

# Aquatic Invertebrates as Indicators Of Stream Pollution

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Year-round field studies of the biology of stream sanitation were initiated by the biology section of the Public Health Service's Environmental Health Center on Lytle Creek in October 1949. The aims of these investigations were:

1. To develop or devise and field test procedures and equipment for biological surveys and investigations of polluted streams.
2. To investigate seasonal and diurnal environmental changes in a stream polluted with oxygen-depleting wastes.
3. To determine how the physical-chemical environment in the various pollutional zones affects the qualitative and quantitative composition of aquatic populations and how these populations in turn affect or change physical-chemical conditions.
4. To relate various qualitative and quantitative compositions of aquatic populations to environmental conditions found in streams polluted with organic wastes and to test the value and use of aquatic organisms as indicators of clean water and various degrees of pollution at all seasons of the year.
5. To determine the rate of satisfaction of biochemical oxygen demand (BOD), the area in which most of the demand is satisfied, and how seasonal and other environmental conditions affect the process.
6. To determine the value of fishes as indicators of pollution and their use in evaluating the effects of pollution.

The composition of the aquatic fauna in

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various sections of the stream and diurnal changes in environmental conditions are discussed here.

Lytle Creek, which was especially selected for the study, is about 45 miles northeast of Cincinnati, Ohio, and is a tributary of Todds Fork, a part of the Little Miami River system. It is a small stream approximately 11 miles long, 3 to 35 feet wide during non-floodstage, and a few inches up to 6 feet deep. The principal natural source of water in the stream is surface drainage from the surrounding area. Some 7 miles above its mouth, the creek receives the effluent from the primary sewage treatment plant of Wilmington, Ohio, a city of about 10,000 people. Lytle Creek is particularly favorable for studies of the pollutional effects of oxygen-depleting wastes because it has only one source and type of pollution (domestic wastes from Wilmington); it has no permanent tributaries below the source of pollution; and it has all degrees of pollution from a definite septic zone through recovery and back to clean water.<sup>1</sup>

## Procedures

One of the early objectives of the Lytle Creek investigations was to evaluate the reliability and possible use of aquatic organisms (chiefly benthic forms) as indicators of the extent and severity of pollution and the degree of stream recovery. Plans were made for a comprehen-

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<sup>1</sup> Zones of pollution, as herein considered for Lytle Creek, are based principally on the amount of dissolved oxygen occurring throughout the stream. The septic zone is the area of stream showing oxygen depletion during at least some portion of the year. It grades gradually into the recovery zone where the minimum oxygen concentrations range from 0.1 to 2 ppm. In the clean-water zone minimal oxygen concentrations may be as low as 2 ppm for short periods of time.

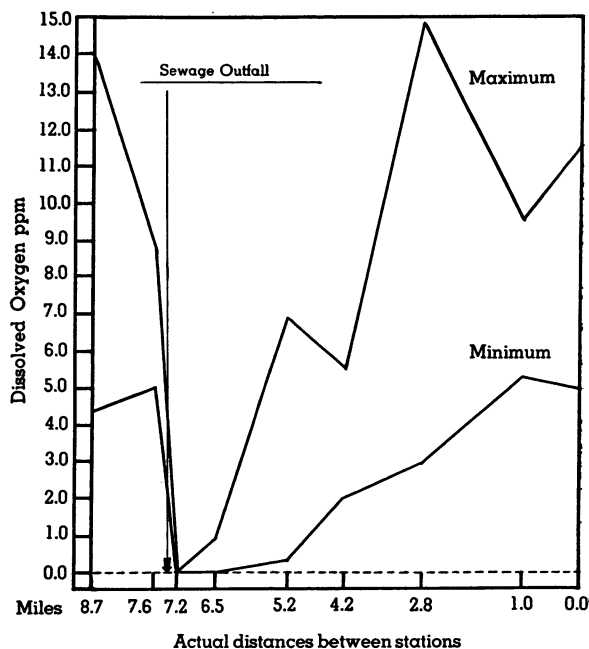
sive study. A detailed survey was made of the stream and a map prepared showing the location and extent of pools, runs, and riffles, and the stream profile and gradient. A sharp crested rectangular contracted weir and continuous float level recorder were placed in the stream about 3 miles above its mouth for the continuous recording of flows.

