

# Respiratory Adaptations to Running-Water Microhabitats in Mayfly Larvae *Epeorus sylvicola* and *Ecdyonurus torrentis*, Ephemeroptera

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## ABSTRACT

The mayfly larvae *Epeorus sylvicola* and *Ecdyonurus torrentis* inhabit either fast-flowing or, for the latter species, calm zones of running water. We studied (1) mechanisms and limitations of oxygen transport in single individuals (oxygen consumption rate, occurrence and rate of gill movements, and heartbeats) in running water of different oxygen concentrations and (2) capacities for anaerobiosis (L-lactate production). Our aim was to look for specific adaptations in the two species to slightly different microhabitats. *Epeorus sylvicola*, whose immovable gills are not able to generate ventilatory convection, proved to be an oxyconformer at both test temperatures (11° and 15°C). *Ecdyonurus torrentis* showed a progressively stronger oxyregulatory behavior at higher temperatures. In this species an onset of gill beating was found at moderate hypoxia (below 16 kPa). Ventilating individuals reached maximum rates (300 min<sup>-1</sup>) of 5–14 kPa. In the case of a further reduction of oxygen partial pressure, the ventilatory rate started to decrease. Ventilatory activity, however, was maintained down to very low oxygen concentrations. Neither in *E. sylvicola* nor in *E. torrentis* was experimental evidence found to confirm the hypothesis of a respiratory function of hindgut movements. During hypoxia, the heart rate was constant in both species (*E. sylvicola*: 80 min<sup>-1</sup>; *E. torrentis*: 60 min<sup>-1</sup>): bradycardia occurred either below 1.5 kPa or below 4 kPa. Anaerobiosis, that is, lactate production, was not detected in either species.

## Introduction

Rheophilous (“living in running water”) mayfly larvae inhabit either lotic (rapidly flowing) or lenitic (slowly flowing) zones of running water. In the former, the concentration of dissolved oxygen is usually near to air saturation. Often, mayfly larvae are sensitive to oxygen depletion (Golubkov et al. 1992), and their occurrence and abundance are used as an indicator for oxygen-rich, unpolluted water. Their habitat requires adaptations in both morphology and physiology. To resist the physical forces of running water (Ambühl 1959; Statzner and Holm 1989) and to avoid being swept away, rheophilous mayfly larvae often possess a dorsoventrally flattened body shape, adhesive organs, or specialized claws (Wesenberg-Lund 1943; Studemann et al. 1987; Wichard et al. 1995). Furthermore, exposure to well-aerated water often reduces the requirement for ventilation.

Nevertheless, oxygen-rich conditions in running waters are not always guaranteed. Organic water pollution or reduced flow velocity near the banks lead to a reduced oxygen availability (hypoxia). To survive hypoxia, efficient respiratory gas transport systems or anaerobic capacities are necessary. In lotic mayfly larvae, the efficiency of respiratory organs is reduced compared with that of lenitic species, which usually possess both an enlarged outer (respiratory) and inner (tracheal) surface (Dodds and Hisaw 1924; Wichard 1979). A complete loss of the ability to ventilate their gills, as in the lotic genus *Epeorus*, deprives these organisms of the possibility to regulate oxygen uptake at varying ambient oxygen partial pressure (Po<sub>2</sub>; Wingfield 1939). We focused on two rheophilous species of the Heptageniidae mayflies, *Epeorus sylvicola* and *Ecdyonurus torrentis*. Both inhabit lotic zones, but *E. torrentis* also lives in lenitic areas. Its tracheal gills consist of pinniform gill trees and lamellar gillplates: the beating gillplates cause ventilatory flows through the gill trees (Eastham 1936). *Epeorus sylvicola* possesses only immovable gillplates.

