellates, chiefly *Euglena* sp., reached a peak of 159 million per square foot in the pools at Station 5.2 and were also in great abundance at Station 2.8.

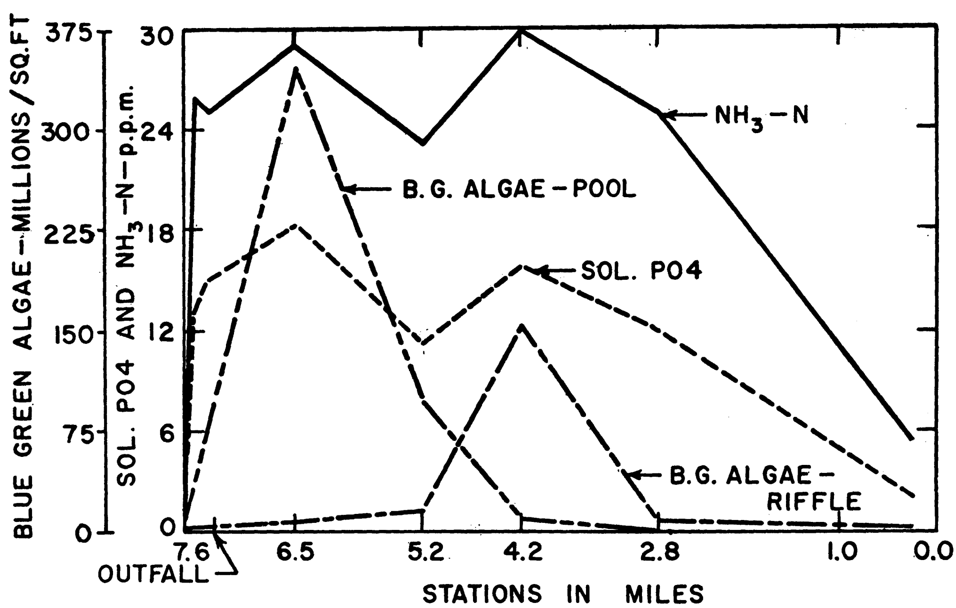

FIGURE 6.—Relation of blue-green algae to nutrients, Lytle Creek, December 1949.

Although flagellates were abundant in the pools from the point of sewage discharge to Station 5.2, very few of them were found in the riffles above Station 4.2. The blue-green algae in the riffles had much the same distribution as the flagellates. The peaks of growth of both groups in their respective habitats corresponded fairly well with high concentrations of ammonia nitrogen and phosphates as determined from the 24-hr. composite samples (Figures 6 and 7). Zoogloeal masses and *Sphaerotilus natans* increased rapidly below the sewage treatment plant, reaching a peak at Station 6.5, below which they decreased rapidly. This decrease was attributed to the reduced food supply and the voracity of the bacteria-feeding rhizopods and ciliates associated with them (Figure 8).

The distribution of bacteria, protozoa, and algae in the stream during December 1949 is believed to be typical of their distribution during other periods of low flow, when definite pollutional zones were observed. As has been previously mentioned, well-defined zones of pollution were present in the stream most of the time from early May to late November or early December each year. By January, however, the increased non-flood flow extended the carpet of *Sphaerotilus* and other bacteria and protozoa characteristic of the septic zone to the formerly clean water zone, three miles downstream. Throughout the period of normal high flows characteristic of the winter months the entire lower section of the stream exhibited pollutional fauna and flora intermixed with clean water forms.

In the Lytle Creek studies emphasis was placed on determinining the role and value of the macro-invertebrates as indicators of pollution. The decision to do this was based on their size and more distinctive morphological characteristics, which facilitate their identification under field conditions. In addition, most representatives of this group have longer life histories than the microbenthic fauna and are thus better fitted for indicating past ecological conditions in any given area.