During the winter and spring, flows average about 6 to 7 cubic feet per second (cfs) with frequent floods of 40 cfs or more. In late summer and fall, flows are generally small, about 0.1 cfs above the sewage plant outfall and 1 cfs below it. During periods of low summer flow, the sewage plant effluent overwhelms the stream with oxygen-depleting wastes and causes a septic zone to be produced immediately below the outfall.

Ten stations were selected along the stream course for periodic sampling in all zones. These stations were designated by their distance in miles upstream from the mouth, as stations: 0, 1, 2.8, 4.2, 5.2, 6.5, 7.2, 7.3 (sewage outfall), 7.6, and 8.7. Monthly, or more frequently, samples were taken at these stations for the determination of dissolved oxygen, pH, CO<sub>2</sub>, methyl orange, and phenolphthalein alkalinity, and temperatures. Quantitative bottom samples were taken at monthly intervals at each station in pools, runs, and riffles. A Surber square-foot sampler was used in riffle areas, while a Petersen or Ekman dredge was used in other areas. Marginal samples were also taken, and reconnaissance surveys of bottom and surface fauna were made along the entire stream. (BOD studies have been made periodically at all stations, as well as plankton, microbottom fauna, and fish population studies.)

In the Lytle Creek investigations emphasis was placed on year-round studies to determine seasonal variations in aquatic populations and ecologic conditions. Attention was also directed to diurnal variations in physical and chemical conditions, especially during the spring and summer when they are more pronounced. Physical-chemical variations were determined by taking hourly samples at each of the stations for a 24-hour period. By these and other studies, information was obtained concerning ecologic conditions and their diurnal and seasonal variations in each of the zones.

These data were then correlated with those obtained by the quantitative and qualitative studies of the benthic, marginal, and surface



Range in dissolved oxygen, Lytle Creek, August 22-23, 1951.

fauna to provide information on (a) aquatic populations which can be expected to develop under certain ecologic conditions, and conversely (b) environmental conditions or variations in environmental conditions which are indicated by various compositions and densities of aquatic organisms.

## Results

The chemical and physical data collected during 6 sampling runs for the 10 stations are enumerated in table 1. These data present the maximum and minimum oxygen concentrations and water temperatures occurring in the stream during the early spring and summer months. Since a wide difference in the amounts of oxygen present in the stream during day and night was noted, special attention was paid to hourly variations in the dissolved oxygen content. The range of these variations for the period 9:00 a. m. August 22 to 8:00 a. m. August 23 is presented in the chart. During the reconnaissance surveys, conducted in connec-

tion with the sampling runs, 68 genera and 79 species of animals were collected and identified. Of these species, 29 percent were Diptera, or fly larvae, a group which is adapted to live under a wide variety of environmental conditions. Fifteen species of organisms were taken in the septic zone, but only six of them, *Chironomus tentans*, *Culex pipiens*, *Eristalis* sp., *Physa integra*, *Limnodrilus* sp., and *Tubifex*

sp., were abundant. Of the 23 species of mayflies, stoneflies, and caddisflies taken, only one species, *Callibaetis* sp., occurred outside of the clean-water zone. The organisms collected at five stations representative of each of the zones are listed in table 2. Their comparative abundance by month and zone is also indicated.

Among the primary requisites for animal existence are oxygen and food. When estab-

