RESPIRATORY FUNCTIONS OF MOTILE TRACHEAL GILLS IN EPHEMEROPTERA NYMPHS, AS EXEMPLIFIED BY *SIPHLONURUS OCCIDENTALIS* EATON

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ABSTRACT

Four questions about the roles of nymphal tracheal gills were posed: do lamellae meet all structural criteria for being gills? How important are they as oxygen intake surfaces? Are lamellae ventilated and, if so, how? Do lamellae aid or enable a nymph to maintain respiratory regulation in the face of dwindling oxygen supplies? *Siphlonurus occidentalis* possesses 7 pairs of extremely large but variously sized, plate-like tracheal gills. Their respiratory importance was assessed by determining oxygen consumption of gilled and gill-less individuals, by measuring beating rate and percentage of time spent beating with changes in dissolved oxygen (DO) and temperature, and through measuring the volume of water passed over the body surfaces by fully-gilled and partially-gilled nymphs. Data substantiate 7 conclusions: (1) gills are important sites of oxygen intake, accounting for about 67% of the total consumed at 12–13°C, and (2) gills take in oxygen proportionate to their fraction of total respiratory surface area. Maximum diffusion gradients are maintained in the face of rising temperature and decreasing DO by increasing (3) gill beating frequency and (4) the percentage of time spent beating. (5) At any particular combination of DO and temperature, beating frequency is fixed; that is, nymphs from whom gills 3–7 have been removed do not beat their remaining gills any faster. Rather, (6) such nymphs further increase the percentage of time spent beating. (7) Even so, individuals with all their paddles (and therefore all respiratory surfaces) force approximately 10% more water over their body than do those with about half such surfaces removed. Thus, by adjusting both beating rate and the amount of time spent beating nymphs control the water volumes moved and in so doing appear to regulate oxygen consumption.

INTRODUCTION

A teeming and diverse fauna inhabits the brown, acid waters of small *Carex*-meadow potholes in the Pioneer Mountains of south-western Montana, U.S.A. Daily physico-chemical fluctuations in these pools include considerable changes in temperature and oxygen concentration, the latter sometimes ranging from supersaturation to 1 mg.l^{-1} in 30 cm deep bottom water. When confronted with such minimal O_2 concentrations, the many resident species of air-breathers have little difficulty in obtaining needed oxygen. However, those requiring dissolved oxygen (DO) face a considerable challenge. Our field work led to the observation that some of the animals in the latter category, such as anostracans and larval anurans, swam near the surface when DO levels were very low, but the often motionless zygopteran, *Lestes disjunctus*, and ever active ephemeropteran, *Siphlonurus occidentalis*, remained distributed throughout the water column.

Lestes and *Siphlonurus*, the most common DO-requiring insects in the potholes, have some of the largest tracheal lamellae (tracheal gills) in proportion to body size of all aquatic insects (Dodds and Hisaw 1924, Edmunds *et al.* 1976, MacNeill 1960). Given the observation that possession of lamellae of such extreme proportions positively correlated with apparent success in habitats of such extreme and variable conditions, a research program was initiated with the goal of understanding the functions and functioning of tracheal lamellae. These organs have been ascribed various roles, among them O_2 intake, ventilation, swimming aids, protection, streamlining, and salt exchange. Undoubtedly many or all of these functions occur in one or another species, but evidence, at least for their respiratory roles, is largely circumstantial, occasionally contradictory, and pieced together from a few studies on widely different organisms. Therefore, we posed the central question: are tracheal lamellae actually gills? Experiments were focused by a sub-set of questions: 1) Do lamellae meet all structural criteria for being gills? 2) How important are they as oxygen intake surfaces? 3) Are lamellae ventilated and, if so, how? 4) Do lamellae aid or enable the animal to maintain respiratory regulation in the face of dwindling oxygen supplies? These questions were addressed for the non-motile caudal gills of the zygopteran *Lestes disjunctus* Selys by Eriksen (1984, 1986). In the present paper we concern ourselves with these same questions, but with regard to the motile, laterally-placed lamellae of the ephemeropteran *Siphlonurus occidentalis* Eaton.

