

The influence of respiration and substrate upon the distribution of burrowing mayfly naiads

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Introduction

This study embodies an attempt to explain the relationships among respiratory demand, type of substrate and distribution, originally described by WAUTIER and PATTÉE (1955). Objectives were to elucidate similarities and differences of two burrowing mayfly species regarding respiration, morphology and behavior in an attempt to show their roles in these insects' differential distribution.

Methods

Nymphs of the burrowing mayflies *Hexagenia limbata* and *Ephemera simulans* were selected for study because they exhibit the experimentally useful characteristic of inhabiting different yet intergrading substrates. Nymphs averaging 20 to 22 mm were taken from environmental water temperatures ranging from 1.0° C to 5.0° C and placed in trays in a 13.0° C constant-temperature room for 24 hours before experimentation. Behavior in the field and laboratory was observed continually. Respiration studies were conducted in distilled water in the dark at 13.0 ± 1.4 ° C. Oxygen consumption was measured by three modifications of the closed-bottle technique (e. g., blank-bottle, substrate-bottle, glass-burrow-bottle; ERIKSEN 1961). Oxygen concentration was measured by the Micro-Winkler method.

In an attempt to determine the importance of gills and body surfaces as areas for oxygen intake, gilled and gill-less nymphs were compared. In order to measure oxygen intake of these animals under similar activities, they were anaesthetized in 0.5% urethane. The role of gills as paddles to actuate a water current over the animal's surface was approached through the use of glass "burrows." With nymphs entrapped, the "burrows" were placed in water of known oxygen concentration and the number of gill beats per minute counted. The volume of water passed under various conditions was measured by adding a carmine suspension to the mouth of a burrow and timing its passage through the burrow.

Gill size as well as leg size was determined by making camera-lucida traces on graph paper.

Experiments involving substrate selection by the nymphs were conducted for three to five days in small plastic aquaria in which rectangles of particular substrate sizes (ϕ sizes, obtained from natural substrates by sieving; CUMMINS 1962) were placed next to each other with no barriers separating them.

Results

Employing the "blank-bottle" method, Fox et al. (1937) showed *Ephemera vulgata* to be a respiratory adjustor. Preliminary experiments by the author (ERIKSEN 1961), using the same method, showed *Ephemera simulans* and *Hexagenia limbata* to also behave as respiratory adjustors. However, experiments involving the substrate-bottle suggested these results to be inapplicable. A bimodal curve indicated the relationship between oxygen consumption and particle size for both species (Fig. 1). In oxygen concentrations averaging approximately 4.85 cc/l, oxygen consumption fell, rose and fell once more as the particle size decreased.

Ephemera's lowest oxygen consumption in a ϕ -1 particle size correlated well with the size of the coarse substrates inhabited in nature (IDE 1935). The fact that *Hexagenia* burrows in fine substrates (LYMAN 1943) and yet had its low oxygen consumption in the ϕ -3 is hard to understand until it is noted that oxygen consumption was considerably higher than *E. simulans* in all substrates, suggesting that the ϕ -3 was just one of an unsuitable series. Oxygen intake was lower in the ϕ -3 because nymphs used interstitial spaces as temporary burrows. Placing *Hexagenia* nymphs in glass-burrows reduced oxygen intake further and was assumed to have simulated natural conditions.

Substrate selection experiments were not conducted with *Hexagenia* since they could not burrow into any of the fractions offered. Work with *Ephemera* showed that preference increased from ϕ -4 to the ϕ -1 particle size, then decreased considerably to ϕ +3 and rose again slightly to ϕ +5 (Fig. 2). The resulting bimodal curve showed a striking inverse correlation to oxygen consumption with particle size. Results suggest that a substrate in which ease of penetrance is least, as shown by oxygen consumption, is preferred and as ease of penetrance becomes more difficult and oxygen consumption therefore rises, preference decreases.

Closer scrutiny of the similar-appearing nymphs showed *Ephemera* to have comparatively narrow prothoracic legs with which they scoop out tunnels in fine sediments or pick at particles of coarse substrates in burrow construction. *Hexagenia*, with prothoracic legs 1.46 times the area of *Ephemera's* (Table 1), apparently can use them only to scoop soft sediments.

