AQUATIC DIPTERA AS INDICATORS OF POLLUTION IN A MIDWESTERN STREAM

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A knowledge of the ecological requirements of aquatic organisms, especially the benthic forms, is of outstanding importance to biologists in determining the degree and extent of pollution in streams. An examination of bottom fauna serves to indicate conditions not only at the time of examination but also over considerable periods in the past. Those organisms having an annual life cycle will by their presence or absence indicate any unusual occurrence which took place during several previous months. Satisfactory use of aquatic organisms as indicators of pollution and self-purification of water is dependent upon a knowledge of the normal habitats of these organisms and their sensitivity to varying environmental factors such as pollution.

Among the aquatic invertebrates, insects such as the mayflies, stoneflies, and caddis flies are primarily restricted to clean water conditions. By comparison, forms such as the pulmonate snails, Tubificid worms, and certain species of leeches can more often be found under conditions where high organic and/or low oxygen content exist. Still other groups such as the Diptera, or true flies, are represented by forms which may be found in all types of stream habitats from the cleanest situation to the most polluted water.

Because aquatic Diptera are to be found in many different ecological niches in both clean and polluted water and many species are highly selective in their choice of habitat, they constitute one of the most important groups of indicator organisms. Year-round field studies of the ecology and distribution of the Diptera associated with the purification of organic wastes in streams were initiated on Lytle Creek in June, 1951. 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. miles, a third of which is contained within the city limits of Wilmington, Ohio, a city of 7,412 people in 1950.

Lytle Creek is a permanent stream, approximately 11 miles long and has an average gradient of 25 ft./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. Its natural flow is augmented, however, at a point about 7.3 miles above its junction with Todds Fork by the effluent from the Wilmington sewage treatment plant. Primary treatment including chemical precipitation is provided at this plant,[1] which treats an average of about 750,000 gallons of sewage per day. The sewerage is of the combined type, and the plant is overloaded during heavy rains which 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 100 percent of the stream flow.

In any ecological study, such as this one, it is important to understand, as far as possible, all of the many factors which influence the distribution of the group of organisms being considered. The occurrence of certain species of Diptera in a given area may be determined by such factors as the direction of stream flow, and the type of bottom materials present. Because of the important influences which the physiography has in determining the fauna of a region, the geology of Lytle Creek is discussed briefly.

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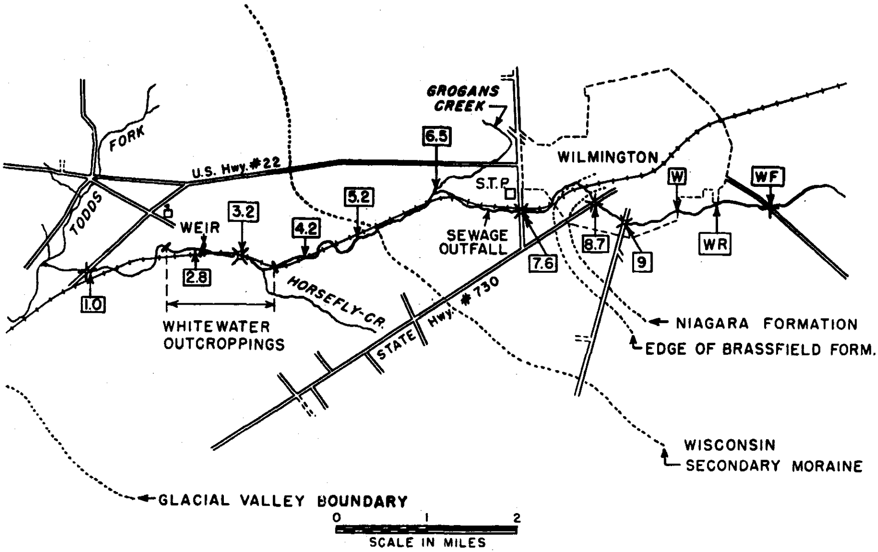
[1] Secondary treatment by means of activated sludge was initiated at this plant in 1954.

THE OHIO JOURNAL OF SCIENCE 56(5): 291, September, 1956.

According to Austin (1930) Lytle Creek flows inside an ancient great valley filled in by the Illinoian Glacial invasion. The subsequent Wisconsin glaciations added a comparatively small amount of till. The stream has done very little cutting in its bed. The basic underlying rocks in the area, which by their dip, have determined the direction of stream flow from east to west, are of Ordovician Age and form the northward extension of the Cincinnati Anticline. There is evidence of a gentle subsidence to the north previous to the Illinoian Glacier with the result that many streams in the drainage basin flowed northward rather than to the south as they do today.