METHODS

Nymphs of *S. occidentalis* were collected from pothole ponds and slow-moving, small rivulets in Mono Meadows, (elevation 2350 m; 45°30'N, 113°6'W) 30-35 km south of Wise River, Beaverhead County, Montana. They were transported to the Wise River Research Laboratory at 1980 m and held in shallow trays at 12.5°C. Experimentation followed within 1–3 days. By grasping gills with forceps and allowing the nymphs to autotomize them at a basal "breaking joint" as they swam away, gills were removed without physiological injury to the animal (Eriksen 1986). At least one hour of acclimation followed ablation before experimentation began.

Lamellar movements were observed through a dissecting microscope and tallied; beating rate (beats.min^{-1}) and percent of time spent beating were calculated from these data. Observations were made while nymphs clung to plastic mesh in small dishes which had been placed in water baths for temperature stabilization. Various experimental temperatures were attained by allowing the water baths to warm slowly to ambient (room) temperature, with counts being made when certain temperatures were reached. To observe the effect of oxygen concentration on these parameters, single nymphs were placed in closed 8.5 ml vials of O_2-saturated, filtered pond water and immersed in a 12.5°C water bath. Nymphs lowered the oxygen concentration in the vials by their own consumption. Rather than DO being monitored, gill beats per minutes and percent of time spent beating were determined at known intervals following introduction.

To determine the volume of water moved at a given beating rate and temperature, nymphs were placed in 5 cm long glass tubes of 5.0 mm ID. A piece of aluminium window screening at each end kept nymphs in without hampering water flow. The tube was then placed against a mm ruler in a water bath and viewed through a dissecting microscope. Small particles were timed as they were swept along a known distance by beating gills.

Metabolic rate was determined by placing 4 nymphs each in 95 ml, or 5 nymphs each in 125 ml, closed bottle respirometers filled with filtered, nearly O_2-saturated (5.0–5.8 mg.l^{-1}) pond water at 12.5 $\pm$ 0.5°C. After a period of time calculated to lower DO about 1 mg.l^{-1}, nymphs were removed and oxygen concentration of the experiments and controls determined with a Southern Analytical Al672 oxygen analyzer or a YSI oxygen meter to $\pm$ 0.05 mg.l^{-1}. Nymphs were dried at 105° for 24 hours and weighed to

0.01 mg on a Sartorius 2404 semi-micro analytical balance. Oxygen consumption (VO_2) was determined as mg $O_2.g^{-1}$ dry weight.h^{-1} $\pm$ 2 SE.

Because the head and thorax are essentially an irregular cylinder, their surface area was calculated from ocular micrometer measurements of diameter and length. The abdomen is a slightly flattened cylinder along the proximal 65% of its length. The distal 35% tapers somewhat to the posterior end. Therefore, surface area of the proximal portion was calculated from its circumference (determined from averaged height and width measurements) multipled by 65% of abdominal length. Given that the distal 35% of the abdomen is a somewhat flattened frustum of a cone, length, and averaged diameters of its anterior and posterior ends were used to calculate the area of its surface. Body surface area therefore equals summed head and thorax, and abdominal surface areas. In order to determine surface areas of lamellae, each was removed from a nymph of known dimensions and placed on a glass slide. These were photographed, and projected images were traced. Surface area was calculated from ocular micrometer measurements, and the known magnification and weights of photocopied images of those tracings.

RESULTS

Body shape and structure

S. occidentalis nymphs grow to approximately 15 mm in length, of which the head and thorax are about 33%, the abdomen 67%. The head and thorax comprise, on average, 40% of body surface area, the abdomen 60%. The exoskeleton of the head and thorax is thickened to support associated muscles. Such thickening makes these areas opaque, which precludes any important observations of internal tracheal structure. By contrast, the abdominal exoskeleton is thin, and internal observations may be made. Such observations reveal that sternal body-wall tracheation provides only 70% of the length of tubing that is found under the same area of the tergum (3.5 vs
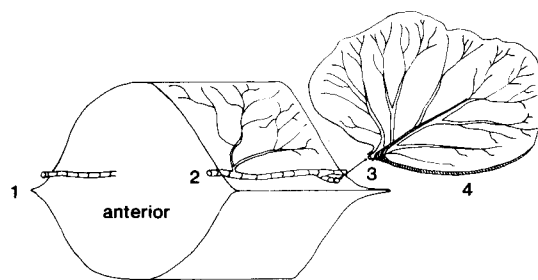

Fig 1. Siphlonurus occidentalis: Structure and tracheation of the sixth abdominal segment and its gill. (1) lateral carina, (2) lateral tracheal trunk, (3) pedicel, (4) cuticular thickening.