Since "optimal substrates" resulted in dramatic reductions in oxygen consumption compared with that in the blank-bottle, and if nymphs are respiratory adjustors as shown by the latter method, it would be difficult to conceive of oxygen consumption decreasing much further with a lowering of oxygen concentration in normally active individuals. It was interesting, therefore, to find

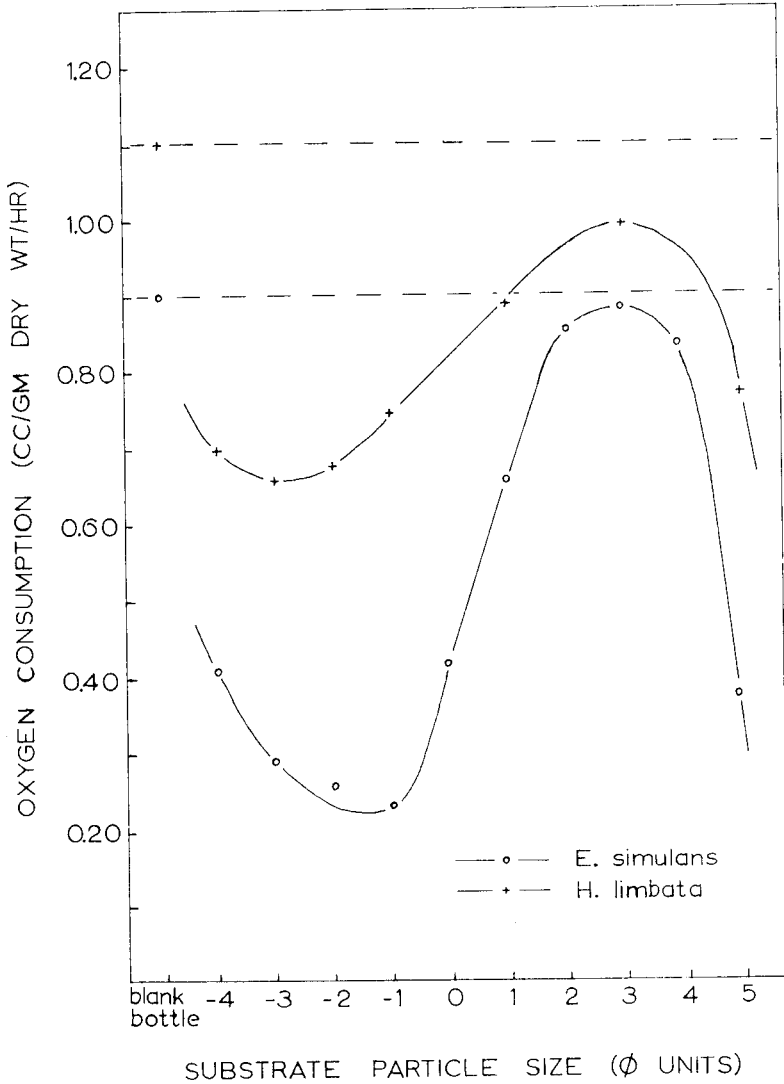


Fig. 1. Oxygen consumption in relation to particle size of the substrate (horizontal lines are blank-bottle maxima).

that *E. simulans* and *H. limbata*, at various oxygen concentrations in optimal substrates, consumed constant amounts of oxygen down to 1.20 cc/l and 0.80 cc/l, respectively (Fig. 3). Thus, in reality, they are respiratory regulators.

After anaesthetizing gilled and gill-less nymphs, and thus making the only known variable the presence or absence of gills, experiments showed that gills absorb approximately 45% of the oxygen consumed. Both species presented similar body surfaces (approximately 220 mm²; Table 1) and, likewise, oxygen

