THE FUNCTION OF THE GILLS OF MAYFLY NYMPHS FROM DIFFERENT HABITATS

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(With Three Text-figures)

I. INTRODUCTION

The majority of mayfly nymphs possess evaginations of the body surface in the abdominal region which are more or less richly supplied with tracheae and to which the term tracheal gills has been applied. Palmen (1877) was of the opinion that these structures were blood gills in which the tracheae served chiefly for mechanical support and played no direct part in respiration. Vayssière (1882), however, assigned a definite respiratory function to the tracheae. He also pointed out that in early instars in which the tracheal gills are not developed, respiratory exchange takes place through the thin body wall. Dewitz (1890) observed that mayfly nymphs from which the tracheal gills had been removed not only survived, but after a few months regenerated their gills. Cuénot (1925), in his work on the mayfly nymph *Cloeon dipterum*, confirmed this observation of Dewitz, and concluded that "les trachéo-branchies, adjuvants de la respiration cutanée générale, peut-être inutiles en hiver, en repos, dans l'eau extrêmement oxygénée, deviennent utiles et indispensables lorsqu'une activité plus grande du corps nécessite une oxydation plus active, lorsque l'eau est moins oxygénée, comme en été". From experiments similar to those of Cuénot, Remy (1925) concluded that oxygen absorption took place chiefly through the tracheal gills and that Cuénot had underestimated the importance of these structures in respiration. Babak & Foustka (1907) found that as the oxygen concentration of the water was decreased the gills of mayfly nymphs moved more rapidly, and they concluded that the gills were special respiratory organs. Working on mayfly nymphs from different habitats, Dodds & Hisaw (1924) demonstrated a definite relation between the gill area and the oxygen content of the water. Pruthi (1927), on the basis of his findings for a single species, *Cloeon dipterum*, came to the general conclusion that "mayfly larvae, and presumably other insects as well, can stand a very low concentration of oxygen, below 1·0 c.c./l.". Morgan & Grierson (1932) observed that in the burrowing mayfly nymph, *Hexagenia recurvata*, the gills were essential to normal life, although gill-less nymphs were found to survive under certain conditions. Eastham (1937) suggested that the chief function of the tracheal gills in *Ecdyonurus venosus* was to bathe with water the respiratory gill tufts attached to their bases.

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Apart from the work of Morgan & Grierson, it appears that no accurate experimental work has been done on the precise respiratory function of the tracheal gills in mayfly nymphs, although many suggestions as to the probable nature of this function have been put forward. Furthermore, nothing is known of any relation between function and habitat.

It appeared desirable therefore to investigate the respiratory function of the tracheal gills of a number of species of mayfly nymphs from different habitats.

II. MATERIALS AND METHODS

The animals used in this investigation were *Cloeon dipterum* (L.), *Baetis* sp., and *Ephemera vulgata* (L.). *Cloeon dipterum* is a typical still-water form, whereas *Baetis* sp. is characteristic of swift streams. *Ephemera vulgata* is a burrowing mayfly nymph found in still waters.

Cloeon dipterum was obtained from three different localities, namely, a pond at Alvechurch, Worcestershire, a pond at Selly Park, Birmingham, and a pond at Newdigate, Surrey. *Baetis* sp. was taken both from a swift stream at Blakedown, Worcestershire, and from a similar stream at Alvechurch, Worcestershire. *Ephemera vulgata* was obtained from the pond at Newdigate, Surrey. The oxygen consumption of specimens of *Cloeon dipterum* from the different localities mentioned above was found to be the same: this was also the case with *Baetis* sp.

The alkaline reserves of the waters from which the animals were taken were as follows: Alvechurch pond, 0·0045 *N*; Selly Park pond, 0·0026 *N*; Newdigate pond, 0·0017 *N*; Blakedown stream, 0·0030 *N*; Alvechurch stream, 0·0048 *N*.

