

Ecological significance of respiration and substrate for burrowing Ephemeroptera

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In an attempt to understand the differential distribution of *Ephemera simulans* and *Hexagenia limbata* in intergrading substrates, the author extended his studies of the ecological roles of the laboratory-derived comparisons of respiration, morphology, and behavior.

Conclusions drawn, mainly from my earlier and present work and also work reported by Lyman in 1943 and Hunt in 1953, seem to be consistent with field distribution. It appears that *H. limbata* is not found in streams unless prevailing conditions include undisturbed, fine sediments, for *Hexagenia* does not burrow into coarse substrates. To the contrary, *E. simulans* can thrive in gravel and pebble stream substrates in which the oxygen content of interstitial water is greater than 1.20 cc/l. In lakes where *Ephemera* can burrow into substrates of gravel, marly sand, and marl, it is limited in the fine sediments by the relative inefficiency of its small gills at low oxygen concentrations (<1.20 cc/l). Again, *Hexagenia* does not occur in coarse substrates. However, in fine sediments such as marl it is found in large numbers and increases in abundance in muds. Most probably its distribution further into lakes is limited by impenetrable peaty substrates or by oxygen stratification (<0.80 cc/l).

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Introduction

Two parameters of great importance to the distribution of aquatic organisms are substrate and oxygen. Although many investigators have considered the various roles that oxygen or substrate independently play, only recently have attempts been made to relate an animal's respiratory physiology to substrate and this, in turn, to its distribution. Wautier and Pattée (1955) took the initial step when they discovered that oxygen consumption of burrowing mayfly nymphs varied with the substrate (sand, pebbles, blank-bottle). The lowest oxygen consumption was registered in the substrate best simulating the type within or upon which the nymphs are generally taken in nature. Thus, they added to a substrate-distribution correlation the possibility that substrate-induced respiratory rates might be factors in an animal's selection of particular bottom types.

Wautier and Pattée's presentation stimulated research leading to the publication of several papers by Eriksen (1963b, 1963c, 1964) dealing with laboratory studies of respiratory physiology and related morphology of burrowing mayfly nymphs. Like Wautier and Pattée, Eriksen showed the effect of substrate particle size—this time of

a greater size range—upon oxygen consumption. Then, by using "optimal substrates", he discovered the true respiratory ability of burrowing mayflies and thereby disqualified earlier ecological conclusions. By knowing their basic respiratory responses, Eriksen subsequently elucidated the various respiratory functions of the structure and behavior of these mayflies. Though information so gained appears ecologically suggestive, studies of the organism in its natural environment must be made before results can be meaningfully applied to a field situation. Therefore, the purpose of this paper, relative to the study of the ecological significance of respiration and substrate for burrowing mayflies, is to discuss laboratory findings (Eriksen 1963b, 1963c) in the light of field collections and observations.

Materials and Methods

Experimental Animals

The morphologically similar nymphs *Ephemera simulans* (Walker) and *Hexagenia limbata* (Serville) were the subjects of this study. They are among the largest (20–35 mm) of the Ephemeroptera, stoutly built and adapted for burrowing. Their prothoracic legs are broadened, allowing them to scoop the substrate. A stout pair of forward-projecting mandibular tusks and a prominent rostrum are also used in burrowing to help push aside the substrate. Large filamentous biramous gills are conspicuous along the sides of the abdomen. Three heavily fringed caudal

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filaments, used more when the animals are swimming than when burrowing, extend from the end of the abdomen.

In the southern Great Lakes region *E. simulans* and *H. limbata* have similar 1-year life histories. Adult swarms usually appear for a short period during the latter part of May. Mating occurs and eggs are deposited. During summer and fall, growth is rapid so that by winter nymphs are about 20 mm long. Activity continues throughout the winter but growth is slight until a spring rise in water temperature. From this time until adults emerge and swarm again, growth and maturation are extremely rapid (Spieth 1936; Hunt 1953; Britt 1962).

Oxygen Determination in the Field

As the experimental animals are burrowing forms, special problems arise when one is attempting to measure the oxygen concentration of their habitat. Ideally, samples should be collected where nymphs obtain their respiratory supply. In lakes, they pump through their burrows water obtained from a very narrow stratum at the substrate-water interface (see Results and Discussion). In streams, indications are that *E. simulans* cannot maintain a burrow open to the flowing water and therefore must obtain its water interstitially (Eriksen 1964). My laboratory and field observations showed that *E. simulans* burrowed little more than 2.5 cm into the sediments. Therefore, small interstitial water samples were obtained at that depth and their oxygen content was determined using the methods described by Eriksen (1963a). The respiratory environment of lake forms was never determined, since apparatus suitable for this purpose does not exist.