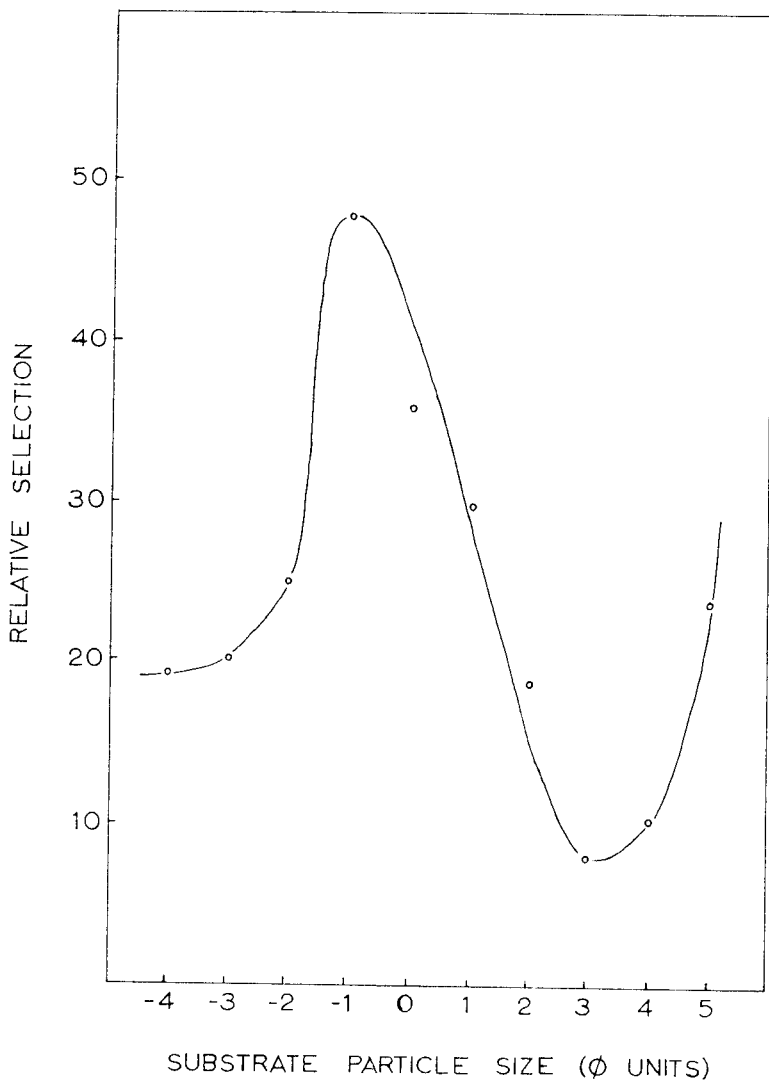


Fig. 2. *Ephemera simulans*: Substrate preference as determined by selection experiments.

consumption by the body (gill-less nymphs) was essentially the same (i. e., 0.17 and 0.16 cc/gm/hr for *Hexagenia* and *Ephemera*, respectively; Table 2). The total area exposed by the gills averaged 245.30 mm² for *H. limbata* and 153.78 mm² for *E. simulans* (Table 1). In spite of this difference, oxygen consumption by gilled nymphs was similar (i. e., *Hexagenia*, 0.32; *Ephemera*, 0.29 cc/gm/hr; Table 2). This similarity is probably basic to the fact that, when anaesthetized, gills lie folded over the abdomen, thus presenting similar surfaces for oxygen intake.

Table 1. Average surface areas in mm² of given body areas.

	<i>Ephemera simulans</i>	<i>Hexagenia limbata</i>	<i>H. limbata</i> larger by factor of
body surface (minus gills) ...	223.20	214.00	0.96
total gill surface	153.78	245.30	1.60
gill bailer surface	44.11	73.28	1.66
burrowing leg surface	1.17	1.71	1.46
(tibia & tarsus)			

Table 2. Average of all oxygen consumption experiments (cc/gm dry wt/hr) of gilled and gill-less nymphs anaesthetized in 0.5% urethane.

<i>Ephemera simulans</i>				<i>Hexagenia limbata</i>			
O ₂ conc. cc/l	gilled	O ₂ conc. cc/l	gill-less	O ₂ conc. cc/l	gilled	O ₂ conc. cc/l	gill-less
5.91	0.29	6.15	0.16	5.94	0.32	6.03	0.17

At oxygen concentrations from approximately 6.00 to 4.00 cc/l, gill beat was slow and increased slowly (Fig. 4). In concentrations ranging from 4.00 to 1.50 cc/l, beats increased rapidly and counts at 2.00 cc/l showed 40 and 107 per minute for *H. limbata* and *E. simulans*, respectively. Just before attaining maximum rate, the curve representing this relationship diverged from its upward trend (1.25 cc/l for *E. simulans*; for *H. limbata* 0.78 cc/l) and assumed a horizontal maximum over a short range of lower oxygen concentration. At yet lower oxygen, gill beats slowed (implied for *H. limbata*), suggesting anoxia.

Both species moved similar water volumes at oxygen concentrations above 1.00 cc/l (Fig. 5). At concentrations greater than 2.00 cc/l, the volume passed was small and difficult to measure. Below 2.00 cc/l, the volume moved by the gills rose rapidly in a straight-line relationship. For *E. simulans*, this straight line held to approximately 1.15 cc/l of oxygen, for *H. limbata*, about 0.75 cc/l. *Ephemera* attained its maximum volume (2.80 cc/min) at 202 beats per minute (Fig. 6). To initiate an equal volume, *H. limbata* required only 84 beats per minute and could pass 3.80 cc/min with 102 beats per minute. This difference is suggested in Table 1 which shows *Hexagenia's* gill-bailer surface (73.28 mm²) to be 1.66 times that of *Ephemera's* (44.11 mm²). Such a difference in bailer surface requires *Ephemera* to beat its gills 2.3 times faster than *Hexagenia* in order to move equal volumes of water. Thus, at oxygen concentrations resulting in death of *Ephemera*, *Hexagenia* continued to increase the water volume and maintain the oxygen gradient necessary for survival.