Intensive qualitative and quantita-

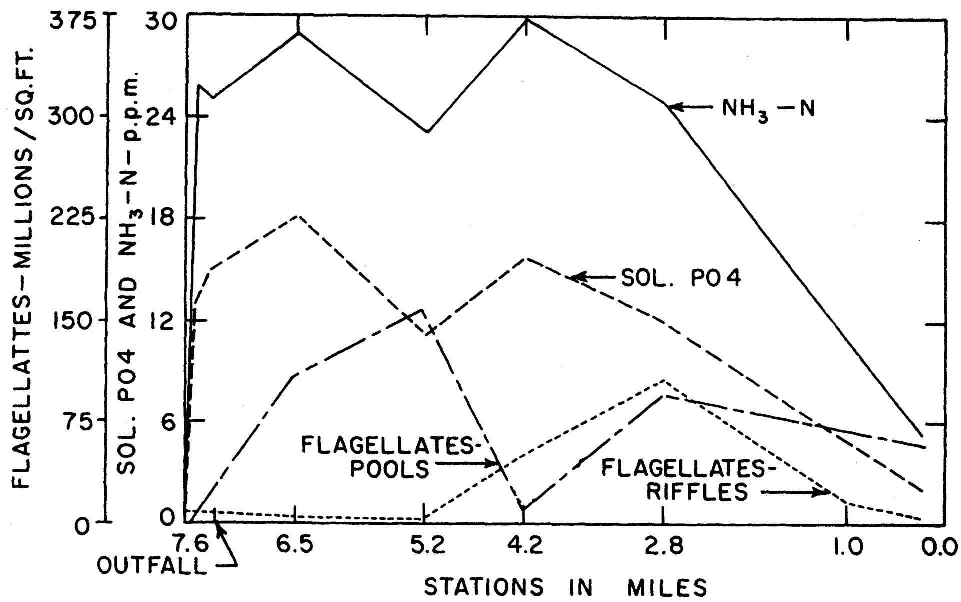

FIGURE 7.—Relation of flagellates to nutrients, Lytle Creek, December 1949.

tive studies of the macro-invertebrate inhabitants of the stream were carried out during all seasons of the year. During the qualitative surveys, conducted in connection with the sampling runs, 144 species representing 110 genera of animals were collected and identified. The studies revealed striking seasonal differences in the numbers of species and faunal associations occurring in the various sections of the stream. The number of species of macro-invertebrates found in different sections of the stream during each of the seasons is shown in Figure 9.

One-third to one-sixth as many spe-

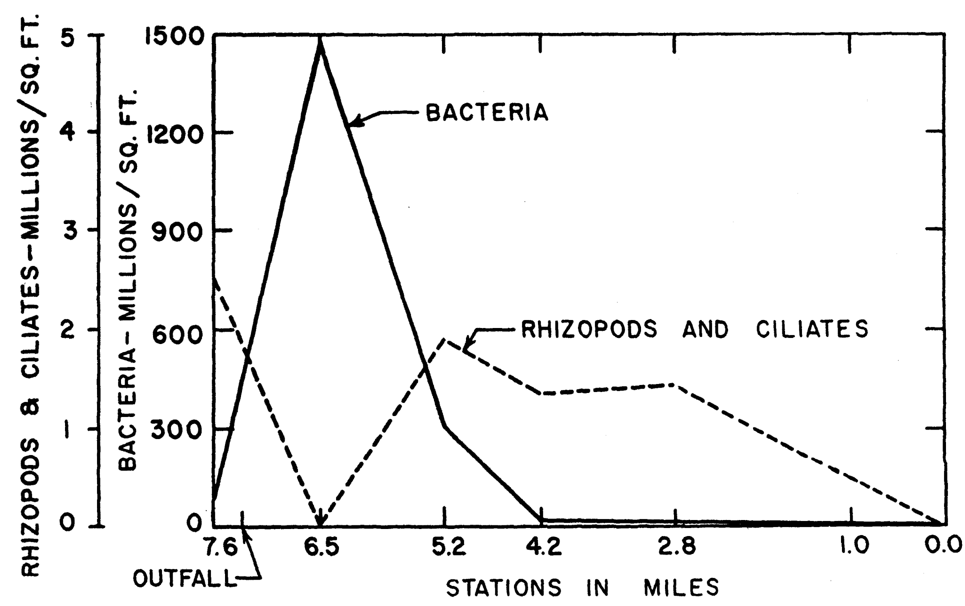

FIGURE 8.—Distribution of bacteria and protozoa, Lytle Creek, December 1949.