The aim of this study was to look for respiratory adaptations to slightly different habitats in the two species. In previous studies, the oxygen consumption of a whole group of individuals was determined or only single-parameter measurements were made (Fox and Simmonds 1932; Fox and Baldes 1934; Fox et al. 1936a, 1936b; Wingfield 1939; Golubkov et al. 1992). Furthermore, a possible respiratory function of peristaltic hindgut movements was discussed (Dewitz 1890; Meyer 1930). By

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using closed respirometry, we studied the specific oxygen consumption rate ( $Mo_2$ ) in single mayfly larvae in relation to the ambient  $PO_2$  at running-water conditions. To probe the fundamental capacity and working range of their respiratory systems, measurements were done over a wide ambient  $PO_2$  range, including hypoxic states probably not occurring in the natural habitat. For similar reasons, measurements were made at natural temperature conditions (11°C) and at a higher temperature (15°C): decreasing ambient oxygen concentration and increasing metabolic rate of animals should further challenge a respiratory system. Under similar conditions to those during respirometry, the occurrence and the rate of gill movements and heartbeats (ventilatory or cardiac activity as well as gill beating rate [GBR] or heart rate [HR]) were measured simultaneously by applying advanced techniques like video microscopy and digital image analysis. Anaerobiosis was tested by measuring lactate production during anoxia.

## Material and Methods

### Animals

The study done on the mayfly larvae *Epeorus sylvicola* Pictet and *Ecdyonurus torrentis* Kimmins lasted from September 1997 to February 1998. The animals were collected in the low mountain range Sauerland from the brook Kleine Schmalenau, a run with swiftly flowing water and a stony bottom. In the laboratory, the animals were kept in aquariums (65 L, pH 8.2–8.3, 9°–12°C) at running-water conditions. The aquariums were filled with tap water, which was also used as experimental medium. The water quality of natural and experimental water was similar: however, brook water was softer and showed a lower conductivity. The collected animals could be kept in the laboratory until emergence (2–3 mo). Experimental animals, however, were tested within 2 wk after collection. The weight range of the tested larvae was 11–40 mg, with body sizes of 5–11 mm.

### Determination of Oxygen Consumption Rate

The oxygen consumption rate was measured in a newly developed closed-respiration chamber (Fig. 1A), which allowed us to investigate single mayfly larvae. The chamber consisted of an optical cuvette (10-mm path length; Hellma 6030, Müllheim/Schräger Baden) and a Perspex stopper, which contained the  $PO_2$  sensor and a small magnetic stirring bar (Spinbar, Pequannock, N.J.). The bar was located in a separate chamber in front of the Clark-style oxygen electrode (1302, Strathkelvin Instruments, Glasgow) to produce a persistent water current in the animal chamber to approach natural flow conditions. A magnetic stirrer (Rank-Brother) was used to rotate the bar. The chamber volume could be varied in a range of 0.7–1.0 mL by the use of Perspex sheets of variable thickness. The closed respirometric chamber was additionally equipped with a small