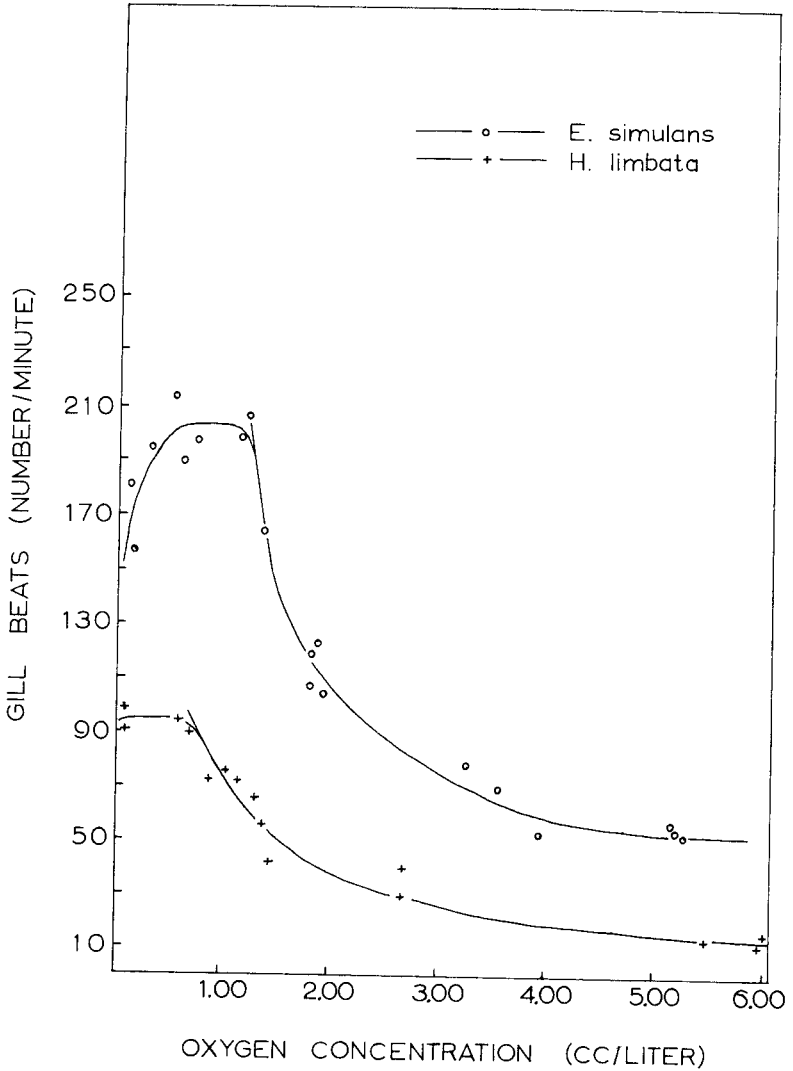


Fig. 4. *Ephemera simulans* and *Hexagenia limbata*: Gill beats per minute under various oxygen concentrations.

Discussion

Ephemera simulans and *Hexagenia limbata*, and *Ephemera vulgata* appeared to be respiratory adjustors when the blank-bottle method is employed (ERIKSEN 1961, Fox et al. 1937). The fact that oxygen consumption varied markedly with substrate particle-size was basis, however, for questioning the applicability of the above findings. In a substrate most like that in which the nymphs are normally found, their oxygen consumption was lowest. When nymphs were given