4.9 mm.mm^{-2}). In both cases tracheae branch repeatedly (Fig. 1), the larger trunks being 15-20 μm outside diameter (OD), the smallest 5 μm.

Gill shape and structure

The abdomen possesses 7 pairs of plate-like gills, the first two of which are composed of double lamellae. Each gill is served by a single tracheal trunk which branches palmately immediately upon entering the gill (Fig. 1). One to several of the branches remain quite large, a structure which, in combination with a V-shaped cuticular thickening that extends from the gill's base to about half its length along its ventral edge and the midline, must give considerable rigidity to the gill (Fig. 1). All but the most anterior lamella of each of the first two pairs of gills have such thickening. The palmate branching, and successive rebranching, supply the gills with abundant tracheae which range from 45-50 μm OD as they enter the gills, are 25-30 μm OD half way out and are 5 μm at the
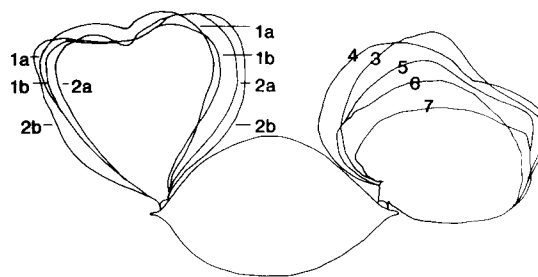

Fig. 2. Siphlonurus occidentalis: Relative positions and shapes of gills 1–7 (a & b are anterior and posterior lamellae of the double lamellar gills).

distal edges. This branching pattern results in about 9.0 mm of tracheae per mm^2 of gill surface (N = 4), a density 2.1 × greater than that of the abdomen. Another result is that no gill tracheae are further apart than 210 μm (n = 22).

Nymphs are commonly seen with lost, regenerating or damaged gills. Gills 4–7 are particularly easily removed. When gills were autotomized, no fluid loss or tracheal flooding was observed. A longitudinal carina demarcates the nymph's lateral abdominal line. Gills are anchored to the body at the posterior dorsal base of this carina (Figs 1, 2) of segments 1–7. Their ventral edges extend nearly horizontally and their lateral edges all nearly align when viewed with their pedicels superimposed (Fig. 2). Gill shape and size varies along the length and fits the shape of the space between the carina and the orsum. For example, the first gill possesses a particularly narrow base allowing it to fit and beat between the lateral carina and the dorsal wing pads. The second gill is wider (Fig. 2) because the wing pads taper medially providing greater space. Gill 3 is positioned at or just beyond the distal tip of the wing pad, is larger because it is not limited in its extension over the abdomen, and possesses a more rounded shape (as do the rest of the gills). Gill 4 is slightly smaller
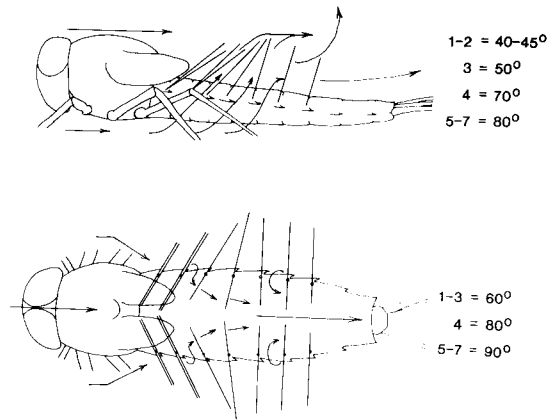

Fig. 3. Siphlonurus occidentalis: Lateral (top) and dorsal (bottom) representations of the attitude (°) of gills 1–7 with the long axis of the body, and the water currents produced by them.

than 3 (Table 1) but extends the furthest over the abdomen's dorsum (Figs 2, 3). Gills 5, 6 and 7 become successively smaller (Table 1), particularly in height and somewhat medially (Fig. 2). All together gills represent 59.4% of the nymph's total surface area. Assuming abdomen and gills provide the only significant respiratory surfaces, the gills account for 70.8% of this (Table 1).