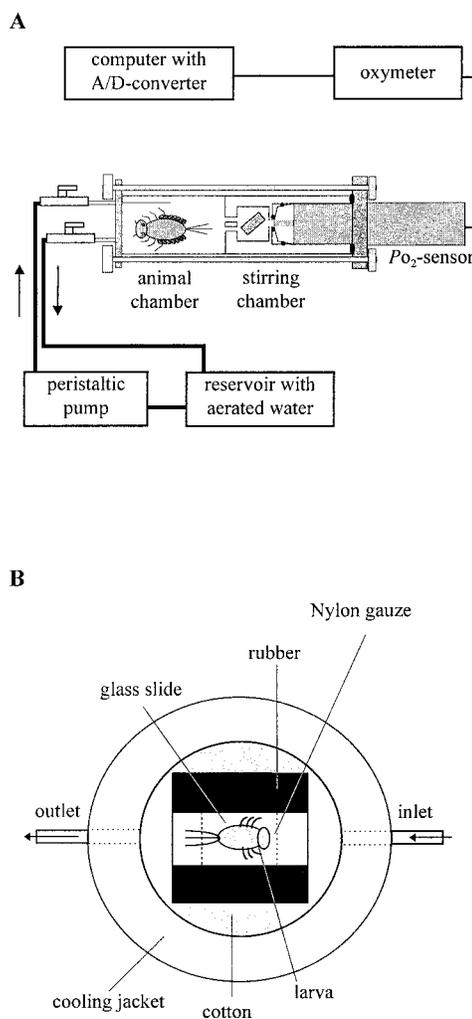


Figure 1. Experimental setup for measuring oxygen consumption rate in single mayfly larvae (A) and the animal chamber (top view) for measurements of heart rate, gill beating rate, and hindgut movements (B). See text for details.

inlet and outlet ( $\approx 0.5$  mm internal diameter) to perfuse the animal with water during normoxic adaptation periods by means of a peristaltic pump (Minipuls 3, M312, Abimed-Gilson, Villiers). During measurement periods, the inlet and outlet were closed by means of stopcocks. The signal of the oxygen electrode was measured with an oxymeter (Model 781, Strathkelvin Instruments, Glasgow) connected to a personal computer (sampling rate: one sample per second). Two-point calibrations of the oxygen electrode were done by perfusing the chamber with anoxic (oxygen completely removed by using sodium dithionite) and normoxic water.

For adaptation, the animals were transferred and kept for 12 h without food in a separate aquarium at the experimental

temperatures (11° and 15°C). One hour before the beginning of the experiment, an individual was introduced into the respiration chamber, which was perfused with normoxic, autoclaved water. Immediately before the experiment, perfusion was stopped, and the  $\text{Po}_2$  signal was continuously recorded. The oxygen consumption rate of the animal caused the  $\text{Po}_2$  to decline and finally to approach anoxia. After the animal had been removed, bacterial respiration was determined. On average, it was approximately 33% of total respiration at 11°C and 23% at 15°C.

The oxygen consumption rate was calculated from the time interval  $\Delta t$ , within which the  $\text{Po}_2$  dropped by 10 Torr (1.3 kPa):

$$\dot{M}\text{O}_2 = V \times \beta \times 10/\Delta t, \quad (1)$$

( $V$ , chamber volume;  $\beta$ , oxygen solubility of the medium calculated by using formulas given by Forstner and Gnaiger 1983). The animal respiration rate resulted from the difference of total and bacterial. After measurement, fresh body mass of the individual was determined the oxygen consumption rate to calculate the mass-specific  $\dot{M}\text{O}_2$  (in  $\mu\text{mol g}^{-1} \text{h}^{-1}$ ).

#### *Measurement of Occurrence and Rate of Gill Movements, Heartbeats, and Hindgut Movements*

Single mayfly larvae were microscopically observed (at  $T = 11^\circ\text{C}$ ) within a thermostated perfusion chamber (Fig. 1B; Paul et al. 1997). To monitor gill movements and heartbeats continuously, we restricted the movements of the larvae by the use of nylon gauze and rubber stripes. The beating pattern of the abdominal tracheal gills proved to be unchanged in the chamber. The chamber's inlet was connected via Tygon tubings to two thermostated glass vessels filled with either normoxic or anoxic (normocapnic) media. The latter was continuously prepared by the use of a gas-mixing pump (Wösthoff, Bochum). Two computer-controlled peristaltic pumps (MCP-CA4 ISM 726, Ismatec, Glattbrugg), which were installed near the inlet, were used to specifically modulate the flow rates of normoxic and anoxic media. This resulted in a constant ( $10 \text{ mL min}^{-1}$ ) perfusion of the chamber with water of specific oxygen content (see Freitag et al. 1998). The medium  $\text{Po}_2$  was checked with an oxygen electrode (TriOximatic 300 plus flow-through device D201 and Oxi 3000 monitor; WTW, Weilheim) installed behind the outlet of the chamber.