All experiments were done in a thermostat at 10° C. Oxygen consumptions were measured on the day following that on which the animals arrived in the laboratory. Oxygen concentrations were determined by the method of Fox & Wingfield (1938). In all experiments on *Baetis* sp. and *Ephemera vulgata*, the experimental technique used was essentially the same as that described in a previous publication (Fox *et al.* 1937): that technique is referred to in this paper as the "normal" technique. The first two series of experiments on *Cloeon dipterum* were also done in this way, but in the second two series a different experimental technique was employed which involved certain structural additions to the thermostat. A framework consisting of one horizontal and two vertical arms was fixed to the inside base of the thermostat. An axle carrying a large wheel was mounted on the vertical arms in such a position that when the thermostat was filled with water the wheel was completely immersed. The wheel, which was perforated by six large holes, was connected through a system of pulleys to an electric motor outside the thermostat. After the animals had been placed in the experimental bottles containing water of known oxygen concentration, a small glass rod (0·5 cm. diameter, 5 cm. long) was introduced and the stopper replaced. The bottles were then inserted horizontally in the holes of the wheel of the apparatus described above and held in position by small pieces of cork. In this way the experimental bottles could be rotated in the thermostat throughout the course of an experiment. The consequent circulation of the glass rod inside each bottle served to set up an artificial water current. This technique, which is termed the "stirring" technique in this paper, ensured that a constant current of water was passed over the body surface of the animals throughout the experiment.

In each of the mayfly species mentioned above, the oxygen consumption of normal nymphs at various oxygen concentrations was compared with that of nymphs from which the tracheal gills had been removed. The removal of the gills was effected as follows. Animals were anaesthetized in 0·5% urethane. Single animals were then transferred to a hollow glass block containing diluted anaesthetic (0·1%) and the gills removed with iridectomy scissors and fine forceps under the low power of a binocular microscope. The animals were then placed in fully aerated water and left to recover; recovery was complete within 30 min. The oxygen consumption of gill-less animals was measured on the same day as the gills were removed and about 3–4 hr. after the operation.

As far as it was possible to judge, the activity of normal and gill-less animals was the same in all experiments except those with *Ephemera vulgata*. In this species the gill-less nymphs were noticeably less active than the normal ones.

III. EXPERIMENTAL

The data for normal and gill-less *Baetis* sp. are shown in Tables I and II, and summarized in Fig. 1. It will be seen from the figure that over a range of oxygen concentrations from 5·0 to 8·0 c.c./l., the oxygen consumption of normal and gill-less animals is alike. No experiments were done at oxygen concentrations lower than 4·0 c.c./l., as it was found that nymphs became moribund and ultimately died if subjected for any length of time to oxygen concentrations lower than this (cf. Pruthi, 1927). It is clear that over the range of oxygen concentrations tested the gills of *Baetis* sp. play no part in oxygen uptake.

Tables III and IV give the oxygen consumption of normal and gill-less *Cloeon dipterum* at various oxygen concentrations using the normal technique. The data are summarized in Fig. 2.[1] It will be seen that the oxygen consumption of normal and gill-less animals remains the same over a range of oxygen concentrations from 8·0 to 3·5 c.c./l. Below the latter concentration the oxygen consumption of gill-less nymphs falls off rapidly, but this marked decrease does not occur in the normal animals until the oxygen content of the water is reduced to approximately 1·5 c.c./l. It is clear that the gills in this species only aid oxygen consumption in water of oxygen concentration lower than 3·5 c.c./l.

In *Baetis* sp. the gills never beat. In *Cloeon dipterum* the gills beat intermittently at high oxygen concentrations, but as the oxygen content of the water falls, the quiescent periods become much reduced, and thus the volume of water passed over the dorsal surface of the nymph in a given time is correspondingly increased. The normal level of oxygen consumption of *Cloeon dipterum* at low oxygen

[1] These results have already been communicated in a preliminary report (Wingfield, 1937).

Table I. *The oxygen consumption of* Baetis *sp. at various oxygen concentrations. Normal animals: normal technique*

Exp. no.	Date	Mean dry weight of 10 animals mg.	Oxygen concentration c.c./l.		Oxygen consumption c.mm./g./hr.	
			In exp.	Mean	In exp.	Mean
1	4. v. 38	11	7·9	7·9	2680	2580
2	,,	10	7·9		2340	
3	,,	13	7·9		2560	
4	,,	10	7·9		2730	
5	12. v. 38	11	7·5	7·5	1990	2210
6	,,	13	7·7		2040	
7	,,	13	7·5		1970	
8	18. v. 38	14	7·6		2310	
9	,,	15	7·5		2360	
10	,,	14	7·5		2050	
11	,,	13	7·6		2120	
1	25. v. 38	13	7·5		2840	
2	,,	12	7·4		2130	
3	,,	12	7·4		2240	
24	5. v. 37	16	6·4	6·5	2870	2630
25	,,	14	6·5		2390	
4	2. vi. 38	16	5·5	5·6	1540	1900
5	,,	18	5·8		1720	
6	,,	17	5·5		2290	
7	,,	15	5·3		1560	
8	,,	15	5·4		1580	
19	28. v. 37	11	5·6		1950	
20	,,	12	5·8		2630	
9	9. vi. 38	14	4·4	4·4	1590	1800
10	,,	12	4·4		1520	
11	,,	11	4·5		2020	
12	,,	14	4·4		1540	
13	,,	11	4·4		2320	