Substrate Sampling

To obtain stream substrates as specific to the nymphs' location as possible, a technique was developed which involved sampling with wide-mouth, half-pint mason jars. Such a jar was placed upside down on the substrate, entrapping surface silt. (The silt fraction is largely lost in most stream sampling techniques, yet may be of considerable importance ecologically (Sanders 1956, 1958).) The jar was pushed into the soil to a depth previously marked (4 cm) and closed by sliding the lid through the substrate and over the mouth. The sample was then raised with little or no disturbance of even surface sediments. All collections were fixed with formalin and stored for later analyses.

Throughout the study period, water was too cold for a swimmer to sample the lake bottom by the half-pint jar method. Therefore, an Ekman dredge was used to secure fairly undisturbed collections and a subsample of each of these was taken for particle-size analysis. As substrates are typically more homogeneous over larger areas in lakes than in streams, the method was considered representative.

Substrate Analysis

Particle-size samples of fine sand and larger fractions may be analyzed fairly easily by sieving, as may silt-sized samples with a hydrometer. Problems

arise, however, when silt and larger fractions occur together. The hydrometer method is not adequate for the large particles, and sieving is unsatisfactory because of clumping of silt particles and their adherence to larger components. An apparatus which can be used to separate these fractions (Lauff *et al.* 1961) was utilized for lotic samples by the procedures referred to by Cummins (1962). A modification of this method was necessary for mud and marl lake samples because, as Beanland (1940) suggested, many particles in a natural sediment are probably larger than are found after harsh laboratory treatment. Marl forms concretions, and burrowers may well use these concretions much as they would use larger particles in a stream. Hence, the samples were carefully washed through a series of sieves and the fraction remaining in each sieve was dried and weighed. Silt and clay were treated as in stream samples. With each component (terminology after Cummins 1962) separated, its percentage by weight of the total sample was determined.

Field Collection Sites and Procedures

Four areas representing a range of bottom types were selected in Fleming Creek in which *E. simulans* was common. These locations are on the property of the University of Michigan Botanical Gardens, Washtenaw County, Michigan. Because of a well-protected watershed and the influx of a number of springs, the volume of flow, hence depth and velocity, remain surprisingly similar throughout the year. These characteristics are favorable to the stability of the bottom which affords a "secure" habitat for the burrowing nymphs. Although this woodland stream may vary from 5 to 10 m in width and contain pools of 1 m or more in depth, *Ephemera* is largely restricted to areas where, because of current, a pebble, gravel, and sand substrate predominates and where water depth seldom exceeds 20–60 cm.

In such shallow portions of the stream selected for study, the procedure was to take, within a restricted area, 3 samples of interstitial water for oxygen analyses and 12 substrate samples. One substrate sample was used in particle-size analysis while all were sorted to determine nymphal density. The information thus accumulated was averaged and considered as one sample (e.g., F220, Table I). The total area covered in this way was relatively small. To minimize possible errors, sieve samples each covering approximately 580 cm² were also taken. Results of the two methods were averaged, giving the value reported as "nymphs/m²" for the substrate in question. In some instances one method, but not both methods, was feasible. Therefore to make the data comparable, an approximate value for the unused technique was obtained. This was accomplished by multiplying a correction factor of 1.4 (determined from substrates on which both techniques were used) by the sieve value to obtain a figure for the half-pint jar method or dividing into the jar value to obtain a figure for the sieve method.

A predominately marl shoal in Big Silver Lake, Washtenaw County, Michigan, known to support

many *E. simulans* and *H. limbata* nymphs, was selected to represent lake conditions. Five transects were established, each passing approximately perpendicular from shore out over the shoal. A cross-sectional view of the shoal along a transect would show a slow but gradual increase in water depth to about 3 m at the edge of the drop-off. Sand and gravel substrates near shore give way rapidly to sands and the latter merge also rather rapidly into a wide expanse, predominately of marl. The marl ultimately grades into mud which extends to the drop-off where the substrate becomes fibrous from detritus. Bottom samples were taken at four stations along each transect. Six collections were obtained at each station with a subsample of one used for sediment analysis and the others used to determine species density.

