THE RELATION OF OXYGEN CONSUMPTION TO SUBSTRATE PARTICLE SIZE IN TWO BURROWING MAYFLIES

By CLYDE H. ERIKSEN*

Department of Zoology, University of Michigan

INTRODUCTION

The French workers Wautier and Pattée (1955) reported a very interesting and perhaps important relationship between oxygen consumption and substrate. Their work demonstrated that nymphs of *Ephemera danica* consumed less oxygen per gram per hour when in a bottle partially filled with sand than when placed in a bottle with several pebbles. In turn, the latter situation resulted in less oxygen being consumed than when the nymphs were placed in a bottle with no substrate at all. They pointed out that the sand in which oxygen consumption was least is the substrate in which *E. danica* is usually taken in nature. This was primarily the extent of their research, but it pointed to a relation between respiration and substrate as a possible cause for selection of a particular bottom type.

The initial findings of Wautier & Pattée stimulated the present study. Methods have been refined to give comparative data concerning small gradations of a considerable range of substrate particle sizes for two different species of burrowing mayflies. Further, and most importantly, an attempt is made to determine why such a relationship exists.

METHODS AND MATERIALS

Experimental animals

The experimental animals were the burrowing mayfly nymphs *Ephemera simulans* Walker and *Hexagenia limbata* (Serville). They are members of the two most morphologically similar genera of the family Ephemeridae. Their prothoracic legs are broadened, allowing them to scoop the substrate into which they burrow.

Throughout most of the experimentation period (19 Feb. to 16 Apr. 1960) water temperatures ranged from 1·0 to 5·0° C. where the animals were collected. Nymphs 16–24 mm. in length were placed in trays in a 13·0° C. 'constant temperature' room for 24 hr. before being used in an experiment.

Oxygen consumption studies

All experiments were conducted in the dark, at $13·0 \pm 1·4°$ C., and in distilled water, which assured a non-toxic, chemically constant medium. The oxygen concentration in all laboratory experiments was measured by the micro-Winkler method. Oxygen consumption was determined by the closed-bottle method, of which there were two variations. They are referred to as the 'blank-bottle' and 'substrate-bottle' methods.

* Present address: Department of Zoology, University of Toronto, Toronto, Canada.

The blank-bottle method simply utilizes 250 ml. glass-stoppered bottles containing only the experimental animals and water. It was used in the experiments as reference for conditions under which oxygen consumption was maximal. Five nymphs were introduced into each bottle except the control and then placed at 13·0° C. for 5 hr. Final results were calculated in terms of ml. of oxygen consumed per gram oven-dry weight per hour.

Table 1. *Description of size fractions of substrates*

Classification after Cummins (1962)	ϕ scale	Size range (mm.)
Boulder	−8	> 256
Cobble	−7	128–256
	−6	64–128
Pebble	−5	32–64
	−4	16–32
Gravel	−3	8–16
	−2	4–8
	−1	2–4
Very coarse sand	0	1–2
Coarse sand	1	0·5–1
Medium sand	2	0·25–0·5
Fine sand	3	0·125–0·25
Very fine sand	4	0·0625–0·125
Silt	5 6 7 8	0·0039–0·0625
Clay	9	< 0·0039

The substrate-bottle method was run similarly. In order to provide the nymphs with a large bottom surface area for burrowing into the 40 c.c. of added experimental substrate, 250 ml. flasks were used instead of bottles. Natural substrates were sieved through a set of Tyler screens by means of a mechanical shaking device (Ro-Tap) to give size fractions in ϕ units (Inman, 1952; − 1 times the natural logarithm of the particle size in mm. expressed as a whole number). Such fractions ranged from $\phi + 5$ (silt) to $\phi − 4$ (pebbles) and afforded a graded series of increasing particle sizes (Table 1) and interstitial spaces. The substrates were washed thoroughly in an attempt to obtain only the inorganic fraction desired.

Substrate selection

Experiments involving substrate selection by the nymphs were conducted in 30 cm. × 10 cm. plastic aquaria. A metal divider partitioned the aquarium into eight compartments and one of four substrate types of alternate ϕ units was placed into each division. The divider was then removed, allowing the substrates to settle together. The experimenter did not have complete freedom, however, in arranging the fractions in random manner, for if $\phi + 3$ particle sizes were placed next to $\phi − 3$, the former would settle into the interstices of the latter. The substrates were arranged to give maximum heterogeneity yet to reduce the settling-in process to a minimum. The use of different nymphs in each experiment eliminated any possibility of learning or conditioning.