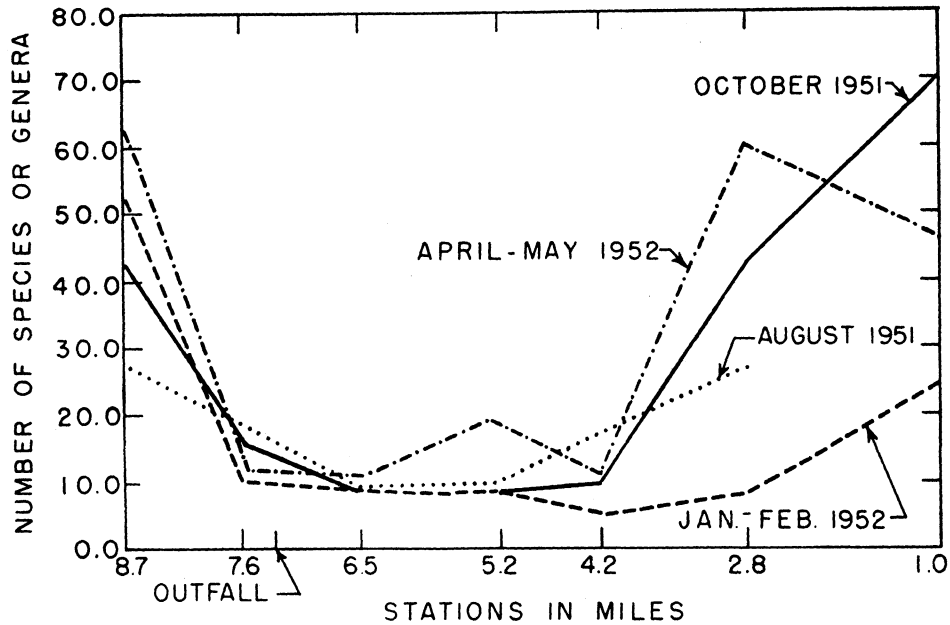

FIGURE 9.—Macro-invertebrate distribution, Lytle Creek.

cies, depending on the season, were found in the septic zone as in the stream above the outfall. This reduction is believed to have been due largely to oxygen depletion during periods of high temperature and low flow and to changes in the nature of the stream bottom. The largest number of species (70) was taken in the lower clean water zone at Station 1.0 during October 1951. The variety and abundance of life present in that section reflect the favorable effects of organic enrichment and relatively constant stream flow during the late summer months.

Analyses of the species composition and abundance of individual organisms in the various zones point to biological phenomena which constitute valuable criteria for evaluating the role and value of such associations as indicators of pollutional conditions in a stream (13)(14)(15)(16)(17)(18)(19)(20)(21). Because the entire stream below the sewage treatment plant was affected to some extent by the effluent which it received, only the two mile section above the outfall displayed what may be considered as clean water conditions at all times. The number and variety of macro-invertebrates occurring in that upper section were typical of the fauna which existed in non-polluted streams in the area.

The fauna at all times consisted of a variety of species representing many different genera, families, and orders of invertebrates. The smallest number of species collected at one sampling was 29, taken in August 1951; the largest number (63) was encountered in April 1952. The latter number, collected at Station 8.7, very well illustrates the diversity of life present. The following species of invertebrates were taken: water fleas (Collembola), 1; true flies (Diptera), 18; beetles (Coleoptera), 13; mayflies (Ephemeroptera), 4; caddis flies (Trichoptera), 6; stoneflies (Plecoptera), 3; dragon flies (Odonata), 4; water bugs (Hemiptera), 3; crayfish and shrimps (Crustacea), 3; snails (Mollusca), 4; segmented worms (Annelida), 1; flatworms (Platyhelminthes), 1; and roundworms (Nematoda), 2. Among this variety of organisms, no one species was found in numbers exceeding 50 per square yard, whereas 45 species were represented by less than five individuals per square yard of bottom area.

The distribution and numbers of species and individuals occurring in the septic and recovery zones contrasted sharply with those of the clean water zone. Only 8 to 10 species of macro-invertebrates were collected at any one time from the septic zone under low water conditions. This number included such forms as the mosquito larva, *Culex pipiens;* rattail maggot, *Eristalis bastardi;* horsefly larva, *Tabanus atratus;* water scavenger beetle, *Tropisternis natator;* water boatman, *Hesperocorixa* sp.; pulmonate snail, *Physa integra;* and sludgeworms, *Limnodrilus* sp. and *Tubifex* sp. As many as 18 species were taken from that section in the spring when the flow was high. However, 8 of that number were drift forms traceable to a nearby tributary.