Spot collections were taken in widely separated areas to compare the particle-size composition of other burrowing mayfly-inhabited localities. These were Whitmore Lake, Livingston County, Michigan; Douglas Lake, Cheboygan County, Michigan; Flathead Lake, Lake County, Montana; the warm fork of Crawfish Creek, Yellowstone National Park, Wyoming; and Uintah River at Highway 40, Uintah County, Utah. Interstitial oxygen samples were also taken at Crawfish Creek.

Laboratory Observations and Experiments

A number of supplemental observations and experiments not hitherto reported, however important to the conclusions of this study, were carried out in the laboratory in aquaria or respirometers. They will be described as necessary in Results and Discussion.

Respiration and Morphology

Physiological studies of burrowing mayflies are not numerous and much of what is assumed to characterize their respiratory physiology emanated from the laboratory of H. M. Fox in the 1930's. In one such paper, Fox *et al.* (1937) described respiratory experiments with, among others, *Ephemera vulgata*. The differential oxygen method was used to determine oxygen consumption in respirometer bottles containing only water (blank-bottle) and experimental animals. By comparing oxygen consumption with environmental parameters, Fox and his associates determined that the greater respiratory demand correlated with a higher environmental oxygen concentration, arguing that a nymph using more oxygen needed to live where greater quantities were available. Using the same method, Eriksen (1963c) found, as had Fox *et al.*, that burrowing mayflies appear to be respiratory adjusters. In turn if the arguments of Fox *et al.* relative to the

ecological meaning of the respiratory data are applied to the present situation with *H. limbata* and *E. simulans*, the former would be thought to require a more highly oxygenated environment since, in the blank-bottle, *Hexagenia*'s oxygen consumption was greatest. However, *Hexagenia* proved to be significantly more tolerant of low concentrations in the laboratory (Eriksen 1963c), and is assumed, by its usual location in a lake, to tolerate more stringent oxygen conditions there as well.

Introduction into the respirometers of a substrate suitable for burrowing resulted in activity more nearly normal for *H. limbata* and *E. simulans* and was accompanied by a significant decrease in oxygen consumption to similar intensities when each was in its respective "optimal substrate". Also, utilization of the nymph's "optimal substrate" over a range of oxygen concentrations produced evidence to show that both species are respiratory regulators, not adjusters as might previously have been assumed (Eriksen 1963c). This information changes the ecological interpretation of the facts as Fox and his colleagues saw them, for the concepts of "regulator" and "adjuster" are basic to an understanding of the manner in which an organism, with respect to its respiration, responds to its environment and they must be considered before distribution is fully understood (Berg and Ockelman 1959).

The work that followed (Eriksen 1963c) showed that by increasing gill beat with lowering oxygen concentration, *Ephemera* and *Hexagenia* were able to maintain respiratory regulation. A marked difference exists in the ability of *H. limbata* to continue to regulate its oxygen consumption to an oxygen concentration (0.80 cc/l) lower than could *E. simulans* (1.20 cc/l). This difference seems to be determined by gill morphology. Beating rapidly, the smaller gills of *Ephemera* produce a respiratory current sufficient for its needs at oxygen concentrations as low as 1.20 cc/l, but not lower. By comparison, *H. limbata* with much larger gills requires about four-tenths the number of beats required of *E. simulans* to produce an equal current. Being able to further increase the beat, and hence the water volume passed, *Hexagenia*

can regulate to concentrations as low as 0.80 cc/l.

Only Eriksen (1963b) has related leg morphology to substrate inhabited, although Lyman's (1943) experiments indicated what *Hexagenia* will and will not penetrate. These works indicate that leg structure determines the substrate into which a nymph can burrow. *E. simulans* nymphs have narrow tibiae and tarsi which allow them to penetrate most substrates although they appear best suited to either pick at individual particles of a coarse ($\phi -1$, $\phi -2$) substrate to construct burrows or scoop out tunnels in marl and sand (Eriksen 1963b). *Hexagenia* with its enlarged prothoracic legs (1.5 times the surface area of *Ephemera*'s) apparently can use them only to scoop out burrows in fine, adhesive sediments containing little peat (Lyman 1943; Eriksen 1963b).

These findings suggest that under otherwise optimal conditions, *Ephemera simulans* should be limited to substrates approximating either $\phi -1$ and $\phi -2$ or $\phi +5$ where oxygen concentrations exceed 1.20 cc/l. *Hexagenia limbata* should occur in only fine adhesive soils (approximately $\phi +5$) where the oxygen concentration is greater than 0.80 cc/l. As conditions of substrate or other affecting parameters change from optimal, thus raising respiratory rates, the minimal oxygen concentrations necessary for survival should rise. It remains, however, to produce corroborating field data for these suggestions and the following discusses information relative to the problem.