Thirty nymphs were used in each aquarium and it was assumed that after 3–5 days they had moved into a preferred substrate. The divider was re-inserted and the percentage of nymphs recovered in each substrate was determined and plotted against size fraction.

Selection experiments were conducted only with *E. simulans*, for *H. limbata* will not burrow into a substrate in which a tunnel-like burrow cannot be maintained. The reason for *Hexagenia*'s inability to burrow was not immediately evident. Therefore, for a critical structural comparison, the burrowing prothoracic legs were mounted on slides and traced in outline using a camera lucida. Surface area was then calculated.

RESULTS

Oxygen consumption with particle size

Wautier & Pattée's finding (1955) that the oxygen consumption of nymphs of the burrowing mayfly *Ephemera danica* is affected by the type of substrate was shown to hold true also for *E. simulans* and *H. limbata*. A bimodal curve (Figs. 1, 2) indicated the relationship between oxygen consumption and particle size for both experimental species. Under oxygen concentrations which averaged 4·80 c.c./l. for *Ephemera* and 4·90 c.c./l. for *Hexagenia*, the oxygen consumption fell, rose and fell once more as the particle size was decreased.

Comparatively, *H. limbata* had an oxygen consumption in all particle sizes considerably higher than did *E. simulans*. *Hexagenia* exhibited its lowest oxygen consumption in the $\phi - 3$ substrate and its greatest in the $\phi + 3$. *Ephemera*, however, registered its lowest oxygen intake in the $\phi - 1$ particle size with its greatest also in the $\phi + 3$. A pronounced drop in oxygen consumption, from the $\phi + 3$ high, occurred in both species in the $\phi + 5$ substrate bottle. A graphical representation of such data does not tell the whole story, however. For example, a substrate varying from optimal should not only become more and more difficult for a burrowing animal to penetrate (expressed by increasing oxygen consumption), but should harbour fewer and fewer numbers of these organisms. Extended to the natural environment, one would expect to find the greatest numbers of burrowing organisms in substrates which most closely simulated the laboratory experimental fraction in which oxygen consumption was minimal. If, by reason of ease of penetrance, oxygen consumption is lowest in what might be termed an 'optimal substrate', why did *H. limbata* show a low oxygen consumption in the $\phi - 3$ substrate (Fig. 2) when in nature it is an inhabitant of soft marls and muds (Lyman, 1943)? Observation made it obvious that the nymphs were unable to dig into any of the experimental substrates, but did use the interstices of the $\phi - 3$ fraction as temporary 'burrows'. For this reason the lowest oxygen consumption was exhibited in a $\phi - 3$ substrate which was only one of an entire series of unsuitable ones. By comparison, the lowest oxygen intake in c.c./g./hr. for *H. limbata* (0·66) was almost threefold the low consumption for *Ephemera* (0·23 c.c./g./hr.; Figs. 1, 2). This high figure for *Hexagenia* was probably due to the fact that all substrates were unfavourable, and not to a higher metabolic rate for *Hexagenia*. Substantiation of this idea came from an experiment with anaesthetized nymphs. Anaesthetized in 0·5 % urethane, nymphs of both species consumed essentially the same amounts of oxygen (*E. simulans* 0·29 c.c./g./hr. and *H. limbata* 0·32 c.c./g./hr.; Eriksen, 1963). It was

reasoned that, as urethane has 'no known specific influence on the respiratory exchange (Prosser, Bishop, Brown, Jahn & Wulff, 1950) and its use as an anaesthetic eliminates movement and gill beating, oxygen consumption of the nymphs could be measured with the only known variable being the species difference.