Lytle Creek rises in a secondary moraine of the Early Wisconsin Age, 2½ miles northeast of Wilmington, flowing through glacial till of that age over buried Silurian strata to Wilmington. This till near and through Wilmington contains great amounts of sand. About 9 miles above its mouth an ancient swamp or forest peat bed is crossed. This bed is decidedly acid (Austin, 1930). The creek continues beyond Wilmington to flow through till and sand, reaching outwash gravel terraces about 1 mile below the sewage treatment plant outfall. These terrace gravels are persistent for 1½ miles and may exert some influence on the 'purification' of the stream. At approximately 3 miles above its mouth, the stream cuts its valley deeply to an 'island' of bedrock of the Richmond group of the Ordovician. Just below this point the creek cuts off into glacial till, again leaving the bedrock, looping around for ⅛ mile to return to this rock, flowing on it until the formation ends about 2½ miles above the mouth. The remainder of the stream bed is glacial till with sand.



LYTLE CREEK DRAINAGE BASIN

PROCEDURES

Twelve stations, representing all zones, were selected along the stream course for periodic sampling (fig. 1). Monthly, or more frequently, samples were taken at seven key stations for the determination of dissolved oxygen, pH, CO_2, methyl orange, and phenolpthalein alkalinity, and temperatures. Diurnal variations in these physical-chemical factors during each of the seasons were determined by taking hourly samples at selected stations for 24-hour periods.

Quantitative bottom samples containing Diptera and other invertebrates were taken at monthly intervals at seven key stations in pools, runs, and riffles as part of a routine sampling program. A Surber square foot sampler was used in riffle areas while an Ekman dredge was used in pools and runs. Marginal samples were taken by means of a handscreen (Needham) and special surface sampler (Tarzwell).

In addition to the specimens taken in the quantitative samples many Diptera were hand picked from specific micro-habitats throughout the stream. Attention was directed toward collecting as many different species of Diptera as possible, determining their habitat preferences, and correlating their distribution with the environmental variations which existed. Adult Diptera were also collected by sweeping vegetation with an insect net and by attracting them to a bright light at night.

The larvae and pupae collected during these reconnaissance studies were brought back to the laboratory alive and individually reared where possible. After emerging, the adults were chloroformed and preserved with their exuviae in vials of 75 percent ethyl alcohol. Some larvae and pupae were also killed and similarly preserved for future study and the correlation of larvae with the adults.

Keys and descriptions by Johannsen, Townes, and other workers were used for identification. Nearly all determinations of adult specimens were checked by Dr. W. W. Wirth of the U. S. National Museum.

RESULTS

Physical-Chemical Data. The Lytle Creek studies revealed that during the summer months when flows were low, septic, recovery, and clean water zones were distinct. From May to November each year variations in dissolved oxygen were at a maximum, and extensive oxygen depletion was characteristic of the two mile section below the sewage treatment plant outfall. During the winter months higher flows and lower temperatures resulted in the life (pollutional) zones changing their location and extent. During the period, December to April, natural purification proceeded at a slower rate, wastes were carried further downstream, and dissolved oxygen was abundant throughout the stream.

Daily maximum and minimum dissolved oxygen values recorded during four representative sampling runs have been shown graphically by Tarzwell and Gaufin (1953). Extreme variations in other important environmental factors affecting the biota in the stream are shown in table 1. Seasonal variations in these different factors throughout the stream have been discussed in detail by Gaufin and Tarzwell in previous papers (1952, 1955).

Biological Data. In addition to the superfamily Tipuloidea, 10 familes of Diptera were collected in Lytle Creek or its tributaries. Of these, 3 families, Psychoididae, Dixidae, and Ephydridae, occurred so rarely as to be of little importance in this study. The other seven, Culicidae, Chironomidae, Heleidae, Simuliidae, Stratiomyidae, Syrphidae, and Tabanidae, were taken on many occasions in various sections of the stream. Because of the variety of species and habits presented and their frequent appearance, the Chironomidae and Simuliidae were especially singled out for intensive consideration as the research progressed.

A total of 94 species of Diptera were collected during the course of this study. By means of their distribution in the stream it is possible to classify them into three categories; first, the pollutional forms, or those able to survive low oxygen and/or a high content of organic materials, second, the facultative or tolerant forms which can live under a wide range of conditions; and third, those forms which require clean waters with abundant dissolved oxygen. A list of the species collected, the location where they were taken, and the pollutional status of the area in which each occurred are given in table 2.

Very few of the larvae and pupae of Tipuloidea can be identified to species with any degree of certainty. In addition, many species develop in moist soil as well as in truly aquatic habitats, and, therefore, many of the adults that may be taken along a stream may have emerged from habitats other than the stream itself. Unless the larvae are individually reared it is thus almost impossible to identify them with certainty. Because of these taxonomic difficulties and a lack of personnel the superfamily Tipuloidea was largely ignored in this study. However, of the seven species taken all occurred in clean water areas only.