Results and Discussion

Stream Sampling

Transect sampling of four areas in Fleming Creek indicated for *Ephemera simulans* a direct relation among particle size, concentration of interstitial oxygen, and nymphal density each increasing with the other (Table I). Laboratory experiments with *E. simulans* have demonstrated that an oxygen concentration of approximately 1.20 cc/l is critical for respiratory regulation, for survival (Eriksen 1963c), and for remaining burrowed (present study). If environmental significance is attached to this value the data can be

divided into groups with greater and less than 1.20 cc/l of oxygen in interstitial water at the 2.5-cm sampling depth. By so doing only 70 *Ephemera* nymphs/m² were averaged in Fleming Creek samples where oxygen registered below 1.20 cc/l. In contrast, the average nymphal density in substrates with greater than that amount of oxygen was 360/m² (Table I). When this apparently critical oxygen concentration is compared with median diameter (Md. ϕ) of the substrate samples, those soils with less than 1.20 cc/l of oxygen in the interstitial water had an average Md. ϕ of 0.0, while for those greater than 1.20 cc/l, it was -2.8 .

Median diameters of samples obtained from widely separated flowing waters inhabited by *E. simulans* averaged $\phi -3.0$ for the Uintah River and 0.0 for Crawfish Creek (Table II). Similarity between the Uintah River and Fleming Creek samples is obvious although there is considerable difference between these two and Crawfish Creek, at least with regard to Md. ϕ . Thus, although a correlation among these parameters exists, with a given level of oxygen being critical and a certain particle size being optimal, it is not always clearly defined in each sample.

Several collections (e.g., F192, F214, F219; Table I) showed large median diameters with a lower oxygen and perhaps also a lower nymphal density than might be expected from the trend. On the other hand F165, with a small Md. ϕ ($+1.9$) and very low oxygen concentration, produced a considerable number of nymphs; and Crawfish Creek, with a 0.0 Md. ϕ , had abundant nymphs in a comparatively high oxygen concentration (Table II). These variant situations show that conditions selected are not always the same nor are they totally described by Md. ϕ and oxygen concentration.

It is, of course, the clues suggested by the exceptions which are most interesting and tend to be most illuminating to the questions asked. In most of these "exceptions", fine sediments compose a disproportionate percentage of the total when compared to "normal" samples (Table I). Finer sediments may blanket or clog substrates and decrease interstitial water flow. Webb and Hill (1958) quantified this relationship in beach soils by

TABLE I

Density of *Ephemera simulans* nymphs in Fleming Creek samples compared with substrate and oxygen concentration (Jan.-May 1960)

Md. ϕ	% fine sand ($\phi + 3$ and smaller)	Interstitial O ₂ concn. (cc/l)	<i>E. simulans</i> (No./m ²)	Sample No.	Substrate description
-4.0	3.37	1.48	201	F220	Less silt than F219
-3.8	4.09	2.44	607	F156	Gravel and pebbles over clay
-3.8	5.18	1.52	809	F159	Pebbles and gravel; scattered cobbles
-3.6	4.81	2.31	490	F186	As F156
-2.9	9.87	2.50	456	F189	As F159
-2.9	12.15	0.88	169	F219	Heavy silt over sand, gravel, and pebbles
-2.9	5.06	1.30	145	F224	As F220
-2.8	10.17	2.13	410	F217	Coarse sand, some gravel
-2.7	10.58	1.30	733	F162	Washed pebbles and gravel in sand bar
-2.5	5.95	1.96	148	F211	Gravel, pebbles, and cobbles
-2.3	13.35	0.27	101	F192	As F162
-2.2	3.73	2.75	150	F208	Gravel and pebbles
-1.8	4.08	1.40	109	F205	Coarse sand and gravel
-1.3	14.49	0.92	120	F214	As F217
-0.6	8.48	1.56	67	F202	Coarse sand and silt
-0.3	7.85	0.96	0	F222	Fine and coarse gravel
-0.0	20.51	0.41	0	F223	Heavy silt over sand; some gravel and pebbles
+1.4	30.99	0.41	37	F199	Sandy mud
+1.7	40.76	0.30	51	F195	Sandy organic mud
+1.9	42.85	0.18	152	F165	As F195
+3.0	76.02	0.00	4	F221	Organic mud

O₂ concn. <1.20 cc/l: av. Md. ϕ = 0.0, av. nymphs = 70/m², av. % $\phi + 3$ and smaller = 28.77.