As *Hexagenia* did not make its way into any of the experimental substrates, the lower oxygen consumption in $\phi + 5$ as compared to $\phi + 1$ and $\phi + 3$ was puzzling. Obviously some factor other than burrowing attempts, swimming and further burrowing attempts must have been responsible for the total oxygen intake with any given substrate fraction. There is no information to explain this variation between ϕ sizes, but perhaps
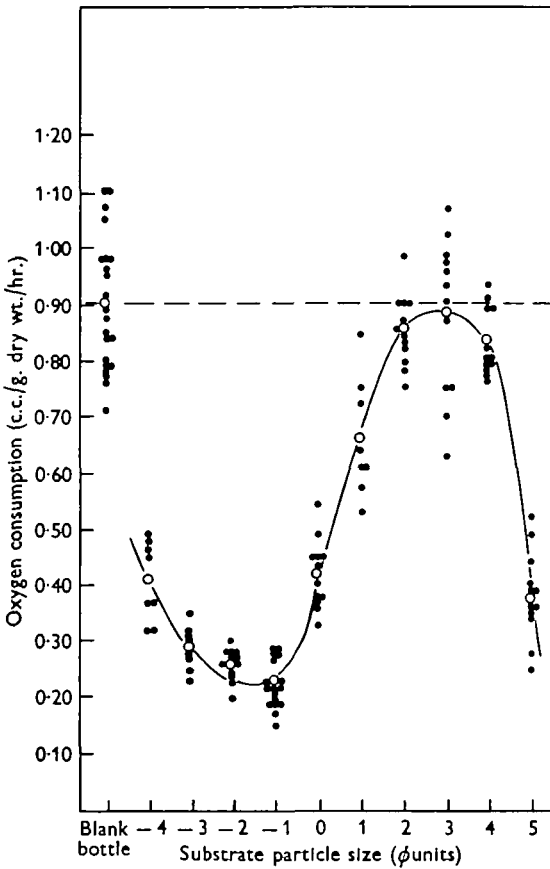

Fig. 1

Fig. 2

Fig. 1. Oxygen consumption of *E. simulans* in relation to particle size of the substrate. ●, Datum from one bottle; ○, average value; – – –, maximum oxygen consumption reference line.
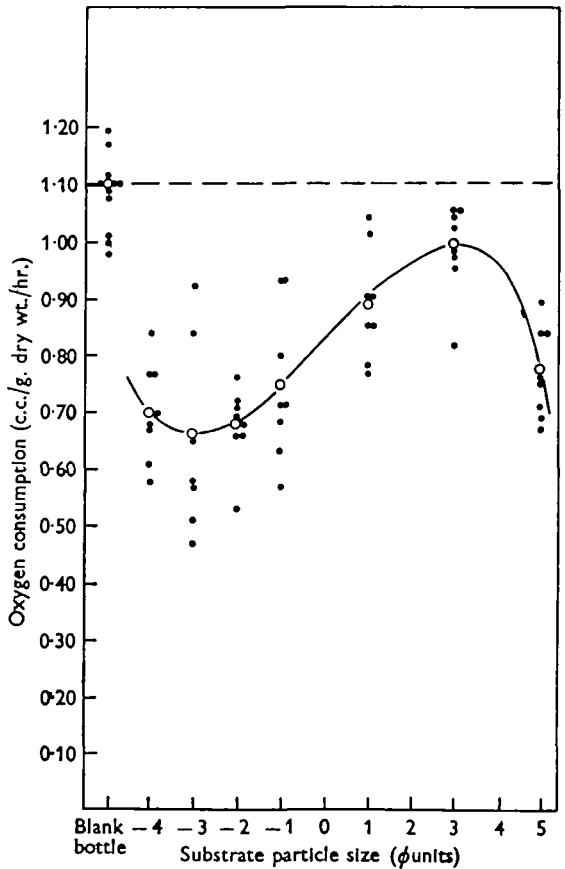
Fig. 2. Oxygen consumption of *H. limbata* in relation to particle size of the substrate. ●, Datum from one bottle; ○, average value; – – –, maximum oxygen consumption reference line.

it might be interpreted as a 'proper particle size' stimulus imparted from the substrate particles to the nymphs by touch receptors in the tarsi. The more favourable the particle size, the less would be the tendency for the nymphs to resort to swimming, resulting in the comparatively lower oxygen consumption of those individuals.