Table 1. Summary of physical and chemical conditions in Lytle Creek, 1951

Station	Range	Dates of hourly sampling over 24-hour period					
		4/24/51	5/1/51	5/9, 10/51	6/28, 29/51	8/15/51	8/22, 23/51
8.7	Maximum {Temp	63°					82.5°.
	{D. O	15 ppm					13.9 ppm.
	Minimum {Temp	45°					59°.
	{D. O	8.6 ppm					4.5 ppm.
7.6	Maximum {Temp	62°					77°.
	{D. O	14.7 ppm					8.8 ppm.
	Minimum {Temp	47°					61°.
	{D. O	7.2 ppm					5.1 ppm.
7.2	Maximum {Temp	59°					71°.
	{D. O	12.1 ppm					0.0 pp.,
	Minimum {Temp	48°					68°.
	{D. O	6.0 ppm					0.0 ppm.
6.5	Maximum {Temp	60°		70°	81°	79°	79°.
	{D. O	11.2 ppm		14.1 ppm	1.2 ppm	0.2 ppm	0.8 ppm.
	Minimum {Temp	50°		58°	71°	64.5°	61°.
	{D. O	4.7 ppm		0.6 ppm	0.0 ppm	0.0 ppm	0.0 ppm.
5.2	Maximum {Temp	60.5°	76°	70°	80.5°	80°	75°.
	{D. O	12.4 ppm	19.1 ppm	19.4 ppm	11.6 ppm	7.3 ppm	6.9 ppm.
	Minimum {Temp	54°	65°	59°	77°	71°	61°.
	{D. O	5.4 ppm	0.8 ppm	2.9 ppm	0.2 ppm	0.0 ppm	0.2 ppm.
4.2	Maximum {Temp	66°	76°			77°	71.5°.
	{D. O	16.4 ppm	18.1 ppm			5.4 ppm	5.5 ppm.
	Minimum {Temp	54°	65°			72°	55°.
	{D. O	6.5 ppm	1.0 ppm			1.4 ppm	2.0 ppm.
2.8	Maximum {Temp	69°	79°		87°	87°	80°.
	{D. O	13.8 ppm	14.3 ppm		10.1 ppm	12.3 ppm	14.7 ppm.
	Minimum {Temp	54°	65°		72°	72°	59°.
	{D. O	8.1 ppm	1.6 ppm		3.6 ppm	1.6 ppm	3.2 ppm.
1	Maximum {Temp	70°		70°			73°.
	{D. O	13.5 ppm		14.2 ppm			9.6 ppm.
	Minimum {Temp	54°		58°			65.5°.
	{D. O	6.9 ppm		5.2 ppm			5.3 ppm.
0	Maximum {Temp						75°.
	{D. O						11.6 ppm.
	Minimum {Temp						63.5°.
	{D. O						5.0 ppm.
Todds Fork	Maximum {Temp						70°.
	{D. O						10.7 ppm.
	Minimum {Temp						62.5°.
	{D. O						5.1 ppm.

Table 2. Distribution of invertebrates in Lytle Creek, May and August 1951

Organisms	Stations <sup>1</sup>									
	8.7		6.5		5.2		4.2		2.8	
	May	Aug.	May	Aug.	May	Aug.	May	Aug.	May	Aug.
<b>DIPTERA</b>										
<i>Chironomus flavus</i> .....	2 P								P	P
<i>Chironomus tentans</i> .....					P	A	P	A	P	P
<i>Chironomus flavicingula</i> .....	P						P		P	
<i>Chironomus quadripunctatum</i> .....		P								
<i>Chironomus</i> sp. A.....		P								
<i>Chironomus</i> sp. B.....		P								
<i>Pentaneura flavifrons</i> .....	P	P								
<i>Dictya</i> sp.....		P								
<i>Anopheles punctipennis</i> .....		P				P				
<i>Culex pipiens</i> .....			P	A	P	A	P	A	P	P
<i>Eristalis</i> sp.....			A	A						
<i>Stratiomyia</i> sp.....			P	P					P	
<i>Nemotelus</i> sp.....	P									
<i>Tabanus</i> sp.....	P		P	P			P	P	P	P
<i>Palpomyia</i> sp.....					P	P				
<i>Stilobezzia</i> sp.....									P	P
<i>Brachydeutera argentata</i> .....					P	P	P		P	P
<i>Simulium vittatum</i> .....	P									P
<i>Hemerodromia</i> sp.....	P									
<i>Pilaria</i> sp.....	P									
<i>Tipula</i> sp.....	P								P	
<i>Eriocera</i> sp.....	P								P	
<i>Paradixa</i> sp.....	P									
<b>COLEOPTERA</b>										
<i>Stenelmis crenata</i> .....	P	P								
<i>Stenelmis</i> sp.....		P								
<i>Simsonia quadrinotata</i> .....	P									
<i>Bidessus</i> sp. A.....		P								
<i>Laccophilus</i> sp.....	P						P		P	P
<i>Berosus</i> sp.....										P
<i>Tropisternus lateralis</i> .....						P				
<i>Tropisternus</i> sp.....	P	P	P	P	P	P	P	P	P	P
<i>Laccobius</i> sp.....				P		P		P		
<i>Peltodytes</i> sp.....	P	P						P	P	P
<i>Gyrinus</i> sp.....										P
<i>Dineutes</i> sp.....										P
<b>EPHEMEROPTERA</b>										
<i>Baetis cingulatus</i> .....		P								
<i>Baetis parvus</i> .....	P								P	P
<i>Caenis</i> sp.....	P	P							P	P
<i>Callibaetis</i> sp.....		P					P			
<i>Isonychia albomanicata</i> .....										P
<i>Stenonema femoratum</i> .....	P	P							P	P
<i>Stenonema ohioense</i> .....	P	P							P	P