Table 1. Siphlonurus occidentalis: Proportional relationships of gills and body surface areas of a terminal instar nymph (n = 8)

	% of			
	Total nymph surface area	Total gill surface area	Respiratory surface area	Paddle surface area
Head & thorax	16.1	–	0	–
Abdomen	24.5	–	29.2	–
Gill 1a	–	10.0	7.0	0
1b	–	10.3	7.2	14.1
2a	–	10.5	7.4	0
2b	–	13.3	9.4	18.3
3	–	15.0	10.6	20.6
4	–	14.0	9.9	19.2
5	–	11.3	8.1	15.6
6	–	8.9	6.3	12.2
7	–	6.8	4.8	0
Gill total	58.4	100.0	70.8	100.0
Gills 1, 2 & abdomen	50.6	–	60.3	32.4

Ventilation

Gill beat forces a posteriorly directed flow of water which allows new water to move in from a cylindrical volume around the body's axis anterior to gill pair 7. The strongest currents appear to come from ventral, up between the gills, then posteriorly along the abdomen. More lateral waterflow is deflected somewhat upward and over the cerci (Fig. 3) by the non-moving, nearly vertically positioned ($\sim 80°$), 7th pair of gills. From a lateral aspect, the first two pair of double-lamellae gills are held 40-45° from the horizontal, gill 3 at about 50°, gill 4 at 70° and gills 5-7 at about 80° (Fig. 3). Given these positions, the first double-gill pair appear as protective shields for gills 2, 3 and perhaps 4. Observed dorsally, gills are hinged and beat at different angles to the body's long axis. For example, gills 1, 2 and 3 lie in a plane $\sim 60°$ off the axis, gill 4 $\sim 80°$ and gills 5-7 $\sim 90°$ (Fig. 3). Thus water is directed posterior-medially by gills 1-4 until currents from opposite sides meet each other in the vicinity of the 5th gill pair then flow parallel to the body's long axis aided by the posteriorly directed beating of gills 5 and 6 (Fig. 3).

Gill beating is sporadic and shallow, often only an occasional flutter, at low temperature and high DO. When beating occurs, gills 1 and 2 are always, and 3 is usually, involved. As temperature rises or DO drops, the others become successively active (except 7 which never beats), and the arc
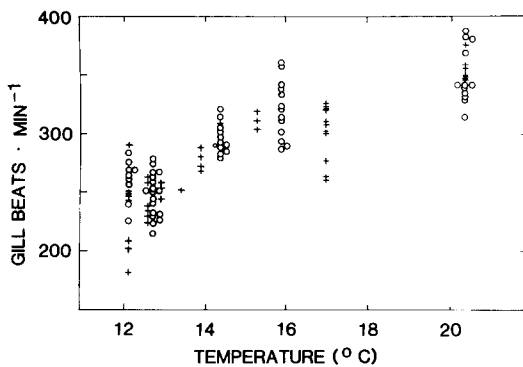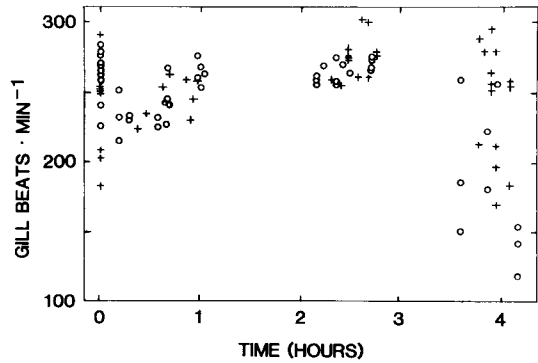

Fig. 5. *Siphlonurus occidentalis*: Gill beating rate, at different time intervals (oxygen concentrations) following placement in closed containers, of nymphs with all gills (+) and those with gills 3–7 autotomized (o).

through which they beat increases rapidly to a maximum 20–22°. Even though they possess 2 lamellae, gills 1 and 2 each work as a single paddle and thus provide only 14.1 and 18.3%, respectively, of the possible paddle surface (Table 1).