TABLE 1

Extreme physical and chemical variations
Lytle Creek 1949–1952

		Stations									
		7.6 Clean Water Zone		6.5 Septic Zone		5.2 Recovery Zone		2.8 Lower Recovery and/or Clean Water Zone		1.0 Clean Water Zone	
Water Temperature	Max.	79°F	8/15/50	80°F	1/8–15/50	81°F	8/15/50	91°F	7/22/52	82°F	7/22/52
	Min.	32°F	12/6–7/49	39°F	12/6–7/49	32°F	12/6–7/49	32°F	12/6–7/49	32°F	12/6–7/49
B.O.D. (5 day) p.p.m.	Max.	2.6	3/14–16/50	82	8/15–18/50	42	12/6–13/49	13.5	12/6–13/49	6.4	12/6–13/49
	Min.	1.2	9/28–29/50	27	9/28–29/50	3.7	9/28–29/50	1.8	9/28–29/50	1.2	9/28–29/50
pH	Max.	8.3	2/25–26/52	8.2	2/25–26/52	8.4	7/26/51	9.1	5/16/51	8.6	7/26/51
	Min.	7.3	7/11–13/50	7.2	8/15/51	7.5	8/22–23/51	7.5	8/22–23/51	7.7	12/6–13/49
Total Alkalinity (as $CaCO_3$ in p.p.m.)	Max.	270	9/28–29/50	306	7/26/51	309	12/6–13/49	310	12/6–13/49	270	9/28–29/50
	Min.	185	3/14–16/50	222	11/28/51	216	3/22/51	223	3/22/51	169	12/6–13/49
Total Phosphate (p.p.m. PO_4	Max.	1.59	12/6–13/49	26.2	12/6–13/49	17.5	12/6–13/49	12.5	12/6–13/49	3.11	12/6–13/49
	Min.	0.55	8/15–18/50	4.0	9/28–29/50	2.4	9/28–29/50	2.3	9/28–29/50	1.32	9/28–29/50
Total Nitrate (TKN-N p.p.m.)	Max.	6.9	9/28–29/50	38.0	12/6–13/49	32.0	12/6–13/49	43.0	9/28–29/50	7.2	12/6–13/49
	Min.	0.04	3/14–16/50	10.0	9/28–29/50	6.9	9/28–29/50	0.95	3/14–16/50	0.45	3/14–16/50
Volume (c.f.s.)	Max.							100+	3/1951		
	Min.							1.0	8/1950		

Only one specimen of Dixidae, *Paradixa* sp., was collected, that being taken in June 1951, in marginal vegetation at Station 2.8 in the lower recovery zone. The family Psychodidae was represented by 2 genera, *Telmatoscopus* and *Psychoda*, and 4 species. Larvae of the first mentioned genus were found only under clean water conditions while the species of *Psychoda* taken were confined to the septic zone and zone of recovery. The immature stages of this genus are often found in the surface film of foul water, in sewage, and in wet, decaying, organic matter. One of the species found in Lytle Creek, *Psychoda schizura*, was also present in large numbers in the filter beds of the Dayton, Ohio, sewage treatment plant. Another family, Ephydridae, which occurred infrequently, but always where there was considerable decaying organic matter, deserves special mention because of the unusual habitats frequented. The larvae of one genus, *Teichomyza*, are found regularly in urine. Larvae and puparia of *Ephydra gracilis* occur in large numbers in the Great Salt Lake, Utah, where the dissolved oxygen content is frequently less than 1.0 p.p.m. and the salt content reaches 25 percent.

TABLE 2

Distribution of Diptera in Lytle Creek

Stations and Bottom Type*

Species	1	2.8	Horsefly Creek	5.2	6.5	Grogan's Creek	8.7	9	W
	Clean water sand & glacial till	End of rec. zone Ord. shaly limestone	Clean water sand to Ord.-shale	Rec. zone Poll. glacial gravel	Poll. zone glacial gravel	Clean water glacial till & sand	Clean water glacial till & sand	Clean water sand	Clean water glacial till
1. *Superfamily Tipuloidea*									
Pseudolimnophila luteipennis Alexander									F
Pilaria (Sintenis) sp. indet.									F
Tipula caloptera	F						F		
Tipula abdominalis (Say)			C				C		
Tipula cf. tricolor gr.	F								
Tipula cf. furca	F								F
Hexatoma cinera	F								F
2. *Family Psychodidae*									
Psychoda alternata (Say)				F	F				
Psychoda larva Johannseni *A*				F	F				
Psychoda schizura? (Fullaway)				F	F				
Telmatoscopus Eaton sp. indet.							F		
3. *Family Culicidae*									
(a) Subfamily Culicinae									
Culex pipiens L.		F	F	C	N	F	F	F	F
Anopheles punctipennis (Say)	F	F							F
(b) Subfamily Chaoborinae									
Chaoborus punctipennis (Say)	C								
4. *Family Dixidae*									
Paradixa Tonnoir sp.		F							
5. *Family Heleidae*									
Bezzia glabra (Coquillett)	F	F	F		F	F			F
Stilobezzia antennalis (Coquillett)				F	F	F			F
Heleidae, gen, et spp. indet.	F	F	F	F	F	F	F		F
6. *Family Chironomidae*									
(a) Subfamily Diamesinae									
Diamesa nivoriunda (Fitch)		F				F	F		F
(b) Subfamily Hydrobaeninae									
Hydrobaeninae gen. et spp. indet.	F			F	F				
Corynoneura scutellata Winnertz							F		
Corynoneura (Thienamanniella) Winnertz LC-72							F		
Hydrobaenus Fries sp. indet.		F				F	F		F
Hydrobaenus LC-81									C
Hydrobaenus LC-88									C
Hydrobaenus LC-98									C
Hydrobaenus (Psectrocladius) sp.		F				F			
Cricotopus absurdus Joh.		F							
Cricotopus bicinctus (Meigen)	C	N	N	F		F	C		C
Cricotopus exilis Joh.		C							
Cricotopus politus (Coquillet)	C	C	C			C	C		C
Cricotopus tricinctus (Meigen)	C	N	C				C		C
Cricotopus trifasciatus (Panzer)	C	N	C	F			C		C
Cricotopus LC-64		C	C						
Cricotopus LC-98							C		
Cricotopus sp. indet. (light adults)	C	C	C						C
Cricotopus sp. indet. (dark adults)	C	C	C						C
(c) Subfamily Pelopiinae									
Pentaneura carnea (Fabricius)		F		F			F		F
Pentaneura illinoensis (Malloch)		C	F						
Pentaneura melanops (Wiedemann)	C	C	C	C	C	C	C		C