Before an experiment was started, the animals were acclimated in the chamber for 30 min. All experimental animals survived the 1.5 h of measurement without any obvious damage. Gill movements and heartbeats were recorded by video microscopy, and the periods of rhythmic events or arrests were continuously noted while the monitor was observed. On-line digital image processing (cf. Colmorgen and Paul 1995; Paul et al. 1997) was used to evaluate the periods of rhythmic gill

movements or heartbeats: periodic brightness changes in selected areas ( $4 \text{ pixels} \times 4 \text{ pixels}$ ) of the digitized images (sampling rate: 25 Hz) caused by tracheal gill movements and heart contractions were evaluated via on-line Fourier analyses. To avoid disturbance of the animals, we restricted the microscopic light to near infrared (IR; RG845, Schott, Mainz) with the IR-blocking filter of the camera removed.

Peristaltic hindgut movements occurred in both mayfly larvae as irregular events. The rate of these events was assessed by the analysis of video sequences recorded during the experiments. The time volume of water flow because of these movements was estimated from the hindgut cross-section area at maximum contraction and dilatation.

Two experimental series were done by applying the following experimental protocols: (1) two cycles of a sinusoidal  $\text{Po}_2$  modulation (30-min period,  $\text{Po}_2$  variation between normoxia and anoxia; Fig. 2) and (2) one  $\text{Po}_2$  profile consisting of 2 kPa (5 min), 21 kPa (5 min), 1 kPa (30 min), and 21 kPa (5 min) to study the effects of severe hypoxia.

#### *Measurement of Whole-Body Lactate*

After either normoxia (30 min; control group) or anoxia (30 min; test group) was applied, three incubated animals each were frozen in liquid nitrogen and homogenized (glyzine-hydrazine-buffer plus 0.02% sodium azide, pH 8). The enzymes were deactivated by heat ( $90^\circ\text{C}$ ). After centrifugation (12,800 g, 15 min), L-lactate concentration was enzymatically analyzed according to Engel and Jones (1978). Six animals (three of each group) were separately tested.

#### *Statistics*

Data are given as mean values  $\pm$  SD, with  $N$  indicating the number of animals, unless stated otherwise. Statistical differences were checked by using an unpaired two-tailed  $t$ -test ( $P < 0.05$ ). The correlation between  $\dot{M}\text{O}_2$  and  $\text{Po}_2$  in *E. sylvicola* was assessed by means of linear regression analysis. Dependencies of  $\dot{M}\text{O}_2$  on temperature (in *E. torrentis*) were tested by a two-factorial analysis of variance (ANOVA;  $P < 0.05$ ). Statements on the significance of differences in the text met the statistical criteria mentioned earlier.

To determine a mean HR or GBR at different  $\text{Po}_2$  despite the occurrence of irregular events, the following procedure was applied (according to Bortz et al. 1990). Within a  $\text{Po}_2$  class width of 1 kPa and within a rate class width of  $10 \text{ min}^{-1}$  (HR) or  $40 \text{ min}^{-1}$  (GBR), the number of events was counted by using all data of each species. After multiplying each of these numbers with the class-specific rate, the maximum product within a  $\text{Po}_2$  class was assumed to represent the mean HR or GBR at a given  $\text{Po}_2$ . Heart arrests or absence of gill beats were considered separately and excluded from distribution statistics. Because the products within one  $\text{Po}_2$  class turned out to be not regularly