Under conditions of oxygen saturation, fully gilled nymphs and nymphs with 68% of their paddle surfaces removed (gills 3–7 autotomized) demonstrated similar and linearly increasing beating rates between the temperatures of 12.5 and 16.5°C. Above 16.5° the rate of increase was not as great, by virtue of reaching what undoubtedly were nearly maximal beating frequencies at 20.5° (Fig. 4). Over this same 12.0–16.5° temperature range nymphs without gills 3–7 beat their remaining gills a greater proportion of time than did those

Fig. 4. *Siphlonurus occidentalis*: Gill beating rate, at different temperatures, of nymphs with all gills (+) and those with gills 3–7 autotomized (o).
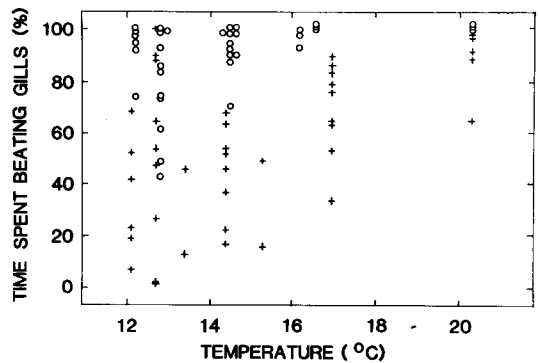

Fig. 6. *Siphlonurus occidentalis*: Percent of time gills beat, at different temperatures, for nymphs with all gills (+) and those with gills 3–7 autotomized (o).

with all surfaces present (Fig. 6). By 20.5° all larvae were beating nearly constantly (99 and 93%, respectively; Fig. 6). Under these conditions, nymphs with only gill pairs 1 and 2 (~68% of their paddle surface removed), showed but a 10.1% reduction in the volume of water moved over their surfaces: 15.9 mm³.sec⁻¹ (n = 115) to 14.3 mm³.sec⁻¹ (n = 126) a difference of only 10.1% (p(F₁₂₃₉ = 4.530) < 0.05).

At 12.5°C and beginning at oxygen saturation, fully gilled nymphs and those with gills 3–7 autotomized demonstrated similar and increasing beating rates as DO dropped for the first 2–3 hours (Fig. 5). However, with longer times no further increase was noted. Actually, fully gilled animals had a somewhat greater beating rate after 3.5–4.5 hours (Fig. 5) because they did not demonstrate as great a variability or reduction in beat due to metabolic impairment from low DO. Over the course of the experiment, as DO decreased the percent of time that gills spent beating increased, reaching 100% by about 3.5 hours. Since oxygen continued to decrease for another hour without the possibility of a compensatory increase in beating rate or the amount of time gills beat, death soon ensued due to asphyxiation.

Metabolism and respiratory surface area

Because the metabolic rate of nymphs is affected by size the data were weight-corrected to either a 3.5 or 8.0 mg nymph for purposes of comparison. Such comparison shows that individuals with 61% of their respiratory surfaces present (gills 3–7 autotomized) demonstrated only a 3.1% decrease in metabolic rate when compared to nymphs possessing 100% of their O_2 intake surfaces (at 12.5° and 5.0–5.8 mg.l⁻¹O_2). When fully gilled nymphs are inactivated by anaesthetic, metabolism is reduced 66.0%. When gill-less nymphs are anaesthetized, O_2 intake declines 66.4% more than that of anaesthetized, but fully gilled individuals (Table 2).