See footnotes at end of table.

lishing biological indices of pollution, such requisites must be taken into consideration. In Lytle Creek from November 15, 1950, to April 1, 1951, the volume of flow was more than 6 cfs on all except three occasions. The time of flow from the sewage outfall to the mouth at Todds Fork never exceeded 48 hours. Water temperatures were under 60° F., and oxygen concentrations approached saturation throughout the

stream. From the standpoint of dissolved oxygen, the stream was satisfactory for most types of bottom organisms throughout its length during the entire period. Chemically, the water appeared to be relatively free from harmful pollution, but the fauna that was present still reflected the critically low oxygen concentrations occurring in the past.

From April 1 to October 1, the volume of flow

Table 2. Distribution of invertebrates in Lytle Creek, May and August 1951—Continued

Organisms	Stations <sup>1</sup>									
	8.7		6.5		5.2		4.2		2.8	
	May	Aug.	May	Aug.	May	Aug.	May	Aug.	May	Aug.
<b>TRICHOPTERA</b>										
<i>Brachycentrus americanus</i> .....										P
<i>Cheumatopsyche</i> sp.....	P	P							P	P
<i>Hydropsyche betteni</i> .....	P	P							P	P
<i>Hydropsyche bifida</i> .....									P	P
<i>Chimarra obscura</i> .....	P								P	P
<i>Dolophilus shawnee</i> .....	P									
<i>Phyacophila lobifera</i> .....	P									
<i>Ochrotrichia</i> sp.....	P									
<i>Hydroptila consimilis</i> .....	P								P	
<i>Hydroptila</i> sp.....	P								P	
<b>PLECOPTERA</b>										
<i>Acroneuria evoluta</i> .....		P								
<i>Allocapnia vivipara</i> .....	P									
<i>Nemoura venosa</i> .....	P									
<i>Perlesta placida</i> .....	P									
<i>Neoperla clymene</i> .....									P	
<i>Isoperla minuta</i> .....	P									
<b>ODONATA</b>										
<i>Plathemis</i> sp.....							P		P	P
<i>Pallthemis</i> sp.....							P			
<i>Argia</i> sp.....	P								P	P
<i>Agrion</i> sp.....	P								P	P
<i>Enallagma</i> sp.....							P			P
<b>NEUROPTERA</b>										
<i>Corydalus cornutus</i> .....										P
<i>Sialis</i> sp.....		P								P
<b>HEMIPTERA</b>										
<i>Belostoma</i> sp.....		P			P		P	P	P	P
<i>Corixidae</i> .....	P	P	P	P					P	P
<i>Gerris</i> sp.....	P	P							P	P
<i>Microvelia</i> sp.....		P								P
<i>Notonecta</i> sp.....								P		P
<i>Ranatra</i> sp.....								P		
<b>CRUSTACEA</b>										
<i>Asellus</i> sp.....	P	P							P	
<i>Cambarus rusticus</i> .....	P	P							P	P
<i>Hyalella</i> sp.....		P								
<b>MOLLUSCA</b>										
<i>Physa integra</i> .....	P	P	P	P	P	A	P	A	P	P
<i>Sphaerium solidulum</i> .....	P	P								
<b>ANNELIDA</b>										
<i>Limnodrilus</i> sp.....			P	A	P	A	P	A	P	P
<i>Tubifex</i> sp.....			P	A	P	A	P	A	P	P
<i>Glossiphonia</i> sp.....								P	P	
Total species per station.....	40	29	9	10	9	10	10	19	37	37

<sup>1</sup> The stations are classified into zones of pollution as follows: clean water, 8.7 and 2.8; septic zone, 6.5 and 5.2; zone of recovery, 4.2.

<sup>2</sup> A = abundant; P = present.

dropped to an average of 1 cfs, the time of flow extended to 7 or 8 days, water temperatures rose to as high as 87° F., and oxygen concentrations became critically low in the 4-mile section of stream below the sewage treatment plant outfall. Definite pollutional zones were established in May and persisted throughout the summer. An abundant food supply and a variable oxygen supply below the treatment plant outfall resulted in a distinctive fauna in each of these zones.

Critically low oxygen concentrations were found in the stream during the night at station 6.5 as early as May 9. Concomitant with the low nocturnal values, remarkably high oxygen concentrations were recorded during the afternoon at the same station. Peak concentrations of oxygen for the season occurred before the deciduous stream-side trees began to exercise a shading influence. A dissolved oxygen value of 19.4 ppm was found at station 5.2 at 3 p. m. on May 9. This peak was produced by a heavy plankton bloom. When marginal vegetation shaded the stream, blooms became less marked and daytime oxygen values were not as high.