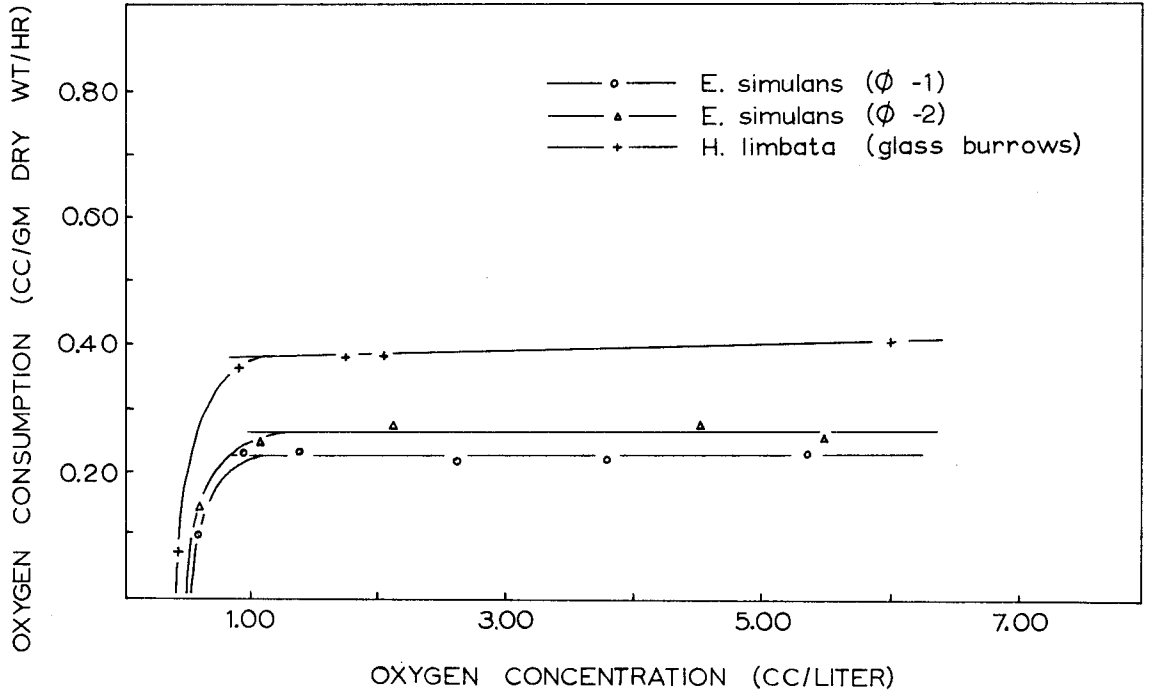


Fig. 3. Oxygen consumption under various oxygen concentrations in "optimal substrates" (*H. limbata* is high due to a spring rise in metabolism: ERIKSEN, in press).

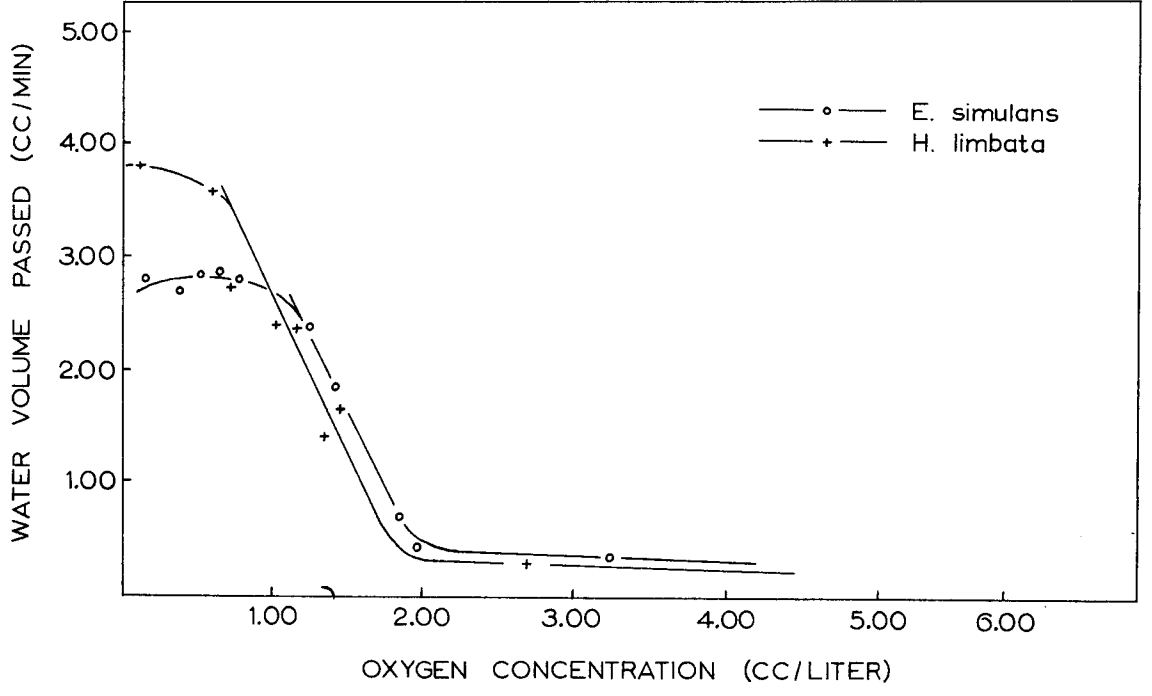
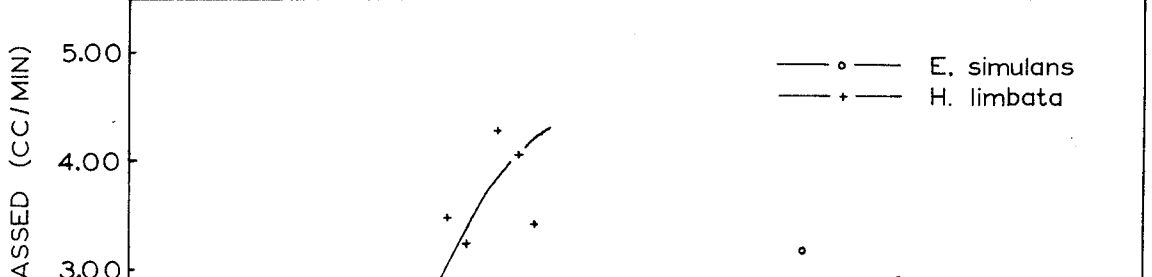