O₂ concn. >1.20 cc/l: av. Md. ϕ = -2.8, av. nymphs = 360/m², av. % $\phi + 3$ and smaller = 6.28.

TABLE II

Substrates and oxygen concentrations of Uintah River and Crawfish Creek where *Ephemera simulans* nymphs are common

Md. ϕ	% fine sand ($\phi + 3$ and smaller)	Interstitial O ₂ concn. (cc/l)	<i>E. simulans</i> (abundance)	Sample No.	Substrate description
Uintah River (18 Aug. 59)					
-4.1	13.82	—	Common in river	U3	Some pebbles and gravel in
-3.5	12.59	—	" " "	U1	coarse sand;
+2.2	56.26	—	" " "	U2	scattered cobbles
Crawfish Creek (22 Aug. 59)					
-0.4	11.81	2-3 cm 4.19	Abundant in creek	CC2	Fine gravel and
+0.4	20.68	3-4 cm 3.17	" " "	CC1	coarse sand
		1-2 cm 3.56			"
		2-3 cm 2.56			"
		4-5 cm 1.11			

demonstrating a correlation among grain size, pore space, and drainage rate with the latter markedly decreasing if more than 20% fine sand was present. Braefield (1964) found that beach soils containing about 10% fine sand were poorly oxygenated (Fig. 1), which supports, he believes, the contention that drainage is a vital factor controlling oxygen

levels in beaches. Braefield also thinks that drainage will be slight regardless of grain sizes as long as more than 10% fine sand is present to fill in interstitial spaces. This phenomenon is demonstrated by the present study in poorly sorted stream substrates and an appreciation of the "clogging" role of fine sediments is most helpful in interpreting

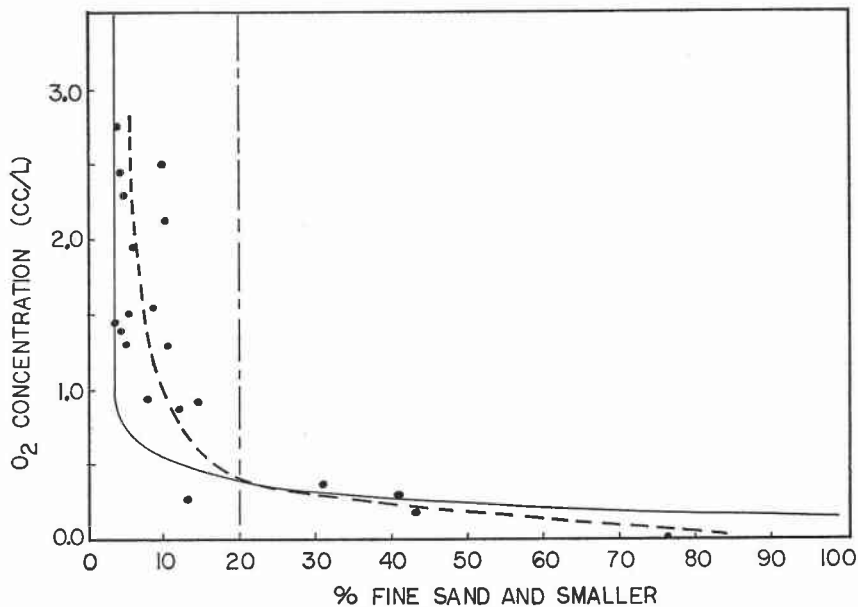


FIG. 1. Interstitial oxygen concentration as related to the percentage of fine sand ($\phi + 3$) and smaller particles in the substrate. --- oxygen vs. % fine sand in Fleming Creek soils. — oxygen vs. % fine sand in beach soils (Braefield 1964). --- % fine sand which significantly reduces drainage (Webb and Hill 1958).

the variant Fleming and Crawfish Creek collections. In fact, the Fleming Creek collections, with but one exception, revealed low oxygen (< 1.20 cc/l) if more than 8–10% fine sand were present, irrespective of the Md. ϕ (Fig. 1). The confusing situation of the Crawfish Creek samples (ϕ 0.0, Table II) also is clarified if the fine fractions are considered. *Ephemera* can certainly burrow into a ϕ 0.0 substrate, although with more difficulty than into its optimal (Eriksen 1963b), but usually does not do so probably because of low interstitial oxygen. In Crawfish Creek, a good deal of oxygen is present in interstitial water, most likely because of a small amount of fine sand for a 0.0 Md. ϕ , making it a suitable habitat.