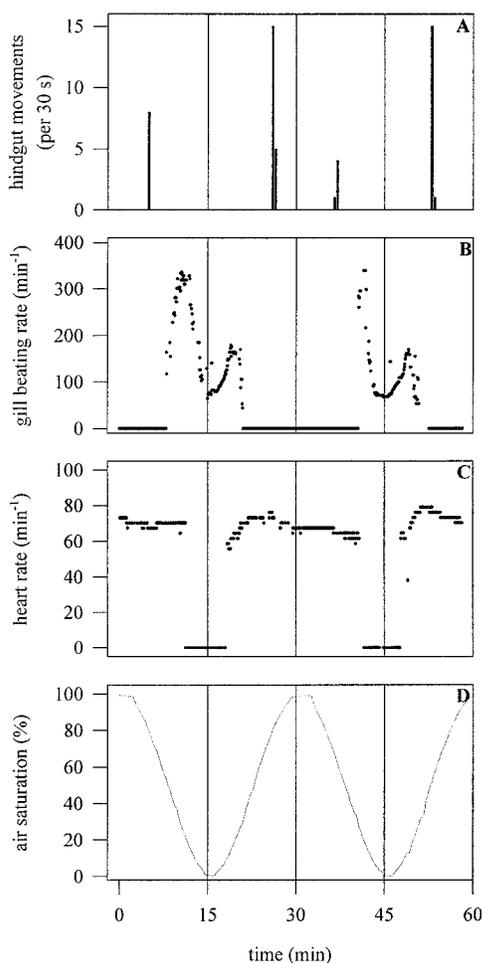


Figure 2. Example of peristaltic hindgut movements (events per 30 s; A), gill beating rate ( $\text{min}^{-1}$ ; B), and heart rate ( $\text{min}^{-1}$ ; C) of *Ecdyonurus torrentis* during sinusoidal  $\text{Po}_2$  modulations (% air saturation; D) versus time (min).

distributed, the variation range was used as a statistical measure of dispersion.

## Results

### Oxygen Consumption Rate

Specific oxygen consumption rates ( $\dot{M}\text{O}_2$ ) were determined during normoxia in the nonventilating *Epeorus sylvicola* and in the ventilating *Ecdyonurus torrentis* at running-water conditions and at two different temperatures (Table 1). Because the mayfly larvae were collected at different points in time, body masses of individuals of *E. sylvicola*, investigated at 11° and 15°C, were different.  $\dot{M}\text{O}_2$  was 224% (11°C) or 144% (15°C) higher in *E. sylvicola* than in *E. torrentis* (Table 1; Fig. 3).

At declining  $\text{Po}_2$ , the  $\dot{M}\text{O}_2$  of both species changed differently.

The oxygen consumption rate declined linearly with decreasing  $\text{Po}_2$  in *E. sylvicola* ("oxyconformer"; Fig. 3A). Significant differences in  $\dot{M}\text{O}_2$  were not detected over the whole range of  $\text{Po}_2$  values at both temperatures. *Ecdyonurus torrentis* proved to be an oxyregulator at 15°C and maintained an almost constant  $\dot{M}\text{O}_2$  down to a critical  $\text{Po}_2$  ( $P_C$ ) of 5.6 kPa (Fig. 3B). The oxygen consumption at 11°C was lower (66% at normoxia) and differed significantly from that at 15°C below 12.3 kPa: oxyregulation was much less pronounced ( $P_C \approx 4.5$  kPa).

### Gill Movements

Because of its rigid gill plates, *E. sylvicola* is not able to generate any ventilatory flow. In *E. torrentis*, the moveable gills responded to variations in ambient  $\text{Po}_2$  (sinusoidal  $\text{Po}_2$  modulations between normoxia and anoxia; 30-min periods) by changing ventilation (Fig. 4). No ventilatory activity was observed at normoxia. When the  $\text{Po}_2$  fell below 16 kPa, the first individual started to ventilate (Fig. 4A, upper panel). More and more of them started gill movements until each individual ventilated at 6 kPa. However, the GBR of already-ventilating individuals reached maximum values (up to  $300 \text{ min}^{-1}$ ) below 14 kPa (Fig. 4A, lower panel). Below 5 kPa, the rate of gill movements started to decline, although all individuals ventilated continuously down to very low  $\text{Po}_2$  values. During the subsequent return to higher  $\text{Po}_2$  values (Fig. 4B), ventilation proved to be weaker than during the previous phase of decreasing  $\text{Po}_2$ . At 4–5 kPa, the GBR reached a maximum of not more than  $130 \text{ min}^{-1}$  on average (Fig. 4B, lower panel). When the  $\text{Po}_2$  was further increased, the GBR gradually became slower, and the gills of more and more individuals stopped beating completely (Fig. 4B, upper panel). Above 11 kPa, ventilatory activity had stopped in all individuals of *E. torrentis*.

