

RESPIRATORY REGULATION IN *Ephemera simulans*  
WALKER AND *Hexagenia limbata* (SERVILLE)  
(EPHEMEROPTERA)

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INTRODUCTION

In 1937 Fox and his associates studied the oxygen consumption of nymphs of *Ephemera vulgata* under varying oxygen concentrations by the closed-bottle method. Their data showed that as the oxygen concentration dropped, oxygen consumption by the nymphs likewise decreased. This phenomenon is called respiratory adjustment. Similar experiments with *Ephemera simulans* and *Hexagenia limbata* (Eriksen, 1961) using similar methods suggested these animals also to be respiratory adjusters.

Investigating further an idea first published by Wautier & Pattée (1955), Eriksen (1963) reported the variation in oxygen consumption with substrate particle size under constant oxygen concentrations working again with *E. simulans* and *H. limbata*. Results of that study showed that: 'A bimodal curve indicated the relationship between oxygen consumption and particle size for both experimental species.' Under oxygen concentrations which averaged 4.85 c.c./l., the oxygen consumption fell, rose and fell once more as the particle size became smaller.

In those experiments the lowest oxygen consumption for *E. simulans*, for example, was 0.23 c.c./g. dry wt./hr. compared with 0.90 c.c./g. dry wt./hr. at the same oxygen concentration in the 'blank-bottle'. Such a low oxygen consumption was in a substrate described by Eriksen as being 'optimal' (most suitable) for *Ephemera* nymphs. If such a low oxygen consumption was measured at 4.85 c.c./l. it was inconceivable to the author how the animals (described as being respiratory adjusters) could continue to adjust and survive to lower oxygen concentrations. Yet, they may survive under oxygen concentrations as low as 1.00 c.c./l. (Hunt, 1953). Subsequent experiments under the more natural conditions were conducted to answer the question and this paper reports findings which show *E. simulans* and *H. limbata* to be respiratory regulators, not adjusters, and how it is that regulation is attained and maintained.

METHODS AND MATERIALS

Many of the methods and materials of this study are the same as those discussed in my previous paper (Eriksen, 1963). Only those aspects specifically pertinent to this study will be discussed here.

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*Experimental animals*

The experimental animals were again the burrowing mayfly nymphs *E. simulans* and *H. limbata*. The nymphs are stoutly built, possessing broadened legs for burrowing and large, filamentous, biramous, tracheal gills completely exposed along the sides of the abdomen. Nymphs varied between 17 and 24 mm., with the majority ranging from 20 to 22 mm.

*Survival in low oxygen concentrations*

Survival in low oxygen concentrations was tested by placing eighteen animals in each of several 22 l. carboys filled with distilled water of a known low oxygen concentration. The carboys were inverted several times every 6-9 hr. to mix the contents thoroughly. After 48 hr. in the dark, the number of animals alive was counted and the oxygen was again determined (following the precedent of Fox, Simmonds & Washbourn, 1935, animals were considered to be alive if the gills were beating).

*Oxygen consumption*

Oxygen consumption of the nymphs was measured at  $13.0 \pm 1.4^\circ$  C. by the closed-bottle method of which there were three modifications. The 'blank-bottle' and 'substrate-bottle' methods were discussed previously. The third modification, the 'glass-burrow-bottle' method, was similar to the other two except that 5 cm. lengths of 1 cm. diameter glass tubing were used as 'burrows' into which *Hexagenia* nymphs were placed and entrapped by screening off the ends. The screen mesh was small enough to prevent the animals from escaping, yet large enough not to affect the water flow actuated by gill movements. Such a method proved necessary since none of a variety of washed substrate size fractions offered to *H. limbata* proved satisfactory (Eriksen, 1963) nor was a natural substrate satisfactory, on account of tremendous oxygen variations in the controls. Hence, it was reasoned that if a burrow be constructed the nymphs might act more as they would in an 'optimal substrate' and oxygen consumption would approach normal under those conditions.

*Gills in respiration*

The relative importance of gills and body surfaces as areas for oxygen intake was approached through a series of experiments comparing normal nymphs and nymphs with gills removed. However, in order to measure oxygen intake of normal and gill-less animals under similar conditions and activities, nymphs were anaesthetized in 0.5% urethane and were maintained in this solution throughout the operation, for a 'recuperation' period of 1 hr. and throughout the experiment. As urethane has 'no known specific influence on the respiratory exchange' (Prosser, Bishop, Brown, Jahn & Wulff, 1950), yet eliminates movement and gill beating, it was reasoned that oxygen consumption of nymphs could be measured with the only known variable being the presence or absence of gills.