Thus, there is considerable evidence to suggest that a value of 1.20 cc/l, which is critical for *Ephemera simulans* in the laboratory, is also critical in the field. Likewise, the “preferred” field substrate (Md. ϕ -2.8, Table I) is similar to the ϕ -1 and ϕ -2 “optimal” laboratory substrates (Eriksen 1963b), that is, similar when one considers the difficulty of evaluating the effect of poor

substrate sorting on the reactions of the nymphs and considers, too, the importance of a small amount of fine sand, which normally increases in amount with lowering Md. ϕ , to the oxygen concentration.

Lake Sampling

In contrast to the coarse sediments inhabited in streams, gravels, sands, and even fine marls are utilized by lake-dwelling *E. simulans* nymphs. This is demonstrated by scattered spot collections as well as the main sampling program on Big Silver Lake. For example, samples from an area of sand and gravel in Whitmore Lake, Michigan, had Md. ϕ diameters of -1.3, +1.7, +2.0, and +2.2. *E. simulans* nymphal density was 155, 207, 34, and 0/m², respectively. In Douglas Lake, Michigan, samples from substrates in which this species is said to occur had Md. ϕ diameters of +1.5, +1.5, and +1.7. These finer substrates compare well with those from Flathead Lake (+1.5, +1.7) and the coarse samples from Big Silver Lake (+2.1, +2.5) where 78 and 103 nymphs/m² were collected, respectively.

TABLE III

Density of *Ephemera simulans* and *Hexagenia limbata* nymphs in Big Silver Lake samples compared with substrate and depth (21, 23 May 1960)

Transect No.	Sample No.	Md. ϕ	Depth (m)	No. nymphs /m ²	
				<i>E. simulans</i>	<i>H. limbata</i>
Station 1: medium sand with thin organic skim					
1	S6	+2.5	0.7	103	0
4	S30	+4.0	1.3	60	17
2	S14	+2.6	1.0	95	0
5	S38	+2.6	1.2	17	0
3	S22	+2.1	1.0	78	9
Av. for station 1		+2.8	1.0	71	5
Station 2: sandy marl					
1	S7	+4.0	1.6	17	78
4	S31	+2.5	1.6	17	26
2	S15	+2.8	1.5	34	9
5	S39	+2.0	1.3	52	0
3	S23	+4.3	1.3	26	0
Av. for station 2		+3.1	1.5	29	23
Station 3: marl with numerous marl concretions					
1	S10	+3.8	2.0	9	9
4	S34	+3.7	1.8	0	112
2	S18	+4.1	1.6	69	60
5	S42	+3.5	1.6	34	43
3	S26	+3.7	1.6	34	112
Av. for station 3		+3.8	1.7	29	67
Station 4: organic mud and some marl					
1	S11	+3.1	3.0	9	86
4	S35	+3.2	2.7	0	103
2	S19	+3.5	3.0	26	95
5	S43	+4.0	2.8	26	138
3	S27	+3.8	3.0	9	112
Av. for station 4		+3.5	2.9	14	107

In contrast to *Ephemera*'s preference for the more coarse soils, *H. limbata* nymphs frequent only marls and muds, being restricted by their burrowing ability (Lyman 1943). In some lakes, such as the main study area, distribution of the two species can be quantitatively compared since they inhabit intergrading bottom types. Information collected from Big Silver Lake (Table III) indicates that with a decrease of Md. ϕ diameter with distance from shore, *Ephemera*'s numbers decrease while those of *Hexagenia* increase. For example, average median diameters were ϕ +2.8, +3.1, +3.8, and +3.5 with greater distance from shore; correspondingly, *E. simulans*' density was 71, 29, 29, and 14/m² and *H. limbata*'s 5, 23, 67, and 107/m². As the station farthest from shore (station 4) showed an increase rather than a decrease in its particle-size composi-

tion while the numbers of *Ephemera* continued to decrease and those of *Hexagenia* increased, it is reasonable to assume that particle size is not the only factor influencing occurrence. Other parameters in the lake, such as origin and composition of sediments and the effect these have on oxygen concentration, need also to be considered.