The nymphs of *E. simulans* were able to enter all of the experimental substrates although with varying degrees of difficulty. In the fractions from $\phi + 1$ to $\phi + 5$, however, the nymphs buried themselves only shallowly. It was not possible to determine whether the ease of penetrance to this depth can explain the entire difference in oxygen consumption (i.e. between $\phi + 4$ and $\phi + 5$). The number of nymphs entering the experimental substrates, and the length of time they remained buried, became more variable with increasing difficulty of penetrance. The trend indicated in Figs. 1 and 2 is of increasing variance around the mean with increasing average oxygen consumption. Thus, the considerable variation in oxygen intake exhibited by *Hexagenia* in all ϕ sizes might further suggest that none of the experimental substrate fractions was favourable.
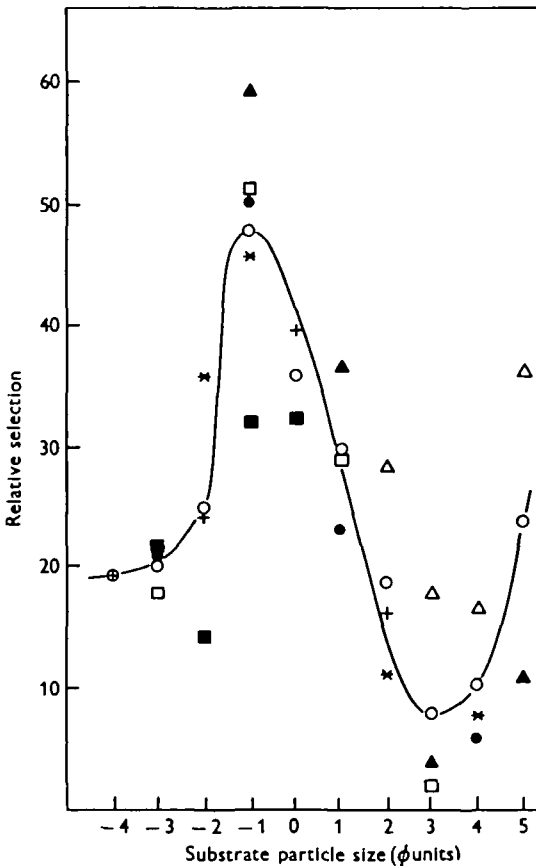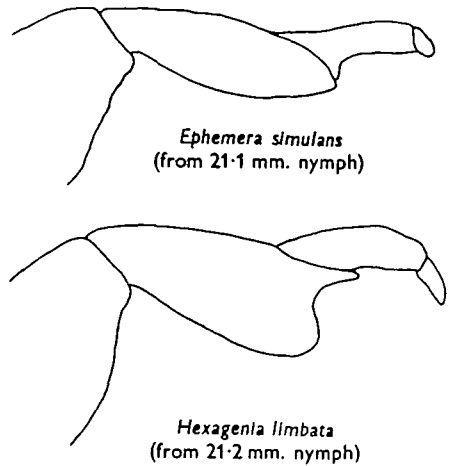

Fig. 3. Substrate preference of *E. simulans* as determined by selection experiments. Substrate groups (ϕ units): -4, -2, 0, 2, $+$; -3, -2, -1, 0, ■; -3, -1, 1, 3, □; -3, -1, 1, 4, ●; -2, -1, 2, 4, ●; -1, 1, 3, 5, ▲; 2, 3, 4, 5, △; average (all experiments), —O—.

Fig. 4. Representative outline traces of prothoracic legs. Scale: $3 \cdot 7$ cm. $= 1$ mm.

Substrate selection

Hexagenia was not used in substrate selection experiments as it could not burrow into any of the fractions presented. Experiments were conducted with *E. simulans* by offering the nymphs a range of substrates laid out in sections in small aquaria. Recovery

of the buried nymphs showed that preference increased from the $\phi - 4$ to $\phi - 1$ particle size, then decreased considerably to $\phi + 3$ and rose again slightly to $\phi + 5$, resulting in a bimodal curve (Fig. 3).

How many factors enter into the final selection of a substrate is difficult to ascertain. No doubt more than just ease of penetration and 'proper particle size stimulus', as measured by oxygen consumption, are important. In any event a striking inverse relationship was obvious when the graphs of substrate selection and oxygen consumption with particle size were compared (Figs. 3, 1). The greatest selection was for the $\phi - 1$ substrate where oxygen consumption was least. The smallest numbers were recovered from the $\phi + 3$ and $\phi + 4$ substrates where oxygen consumption was greatest.