The current-producing role of the gills was approached through the use of glass model burrows of a size and shape simulating natural burrows. Nymphs were forced to remain in place by wire screens as described in the glass-burrow-bottle experiments. The burrows were placed in a small plastic observation aquarium which contained

Water of known oxygen concentration. In this way the number of gill beats per minute at a given oxygen concentration was counted. The volume of water passed by a certain number of gill beats at a determined oxygen concentration was measured by adding a carmine suspension to the mouth of a burrow and timing its passage through the burrow.

Surface area measurements were made from camera lucida traces of mounted gills (Fig. 7). A value obtained in this way was considered as the paddle area of each gill lamella. Assuming that the entire gill area absorbs oxygen, the respiratory surface area was obtained by doubling that of the axis (the gill presents two surfaces) and adding to this a value four times the filament surface trace (lamella minus axis). The

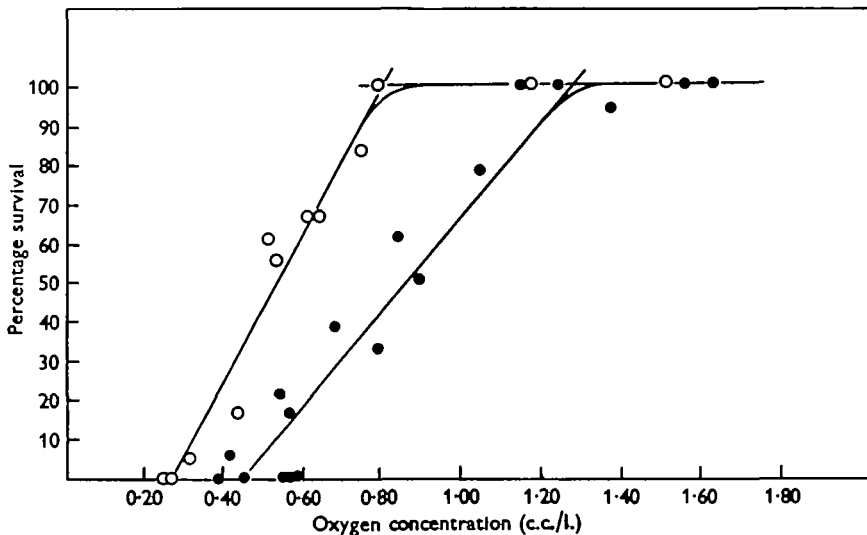


Fig. 1. Relationship of the survival of nymphs to oxygen concentration. O, *H. limbata*; ●, *E. simulans*.

latter assumes that a filament is square in cross-section and measures the surface area between adjacent filaments as well as that on the 'top' and 'bottom'. Measurements obtained for each gill lamella were totalled for each animal. Assuming the body to be a cylinder, the entire surface of which absorbs oxygen, its area was calculated from measurements of length and width. These methods of calculating surface area are only approximate but they provide data which are comparable from one individual to another.

## RESULTS

### *Survival at low oxygen concentrations*

Results of survival experiments at low oxygen concentrations (Fig. 1) showed that *H. limbata* was able to survive with less oxygen than *E. simulans* under otherwise similar conditions. The intercepts with the 100% survival axis of the regression lines fitted mathematically to the data were taken as indicating oxygen concentrations above which there was total survival, but below which oxygen became critical and the less resistant nymphs began to be eliminated. Thus the data showed that *Ephemera* began

to die below 1.29 c.c./l. of oxygen, but at that concentration, or above, there was total survival. On the other hand, it was not until the oxygen concentration fell below 0.81 c.c./l. that death began to eliminate *Hexagenia*. All *Ephemera* nymphs had died by the time it reached 0.44 c.c./l. and *Hexagenia* by 0.27 c.c./l. The latter concentrations