Table II. *The oxygen consumption of* Baetis *sp. at various oxygen concentrations. Gill-less animals: normal technique*

Exp. no.	Date	Mean dry weight of 10 animals mg.	Oxygen concentration c.c./l.		Oxygen consumption c.mm./g./hr.	
			In exp.	Mean	In exp.	Mean
1	3. ii. 37	11	7·9	7·8	2040	2180
2	,,	12	7·9		2240	
3	11. ii. 37	12	7·7		1900	
4	,,	9	7·8		2550	
7	14. iv. 37	19	7·5	7·5	2260	2220
8	,,	20	7·5		2040	
9	,,	21	7·5		1840	
10	16. iv. 37	17	7·4		2340	
11	,,	16	7·4		2320	
12	,,	15	7·5		2520	
13	21. iv. 37	12	6·3	6·4	2600	2550
14	,,	11	6·3		2740	
15	,,	13	6·3		2730	
20	5. v. 37	11	6·4		2210	
21	,,	13	6·5		2560	
22	,,	14	6·4		2500	
17	28. iv. 37	14	5·8	5·8	2370	2260
18	,,	11	5·8		2150	
1	15. vi. 38	9	5·1	5·0	1570	1580
2	,,	10	5·0		1390	
3	,,	9	4·8		1780	

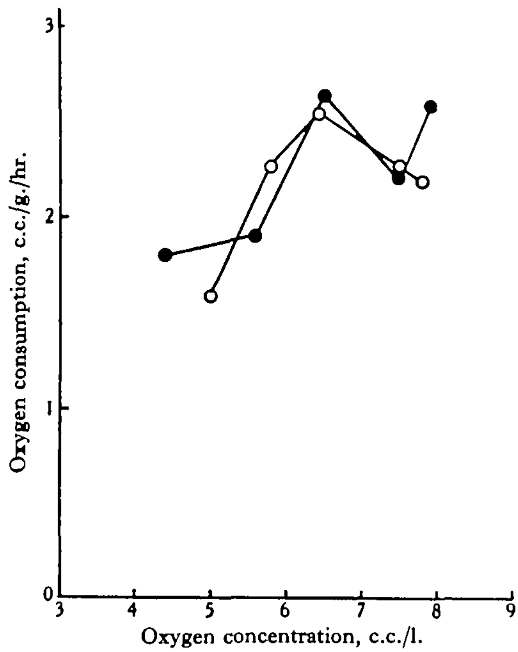

Fig. 1. The oxygen consumption of normal and gill-less *Baetis* sp. at various oxygen concentrations.

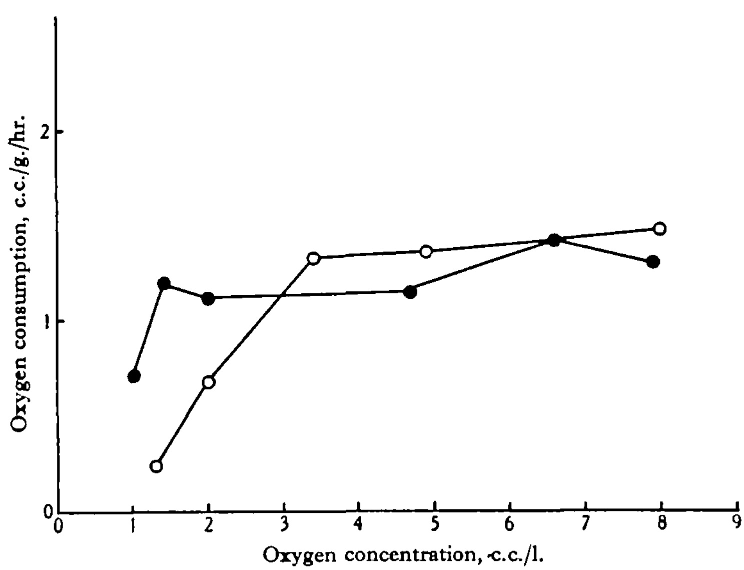

Fig. 2. The oxygen consumption of normal and gill-less *Cloeon dipterum* at various oxygen concentrations. Normal technique.