Fig. 5. *Ephemera simulans* and *Hexagenia limbata*: Effect of oxygen concentration upon the water volume passed by the gills.



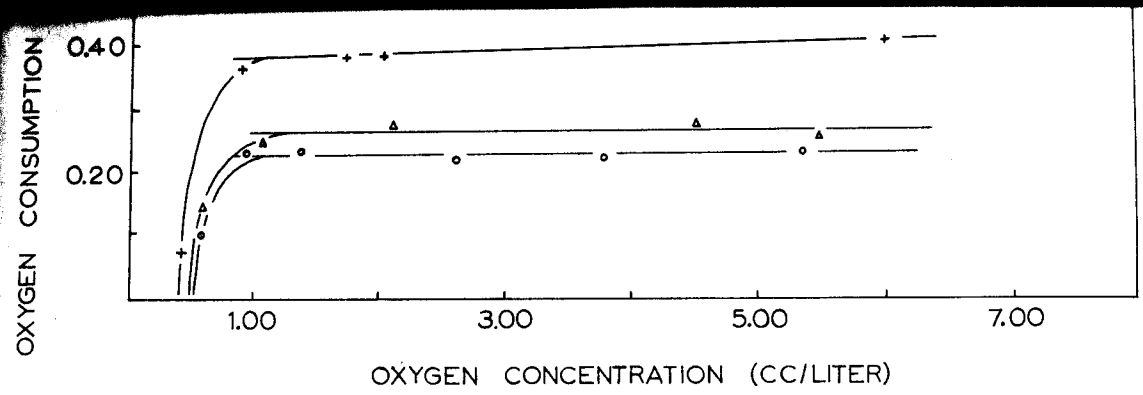


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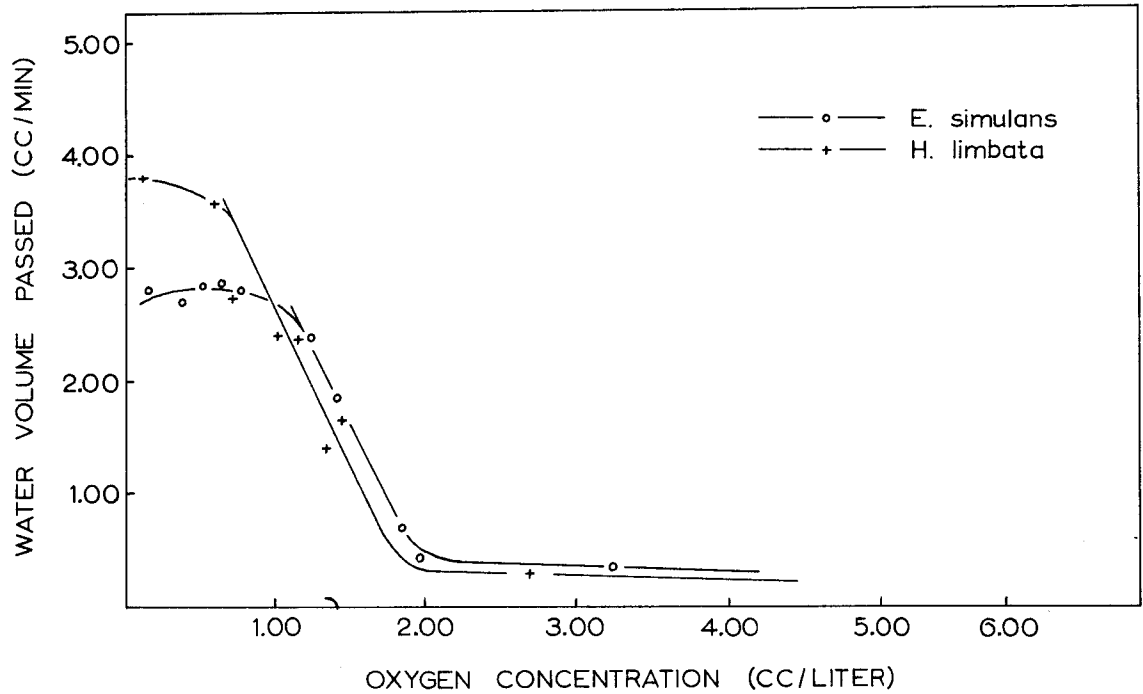