TABLE 2 (*Continued*)

	Stations and Bottom Type*								
Species	1	2.8	Horsefly Creek	5.2	6.5	Grogan's Creek	8.7	9	W
	Clean water sand & glacial till	End of rec. zone Ord. shaly limestone	Clean water sand to Ord.-shale	Rec. zone Poll. glacial gravel	Poll. zone glacial gravel	Clean water glacial till & sand	Clean water glacial till & sand	Clean water sand	Clean water glacial till
Pentaneura vitellina (Kieffer)									F
Pentaneura sp. indet.	F	F							F
Anatopynia dyari (Coquillett)	C	C	C	C	C	C	C	C	C
Anatopynia sp. indet.				F					
Pelopia stellata (Coquillett)	C	C		C	C	C	C		C
Pelopia punctipennis? Meigen	F	F		F			F		
Procladius culiciformis (L)		C							
Procladius LC-18	F	C		F		F	F		
Clinotanypus caliginosus	F								
Clinotanypus (pupal exuviae, indet)		F							
Coelotanypus concinnus (Coquillett)		C				C	F		F
(d) Subfamily Chironominae									
Calopsectra johannseni (Bause)		C							
Calopsectra neoflavella (Malloch)	C	C	C				C		C
Calopsectra nigripilus (Joh.)							C		C
Calopsectra LC-7	C	C					C		C
Calopsectra, sp. indet.	F								
Pseudochironomus richardsoni Malloch	F	F					F		
Polypedilum illinoense (Malloch)	F	N	N						
Polypedilum vibex? Townes		F							
Polypedilum fallax Joh.				C			C		
Polypedilum LC-15		F		F		F	F		F
Polypedilum sp. indet.	F			F		F			F
Tanytarsus LC-61							F		
Endochironomus nigricans Joh.		F					F		C
Xenochironomus scopula Townes		F							C
Cryptochironomus fulvus Joh.							F		F
Cryptochironomus LC-55	F	F	F	F		F	F		F
Chironomus neomodestus ? Malloch	F	C	F	F			F		F
Chironomus dux Joh.		F							F
Chironomus decorus Joh.	C	N	C	C	C	C	C		F
Chironomus riparius Meigen		F		N	N		F		F
Chironomus LC-26									
Chironomus LC-100		F							
Glyptotendipes lobiferus (Say)	F	F					F		
Glyptotendipes LC-31		F		C	C		F		
Harnischia tenuicaudata (Malloch)									F
Genus Incertus Mallochi D		F							
Microtendipes pedellus (DeGeer)	F	F					C		F
Paratendipes albimanus (Meigen)		F					F		
Stictochironomus varius Townes	F	F	C	F		C	N	N	N
Stenochironomus macateei (Malloch)		F							
7. *Family Simuliidae*									
Prosimulium johannseni (Hart)							F		
Simulium vittatum Zetterstedt	N	N	N	F			F		F
Simulium venustrum (Say)							F		F
Cnephia pecuarum (Riley)							F		
8. *Family Tabanidae*									
Tabanus atratus Fabricius	F		F	F	F	F			
Tabanus stygius Say	F		F	F	F				
Tabanus benedictus ? Whitney					F				
Tabanus giganteus DeGeer			F						
Tabanus lineola ? Fabricius					F				
Tabanus variegatus Fabricius			F						
Tabanus sp. indet.	F	F					F		F

TABLE 2 (*Continued*)

Species	Stations and Bottom Type*								
	1	2.8	Horsefly Creek	5.2	6.5	Grogan's Creek	8.7	9	W
	Clean water sand & glacial till	End of rec.zone Ord. shaly limestone	Clean water sand to Ord.-shale	Rec. zone Poll. glacial gravel	Poll. zone glacial gravel	Clean water glacial till & sand	Clean water glacial till & sand	Clean water sand	Clean water glacial till
9. *Family Stratiomyidae*									
Odontomyia cincta		F		F	F				
Stratiomys meigeni		C		C	C				
Stratiomys discalis				F	F				
10. *Family Syrphidae*									
Eristalis aeneus Fabricius					F				
Eristalis bastardi Macquart		F		C	N				
Eristalis brousi Williston					F				
Syrphus americanus Weidemann				F					
Chysogaster pulchella (Williston)				F					
Chilosia prima ? (Hunter)				F					
11. *Family Ephydridae*									
Brachydeutera argentata				F	F				

*Prefix LC before a number means the larva specimen is unidentifiable from available descriptions or keys and is found in Lytle Creek Basin.