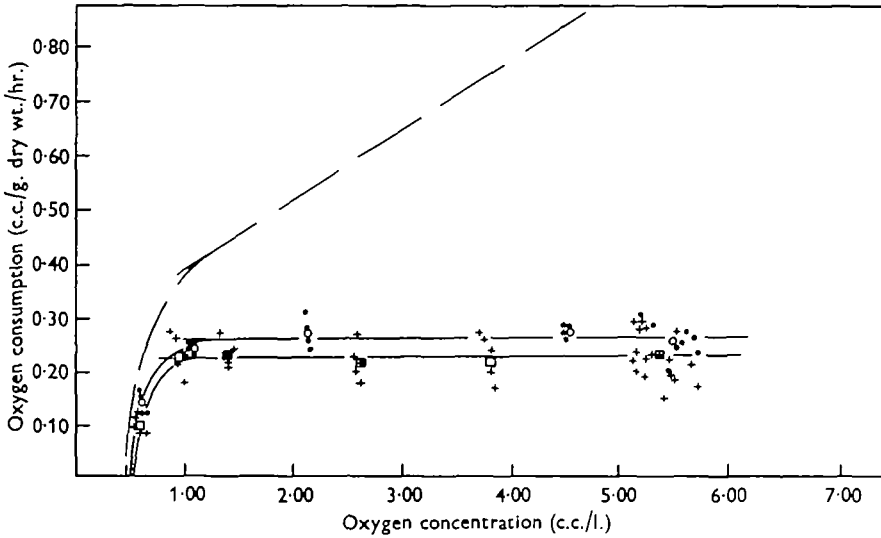


Fig. 2. *E. simulans*: oxygen consumption under various oxygen concentrations in  $\phi-1$  and  $\phi-2$  substrates ('optimal substrates') as compared with its oxygen consumption in blank-bottles. --, Oxygen consumption in blank-bottles; —, oxygen consumption in substrates; ●, datum from one bottle of  $\phi-2$  substrate; ○, average value; +, datum from one bottle of  $\phi-1$  substrate; □, average value.

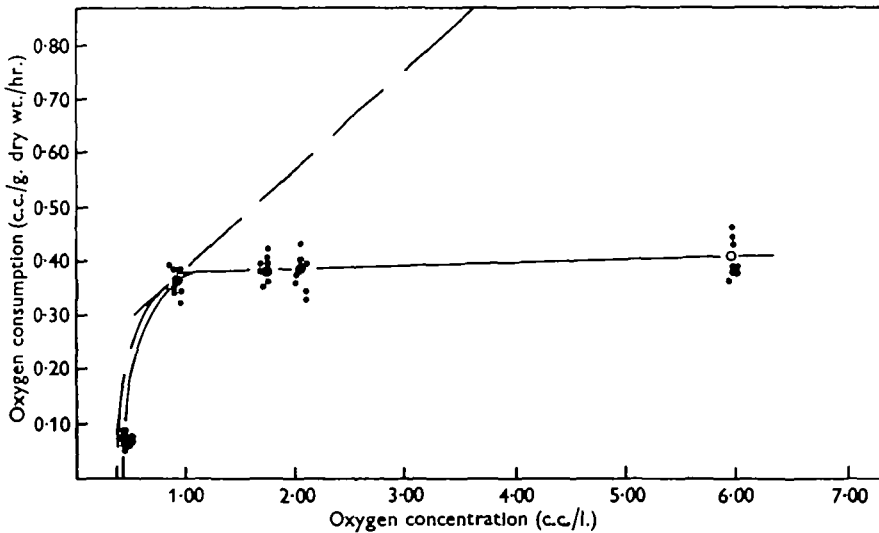


Fig. 3. *H. limbata*: oxygen consumption under various oxygen concentrations in glass-burrows ('optimal substrate') as compared with its oxygen consumption in blank-bottles. --, Oxygen consumption in blank-bottles; —, oxygen consumption in substrates; ●, datum from one bottle; ○, average value.

are interesting purely from a standpoint of comparative physiology, but probably are of little significance in the natural environment.

#### *Oxygen consumption in blank-bottles*

Oxygen consumption experiments using a blank-bottle method showed a decrease in oxygen consumption by both species with a decrease in oxygen concentration. Similar results were reported by Fox & Wingfield (1937) using *E. vulgata*.

The simplest interpretation of the oxygen consumption data for *E. simulans* and *H. limbata* assumed that a straight-line relationship held over the greater range of oxygen concentration (Figs. 2, 3). At lower concentrations (approximately 1.00 c.c./l.), the line curved downward sharply as the oxygen consumption decreased rapidly with small reductions of oxygen concentration. Regression lines were constructed for the straight-line sections and curves drawn freehand represented the trend on the divergent portions.

*H. limbata* exhibited the steeper of the two slopes because of greater activity and hence greater oxygen consumption at the higher oxygen concentrations, although both showed similar oxygen consumptions at 1.25 c.c./l. It will be noted that the concentrations on the oxygen-intake graph at which the curves begin to diverge from a straight line (*E. simulans* 1.20 and *H. limbata* 0.80 c.c./l.; Figs. 2, 3), are strikingly close to those necessary for total survival of the two species (1.29 and 0.81 c.c./l. respectively; Fig. 1).

Prosser *et al.* (1950) define two types of physiological adaptation, distinguished as adjusting (altering to correspond to environmental conditions) and regulating (maintaining an internal constancy independent of the environmental situation).