Of the 8 to 10 species of macro-invertebrates occurring in the septic zone during the late summer and autumn, not one species was restricted to that section of stream. All occurred, but in far smaller numbers, in both the recovery and clean water zones. In these latter areas, however, they were very restricted in their distribution, being largely confined to quiet water micro-habitats having dissolved oxygen, food supplies, and bottom conditions similar to those which existed in the septic zone.

Analysis of the macro-invertebrate population characteristic of the septic zone in August 1951 reveals that 40 per cent were Diptera, 20 per cent Coleoptera, 20 per cent Annelida, 10 per cent Hemiptera, and 10 per cent Mollusca. All of the insects generally found in this zone were characterized by the possession of special adaptations for obtaining oxygen, such as the caudal respiratory tubes of the mosquito larva and rattail maggot, or the air space under the elytra of the beetles.

During summer and early autumn, an abundant food supply and other favorable conditions for reproduction and growth enable several species occurring in the septic and recovery zones to attain great abundance. As many as 3,000 mosquitoes, *Culex pipiens,* and 20,000 Tubificids were collected in a square foot sample from the septic zone during these months. Rattail maggots, *Eristalis bastardi,* were so numerous at times that their respiratory tubes had the appearance of blades of grass.

The variety and number of organisms in the recovery zone were much less constant than they were in either the clean water or septic zones. Environmental conditions ranged from near septicity in the upper end of the recovery zone to practically clean water in the lower portion. The limits of the zone also shifted frequently with the seasons and change in flow. The least number of species encountered in the middle section of the recovery zone was 9, found in October 1951; the greatest number was 32, collected in April 1952. During the former month the fauna consisted of species typically found farther upstream, while during the latter month the fauna was more typical of clean water conditions. The community of organisms encountered in April included seven species of relatively sensitive mayflies, stoneflies, and caddis flies which apparently had drifted into the main stream from tributaries. Each of these species had been able to survive in numbers up to five per square yard. Such seeding of additional fauna did not occur in the autumn of 1951 when all the tributaries were dry.

Analysis of the distribution of the major groups of aquatic macro-invertebrates in the stream reveals that no association was made up entirely of clean water representatives. The mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddis flies (Trichoptera), all gilled forms, were more nearly restricted to and comprised a larger portion of the clean water association than any of the other groups. Members of these orders and other gill breathing immature insects scattered through several orders, were most affected and restricted in their distribution by the very low dissolved oxygen concentrations often present in the polluted zones.

There are, however, exceptions. Two species of mayflies, *Stenonema femoratum* and *Callibaetis* sp., were taken on several occasions in sections of the recovery zone where the dissolved oxygen content was reduced to as low as 1 p.p.m. at night. It is possible that the oxygen concentrations in the niches occupied by the two species, of which the former occurred in the fastest riffles and the latter in marginal or shoal areas, where the water was less than 6 in. deep, were higher than the value recorded for the stream section. However, both species displayed much greater tolerance to low oxygen levels than has heretofore been thought possible (22).

Further, representatives of one family of Trichoptera, the net building *Hydropsychidae,* also were found commonly in areas where the oxygen supply at night was often reduced to less than 3.0 p.p.m. In all such cases, however, the larvae or pupae were located in the most rapid portions of riffles where they could obtain the maximum available supply of oxygen as well as food.

With only two exceptions the stoneflies were entirely restricted to clean water areas. *Allocapnia viviparia* and *Perlesta placida* were taken both as nymphs and adults in limited numbers from the upper recovery zone of the stream during the winter and early spring months when conditions were temporarily favorable for their existence. The minimal dissolved oxygen value recorded during the period when they were collected was 7.8 p.p.m. All evidence indicated that they were contributed to the main stream from nearby tributaries where they occurred in large numbers. Since both species pass through the summer and early fall in either the egg stage or as very small inactive nymphs, it is debatable whether or not they could have lived and completed their earlier development in the stream proper when much lower dissolved oxygen levels existed.