As the summer advanced, oxygen depletion became of progressively greater duration and extent. The result was a well-defined oxygenless zone extending from the sewage treatment plant outfall to a point 2 miles downstream, as indicated by studies carried out on the night of August 22. This depletion persisted throughout the day at stations 7.2 and 6.5. The range in hourly dissolved oxygen values found late in August at the various sampling stations along the stream is shown in the chart.

## Discussion

One of the primary objectives of the Lytle Creek studies has been to arrive at a better definition of indicator organisms. The finding of certain organisms, such as rat-tail maggots, *Eristalis* sp.; sludgeworms, *Tubifex tubifex*; and bloodworms, *Chironamus tentans*, presents important evidence of the pollutional condition of a stream (1), while the absence of cleaner water forms is likewise a valuable measure of existing conditions.

With favorable ecologic conditions, such as high temperatures, uniform flow, freedom from

floods, and abundant food, prevailing throughout the summer of 1951, a surprisingly large and varied macrobottom fauna developed in the stream. This condition was observed during May and again in August when special collections of aquatic organisms were made at all sampling points along the stream, and their distribution correlated with the diurnal physical and chemical conditions prevailing at the various stations.

In evaluating aquatic organisms as indicators of pollutional conditions, great caution must be used because of several complicating ecologic conditions. First, many organisms which occur in large numbers in extremely polluted water may also be found in limited numbers in cleaner situations. Several species of invertebrates, such as the mosquito, *Culex pipiens*; beetle, *Tropisternus* sp.; and sludgeworms, *Limnodrilus* and *Tubifex* spp., which occurred in abundance at stations 6.5 and 5.2, also occurred in the clean-water zones. Second, many species listed in table 2 occurred in such small numbers as to discourage their individual use as indicators. Third, several ecologic factors other than the presence of a pollutant may limit the distribution of certain species; for example, erosion, floods, the size of the stream, the type of bottom, the flight range of the insect, and the portion of the stream under study. It is believed that the moderate abundance of single species should not be considered as biological indicators of pollution because organisms such as *Tubifex* usually associated with polluted areas are also found in clean waters. It is the complex or association of organisms which is important for indicating clean or polluted water. All organisms present and their relative abundance must be considered.

In satisfactorily using associations of aquatic organisms as indicators of pollution, the absence or much reduced numbers of formerly present clean-water species in an area may be as important, or more so, as numbers of known pollutional forms (2). In this connection, table 2 shows that such insects as mayflies, caddisflies, stoneflies, and hellgramites were almost entirely limited to the clean water occurring at stations 8.7 and 2.8. The only exception was a mayfly, *Callibaetis* sp. This form was collected in a marginal surface sample and evidently had

been able to obtain sufficient oxygen from the surface film to survive.

The absence of organisms, which are indicative of clean-water conditions, from a section of stream should not always be taken as definite indication of pollution. A knowledge of the life histories of the various groups of aquatic insects often is helpful in interpreting the meaning of their distribution. For example, in the collection of invertebrates made in August, only one species of stonefly and five species of caddisflies were represented. In the May collection five species of stoneflies and nine species of caddisflies were present. Their absence later on was due to their emergence as adults during the intervening months. Similarly, the absence of some beetles, dragonflies, and damselflies in the earlier collections was partly due to their presence in immature stages so that they were missed or overlooked.

Apart from the fact that pollutional organisms must be found and clean-water species must be missing in delineating the zones of pollution in a stream, the mode of occurrence of the organisms must be taken into account (3). For example, the assemblage of organisms found in the polluted zones of Lytle Creek presented the following characteristics: (a) very large numbers of individuals; (b) few species represented in the fauna; (c) principally scavenger types present; and (d) the presence of forms having low oxygen requirements or special adaptations for obtaining their oxygen supply. Not over 10 different species of macroinvertebrates were taken from stations 6.5 and 5.2 in the septic zone at any one time, while as many as 40 species were found in the clean-water zones (table 2). However, as many as 3,000 *Culex pipiens* or 20,000 Tubificids were collected per square foot sample in the septic zone, while no species exceeded 500 individuals per square foot in the clean-water area. All the insects occurring in the septic zone have special adaptations for obtaining oxygen, such as the caudal respiratory tubes of the mosquito and rattail maggot or the air space under the elytra of the beetles. With such adaptations, depletion of oxygen in the water does not serve as a barrier to their distribution. An abundant food supply enables

these forms to attain a great abundance in polluted areas.