Table 2. Siphlonurus occidentalis: VO₂ (mg·g⁻¹·h⁻¹) of normal and anaesthetized nymphs, weight-adjusted to 3.5 and 8.0 mg, possessing differing numbers of gills (12.5 ± 0.5 °C)

Gills present	Normal	Anaesthetized
	(3.5 mg O₂)	
All	(a) 1.971 (n = 31)	(b) 0.868 (n = 26)
None	–	(c) 0.292 (n = 27)
	(8.0 mg O₂)	
All	(d) 1.212 (n = 31)	–
1 & 2	(e) 1.175 (n = 12)	–

$\dfrac{b}{a}$ = 66.0% decrease

$\dfrac{c}{d}$ = 66.4% decrease

$\dfrac{e}{d}$ = 3.1% decrease

DISCUSSION

Criteria for being gills

In the absence of internal or cross sectional studies of mayfly tracheal gills, one must assume that they are structurally similar to the caudal lamellae of Zygoptera, first described by Tillyard (1917) and reviewed by Eriksen (1986). The gills of *S. occidentalis* are undoubtedly flattened outgrowths of the abdominal wall, being therefore outer layers of cuticle secreted by underlying hypodermis in turn sandwiching tracheae and tracheoles yet leaving some small spaces for circulation of hemolymph. *S. occidentalis* gills, which comprise 59% of the total body surface area, demonstrate various plate-like shapes. Each attaches by a narrow pedicel to the posterior, dorsal base of the lateral carina where its tracheal system connects to one of the two main lateral tracheal trunks of the body (e.g. Soldán 1979) (Fig. 1). The tracheal trunk that enters a gill is quite large (45–50 μm) but then palmately branches and rebranches into the gill structure, being only 5 μm OD near the lamella's distal edge. Since these

measurements approximate those of Tillyard (1917) for *Lestes*, we assume gill thickness also approximates that of a lestid gill, namely 45–65 μm. Diffusion distance into the tracheae of the gills would be as little as 10–20 μm (Eriksen 1984). Given the dense tracheal branching pattern, an O_2 molecule, once through the cuticle, would have to diffuse or be moved laterally by hemolymph a maximum 105 μm to a tracheole. Such thin structures, with length and width measurements up to 2,000 μm, obviously meet the structural criteria for being gills (Eriksen *et al.* 1984): large surface area, minimal bulk, and highly vascularized with a short diffusion path from the outside surface to the tracheal system. Finally, gills should be ventilated in some way. Given that the beating of these structures passes a continuous stream of water over their surfaces, they meet this final criterion as well.

Oxygen intake surfaces

Any structure which meets the described criteria for a respiratory surface can be considered an O_2 intake site. Thus the abdomen, with its thin cuticle, considerable tracheation (particularly the dorsum) (Fig. 1) and strong ventilation by the gills, must also be important in respiration. In contrast, although ventilated as the gills draw water toward the abdomen from anterior, the head and thorax with their thick cuticle and probable lack of significant body-wall tracheae (at least they cannot be seen given the thick cuticle) seem unimportant sites of oxygen intake. Respiratory surface area should therefore be considered only that of the gills and abdomen.

When gilled and gill-less nymphs of *S. occidentalis* were anaesthetized to eliminate any differential effects of activity and ventilation, the role of different amounts of surface area in supplying O_2 for the needs of metabolism could be compared. Under this circumstance, and with DO near saturation, fully gilled nymphs consumed 66.4% more oxyggen than did gill-less individuals (Table . The gills of *S. occidentalis* comprise 70.8% of the total respiratory surface (Table 1), suggesting that oxygen consumption is proportional to respiratory

surface area. Thus the tracheal gills of *Siphlonurus* are very important O_2 intake sites and since they are taking in an amount proportionate to their surface area at relatively low temperatures and high DO, it is doubtful that they could increase their proportion of O_2 intake significantly as temperatures rise or DOs fall. By contrast, Eriksen (1986) demonstrated that the tracheal gills of the damselfly, *Lestes disjunctus*, work quite differently. When 1, 2 or all 3 *Lestes* gills were removed, and nymphs were provided with a reasonably high oxygen supply, a positive relationship between oxygen intake and the amount of gill surface area was suggested. However, the gills were responsible for only 20–30% of total O_2 intake even though they comprised about 75% of the respiratory surface area. Only as O_2 levels dropped, did their importance approach, and finally attain, their respiratory surface area proportion.