By these definitions the work of Fox & Wingfield (1937) on *E. vulgata* and the experiments with *E. simulans* and *H. limbata* discussed to this point show these animals to be respiratory adjusters. When considering only the blank-bottle method, such conclusions are acceptable. In comparison with the substrate-bottle method, however, a striking peculiarity is noted. Whereas *E. simulans* registered an oxygen consumption of 0.90 c.c./g./hr. in the blank-bottle at an oxygen concentration of 4.80 c.c./l., at the same oxygen concentration only 0.23 c.c./g./hr. was consumed in a  $\phi-1$  (2-4 mm.) substrate. Such a low value was not recorded from the blank-bottle until the oxygen concentration had fallen to 0.63 c.c./l., and this concentration would soon have resulted in death. Nymphs, however, survived well and were vigorous at this minimal oxygen consumption in the  $\phi-1$  substrate. If the nymphs are truly respiratory adjusters, it would be difficult to conceive of oxygen consumption decreasing much further in normally active individuals.

Oxygen consumption of *E. simulans* in a  $\phi-1$  substrate was maintained at a constant level over oxygen concentrations ranging from approximately 6.00 to 0.94 c.c./l. (e.g. about 0.23 c.c./g./hr.). When the environmental oxygen supply dropped below 0.94 c.c./l., the oxygen consumption rapidly decreased indicating a disruption of respiratory functions (Fig. 2). The results were essentially the same for *Ephemera* in a  $\phi-2$  (4-8 mm.) substrate (Fig. 2). The same trend was apparent for *Hexagenia* in 'glass-burrows' (Fig. 3) even though it is probable that a spring rise in metabolism had increased its oxygen consumption over the winter rate (Eriksen, 1961). These results necessarily alter the present view as to the means of physiological adaptation

typical of the burrowing mayflies. Therefore, at least these two species, and probably *E. vulgata* also, should be looked upon, under normal environmental conditions and activities, as respiratory regulators and no longer as respiratory adjustors.

### Gill function

The history of the investigations of gill function has been adequately covered by Wingfield (1939) who concluded that *E. vulgata* used its gills as accessory respiratory structures (accessory to body surface) as well as paddles to produce a current over its surface. These two functions have long been argued. The role of the gills as respiratory structures was evaluated in the present study by comparing the oxygen consumption (under anaesthesia) of normal nymphs and of nymphs from which the gills had been amputated (gill-less). At an oxygen concentration of approximately 6.00 c.c./l., normal *Hexagenia* nymphs (anaesthetized) consumed an average of 0.32 c.c./g./hr.

Table 1. *Hexagenia limbata*: oxygen consumption (c.c./g. dry wt./hr.) of normal and gill-less nymphs anaesthetized in 0.5% urethane

Normal			Gill-less		
Date, 1960	O <sub>2</sub> conc. (c.c./l.)	O <sub>2</sub> consumed	Date, 1960	O <sub>2</sub> conc. (c.c./l.)	O <sub>2</sub> consumed
17 Apr.	6.16	0.37	16 Apr.	6.00	0.16
	6.19	0.30		6.01	0.17
	6.22	0.34		6.01	0.21
	6.23	0.26		6.02	0.18
	6.24	0.29		6.02	0.17
15 Apr.	5.65	0.35		6.03	0.16
	5.65	0.33		6.03	0.20
	5.67	0.31		6.03	0.16
	5.69	0.35		6.04	0.14
	5.70	0.33		6.05	0.16
Av.	5.94	0.32		6.05	0.15
			6.06	0.14	
			Av.	6.03	0.17

compared to 0.29 c.c./g./hr. for *Ephemera*. Under the same conditions gill-less nymphs of *H. limbata* (anaesthetized) consumed 0.17 c.c./g./hr. while *E. simulans* registered 0.16 c.c./g./hr. (Tables 1 and 2). Thus it appears that the general body surface takes in approximately 55% of the oxygen while the gills absorb the remaining 45%. These values are similar to those determined for *Hexagenia recurvata* by Morgan & Grierson (1932), while Wingfield (1939) reported an oxygen consumption four times as great by normal nymphs.

The potential surface area afforded by the body and gills for oxygen consumption was calculated for nymphs measuring approximately 20 mm. Both species presented similar body surface areas (approximately 220.0 mm.<sup>2</sup>; Table 3) and, likewise, oxygen consumption by the body surfaces was essentially the same (i.e. 0.17 and 0.16 c.c./g./hr. for *Hexagenia* and *Ephemera*, respectively). The total area exposed by the gills (Table 4) averaged 245.3 mm.<sup>2</sup> for *H. limbata* and 153.8 mm.<sup>2</sup> for *E. simulans*. In spite of this difference, oxygen consumption by normal nymphs was similar (i.e. *Hexagenia*, 0.32; *Ephemera*, 0.29 c.c./g./hr.). This similarity no doubt is basic to the fact that, when the

animal is anaesthetized, the gills do not beat and are folded over the dorsum of the abdomen, thus presenting similar surfaces for oxygen intake.