Fig. 5. *Ephemera simulans* and *Hexagenia limbata*: Effect of oxygen concentration upon the water volume passed by the gills.

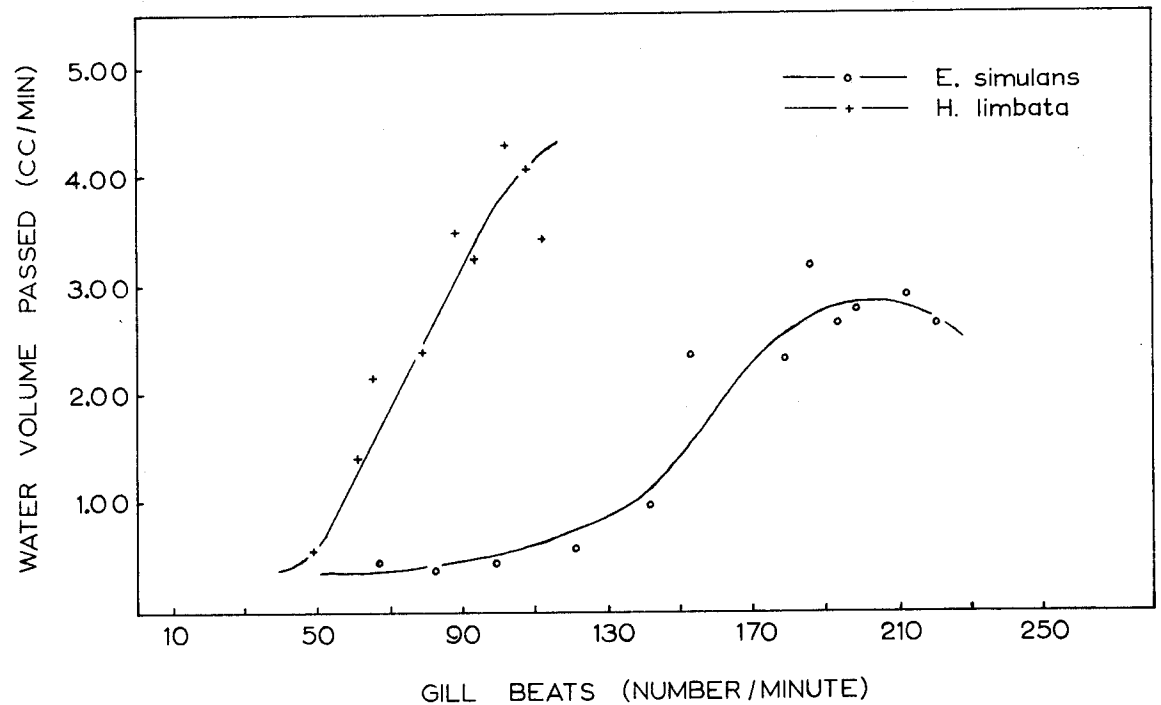


Fig. 6. *Ephemera simulans* and *Hexagenia limbata*: Effect of various beating rates upon the water volume passed by the gills.

an opportunity to select substrates, preference was inversely correlated with oxygen consumption, which is to say that the greater the oxygen consumption, the less was the selective preference. How nymphs in their "optimal substrate" (low oxygen consumption) could further adjust oxygen intake to lower oxygen concentrations could not be visualized. Subsequent experiments over a wide range of oxygen concentrations proved that, under "natural conditions", both species are respiratory regulators.