These differences in ability or response between *Lestes* and *Siphlonurus* are probably explained by several major differences in respiratory structure and behaviour. Although the gills of both species present similar porportions of respiratory surface area (~ 71 and 75%, respectively), mayfly gills are individually smaller and stationed along the abdomen quite close to sites of metabolic need. Therefore, the rate of supply is much elevated over damselfly gills whose length and terminal placement mean that the diffusion distance is considerably lengthened and therefore the rate of supply of O_2 comparatively diminished. Secondly, the gills of *S. occidentalis* are well ventilated, and the volume of water by which they are ventilated is controlled by a change in pumping frequency, the length of time ventilation occurs, or both. *Lestes* utilizes a poor ventilation mechanism (Eriksen 1984), which though somewhat helpful, is not nearly as efficient as that of *S. occidentalis*. (An analagous situation with Plecoptera and Ephemeroptera is discussed by Nagell, 1973). The importance of *Siphlonurus*' gills as O_2 intake surfaces seems also suggested by the fact that individuals with greater surface area can withstand somewhat lower oxygen concentrations; that is, they do not die quite as soon as partially degilled nymphs (Figs 4, 6) (a situation similar to *Lestes*; Eriksen

1984). However, this result is also somewhat affected by nymphs with fewer gills passing about 10% less water over their bodies as do fully gilled individuals (Table 2).

That mayfly gills are important oxygen intake sites is quite in contrast to the conclusions reached by Wingfield (1939) that the nonbeating gills of *Baetis* sp. (a flowing water species) and the beating gills of *Cloeon dipterum* (a still water form) were unimportant as O_2 intake surfaces. However Wingfield's work was flawed with numbers too few, variation too great, gills cut off rather than autotomized, no distinction in stirred water between a gill-less nymph and a gilled nymph which could also beat its gills, and no observations of activity or behaviour to help interpret data. Even if his conclusions are correct, they probably hold only for the 10°C at which he ran his experiments. Eriksen (1986) showed that at this temperature or below, the gills of *Lestes disjunctus* played no role in oxygen intake either, but at higher temperatures and variable DOs, they were responsible for 20–80% of O_2 intake, and this for an apparently somewhat cooler water species than *C. dipterum*.

Ventilation

Ventilation is accomplished by the beating of the gills as was shown in several papers by Eastham (1932, 1936a, b, 1958). The double-lamellar gills of *Leptophlebia* allow each gill to increase its efficiency of pumping water because it not only acts as a paddle, but also squeezes water from between the double lamellae. In this way, *Leptophlebia* can have gills attached at different attitudes with the body and thus move water from different levels around the nymph (Eastham 1936a). When lamellae are single, they function best when they all share the same attitude with the body because the metachronal beat means that water is pushed by each gill as well as squeezed from between adjacent gills, as in *Ecdyonurus* (Eastham 1936b). *Cloeon dipterum* (Eastham 1932, 1958) and *S. occidentalis* combine some of each of these features. *Cloeon*'s first 6 pairs of gills and *Siphlonurus*' first 2 pairs are double and thus can paddle water as

well as squeeze water from between the double lamellae. However, all the gills, double ones included, work in a metachronal manner to produce current, thus summing the effect of the two methods. Perhaps this is part of the reason that ventilation by *Siphlonurus* nymphs missing gills 3–7 was not impeded nearly as much as might have originally been hypothesized.

The gill beating rate of *S. occidentalis* increases with rising temperature or falling DO (Figs 4, 5) and it appears that this is the case for all mayflies which beat their gills (e.g., Babak and Foustka 1907 cited in Wingfield 1939, Eastham 1936a, b, Eriksen 1963, Ookeaw this volume). The single exception, Kamler 1971, is based on too few data to draw any conclusion. If *Siphlonurus occidentalis* is typical, beating rates are fixed at a given temperature and oxygen combination, for gill beat could not be shown statistically to vary with the amount of respiratory surface area available (e.g., nymphs with gills 3–7 removed beat at the same rate as fully gilled individuals). Nymphs did compensate for gill loss, however, in a manner also used to help combat the effects of increasing temperature and decreasing oxygen, that is by lengthening ventilation time (Figs 6, 7). Nymphs of *Cloeon dipterum* also do this (Kamler 1971).