As obtaining oxygen is strictly a physical process dependent upon the gradient across the respiratory surfaces, oxygen consumption would decrease with a lowering of the

Table 2. *Ephemera simulans*: oxygen consumption (c.c./g. dry wt./hr.) of normal and gill-less nymphs anaesthetized in 0.5% urethane

Normal			Gill-less		
Date, 1960	O <sub>2</sub> conc. (c.c./l.)	O <sub>2</sub> consumed	Date, 1960	O <sub>2</sub> conc. (c.c./l.)	O <sub>2</sub> consumed
21 Mar.	5.76	0.29	8 Apr.	6.04	0.19
	5.78	0.28		6.05	0.19
	5.79	0.31		6.05	0.15
	5.79	0.25		6.06	0.19
	5.80	0.24		6.06	0.13
	5.81	0.26		6.09	0.14
8 Apr.	5.97	0.31		6.09	0.13
	5.97	0.31		6.11	0.11
	5.98	0.29	17 Apr.	6.25	0.18
	6.00	0.35		6.26	0.16
	6.01	0.27		6.28	0.18
	6.05	0.23		6.28	0.18
	6.06	0.30		6.29	0.15
8 Apr.	5.99	0.31	Av.	6.15	0.16
	6.00	0.26			
	6.01	0.35			
	6.02	0.27			
	6.02	0.25			
	6.04	0.30			
	6.07	0.20			
16 Apr.	5.67	0.42			
	5.72	0.36			
	5.78	0.29			
Av.	5.91	0.29			

Table 3. *Body measurements in mm. and mm.<sup>2</sup>*

<i>Ephemera simulans</i>				<i>Hexagenia limbata</i>			
Nymph no.	Length	Width	Surface area	Nymph no.	Length	Width	Surface area
1	20.4	3.2	211.1	10	18.9	3.0	192.2
2	20.9	3.0	211.0	11	24.0	2.9	231.7
3	21.3	3.0	214.8	12	19.9	3.0	201.6
4	21.3	3.8	276.8	13	19.7	3.1	206.8
5	21.6	3.0	217.6	14	20.1	2.9	196.2
6	21.2	3.1	221.5	15	22.9	3.0	229.8
7	22.0	3.2	237.1	16	17.5	3.5	211.6
8	19.9	3.1	208.8	17	19.2	2.9	188.0
9	20.5	2.9	199.9	18	21.5	2.8	201.3
				19	21.6	3.0	217.6
				20	22.8	3.2	245.2
				21	21.2	3.0	213.8
				22	22.5	3.0	226.1
				23	21.8	3.2	235.1
				24	21.1	3.0	212.9
Av.	21.0	3.1	223.2	Av.	21.0	3.0	214.0

Table 4. Average total area of axes, lamellae and respiratory surfaces in mm.<sup>2</sup> for the second to seventh pair of gills inclusive

<i>Ephemera similans</i>				<i>Hexagenia limbata</i>			
Nymph no.	Axes	Lamellae	Resp. surface	Nymph no.	Axes	Lamellae	Resp. surface
1	13.89	49.79	171.39	10	21.65	56.80	183.90
2	13.36	50.50	175.28	11	25.79	82.11	276.85
3	11.04	37.14	157.28	12	28.44	86.07	287.40
4	13.10	50.15	174.39	13	24.98	68.61	274.17
5	16.03	53.84	183.30	14	21.13	62.40	207.34
6	15.45	49.46	166.93	15	14.08	45.30	153.02
7	9.95	28.87	95.64	16	27.47	78.74	260.00
8	12.31	41.60	141.76	17	24.39	68.43	224.93
9	12.31	35.67	118.04	18	29.45	86.55	287.30
				19	18.01	46.98	151.90
Av.	13.05	44.11	153.78	20	35.39	117.07	397.46
				21	23.84	65.21	213.22
				22	29.23	81.01	265.58
				23	30.76	73.46	232.31
				24	30.04	80.53	262.06
				Av.	25.64	73.28	245.30