● Normal animals; ○ Gill-less animals.

Table III. *The oxygen consumption of* Cloeon dipterum *at various oxygen concentrations. Normal animals: normal technique*

Exp. no.	Date	Mean dry weight of 10 animals mg.	Oxygen concentration c.c./l.		Oxygen consumption c.mm./g./hr.	
			In exp.	Mean	In exp.	Mean
1	16. xi. 36	28	7·9	7·9	980	1290
2	,,	29	7·9		1470	
3	,,	31	7·9		1480	
4	,,	25	7·9		1110	
5	,,	27	7·9		1290	
6	,,	28	7·9		1410	
7	2. xii. 36	31	7·0	6·6	1390	1410
8	,,	27	6·9		1370	
9	3. xii. 36	35	6·5		1550	
10	,,	39	6·4		1570	
11	,,	30	6·4		1530	
12	,,	38	6·5		1350	
13	,,	36	6·4		1270	
14	,,	35	6·5		1260	
15	20. xi. 36	40	4·6	4·7	1250	1150
16	,,	39	4·7		1280	
17	,,	39	4·6		930	
18	,,	32	4·7		1150	
19	21. xi. 36	35	1·9	2·0	990	1110
20	,,	40	1·9		1090	
21	25. xi. 36	36	2·1		1020	
22	,,	38	2·2		1230	
23	,,	34	2·1		1210	
24	,,	34	2·1		1110	
25	28. xi. 36	37	1·3	1·4	1330	1190
26	,,	35	1·3		1000	
27	,,	30	1·5		1430	
28	,,	32	1·4		1000	
29	26. xi. 36	37	1·1	1·0	770	710
30	,,	38	1·1		710	
31	28. xi. 36	36	0·9		600	
32	,,	38	1·0		750	

concentrations might be maintained by either of the following processes. The gills might function as true respiratory organs, actual gaseous exchange taking place at the surface of the gills, or, alternatively, the gills might act as paddles passing a greater volume of water over the normal respiratory surfaces of the animal. I have tested the possibility of the second alternative by measuring the oxygen consumption at various oxygen concentrations of normal and gill-less animals in an artificial water current produced by rotating the experimental bottles in the stirring apparatus mentioned above. The results are given in Tables V and VI, and summarized in Fig. 3. It should be noted that the oxygen consumption of normal and gill-less animals at relatively high concentrations of oxygen was approximately the same using either technique (cf. Figs. 2 and 3).

It will be seen from Fig. 3 that, using the stirring technique, the oxygen consumption of normal and gill-less nymphs remains the same over the whole range of oxygen concentrations studied. It is clear then that the rapid decrease in oxygen consumption of the gill-less nymphs at oxygen concentrations lower than 3.5 c.c./l.,

Table IV. *The oxygen consumption of* Cloeon dipterum *at various oxygen concentrations. Gill-less animals: normal technique*

Exp. no.	Date	Mean dry weight of 10 animals mg.	Oxygen concentration c.c./l.		Oxygen consumption c.mm./g./hr.	
			In exp.	Mean	In exp.	Mean
1	10. xii. 36	40	8·0	8·0	1430	1470
2	,,	28	8·0		1560	
3	11. xii. 36	25	8·0		1550	
4	,,	33	8·0		1420	
5	12. xii. 36	23	7·9		1410	
6	9. i. 37	31	4·9	4·9	1280	1350
7	,,	26	4·9		1580	
8	,,	31	4·9		1200	
9	7. i. 37	30	3·3	3·4	1500	1320
10	,,	30	3·3		1070	
11	8. i. 37	33	3·5		1470	
12	,,	28	3·5		1240	
13	16. i. 37	30	2·1	2·0	690	680
14	,,	31	2·1		590	
15	29. i. 37	26	1·9		900	
16	,,	21	1·9		620	
17	,,	19	1·9		620	
18	15. i. 37	35	1·3	1·3	290	240
19	,,	31	1·3		250	
20	28. i. 37	28	1·3		200	
21	,,	27	1·3		270	
22	,,	32	1·3		190	