While there are exceptions, such as those just considered, in general an association of mayflies, stoneflies, and caddis flies in a stream is indicative of clean water conditions, and their absence often denotes the presence of pollution and/or a low oxygen supply if the physical nature of the habitat is otherwise suitable. Usually the presence or absence of representatives of other orders of aquatic insects which breathe by gills, and which are, therefore, dependent upon oxygen dissolved in the water for their respiratory needs, has similar indicator significance. For example, while most aquatic beetles can renew their oxygen supply directly from the atmosphere and are thus unaffected by oxygen-depleting wastes, the larvae, pupae, and adults of those species which are entirely aquatic are dependent upon dissolved oxygen and are restricted to clean water streams which are well aerated. In Lytle Creek several species of riffle beetles, such as *Stenelmis crenata* and *Stenelmis sexlineata,* were found only in the upper and lower clean water zones. Their distribution in the stream indicates that the family, Elmidae, to which they belong, is a member of the clean water association.

While most gill-bearing aquatic insects are limited in their distribution by low dissolved oxygen supplies, some forms which have more than one means of respiration, such as the dragonflies and damselflies, display considerable tolerance to low levels of dissolved oxygen. Their greater adaptability to environments low in dissolved oxygen is made possible by the possession of respiratory structures which are the most highly developed of the various gill systems (22). These insects can carry on respiration by means of four different structures; namely, (1) caudal tracheal gills; (2) rectal folds; (3) the integument; and (4) spiracles. Since all four organs may function at the same time and many of the stream forms occur in either riffles or shallow marginal areas, the group is remarkably well-adapted to withstand the oxygen depleting effects of organic pollution. As a result of this adaptability, the nymphs of both dragonflies and damselflies were often taken in Lytle Creek in sections of the recovery zone where the dissolved oxygen supply during the summer was as low as 1.0 p.p.m. for a short time during the night or in the early morning hours.

In Lytle Creek insects of the orders Hemiptera, Coleoptera, and Diptera had the most varied representation, were the most widespread in their distribution and were least affected by dissolved oxygen concentration. Representatives of these orders were found in all stream habitats representing all degrees of pollution and stream recovery. Some species from each group were found fairly widely distributed through the stream; others, while not restricted to either a clean water or polluted area, showed by their abundance a strong preference for one or the other type of habitat. Still other species, particularly among the Diptera, were restricted to clean water or to water rich in organic materials.

Of the three orders, the Hemiptera and Coleoptera were the poorest indicators of organic pollution and oxygen depletion in the stream. With the exception of the Elmidae, or riffle beetles, other species of beetles and all of the species of water bugs collected were found throughout the stream, usually occurring most abundantly in the polluted areas where they found an abundant food supply. The ability of members of these two orders to withstand the oxygen-depleting effects of organic pollution is due to special modifications of their tracheal system. These modifications serve to increase the internal air capacity of the tracheal system, supplement tracheal diffusion by ventilation movements when the insects come to the surface for air, and provide supplementary external air stores. Common to all of these forms are the modification of the body surface for breaking the water surface film, and changes in the wings and body surface for capturing and holding stores of air, and in the tracheal system for surface ventilation and connection with the external air stores. In oxygen-deficient waters members of these two groups have only to increase the frequency of their visits to the surface to cope with decreasing oxygen supplies.

The efficiency of these modifications for obtaining and storing atmospheric oxygen is well illustrated by *Dytiscus,* one of the diving beetles. Reeder (22) reports that this beetle can remain submerged for 36 hr. without coming to the surface to renew its oxygen supply. To obtain a supply of oxygen *Dytiscus* breaks the surface periodically with hydrofuge hairs, and ventilates violently by means of accessory respiratory muscles. The fore wings, elytra, have a locking mechanism to trap the atmospheric air, and the abdominal and thoracic spiracles are displaced so as to open into this respiratory air store.