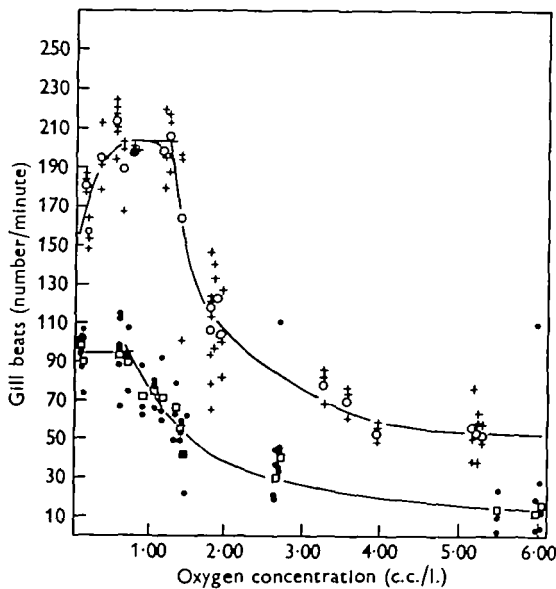


Fig. 4. Gill beats per minute of *E. similans* and *H. limbata* under various oxygen concentrations. *E. similans*: +, average of three counts for a given individual; O, average value. *H. limbata*: ●, average of three counts for a given individual; □, average value.

environmental concentration if the nymphs were unable to regulate oxygen intake. The obvious means of regulating is through a more rapid gill beat, thereby forcing increased volumes of water over the body surface. Inasmuch as gills do create a current of water over the body (Eastham, 1937), and do beat more rapidly as the oxygen concentration falls (Wingfield, 1939), it appears that the nymphs regulate oxygen intake in this manner.



At oxygen concentrations falling from approximately 6.00 to 4.00 c.c./l. the number of gill beats per minute increased slowly (Fig. 4). There was, however, an appreciable difference between the two species in the number of beats. Whereas, at an oxygen

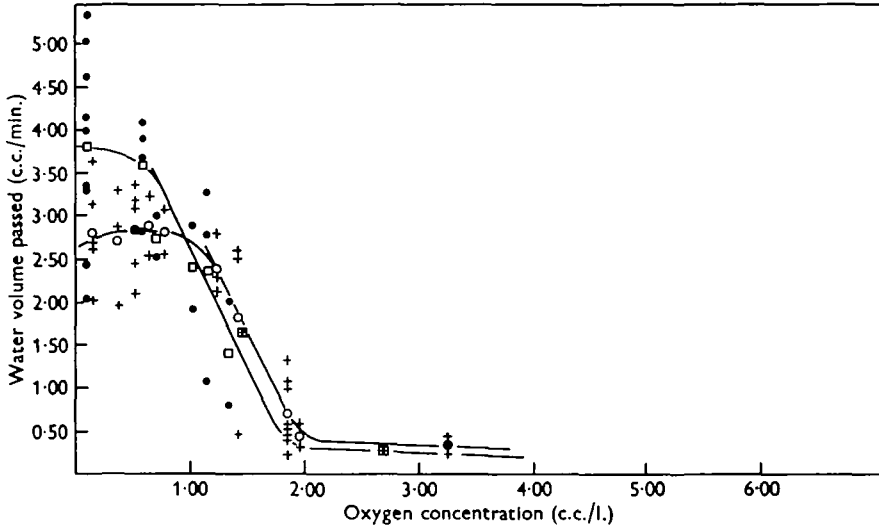


Fig. 5. Effect of oxygen concentration upon water volume passed by the gills of *E. simulans* and *H. limbata*. *E. simulans*: +, average value for single individual; O, average value. *H. limbata*: ●, average value for single individual; □, average value.