When conditions are favorable for organisms which can adapt to pollution, they thrive and build high populations (4). For this reason, the society of organisms found in zones of pollution is highly significant in offering clues to the intensity of pollution and the degree of recovery. If changing conditions brought about by pollution are unfavorable, organisms must resist these changes, migrate, or be destroyed. Since many of the species of insects found in Lytle Creek are found in both the lower and upper sections of the stream but are missing in the pollutional zones, and since such organisms are continuous in other nonpolluted streams in the area, it seems probable that pollution is responsible for their discontinuous distribution in Lytle Creek.

In a stream polluted with organic wastes, a certain reduction of the supply of dissolved oxygen may occur in a certain section throughout the year, during the summer months, or for only a few days in the year. Because pollution surveys frequently cannot be made during the period of critical conditions, and chemical studies give information on physical-chemical conditions only at the time of sampling, there is need for additional methods which can be used throughout the year for determining over-all conditions and the extent and severity of brief critical or limiting environmental factors. The qualitative and quantitative composition of an aquatic population is determined by recurring critical conditions, even though of short duration, as well as the more stable or long-time environmental factors. Therefore, the complex of organisms which develops in a given area is in turn indicative of environmental conditions which have occurred during its development. Organisms having life histories of a year or more will thus serve to indicate unfavorable or limiting conditions that have occurred several months previously. Because aquatic populations are a result of past environmental conditions, they serve as a means for determining such conditions in a stream. They are especially valuable because they can be used during fall, winter, or spring months, when flows are large, dilution is at a maximum, dissolved oxy-

gen is near saturation, and visual evidence of pollution at a minimum, to delineate former septic areas or to indicate critical conditions of short duration.

In order to utilize aquatic populations in this way, however, it is essential to have a knowledge of the species composition and abundance of the various organisms in the population under the various ecologic conditions which prevail in clean and in polluted waters. Further, consideration must be given to the fact that pollution with organic wastes is only one of the several environmental factors determining the composition of aquatic populations. Other environmental factors, such as type, size and character of the stream, variations in flow, severity of silting, floods and erosion, water temperatures and the character of the watershed, are all important in determining the make-up of aquatic populations. Occasionally, there is a sparsity of bottom organisms which makes the drawing of any conclusions in regard to organic pollution difficult. However, one who has knowledge of and experience with aquatic organisms can, by a reconnaissance survey, learn a great deal about the severity and extent of pollution and the extent of stream recovery even though the survey is made at a time of year when the physical-chemical indicators of pollution are least evident or largely absent.

## Conclusions

1. Single species of organisms such as *Tubifex tubifex*, or *Chironomus tentans* cannot be used safely as indicators of pollution unless their relative abundance is considered.
2. The absence or much reduced numbers of formerly present clean-water species constitutes an important index in evaluating the degree of pollution.
3. The quantitative and qualitative composition of an aquatic population constitutes a valu-

able index in delineating zones of pollution in a stream.

4. In interpreting the distribution of organisms in a stream as an index of pollutional conditions, all environmental factors should be considered.

5. Pollutional associations are characterized by few species but large numbers of individuals.

6. The association of organisms normally present under most septic conditions is characterized by the absence of plant and animal eaters and the presence of scavengers.

7. Organisms characteristic of septic zones are those which can exist under conditions of very low oxygen or have adaptations for breathing atmospheric oxygen.

8. Nocturnal deficiencies in dissolved oxygen are often the critical environmental factor which determines the distribution of organisms in a stream.

9. Under conditions of organic pollution diurnal-nocturnal deficiencies in oxygen are sometimes concomitant with supersaturation of dissolved oxygen.

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## REFERENCES

- (1) Bartsch, A. F.: Biological aspects of stream pollution. *Sewage Works Journal* 20: 292-302 (1948).
- (2) Richardson, R. E.: The bottom fauna of the Middle Illinois River, 1913-1925. *Ill. Nat. Hist. Surv. Bull.* 17: 387-475 (1928).
- (3) Whipple, G. S.: The microscopy of drinking water. Ed. 4. New York, John Wiley and Sons, 1948.
- (4) Lackey, James B.: Stream microbiology. In *Stream sanitation*, by Earle B. Phelps. New York, John Wiley and Sons, 1944.