In shore and shoal areas where wave action influences the bottom, general conditions are somewhat similar to those in the coarse soils of streams. In these gravel and sand substrates, only *E. simulans* is found, apparently because of burrowing ability (Lyman 1943; Eriksen 1963b). In somewhat deeper water *Ephemera* and *Hexagenia* reside together in marl, but in yet deeper water where the percentage of mud increases, the numbers of *Ephemera* diminish while those of *Hexagenia* remain high. Yet farther

out into a lake *Hexagenia* is limited either by peaty substrates which it cannot penetrate or by oxygen stratification (Lyman 1943).

It would not appear that the particle-size of the fine sediments has changed sufficiently from station 4 to 5 to affect species distribution, but the organic nature of the sediments and their effect on the oxygen concentration present another series of controlling factors to be considered. Because interstitial oxygen is either very low or absent in fine and organic sediments, and because of the lack, at that depth, of turbulence severe enough to transport bottom sediments, burrows must and can be maintained open to the substrate-water interface and respiratory water is taken from this environment. From his study of tubificid respiratory behavior, Alsterberg (1922) concluded that an oxygen microstratification exists at the mud-water interface. Mortimer (1941, 1942), by means of redox potential studies, and Joeris (1964), using a horizontal Kemmerer bottle to obtain water samples, demonstrated that such microstratification exists. Oxygen utilized in the sediments' biochemical oxygen demand (BOD) is derived from water immediately above as shown by Hayes and MacAuley (1959), as well as Knowles *et al.* (1962), who studied the respiration of natural sediments. This consumption can affect the oxygen content of the water at the interface and must vary with particle size and organic content.

In the laboratory a carmine suspension was observed being drawn into glass "burrows" through the bailing action of abdominal gills. Such observations demonstrate that the layer of water above the substrate available to the nymphs for respiratory purposes is very thin (6–7 mm). Hence microstratification of oxygen, the severity of which depends upon substrate and water circulation, can indeed be important to the nymphs. Oxygen consumed from water at this interface, by the BOD of the sediments, would be minimal over inorganic sediments (e.g., marl from Big Silver Lake, stations 2 and 3) and hence oxygen could be sufficient for the requirements of *E. simulans* and *H. limbata*. By comparison, oxygen reduction at an organic mud-water interface (station 4) might develop

sufficiently to limit the more demanding requirements of *Ephemera*, yet suffice for *Hexagenia*; or at the extreme, exclude *Hexagenia* also.

The greater the oxygen stratification at the substrate-water interface (the lower the oxygen concentration), the more water that must be pumped through the burrows to maintain respiratory regulation (Eriksen 1963c). Similarly, the deeper or longer the burrow, the more water that must be moved by the gills to obtain sufficient respiratory supply. Recalling gill structure and low oxygen requirements (Eriksen 1963c) perhaps explains the different burrow sizes (Table IV) as a behavioral response necessitated by a differential ability to pump water through burrows particularly at low oxygen concentrations. In the laboratory, maximum burrow depth for *Ephemera* was 2.9 cm and averaged only 1.5 cm. In comparison, Hunt (1953) reported a maximum burrow depth for *Hexagenia* of 12.7 cm although in my observations burrow depth did not exceed 6.4 cm, averaging only 4.2 cm. Burrow length was likewise greater for *H. limbata* than *E. simulans* (e.g., average surface distance between openings: 9.3 and 4.7 cm, respectively).

TABLE IV
Burrow measurements, in centimeters

<i>Ephemera simulans</i>		<i>Hexagenia limbata</i>	
Depth	Surface distance between burrow's openings	Depth	Surface distance between burrow's openings
0.4	—	2.3	—
0.7 (2)*	—	2.6	5.5
1.0 (4)*	—	2.9 (2)*	—
1.3	2.9	3.2	—
1.3	—	3.9	7.1
1.6	4.5	3.9	—
1.6 (7)*	—	5.5	10.6
1.9	6.7	5.8	9.0
1.9 (2)*	—	6.1	14.1
2.3 (2)*	—	6.4	—
2.6	—		
2.9	—		
1.5	4.7 Av.	4.2	9.3

*Number in parenthesis refers to the number of burrows with that measurement.