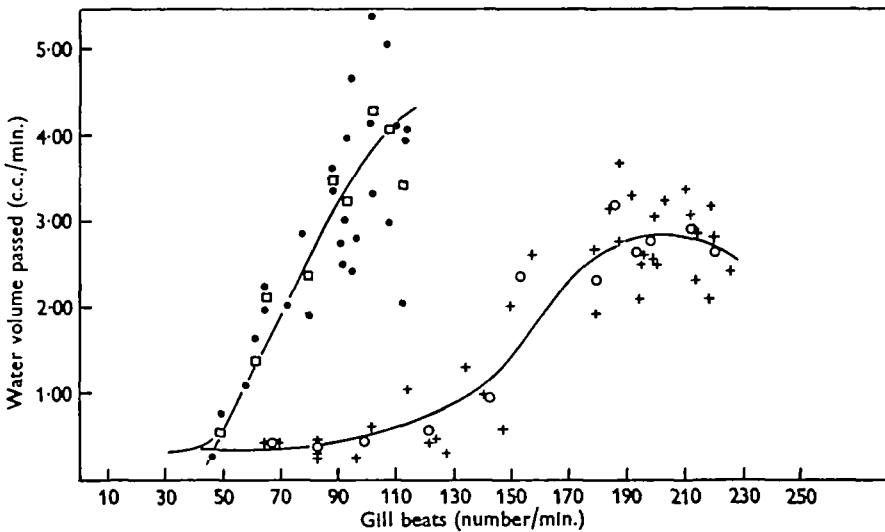


Fig. 6. Effect of various beating rates upon the water volume passed by the gills of *E. simulans* and *H. limbata*. *E. simulans*: +, average value for a single individual; O, average value. *H. limbata*: ●, average value for a single individual; □, average value.

concentration of 5.25 c.c./l. *H. limbata* beat its gills approximately 18 times a minute, *Ephemera* did so 55 times. In oxygen concentrations falling from 4.00 to 1.50 c.c./l. beats increased rapidly, and comparative counts at 2.00 c.c./l. showed 40 and 107 per

minute for *H. limbata* and *E. simulans*, respectively. Beats increased even more at oxygen concentrations less than 1.50 c.c./l. Just before a maximum rate of beating was attained the curve representing this relationship diverged rapidly from its upward trend and continued horizontally over a short range of lower oxygen concentration. At still lower oxygen concentration the gill beats began to slow (implied for *H. limbata*), suggesting anoxia.

Those oxygen concentrations where the curve representing gill beat began to lose its rapid upward trend (1.25 c.c./l. for *E. simulans*; 0.78 c.c./l. for *H. limbata*) are, no doubt, of greatest importance to respiratory function.

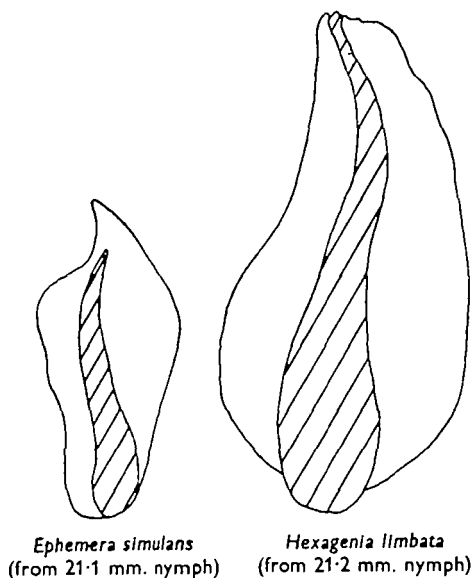


Fig. 7. Representative structure of gill lamellae (outline trace of lamellae from third gill).  
 ▨, Axis of lamella; □, filaments of lamella; scale: 3.7 cm. = 1 mm.

*Hexagenia* and *Ephemera* moved similar volumes of water at oxygen concentrations above 2.00 c.c./l., but the volume passed was small (approximately 0.30 c.c./min.). Such quantities were difficult to measure by the methods used and, in fact, with the few beats exhibited by *Hexagenia*, only one measurement of volume (0.27 c.c./min. at 2.70 c.c./l. of oxygen) was obtained. The significance of one value without supporting data is questionable. However, Fig. 5 shows that the value obtained was at least reasonable if any similarity to the curve for *Ephemera* could be expected.

As the oxygen concentration was lowered below 2.00 c.c./l. the water volume moved by the gills rose rapidly in a straight-line relationship for both species. For *E. simulans* this straight line held to approximately 1.15 c.c./l. of oxygen. The relationship diverged from the straight line below this concentration until it indicated a constant maximum of 2.80 c.c./min. from 0.85 to 0.40 c.c./l. Below the latter oxygen concentration the volume of water moved by the gills decreased. On the other hand, *H. limbata* maintained the straight-line relationship of volume to lowering oxygen, past *Ephemera*'s maximum volume, until it too broke away from the straight line at approximately

75 c.c./l. of oxygen. A maximum volume of 3.80 c.c./min. was reached at approximately 0.10 c.c./l.

*Hexagenia* moves more water over its body per beat than does *Ephemera* (Fig. 6). For example, the latter reached its maximum volume (2.80 c.c./min.) at 202 beats/min. To move the same volume *H. limbata* required only 84 beats/min. and could consistently pass 3.80 c.c./min. with 102 beats/min. (about maximum). Such a difference is perhaps suggested in Fig. 7 and Table 4, which show *Hexagenia*'s gill bailer surface (73.28 mm.<sup>2</sup>) to be 1.66 times the area of *Ephemera*'s (44.11 mm.<sup>2</sup>). This difference in surface requires *Ephemera* to beat its gills 2.3 times/min. faster than *Hexagenia* in order to move an equal volume of water. At a beating rate faster than 202/min., *Ephemera* passed a lessened volume. This trend is perhaps indicative of a drop in gill efficiency so that a reduced volume is moved even though the gills beat faster. Such a condition could be caused by shorter strokes or perhaps by interference between forward and backward strokes.