In these combined ways nymphs undoubtedly force over the body greater quantities of water from which to extract O_2 in an apparent attempt
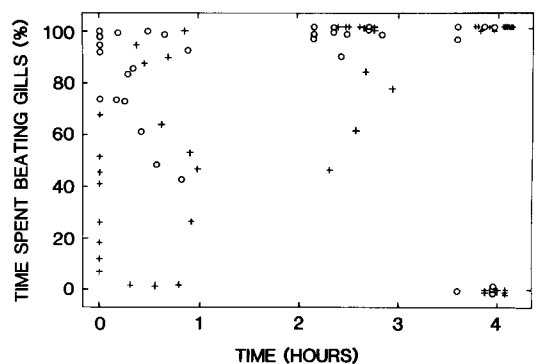

Fig. 7. Siphlonurus occidentalis: Percent of time gills beat, at different time intervals (oxygen concentrations) following placement in closed containers, of nymphs with all gills (+) and those with gills 3–7 autotomized (o).

to regulate oxygen intake. If the ultimate proof of a nymphs' ability to regulate its respiration lies in the measurement of its oxygen consumption over a range of DO, then only several workers have undertaken the test. Fox, Wingfield and Simmonds (1937) demonstrated some semblance of regulation in *Cloeon dipterum, Leptophlebia vespertina* and *Baetis scambus* although it is difficult to understand how *Baetis* manages to do so out of its natural current flow and with immobile gills, and when young individuals do not have this ability (Fox et al. 1937, Wingfield 1939). In the latter paper, Wingfield reconfirmed respiratory regulation in *Cloeon dipterum*, an ability amply documented by Nagell (1974). Finally, the burrowing mayflies *Hexagenia limbata* and *Ephemera simulans* were shown to be capable regulators by Eriksen (1963).

If it is necessary to demonstrate further that decreasing DO results in increasing beating rate, greater volumes of water moving past gills, and a constant VO_2, then this has only been demonstrated with *Hexagenia limbata* and *Ephemera simulans* (Eriksen 1963). Given what is known about the physical requirements for diffusion and respiratory regulation, it is reasonable to assume that if one of the parameters changes, the remainder can be extrapolated. Therefore, the increasing gill beat and greater percent of time spent ventilating with decreasing DO suggests that *Siphlonurus* passes increasing quantities of water and thus maintains regulation. In like manner, the increasing beating frequency of *Cloeon dipterum* (Wingfield 1939) must result in greater water flow and the respiratory regulation documented by Fox et al. (1937), Wingfield (1939), and Nagell (1973, 1974). *Leptophlebia*'s regulation (Fox et al. 1937) must also stem from a greater beat frequency and increased volume of water passed with lowering DO.

It might be argued that nymphs with lost (autotomized) or damaged gills should move even more water than fully-gilled individuals to compensate for lesser respiratory surface. Since those with gills 3–7 autotomized actually move a 10% smaller volume, at least at 21.5°C (the conditions necessary to compare constantly beating gilled and

partially de-gilled nymphs), one or a combination of several explanations would probably resolve the results. For example, with less respiratory surface a steeper diffusion gradient would be established (lower O_2 inside the body) with the result that more O_2 would pass through the respiratory surfaces available. Additionally or alternatively, because they are beating their gills a greater proportion of the time, partially de-gilled nymphs may in fact pump somewhat larger water volumes than fully gilled individuals, but only up to the point where they begin beating 100% of the time (~17°C, Fig. 5). As evidence that this may indeed be occurring, at 12.5° metabolic rates of nymphs with all gills or only gill pairs 1 and 2 differ by only 3.1% (Table 3). Although this small difference is statistically significant, it demonstrates that compensation for loss of a number of gills is almost complete at this temperature. However, if temperature continues to rise or DO falls, beating rate will continue to increase, but while the fully gilled nymph can continue to increase the percent of time spent beating up to ~21°C, the partially gilled nymph will be constantly beating by 17°C and therefore should not be able to extract quite as much oxygen as a normal animal above this temperature.

ACKNOWLEDGEMENTS

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