Aquatic Diptera were found in the stream in many different ecological niches in both the clean water and polluted zones. However, with the exception of only a few species, representatives of this order were highly selective in their choice of habitat. A number of species such as *Diamesa nivoriunda,* *Cricotopus absurdus,* and *Calopsectra neoflavella,* were found only in the cleanest, most highly aerated sections of the stream, while others such as the mosquito, *Culex pipiens,* and rattail maggot, *Eristalis bastardi,* while found in limited numbers in clean water areas, showed a decided preference for the polluted sections. The variability in choice of habitat and in the range of distribution of the species taken was determined largely by the food-getting and respiratory requirements and adaptations of the different individual species. The larvae and pupae of the mosquito and rattail maggot, with their special respiratory tubes, were unaffected by low oxygen supplies, as evidenced by the extremely large numbers of each taken in the most septic areas. Certain red-blooded Chironomids, such as *Chironomus riparius,* also demonstrated a remarkable ability to thrive in the septic and recovery zones. Walshe has shown that the hemoglobin possessed by midge larvae such as *Chironomus riparius, Chironomus plumosus,* and closely related species, apparently acts in both the transportation and storage of oxygen (23) (24). Its greatest transport role is during anaerobiosis, when it permits the larva to continue filter feeding in low oxygen tensions and thereby increases the rate of recovery from exposure to such conditions.

As in the case of the insects, the other groups of macro-invertebrates in the stream showed considerable variation in their distribution and adaptability to varying degrees of pollution. Certain groups such as the sludge worms, Tubificidae, were found in very large numbers in bottom sludges of high organic content in the lower end of the septic zone and the upper end of the recovery zone. Their numbers were found to decrease rapidly as the nature of the bottom sediments changed. The ability of two genera of these worms, *Tubifex* and *Limnodrilus,* to utilize the rich supply of organic material in the polluted zones, under practically anaerobic conditions, made them important and conspicuous members of one of the stream's most characteristic pollutional communities.

Another species of invertebrate which occurred throughout the stream, but which formed a large and conspicuous component of the most common pollutional association in Lytle Creek, was the pulmonate snail, *Physa integra.* The ability of this species to obtain atmospheric oxygen directly enabled it to take advantage of the abundant food supply in the polluted zones. As a result, this species was several hundred times as abundant in the pollutional enriched areas as in the clean water sections. The mere presence or absence of this species in a given area is a poor criterion of organic pollution and oxygen depletion, but when its mode of occurrence, relative abundance, and faunal associates are considered, its index value as an indicator of the degree and extent of organic enrichment in Lytle Creek was very great.

Discussion

Some investigators, such as Kolkwitz and Marsson (20)(21), Weston and Turner (5) and Richardson (19), have attempted to associate definitely individual species with different degrees and types of pollution, and have published lists assigning such species to different zones or subzones of pollution. Careful comparison of these different lists will show that there is an appreciable lack of agreement as to the true status of many of the organisms. A species which one worker may list as being characteristic of polluted water may be reported by others as being found only under clean water conditions.

It is apparent that there has been an oversimplification of the problem by the supplying of lists which might be used by many without adequate investigation of the clean water communities. There are several reasons for the lack of agreement in the different lists. Perhaps foremost is the fact that, in making use of such environmental classifications for the setting up of lists, little reliance can be placed upon the mere occurrence of a single species in a given locality (19)(26). Many organisms which occur in large numbers in extremely polluted water also are found in limited numbers in cleaner situations. For example, all of the species of invertebrates which occurred in the septic or recovery zones of Lytle Creek were also found in limited numbers in similar microhabitats in the clean water zones. Conversely, many aquatic organisms that are intolerant of persistent organic pollution can live for a short period of time in a polluted area when pollutional effects are at a minimum. Such occurrences were particularly evident in Lytle Creek during periods of high flow in the winter and spring months when temperatures were low and dissolved oxygen concentrations were high. Under such conditions several species of mayflies, stoneflies, and caddis flies, which drifted into the septic and recovery zones of the stream from nearby tributaries, were able to remain alive for periods sufficient to allow them to emerge as adults. In all such instances, however, the numbers of such clean water forms were distinctly limited when compared with the populations of organisms usually found in the stream.