F = Few, occasional specimen to 2 per square yard

C = Common, 3-50 per square yard

N = Numerous, 51 or over per square yard

As shown in table 2, the larvae of those species which can utilize atmospheric oxygen directly were all unaffected by low dissolved oxygen concentrations in the stream and occurred frequently in large numbers in the septic and recovery zones. The members of the families Culicidae, Syrphidae, and Stratiomyidae have special adaptations for obtaining oxygen from the surface, such as the terminal siphons of the mosquito and the "rat-tails" of the rattail maggot. Because of these adaptations, depletion of dissolved oxygen did not serve as a barrier to the distribution of these forms. Limited numbers of all species of these three families were also found in the clean water areas but they attained their greatest abundance in the polluted sections where food was abundant. The Tabanidae, the larvae of which enter and leave the water frequently or swim on the surface (Schwardt 1930), and the Heleidae, which are also reported to do so (Foote and Pratt 1953), were also found to be unaffected by pollution in the stream. The family Chironomidae (Tendipedidae) was represented by forms which display all degrees of habitat preference from the cleanest to the most polluted situation. The four species of Simuliidae that were taken were all confined to riffles or fast running water. Three of the species occurred only in the upper clean water sections of the stream. The fourth, *Simulium vittatum*, was found at all stations except in the septic zone. At Station 5.2 in the recovery zone it was often abundant in the riffles in a section of stream where the dissolved oxygen content frequently went below 1.0 p.p.m. at night.

Among the Chironomidae, *Chironomus riparius* was the only species taken regularly in the septic zone during the course of the study. It also occurred in large numbers in the recovery zone but was very rarely present in any clean water sections. By contrast, a closely similar species, *Chironomus decorus*, whose larvae are also red and possess ventral gills, occurred frequently in the clean water sections but was far less common in the polluted zones. These two species and a third member of the group, *Chironomus tentans*, are frequently referred to in the literature as being positive indicators of organic pollution. All three species are often confused in the immature stages and are almost impossible to separate as larvae. In fact, Gaufin and Tarzwell (1952) considered *Chironomus tentans* rather than *Chironomus riparius* as being the characteristic species found in the septic and recovery zones of Lytle Creek.

Because these and other red-blooded Chironomids are commonly confused and associated only with habitats of high organic content a special effort was made to determine their taxonomic status and ecological adaptations. Specimens which were reared revealed that four of the species of red-blooded chironomids found in the stream, *Chironomus riparius* and *Chironomus decorus*, both of which possess 4 ventral gills, and *Stictochironomus varius* and *Microtendipes pedellus* which lack these gills, had such distinctly different habitat adaptations that they deserved considerable further study.

Of a total of 5,325 specimens of *Chironomus riparius* collected in 1951, all were taken from the polluted zones of the stream. By contrast, of the *Chironomus decorus* collected, 1,803 specimens were from clean water sections and 999 from the septic and recovery zone; while *Stictochironomus varius* produced 1,106 specimens in clean water and only 13 in the recovery zone. Of the 61 larvae of *Microtendipes pedellus* taken, all occurred in cleaner water areas. In addition to the latter two species, a number of others were largely, if not entirely, restricted to clean water areas.

Polypedilum illinoense, *Cricotopus absurdus*, and *Cricotopus politus* might be classified as clean water species since they were restricted to the clean water sections of the creek.

A difference in the response of several of the red-blooded species of chironomids to pollution was revealed by an experiment performed in the laboratory. Larvae of several species of Diptera clinging to stones in a riffle and a pool at Station 5.2 below the mouth of a clean-water tributary, were collected and placed in well aerated jars of stream water from this station. At the time of collection (3:00 P.M.) the D.O. in the riffle was 6.5 p.p.m. while in the pool it was 5 p.p.m. The water had a characteristic sewage odor and the rocks were covered with a good growth of algae. Within 24 hours all larvae of *Stictochironomus varius*, *Calopsectra* sp. indet., and *Simulium vittatum*, and all but one of *Paratendipes* sp., all taken from the riffles, were dead. Half of the larvae of the *Chironomus decorus* group were alive and in good condition, some having pupated and emerged. The dead larvae of the *C. decorus* group were mostly *C. decorus*. Those which pupated and emerged and, most of those still living as larvae, were *C. riparius*.

Several species of Diptera appeared to be restricted by the rate of movement of the water, the type of substratum, or the kind of food present more than by the effects of pollution. The species of Stratiomyidae and Tabanidae, which were not dependent upon the amount of oxygen dissolved in the water, were found scattered throughout the drainage area according to the abundance of their particular food preference. *Stratiomys meigeni*, for example, was as abundant in mats of algae at Station 2.8 as at Station 6.5. *Tabanus atratus* and *Tabanus stygius* were scattered in distribution but were most common where there were cattle along the creek upon which the adults could feed. *Stictochironomus varius* was most common at Station 8.7 and in the intermittent tributaries of the main stream, where it was restricted almost entirely to a sandy stream bottom.