Why the nymphs of *E. simulans* and *H. limbata*, which at first glance are morphologically similar, should vary so greatly in their ability to burrow into similar substrates was puzzling. The manner of burrowing has adequately been described for *H. limbata* by Lyman (1943). Observation has revealed that the same description could apply equally to nymphs of *E. simulans* burrowing in marl or sand. The difference in burrowing ability seems to be related to morphological differences of the burrowing prothoracic legs.

Table 2. *Area trace of tibia and tarsi of burrowing prothoracic legs in mm².*

Ephemera simulans. 21·1 mm. nymphs				*Hexagenia limbata.* 21·5 mm. nymphs			
Nymph no.	Tibia area	Tarsus area	Total area	Nymph no.	Tibia area	Tarsus area	Total area
2	0·86	0·26	1·12	18	1·41	0·31	1·72
2	0·89	0·23	1·12	18	1·33	0·32	1·65
3	0·94	0·22	1·16	23	1·42	0·31	1·73
3	1·02	0·26	1·28	23	1·39	0·35	1·74
Av.	0·93	0·25	1·17	Av.	1·39	0·32	1·71

E. simulans nymphs have comparatively narrow tibia and tarsi (Fig. 4; Table 2) with which they can either scoop out tunnels in marl and sand or pick at individual particles of a coarse substrate in order to construct burrows. *Hexagenia*, with its enlarged prothoracic legs (1·5 times the surface area of *Ephemera's* prothoracic legs), apparently can use them only to scoop out burrows in soft sediments.

DISCUSSION

The data show that oxygen consumption of the two burrowing mayfly nymphs varies considerably with particle size (Figs. 1, 2). Further, particle size selection by the nymphs shows an inverse relationship to oxygen consumption (Figs. 3, 1). Seemingly, they select as 'optimal substrates' ones in which their oxygen consumption is least, which is to say, substrates best suited to their burrowing modifications. As the bottom type becomes less favourable for the nymphs' particular modifications, penetration and further movement become increasingly difficult and oxygen consumption rises. Hence, physiologically, it becomes more difficult for the organism to compete and survive and the numbers, therefore, decrease. Thus, the laboratory and field data collected, and the situations observed, strongly indicate that leg structure determines the substrate into which a nymph can burrow. Concerning only the aspect of penetration, fine adhesive substrates appear to be the only ones which *H. limbata*

can enter with its broadened burrowing legs (Fig. 4). *E. simulans* penetrates almost any substrate with its comparatively narrow prothoracic legs (Fig. 4), although gravel ($\phi - 1$ and $\phi - 2$) and the fine adhesive materials seem to be the most suitable.

This study also shows that oxygen consumption can be used as a means of assessing the suitability of a given substrate. However, it points out as well that care must be taken with interpretation of results and that behavioural observations must be included.

SUMMARY

1. This study is concerned with the relation of oxygen consumption to substrate particle size in the morphologically similar burrowing mayflies *Ephemera simulans* and *Hexagenia limbata*.

2. Nymphs, 16–24 mm. in length, were placed at 13·0° C. for 24 hr. before experimentation. Oxygen consumption experiments employed the standard 'oxygen consumed in a closed bottle' technique and a modification. The standard technique was referred to as the 'blank-bottle' method, while the modification was called the 'substrate-bottle' method (washed substrate fractions were placed in the bottle).

3. The relationship between substrate particle size and oxygen consumption was indicated by a bimodal curve. The oxygen consumption fell, rose and fell once more as the particle size decreased from $\phi - 4$ to $\phi + 5$.

4. Substrate selection experiments with *E. simulans* nymphs revealed an inverse relationship between selection for particle size and oxygen consumption.

5. The burrowing front legs of the nymphs seem to be modified for a given substrate (coarse for *E. simulans*; fine for *H. limbata*) and, the less favourable the substrate, the more work that must be done to gain admittance or to burrow further. The particle size in which the nymphs showed the least oxygen consumption was considered to be an 'optimal substrate' ($\phi - 1$ for *E. simulans* and probably silt for *H. limbata*).

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