A second reason for the lack of agreement as to the indicator value of certain aquatic organisms is that several environmental factors other than the presence of a pollutant may affect or limit the distribution of certain species (25). Chief among these are geographical location, erosion, floods, the size of the stream, the type of bottom, and the flight range of the insect. Species lists compiled for one section of the country or world may be of little value in another region, because of the fact that many aquatic organisms are very restricted in their distribution. In order for any comprehensive list or grouping of indicator organisms to be applicable to all sections of the country, the pollutional status of typical aquatic organisms throughout the country must be studied and such information incorporated into the list.

Type of bottom, speed of current, depth of water, and many other factors affect the distribution of the stream fauna in any given section. Due to the differing physiological requirements and morphological adaptations of aquatic organisms, the populations found in soft muck, sand, or gravel bottoms, and in riffles, pools, and marginal areas are quite distinct.

A third major reason for lack of agreement among lists of indicator species is the absence of keys and descriptions for the fauna in many areas and want of sufficiently distinct morphological characteristics to provide for species separation. Because of considerable lack of agreement which exists concerning the pollutional status of many aquatic organisms, little reliance can be placed, at the present time, upon the occurrence of a single species as an indicator of pollution. In evaluating the reliability of aquatic organisms as indicators of pollutional conditions, one must consider the different indicator organisms not separately, but as biological associations or populations. The authors believe that the organisms should be considered in groups according to their morphological adaptations and physiological requirements. This became very evident during the course of the Lytle Creek studies and has led them to the conclusion that few definite statements can safely be made concerning the indicator value of the mere occurrence of specific organisms in the stream. However, fairly definite conclusions can be formulated as to the value of certain associations of aquatic invertebrates for indicating degrees of organic pollution in the stream.

In general, the community of aquatic invertebrates that was most characteristic of the septic zone in Lytle Creek consisted of such pollution tolerant organisms as sludge worms, *Tubifex* spp. and *Limnodrilus* spp.; pulmonate snails, *Physa integra;* rattail maggots, *Eristalis bastardi;* mosquitoes, *Culex pipiens;* and midge larvae, *Chironomus riparius.* All of these species occurred in large numbers during the summer and early autumn. During the winter months, only the sludge worms, pulmonate snails, and midge larvae were commonly represented. In addition to the several species mentioned, the association of organisms in the septic zone, particularly during the summer months, included limited numbers of diving and water scavenger beetles, *Laccophilus maculosus* and *Tropisternus natator;* whirligig beetles, *Dineutes americanus;* and various species of Hemiptera, such as *Corixa* spp., *Gerris* spp., and *Belostoma* spp. Since all of these species of beetles and water bugs obtain their oxygen supply directly from the atmosphere and are largely confined to the surface or marginal areas of a stream, they were distributed in fairly uniform numbers throughout Lytle Creek. Their presence, then, cannot be regarded as having any index value for the degree of oxygen depletion.

In the recovery zone the population of invertebrates varied from septic associations at the upper end of the zone to practically clean water communities in the lower section. The community of organisms most characteristic of average conditions in the recovery zone consisted of lesser numbers of the species found in the septic zone plus variable numbers of the more tolerant clean water species. The latter group included a variety of invertebrates. The most common of these species were blackfly larvae and pupae, *Simulium vittatum;* dragonfly nymphs, *Libellula lydia;* midge larvae and pupae, *Pelopia stellata;* mayfly nymphs, *Callibaetis* sp.; and caddis fly larvae and pupae, *Cheumatopsyche* sp.

In the clean water zones there was a great variety of invertebrate communities, each consisting of many different species. With ecological conditions less restrictive, and greater competition for food and space existing between the different organisms that made up the population, no one species was able to increase in numbers and be represented by the large numbers of individuals characteristic of the septic zone. Each community was a dynamic unit, consisting of herbivores, carnivores, and omnivores; prey and predators; lung, tracheal tube, and gill breathers. Each organism was adapted to its own ecological niche and offered check reins against too rapid growth or reproduction of the others. Most of the species which occurred in the septic and recovery zones were also found in very limited numbers in the clean water zones. In addition, there was also present a wide variety of forms which were intolerant of conditions in the polluted zones. Most of these were the gill-breathing, immature stages of such insects as the mayflies, stoneflies, caddis flies, and alder flies, plus clean water representatives of the other groups of aquatic invertebrates.