Another very important factor influencing the distribution of Diptera in Lytle Creek was the amount of precipitation. The normal rainfall at the Wilmington Station of the U. S. Weather Bureau for the 6-month period of April through September is 24.79 in. The year 1950 was near normal in summer rainfall after a very wet winter. The years 1951 and 1952 had dry summers which decreased both the flow of Lytle Creek and the normal dilution of sewage. Some species taken in pollution zones in 1950 were not found in 1951 and 1952. The number of specimens of some species taken in 1950 was greater than that of the same species taken in 1951 and 1952. This may have been caused by non-uniform sampling, however. Tarzwell and Palmer (1951) state that the amount and character of rainfall exerts a definite and important effect on the growth of algae in a stream. Patriarche and Ball (1949) found that an increase of algae in ponds was followed by a great rise in chironomid population. This same influence undoubtedly affected the chironomid distribution in Lytle Creek. Large numbers of larvae and pupae occurred in the stream wherever there were mats of algae.

The physiography and geological history of the region also exert an influence, but slightly appreciated so far, on the fauna of Lytle Creek and its reaction to sewage pollution. The beds of gravel found throughout the creek provide possible habitats for many species of Diptera as well as strata for the holdfasts of algal, fungal, or bacterial growth. Also the climate was cool throughout the interglacial periods in this region (Austin 1930). These factors may have been determinants of the present abundance of species such as *Stictochironomus varius*, which based upon its distribution, seems to be a species of northern or cool climates (Townes, 1945).

The Whitewater formation which forms the bedrock bottom for a short distance at Station 2.8 is of more than passing interest.

This particular formation is an isolated one, no other outcrop being found anywhere else along the creek. Further, on both sides of this outcrop, drilling has failed to reach other bedrock at 77 ft. below the surface (Austin, 1930). Lytle Creek has cut but little into this bedrock (about 2 ft.) although it is relatively soft; but where it has formed cascades and piled up broken and eroded fragments, special habitats have been formed which have been utilized to a surprising degree by different genera of the family Chironomidae. For example, the cascades provide habitat for a few species of *Cricotopus* which have spread over them in great numbers, to the exclusion of practically all other species. In an approximate square foot sample taken in the Cascades June 10, 1952, the following specimens were found:

A). Larvae preserved (killed)—	
Cricotopus bincinctus	853
Cricotopus trifasciatus group	73
Hydrobaeninae, indet. (prob. *Cricotopus*)	37
B). Emergences from larvae kept alive and placed in culture jars—	
Cricotopus absurdus	11
Cricotopus trincinctus	56
Critotopus trifasciatus	43
Cricotopus bicinctus	175
Cricotopus, sp. indet	13
Total of known specimens	1261

In a zone of glacial boulders and piled rock in this area the species predominating to the exclusion of practically all others are *Simulium vittatum* and *Polypedilum illinoense*, thus demonstrating that in any given section the type of bottom may be very important in determining the type of fauna which is present.

DISCUSSION

As has been previously mentioned, the aquatic Diptera of Lytle Creek can be grouped into three categories relative to their pollutional responses—those found only in cleaner water, those usually found in polluted waters, and those which appear to show no preference for either polluted or clean water. In using these data for determining the degree of pollution or its presence or absence in streams, consideration must be given to the physiology of the insects themselves, their relationship to the geology of the region, and to other environmental factors. These considerations are essential because there is a difference in habitat preference between the species, genera, families, and even suborders. All aquatic insects secure oxygen in one or more of three ways; directly from the atmosphere, by absorption of dissolved oxygen from the surrounding water, or by absorption of free oxygen from the aerenchyma of aquatic Tracheophytes which they puncture (Edwards, 1953a, b). Some of the species of Chironomidae are apparently able to carry on respiration even in oxygen depleted streams for a relatively long period of time (Walshe, 1950, 1951).

All of the "air-breathers" possess integuments which are practically impervious to water and salts. On the other hand, larvae of the Chironomidae and Simuliidae absorb dissolved oxygen from the surrounding water through the integument. In the pupae of these families it has been shown that the so-called thoracic respiratory organs are not used to extract dissolved oxygen from water. In those of the Simuliidae, however, the internal structure of these organs suggests possible adaptation for atmospheric absorption (Wigglesworth 1939). The direct air-breathers are equipped with siphons, open "trumpets," or tubes to maintain frequent contact of the tracheal system with atmospheric oxygen. Because the direct air-breathers are not dependent upon dissolved oxygen the lack of such oxygen, which is usually concomitant with zones of high organic pollution, does not deter them from utilizing these waters as habitats. Such conditions are advantageous for these organisms because food is plentiful and enemies and competition are restricted by the low oxygen supply. For example, the ***Psychoda*** population of sewage plant trickling filters is large and mosquito larvae and pupae occur in great numbers in septic zones. As many as 20,000 ***Culex pipiens*** larvae have been taken in a square foot marginal sample at Station 6.5.