The behavior of nymphs in very low oxygen was observed a number of times in the laboratory. As oxygen in the burrows approached critical low levels, nymphs took up positions at the mouths of their burrows, then partially out on top of the substrate where oxygen must have been in somewhat greater supply. With a further decrease of oxygen concentration they left the bottom to swim toward the water surface, and finally die. Under similar conditions in nature it is probable that many nymphs fall prey to fish. Burrowing mayflies are very important in the diet of many fish as was well shown by the studies of Clemens *et al.* (1923), Dymond (1926), Rawson (1930), and Britt (1962). Hunt (1950) saw bluegills (*Lepomis macrochirus*) and yellow perch (*Perca flavescens*) grubbing into the bottom. To what depth these fish can grub is unknown, but stomach analyses showed considerable quantities of burrowing nymphs present. Nymphs in coarse substrates or burrowed deeply in mud would be difficult to rout out; but if burrowed shallowly, or if exposed, predation might decrease the population considerably.

Ephemera nymphs were never found burrowed in laboratory substrates with oxygen concentrations less than 1.07 cc/l and probably the lower limit was somewhat higher. They would not attempt to burrow if oxygen was less than 1.10 cc/l and, of course, since respiratory regulation is disrupted at 1.20 cc/l, continuous swimming at concentrations below the latter would be impossible and death would ultimately result (Eriksen 1963c). *Hexagenia* nymphs could not escape from the experimental glass-burrows; hence, their behavior in this regard could not be determined. However, nymphs which were placed in an aquarium with a bottom of mud and marl remained active in their burrows and finally emerged as subimagos at an oxygen concentration of 0.90 cc/l. A breakdown of respiratory regulation at 0.80 cc/l ultimately resulting in death (Eriksen 1963c) and survival and emergence at 0.90 cc/l suggest that only a slightly lessened concentration probably would have resulted in *Hexagenia* nymphs leaving their burrows and swimming toward the surface. Hunt (1953) observed *H. limbata* nymphs behaving in

this manner in the laboratory under the influence of 0.84 cc/l of oxygen. Britt (1955) wrote of the effect of low oxygen on the natural population of *Hexagenia* in western Lake Erie. Although the western portion of the lake is shallow, usually assuring a total circulation of the water mass, several instances of short-term stratification have occurred. During one of these periods (Britt 1955), oxygen concentrations of the bottom water fell as low as 0.49 cc/l. Extensive dredging yielded no living *Hexagenia* after 3 days of stagnation although numerous dead nymphs were recovered. Thus the oxygen concentrations at which the respective species come to the substrate surface undoubtedly have great significance for distribution, for under these conditions predation, displacement by swimming, or death resulting from insufficient oxygen would serve to remove the animal from an unsuitable respiratory area where it may have become established under more favorable conditions.

Conclusions

Nymphs of *Ephemera simulans* are found in nature in a variety of substrates, including marl with *Chara* (Rawson 1930), sand (Needham 1920; Adamstone and Harkness 1923; Berner 1950; Hunt 1953), gravel and marl (Ide 1935), and coarse gravel and sand (Britt 1962). In contrast, nymphs of *Hexagenia limbata* occur only in soft marls and muds (Needham 1920; Adamstone 1924; Rawson 1930; Lyman 1943; Hunt 1953; and many others). These citations as well as the distribution of *E. simulans* and *H. limbata* in the substrates of Big Silver Lake demonstrated in this paper show a different though intergrading distribution. Since the nymphs are similar in appearance it is not immediately obvious why they occur in generally different habitats.

A means of accounting for their distribution is herein considered. Although a number of laboratory investigations could not be directly supported by field work, laboratory-derived conclusions appear to be consistent with field distribution. From such information obtained in the present and other investigations (mainly, Lyman 1943; Hunt

1953; Eriksen 1963b, 1963c) it appears that *H. limbata* is not found in streams unless prevailing conditions include at least undisturbed, fine sediments, in which burrows can be maintained open to the substrate surface, for *Hexagenia* does not burrow into coarse substrates. To the contrary, *E. simulans* can thrive in gravel and pebble stream substrates with a Md. ϕ in the -1 to -3 range in which the oxygen content of interstitial water is greater than 1.20 cc/l. In lakes where *Ephemera* can burrow into substrates of gravel, marly sand, and marl, it is limited in the fine sediments by the relative inefficiency of its comparatively small gills at low oxygen concentrations (<1.20 cc/l). Again, *Hexagenia* does not occur in coarse substrates. However, in fine sediments (e.g. Md. ϕ +3 to +5) such as marl it is found in large numbers and increases in abundance in muds. Most probably it is limited in its distribution further into the lake by impenetrable peaty substrates or by minimal oxygen or oxygen stratification (<0.80 cc/l) of the lake.

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