In considering the distribution of organisms in the stream, those species of aquatic invertebrates which obtain their oxygen supply by means of gills only, with the exception of *Callibaetis* sp., *Stenonema femoratum, Cheumatopsyche* sp., and *Allocapnia viviparia,* were found to be most sensitive to the organic pollution in Lytle Creek. With those exceptions, all strictly gill-breathing forms were restricted, by the oxygen depleting effects of the organic wastes, to the clean water zones of the stream. They constituted the principal members of the varied communities characteristic of the clean water areas.

By contrast, all of the invertebrates which occurred most commonly in the polluted zones had special adaptations for obtaining atmospheric oxygen or had low oxygen requirements. With such adaptations, depletion of oxygen in the water did not serve as a barrier to their distribution.

In conclusion, pollution of Lytle Creek by organic wastes was only one of several environmental factors determining the composition of the aquatic populations. Before any definite conclusions concerning the effects of organic pollution on the stream's biota could be drawn, it was necessary to consider such factors as variations in flow and water temperatures, the nature of the watershed, the type of bottom, the life histories of the organisms concerned, the drift of organisms downstream, seeding from tributary streams, and the morphological adaptations and physiological requirements of the organisms. Only after considering all these factors was it possible to arrive at the various conclusions stated in this paper.

Summary

Intensive studies were carried out on Lytle Creek to discover the effects of sewage pollution on the aquatic communities of the stream and the value of these populations as indicators of present and past pollutional conditions. In these investigations physical-chemical conditions were related to the qualitative and quantitative composition of the aquatic populations in the different life zones.

The distribution and abundance of bacteria, protozoa, and algae in the stream were largely determined by nutrients and predator-prey relations. Approximately 99 per cent of the coliform bacteria in the stream below the sewage outfall were contributed by the sewage. In the first two miles of stream below the outfall, coliforms and enterococci decreased, whereas the total bacterial population increased. Zoogloea, *Sphaerotilus,* and certain protozoa were most abundant in the section immediately below the sewage outfall while pollution-tolerant blue-green algae increased progressively in the pools for a distance of two miles downstream with the diatoms reaching their peak below this section.

Seasonal diurnal changes were great and were most marked in the recovery zone. Diurnal fluctuations in dissolved oxygen and pH were greatest in late spring and early summer.

Very few definite statements can safely be made concerning the indicator values of specific organisms, but definite conclusions can be formulated as to the value of certain associations or populations of aquatic invertebrates for indicating the severity and extent of pollution and the degree of stream recovery.

Little reliance can be placed upon the mere occurrence of a single species in a given locality. In Lytle Creek the 9 species characteristic of the septic zone also occurred in the recovery and clean water zones, but in much smaller numbers. The distribution and number of species and individuals in the septic zone differed greatly from that in the clean water areas. The septic zone had less than one-fifth as many species as the clean water zone, but the number of individuals of each species and the total number of organisms per unit area were many times greater. The septic zone was characterized by species adapted to live under low D.O. conditions (less than 1 p.p.m.) as *Tubifex* and *Limnodrilus,* or those able to secure their oxygen directly from the air as do *Culex pipiens, Eristalis bastardi,* and *Physa integra.* Hemiptera and Coleoptera were found in the septic zone, but these were also equally numerous in other zones. The modification of their tracheal system and general body surface which increases the internal air capacity of the tracheal system and enables them to use external air stores which serve like

physical gills, enabled them to withstand very low D.O. levels (1 to 0.5 p.p.m.), and made their mere presence a poor indicator of dissolved oxygen content.

The community of organisms most characteristic of average conditions in the recovery zone consisted of lesser numbers of the species found in the septic zone plus variable numbers of the more tolerant forms found in clean water, especially those having a variety of methods for securing oxygen. These included certain larvae of the blackfly, *Simulium vittatum;* midge, *Tanypus stellatus;* and caddis fly, *Cheumatopsyche* sp.; and nymphs of the mayfly, *Callibaetes* sp., and dragonfly, *Libellula lydia.*

Clean waters were characterized by a great variety of invertebrate communities consisting of herbivores, carnivores, and omnivores; prey and predators; lung, tracheal tube, and gill breathers. In general a population containing abundant gill-breathing forms, mayflies, stoneflies, and caddis flies was indicative of clean water conditions and their absence denoted the presence of pollution and/or low oxygen.

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