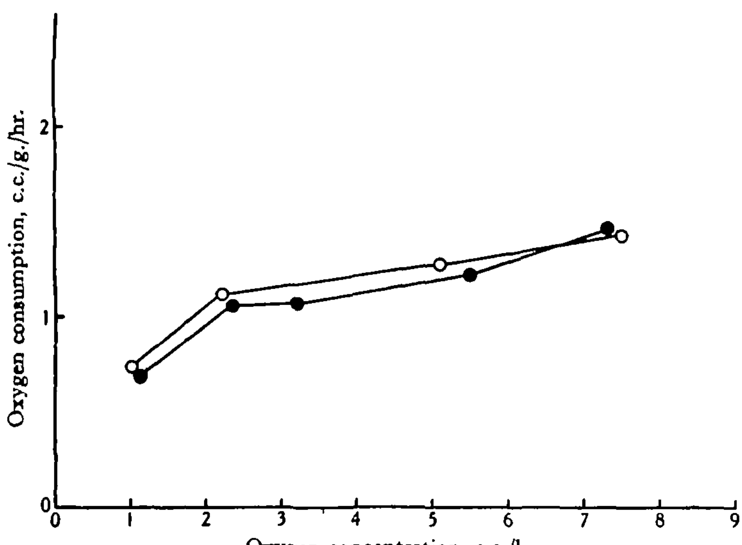

Fig. 3. The oxygen consumption of normal and gill-less *Cloeon dipterum* at various oxygen concentrations. Stirring technique.

● Normal animals; ○ Gill-less animals.

Table V. *The oxygen consumption of* Cloeon dipterum *at various oxygen concentrations. Normal animals: stirring technique*

Exp. no.	Date	Mean dry weight of 10 animals mg.	Oxygen concentration c.c./l.		Oxygen consumption c.mm./g./hr.	
			In exp.	Mean	In exp.	Mean
1	29. vi. 38	13	7·3	7·3	2090	1460
2	,,	10	7·2		1460	
3	,,	9	7·2		1190	
4	,,	10	7·2		1670	
5	1. vii. 38	11	7·2		1480	
6	,,	12	7·2		1330	
7	5. vii. 38	14	7·4		1250	
8	,,	10	7·3		1580	
9	,,	13	7·4		1230	
10	12. vii. 38	12	5·5	5·5	1220	1230
11	,,	10	5·5		1310	
12	,,	12	5·4		1150	
13	9. vii. 38	9	3·2	3·2	1010	1080
14	,,	10	3·2		1030	
15	,,	10	3·3		1210	
16	14. vii. 38	11	2·3	2·3	1050	1070
17	,,	12	2·2		1080	
18	7. vii. 38	10	1·0	1·1	700	700
19	,,	13	1·1		790	
20	,,	11	1·1		620	

Table VI. *The oxygen consumption of* Cloeon dipterum *at various oxygen concentrations. Gill-less animals: stirring technique*

Exp. no.	Date	Mean dry weight of 10 animals mg.	Oxygen concentration c.c./l.		Oxygen consumption c.mm./g./hr.	
			In exp.	Mean	In exp.	Mean
3	16. vii. 38	13	7·5	7·5	1520	1430
4	,,	11	7·4		1470	
5	,,	12	7·4		1320	
6	,,	11	7·5		1410	
7	19. vii. 38	9	5·1	5·1	1310	1280
8	,,	12	5·2		1250	
9	,,	11	5·1		1270	
10	21. vii. 38	10	2·1	2·2	1210	1110
11	,,	11	2·2		900	
12	,,	11	2·3		1150	
13	,,	9	2·3		1170	
14	23. vii. 38	11	0·9	1·0	770	730
15	,,	9	1·0		680	
16	,,	9	1·0		740	

which was found when the "normal" technique was used, is due to the absence of a water current over the body surface of the animals. Thus in *Cloeon dipterum*, the gills do not function as true respiratory organs even at low oxygen concentrations and, under these conditions, the normal level of oxygen uptake is maintained by the increased movement of the gills which causes a larger volume of water to be passed over the respiratory surfaces of the animal. As no indication was found of the presence of open spiracles in this species, it appears probable that gaseous exchange takes place through the integument.

Remy (1925) explained the survival of gill-less nymphs by supposing that the air stored in the tracheae was sufficient for normal respiration to be continued for some time after the removal of the gills. The normal rate of oxygen consumption of gill-less *Cloeon* and *Baetis* at high oxygen concentrations might be explained in a similar way. Such a supposition would not however explain the difference in oxygen consumption between normal and gill-less *Cloeon* at low oxygen concentrations (normal technique) and, moreover, the disappearance of that difference when the stirring technique is used.