Gills served as surfaces for approximately 45% of an oxygen intake which was similar for both experimental species. Hence, ecologically important differences cannot be distinguished on these bases. However, gills proved to have another function. By increasing beating rate with lowering oxygen concentration, nymphs were able to maintain respiratory regulation. A marked difference existed in the ability of *H. limbata* to continue to regulate oxygen consumption to an oxygen concentration lower than could *E. simulans*. This difference seems to be determined by gill morphology. Beating rapidly, the smaller gills of *Ephemera* produced an average maximal current of 2.5 cc/min which was apparently sufficient for its needs at oxygen concentrations as low as 1.20 cc/l, but not lower. With larger gills, *H. limbata* required about four-tenths the number of beats to produce a current equal to *Ephemera*'s. Further increasing the beat, *Hexagenia* moved a maximum water volume of about 3.8 cc/min, enabling it to survive in oxygen concentrations as low as 0.80 cc/l.

The work accomplished suggests that leg structure determines the substrate into which nymph can burrow. Thus, *H. limbata* and *E. simulans* are partially separated on a purely morphological basis. Fine adhesive substrates appear to be the only ones which *Hexagenia* can enter (LYMAN 1943) with its broadened burrowing legs. Therefore, it is not found in streams unless conditions similar to those inhabited in lakes are present. *Ephemera* penetrates most substrates with its comparatively narrow prothoracic legs, although its largest populations are in gravel and marl (IDE 1935). In streams, mayfly burrows probably cannot be maintained open to the surface owing to conditions of current and turbulence. Nymphs are limited, therefore, to an interstitial respiratory environment. As particle size decreases, interstitial water is less easily renewed, resulting in reduction of oxygen. In some finer sediments ($\phi +1$ to $\phi +4$ for *E. simulans*), penetration and burrowing are more difficult, resulting in greater oxygen consumption. These two features being antithetical, combined with a low limit for survival of 1.20 cc/l of oxygen for *E. simulans*, rapidly place limits on the species' occurrence in streams.

In shore and shoal areas of lakes, conditions are probably similar to those mentioned concerning coarse substrates of streams and here *E. simulans* may again be found at the exclusion of *H. limbata*. *Ephemera* and *Hexagenia* appear together in considerable numbers in marl but in deeper water, where marl merges with mud, the numbers of *Ephemera* diminish owing to the relative in-

efficiency of its gills at low oxygen concentrations while those of *Hexagenia* remain high. *Hexagenia* seems to be limited in its lakeward distribution either by peaty substrates which it cannot penetrate (HUNT 1953) or by oxygen stratification of the lake (LYMAN 1943). Because interstitial oxygen is absent in fine sediments, burrows are maintained open to the mud surface and respiratory water is drawn from the mud-water interface. Marl substrates have a rather low biological oxygen demand and probably effect only a slight oxygen microstratification in the water at the substrate-water interface. Thus, water drawn through the burrows is sufficient to supply the respiratory needs of both species. The greater oxygen demand of organic mud, coupled with reduced turbulence at increased water depth, probably assists in establishment of an oxygen microstratification at the mud-water interface which affects respiration appreciably (BRUNDIN 1951). Oxygen concentrations in the burrow of only slightly less than 1.20 cc/l will eliminate *Ephemera* since it can regulate respiration only to this concentration, whereas *Hexagenia*, requiring but 0.80 cc/l before regulation is interrupted, would have little difficulty in surviving.

Acknowledgment

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Discussion

GAUFIN: What influence did temperature have on the respiratory rates?

ERIKSEN: I conducted several early experiments at higher temperatures and, as you would expect, metabolic rates were increased. All subsequent experiments were at 13° C.

GAUFIN: What effect did current have on the respiratory rates?

ERIKSEN: The effect of current on respiratory rate was not determined since under natural conditions it is not a factor. Burrowing mayflies remain in burrows with the only water movement being that initiated by beating gills of the nymph.

FETTEROLF: Have you ever noted the nymphs coming out of their burrows either during the day or night, or during periods of low oxygen? If they do not come out, how can fish capture them?

ERIKSEN: In the laboratory or field, I have never seen either species come out of their burrows except in response to low oxygen stress. HUNT also reports this for *H. limbata*. LEONARD wrote that he has seen blue gills (*Lepomis macrochirus*) grubbing into the bottom. Perhaps fish are able to get shallowly buried nymphs in this manner or perhaps they can suck nymphs out of burrows.

HARTLAND-ROWE: An African burrowing mayfly, *Povilla* (Polymitarcidae), remains in its burrow during the day but leaves it every night. The larvae are extremely sensitive to light and could only be observed by means of an infra-red viewer.