Generally, the larvae and pupae of the air-breathing aquatic Diptera show an oxygen consumption that is independent of the dissolved oxygen content of the surrounding medium. This may result from morphological or physiological adaptations. The long "rat-tail" siphons of the Syrphidae, which may inhabit highly polluted waters, is a morphological adjustment of the air-breathing insect which makes it able to be completely independent of the oxygen of the surrounding water. Physiological adjustment is shown in several species of Chironomidae. The exact nature of this physiological adjustment is not known, but it has been found by various workers that some red Chironomidae containing erythrocruorin ("haemoglobin") can exist in the complete absence of dissolved oxygen for 30 to 120 days (Lindeman 1942). Walshe (1947b) also found that a colorless European species, ***Prodiamesa*** sp., survives under frequent low oxygen pressure tensions. The main advantage which "haemoglobin" seems to possess, is that it does not give up its 0_2 until a $p0_2$ of 7 mm. Hg. is reached; it enables fast recovery from a period of anoxia, and it is vital in the process of feeding (Walshe 1951, Wigglesworth 1939, Leitch 1916). There does not seem to be any ascribable function to haemoglobin when the dissolved oxygen is at zero p.p.m.

While many species of chironomids are adapted to low concentrations of dissolved oxygen and are able to withstand complete anoxia under laboratory tests for many days, our studies indicate that only some predatory species and those possessing ventral bloodgills persist in those areas of Lytle Creek which have continued low $p0_2$ or frequent oxygen depletion. In laboratory studies of ***Chironomus plumosus***, Walshe (1947a) found that during a period of prolonged anoxia when the insect lived anaerobically the water surrounding it showed a great increase in the amount and number of organic acids. Wigglesworth (1939) believes that the ventral gills are organs of excretion; *i.e.*, that these allow the excape of lactic acid and other organic acids into the surrounding water medium, with the result that there is no "oxygen debt" since no lactic acid remains in the system to be oxidized. While all larvae will die under prolonged anoxia, the length of time varying according to species, Walshe (1950, 1951) states that in certain instances death may be caused by factors other than lack of oxygen before asphyxiation takes place. The work of this investigator (Walshe 1951) indicates that starvation may be the direct cause of death in specimens surviving 30 days or more, because when the D.O. is lowered, feeding is abandoned in favor of undulatory efforts at aeration. She describes the process and states further that the larva when in great duress through anoxia, leaves its tube and will die after a period outside the tube if no dissolved oxygen is obtained. In such a situation feeding is abandoned completely and the respiratory efforts (undulating movements) are increased.

Miall (1912) and Miller (1941) postulate that predatory species probably rise to the surface of the water to obtain oxygen at the interface.

All these considerations of physiology are important because Lytle Creek contains 3 species of red-blooded Chironomidae each bearing ventral gills—*Chironomus decorus*, *C. riparius*, and *Glyptotendipes* LC-31. *Chironomus decorus* and *C. riparius* have 2 or more generations a year in Lytle Creek, with each generation having several broods. The second generation of *C. decorus*, which emerges when stream conditions are at their worst in September and October, has not been found in the septic zone, while the first generation was frequently collected. By comparison, both generations of *C. riparius* frequently occur in that area and larvae are very rarely taken at any other regularly sampled stations (4 larvae were collected over a period of 3 years at Station 2.8 and 3 larvae over the same period at Station 8.7

During a 24-hour study carried out in October 1952, at Station 2.8 where the water was 9 in. deep, it was found that the dissolved oxygen was consistently but slightly higher near the air-water interface, than that near the bottom. This finding may be important when it is considered that the "haemoglobin" of the red species is still ½ saturated with 0_2 when the oxygen tension (external oxygen pressure) = 4 mm. Hg. (Leitch, 1916) and that the predatory species move toward the surface at low $p0_2$ (Miller 1941). In comparison the haemoglobin of human blood begins to give up its 0_2 when the external $p0_2$ falls to 90 mm. Hg. and 50 percent is lost when $p0_2$ falls to 25 mm. Hg.

Another factor which may be limiting to the biota in a stream is the food supply. The food of aquatic Diptera may consist of materials orally ingested or dissolved materials absorbed through specialized structures elsewhere on the body. From the works of Wigglesworth (1939), Hers (1942), and others, it appears that the anal gills of Culicinae and Chironomidae and the rectal gills of Simuliidae and Corethrinae are used for the absorption of Cl^- from the surrounding water. Other foods, required for their metabolism are various, but in most instances the Chironomidae, Culicinae, and Simuliidae, consume microorganisms ranging from protozoa and algae to the small Arthropoda. Some Chironomidae and the Corethrinae are predators, feeding on the larvae of other members of the families. Still other Chironomidae are scavengers, among these is *Chironomus riparius*; Branch (1923) found this species appearing in great numbers in a sluiceway from a milk processing plant in New York. Due to the fact that some of the terrestrial Chironomid larvae of the subfamily Hydrobaeninae live in and feed on dung, it is not surprising to find some aquatic species adapted to feeding on sewage.