The oxygen consumption of normal and gill-less *Ephemera vulgata* at about the same oxygen concentration (7·1 and 6·9 c.c./l.) is shown in Tables VII and VIII. Further experiments at lower oxygen concentrations were not possible as the requisite number of animals could not be obtained. It will be seen from the tables that the oxygen consumption of gill-less animals is about one-quarter that of normal animals. In this species it appears that the gills aid respiration even at high oxygen concentrations. Morgan & Grierson (1932) obtained a similar result with *Hexagenia recurvata*, a near relative of *Ephemera vulgata*.

Cloeon dipterum, which has plate-like tracheal gills well supplied with tracheae, is found in ponds where the oxygen content falls very low (unpublished data). Fig. 2 shows that at low oxygen concentrations (1·5–3·5 c.c./l.) the normal level of oxygen consumption cannot be maintained by the body surface alone. Under these conditions, which are often experienced in nature, the motile tracheal gills furnish an accessory respiratory mechanism which enables normal respiration to be continued.

Baetis sp. is found in swift streams where the oxygen content rarely falls below 4 c.c./l. (unpublished data): usually it is considerably higher. The structure of the gills is similar to that of *Cloeon dipterum*, except that whereas in the latter species each gill is composed of two lamellae, in *Baetis* sp. it consists of only a single lamella. It will be seen from Fig. 1 that the respiration at the body surface in *Baetis* sp. is sufficient to maintain normal respiration at all oxygen concentrations which would be experienced in its natural habitat. Under natural conditions therefore there is no need for any accessory respiratory mechanism in this species, and the non-motile gills do not function in respiration.

The burrowing mayfly nymph *Ephemera vulgata* is also found in ponds where the oxygen content may drop very low; its motile gills are filamentous and sparsely provided with tracheae. Tables VII and VIII show that in this species, although some respiratory exchange appears to take place through the integument, the body surface is insufficient to maintain normal respiration even in water saturated with

Table VII. *The oxygen consumption of* Ephemera vulgata.
Normal animals: normal technique

Exp. no.	Date	Mean dry weight of 10 animals mg.	Oxygen concentration c.c./l.		Oxygen consumption c.mm./g./hr.	
			In exp.	Mean	In exp.	Mean
6	12. xii. 36	170	7·9	6·9	1470	1460
7	"	180	7·8		1450	
8	"	150	7·8		1480	
9	"	150	7·9		1490	
10	14. ii. 35	190	6·5		1960	
11	"	180	6·5		1890	
12	"	180	6·5		1820	
13	20. ii. 35	170	6·3		1210	
14	"	190	6·4		1020	
15	10. iv. 35	200	6·5		1390	
16	"	200	6·5		1190	
17	"	230	6·5		1170	

Table VIII. *The oxygen consumption of* Ephemera vulgata.
Gill-less animals: normal technique

Exp. no.	Date	Mean dry weight of 10 animals mg.	Oxygen concentration c.c./l.		Oxygen consumption c.mm./g./hr.	
			In exp.	Mean	In exp.	Mean
2	19. i. 37	180	7·1	7·1	450	400
3	"	140	7·1		370	
4	"	170	7·1		450	
5	"	180	7·1		350	
6	25. i. 37	160	6·9		300	
7	"	130	7·1		530	
8	"	170	7·1		350	

air. It appears that the accessory respiratory mechanism in the form of gills is very well developed in this species. This can be related to the very low oxygen concentration of the water which may often be experienced inside the burrows of these nymphs in nature. The exact nature of the accessory respiratory mechanism in *Ephemera vulgata* is unknown, but in view of the difference in gill structure between it and both *Cloeon* and *Baetis*, it is possible that the gills may act as true respiratory organs as well as paddles producing a respiratory current.

SUMMARY

1. The oxygen consumption of normal and gill-less nymphs of the mayflies *Baetis* sp., *Cloeon dipterum* and *Ephemera vulgata* has been measured at various oxygen concentrations.

2. It has been found that over the complete range of oxygen concentrations studied, the tracheal gills do not aid oxygen consumption in *Baetis* sp. In *Cloeon dipterum*, at all oxygen concentrations tested, no gaseous exchange takes place through the gills; at low oxygen concentrations, however, the gills function as an accessory respiratory mechanism in ventilating the respiratory surface of the body and so aid oxygen consumption. In *Ephemera vulgata* the gills aid oxygen consumption

even at high oxygen concentrations. In this species the gills may function both as true respiratory organs and as a ventilating mechanism.

3. It is shown that the differences in gill function can be related to the oxygen content of the habitat of each species.

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