#### DISCUSSION

With the so-called 'blank-bottle' method, *E. simulans* and *H. limbata* appeared to be respiratory adjustors (as defined by Prosser *et al.* 1950; Figs. 2, 3). Essentially similar results were obtained by Fox & Wingfield (1937) using the same method with another of the burrowing mayflies, *E. vulgata*. This and other papers by Fox have been cited frequently and used as the bases for understanding the respiratory physiology of the aquatic stages of mayflies. Comparing oxygen consumption data with environmental parameters, Fox concluded that the greater respiratory demand could be correlated with the higher environmental oxygen concentration, arguing that a nymph using more oxygen needed to live where greater quantities were available.

In this light it would be expected that *Hexagenia* would require a more highly oxygenated environment than *Ephemera*, since in the blank-bottle method *H. limbata* had greater oxygen consumption than *E. simulans*. However, *Hexagenia* proved to be significantly more tolerant of low concentrations (Fig. 1). The higher oxygen consumption was apparently a consequence of greater activity, for both species have similar minimal demands (Eriksen, 1963; present paper).

Introduction into the experimental bottles of a substrate suitable for burrowing resulted in activity more nearly normal and a significant decrease in oxygen consumption for both species. Utilization of the nymph's 'most suitable' substrate over a wide range of oxygen concentrations produced evidence that both *E. simulans* and *H. limbata* are respiratory regulators (Figs. 2, 3). This conception of their physiology was masked heretofore only by experimental design.

In an unfavourable substrate (i.e.  $\phi + 3$  or a blank-bottle) the oxygen requirements at all concentrations were so great that the nymphs were unable to regulate. At such activity and oxygen demand they were thus apparent adjustors (Figs. 2, 3) as the necessary oxygen gradient across the respiratory surfaces was then dependent upon the environmental concentration modified only slightly by the regulatory apparatus. Therefore, as the oxygen concentration fell, activity was reduced due to the lessened oxygen consumption. Reduction of activity continued until the respiratory system became entirely ineffective and oxygen consumption fell off rapidly.

Since water is moved over the body by the nymphs' beating gills, and since the

amount moved is directly proportional to the number of gill beats (Fig. 6), *E. simulans* and *H. limbata* are able, by increasing the gill beat with a lowering oxygen concentration, to maintain respiratory regulation. If gill movement cannot be maintained at the same rate of increase with a further lowering of the oxygen concentration, the necessary oxygen cannot be obtained and death will result. A marked difference exists in the ability of *Hexagenia* to continue to regulate its oxygen consumption to an oxygen concentration (0.80 c.c./l.) lower than could *Ephemera* (1.20 c.c./l.; Figs. 2, 3). This difference is probably determined by gill morphology (Fig. 7). Beating rapidly, the smaller gills of *Ephemera* can produce an average maximal current of 2.80 c.c./min. This value is apparently sufficient for the needs of the nymphs at an oxygen concentration of 1.20 c.c./l., but not lower. On the other hand, with its much larger gills, *H. limbata* requires about four-tenths the number of beats required of *E. simulans* to produce an equal current. Being able further to increase the beat *Hexagenia* can move a maximum water volume of about 3.80 c.c./min., enabling it to regulate and survive at a low oxygen concentration of 0.80 c.c./l.

## SUMMARY

1. This study shows that *Ephemera simulans* and *Hexagenia limbata* are respiratory regulators and how it is that regulation is attained and maintained.
2. Nymphs, 17–24 mm. in length, were acclimated in the laboratory at 13.0° C. for 24 hr. Oxygen consumption experiments employed the 'closed-bottle' technique and several modifications.
3. When studied in bottles containing no substrate, both species appeared to be respiratory adjustors.
4. Using 'optimal substrates' and a wide range of oxygen concentrations, both species proved to be respiratory regulators.
5. As the oxygen concentration decreased, respiratory regulation was attained by initiating and increasing a flow of water past the body surfaces by the bailer action of beating gills. The volume passed was proportional to the number of gill beats which, in turn, was influenced by the size of the gills. The larger gills of *Hexagenia* beat at a slower rate to produce the same current as *Ephemera* and potentially they were capable of producing a significantly greater current and, hence, could regulate to a lower oxygen concentration.

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