In controlled pond cultures, some fertilized with sheep manure and others unfertilized, Sadler (1935) found that *Chironomus tentans* ingests sheep manure in great quantities when it is available. Unfortunately, not enough work has been done on the larval food preferences of many Chironomid species to enable workers to use such information in determining habitat preferences. However, it is known that all aquatic larvae of insects must depend directly or indirectly upon microorganisms such as bacteria, yeasts, and protozoa for the accessory factors such as vitamins which are essential for their growth.

While the availability and amount of food and oxygen were the most important factors determining the distribution of the majority of species of Diptera in various sections of the stream, other factors such as speed of current and bottom type should not be overlooked. For example, all four species of Simuliidae that were taken were confined to riffles or fast running water. Current can also limit the fauna as during the fall and winter months floods scour the bottom in some sections, carry away many Diptera, and intermix them with other populations downstream.

The nature of the stream bottom was also of considerable importance in limiting the distribution of Diptera in the stream. *Stictochironomus varius*, a common inhabitant of the upper clean water section and the intermittent tributaries, was restricted almost entirely to a sandy bottom. The bedrock cascades at Station 2.8 were inhabited by four species of *Cricotopus* to the exclusion of practically all other species. In a zone of glacial boulders and piled rock in this same area the predominate species were almost entirely *Simulium vittatum* and *Polypedilum illinoense.*

However, while all of these latter factors were important and should not be overlooked, the availability and amount of food and oxygen were the most important factors determining the distribution of Diptera in this stream.

SUMMARY AND CONCLUSIONS

1. A total of 94 species of Diptera representing 11 families and one superfamily were collected and identified from Lytle Creek or its tributaries during this study. These species were found in many different ecological niches in both clean and polluted sections of the stream, with many being highly selective in habitat.

2. All species with special adaptations for obtaining oxygen from the water surface such as the larvae of the families Culicidae, Syrphidae, and Stratiomyidae were unaffected by low dissolved oxygen concentrations in the stream and occurred frequently and in large numbers in the septic and recovery zones. Larvae of the tha families Tabanidae and Heleidae which can enter and leave the water frequently or swim on the surface were also found to be unaffected by pollution in the stream.

3. Representatives of the family Chironomidae were particularly adaptable to many different habitats in the stream, with a number of species showing a marked selection for specific ecological niches in each of the life zones. Because of their adaptability and selectivity of habitat they constituted the most important group of indicator organisms in Lytle Creek among the Diptera.

4. A number of ecological factors other than pollution were responsible for noticeable variations in the composition of the Diptera biota at different times and must be taken into account in interpreting the distribution of organisms in the stream. For example, normal decreases in the larval populations of certain species or the complete absence of others at certain times were caused by mass emergences of adults. Floods on occasion scoured out the bottom in some places intermixing the fauna or wiping it out completely.

5. The presence of *Chironomus riparius*, *Glyptotendipes* LC-31, *Eristalis bastardi*, and *Culex pipiens* in large numbers was very characteristic of the septic and recovery zones and their abundance in other similar streams may be interpreted as an indication of organic pollution.

6. The predatory chironomids had a wider range and greater adaptability to different environmental conditions than any other species, except *Chironomus decorus*, and because of their widespread distribution, their use as indicator organisms is distinctly limited. In addition *Anatopynia dyari*, *Pelopia stellata*,

Pentaneura melanops, *Pentaneura carnea*, and the families Heleidae and Tabanidae can be considered in this category.

7. A considerable difference in the pollutional tolerances of four red-blood chironomids was found to exist with *Chironomus riparius* being confined to the septic and recovery zones; *Stictochironomus varius* and *Microtendipes pedellus* to the clean water areas; and *Chironomus decorus* somewhat ubiquitous.

8. Species restricted to definite and narrow habitats constituted much better indicator organisms than those species adaptable to both clean water and polluted conditions. Because all of the species found in the septic and recovery zones were also found in limited numbers in the clean water areas, those species restricted to the latter areas have more value as indicators. On the basis of the Lytle Creek studies, *Polypedilum illinoense*, *Microtendipes pedellus*, *Coelotanypus concinnus*, *Endochironomus nigricans*, and possibly *Stictochironomus varius* and *Chironomus neomodestus* are positive indicators of an unpolluted habitat when they are present.

ACKNOWLEDGMENTS

Gratitude is expressed to Dr. C. M. Tarzwell and other staff members of the Aquatic Biology Section for help and suggestions during the course of this study. Special thanks are extended to the following people for help in the identification of specimens: Dr. O. A. Johannsen, Cornell University, Dr. H. J. Townes, Jr., Entomologist, U. S. Air Force, and Dr. W. W. Wirth, U. S. National Museum. Lawson Moyer of the U. S. Weather Bureau, Columbus, Ohio, kindly furnished the climatological data, and Wilmington College information concerning the geology of the region. Dr. Barbara Walshe-Maetz furnished valuable physiological data on the Chironomidae.

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