Further experiments (Fig. 5) tested whether *E. torrentis* was able to sustain ventilation during 30 min of severe hypoxia (1 kPa) and not only during short-term expositions to hypoxia (one sinusoidal  $\text{Po}_2$  cycle in 30 min; Fig. 2). All individuals showed a reduced but more or less constant GBR of  $100 \text{ min}^{-1}$  under this condition.

### Heartbeats

The cardiac responses to various ambient oxygen partial pressures were studied: HR was found to be nearly constant over a wide range of hypoxic  $\text{Po}_2$  values in both species (Fig. 6, lower panels), but in *E. sylvicola*, it was significantly higher than in *E. torrentis* (80 vs.  $60 \text{ min}^{-1}$ ). Below 1.5 (in *E. sylvicola*) or 2.5 kPa (in *E. torrentis*), HRs decreased, reaching zero values between 0.7 kPa and 1.5 kPa. However, below 7 kPa (*E. sylvicola*) or 10 kPa (*E. torrentis*), single individuals already started to arrest (Fig. 6, upper panels). At a specific  $\text{Po}_2$ , the heartbeat stopped rather instantly in individual mayflies (see Fig. 2C). When  $\text{Po}_2$  steps (and not  $\text{Po}_2$  modulations) were applied, the

Table 1: Specific oxygen consumption rate at normoxia

T (°C)	N	$\dot{M}O_2 \pm SD$ ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ )	Mean body mass $\pm$ SD (mg)	Time of measurement
<i>Epeorus sylvicola</i> :				
11 .....	5	13.0 <sup>a</sup> $\pm$ 2.1	13.7 <sup>b</sup> $\pm$ 2.3	12/97
15 .....	6	12.7 $\pm$ 4.3	25.8 <sup>b</sup> $\pm$ 2.3 $\pm$ 9.7	1/98
<i>Ecdyonurus torrentis</i> :				
11 .....	8	5.8 <sup>a</sup> $\pm$ 2.1 $\pm$ 3.8	17.6 $\pm$ 12.26	11–12/97, 1/98
15 .....	6	8.8 $\pm$ 3.9	27.5 $\pm$ 13.3	1/98

<sup>a</sup> Significant difference between oxygen consumption rates.

<sup>b</sup> Significant difference between body masses.

$P_{O_2}$  below which bradycardia in *E. sylvicola* occurred seemed to be a little higher (Fig. 6A, open circles). Cardiac responses were, in contrast to ventilatory responses (Fig. 4, *E. torrentis*), independent of the direction of the  $P_{O_2}$  modulation (between normoxia and anoxia or vice versa).

In addition, we looked for heartbeats during a longer period of severe hypoxia (1 kPa; 30 min) and for survival of this condition. After  $P_{O_2}$  was switched from normoxia to severe hypoxia, all individuals of both species showed, after a short transitional period (3–5 min), heart arrest, which was interrupted only by a few spontaneous heartbeats during the whole hypoxic period (Fig. 5). When  $P_{O_2}$  returned to normoxia again, HR slowly increased, reaching more or less the prehypoxic (normoxic) level.

#### Hindgut Movements

Both mayfly larvae showed peristaltic movements of the hindgut (Fig. 2) that caused water to flow between the ambient medium and the gut lumen. However, the peristaltic contraction rate (showing varying values between 70  $\text{h}^{-1}$  and 160  $\text{h}^{-1}$ ) and the ambient  $P_{O_2}$  (0–21 kPa) did not correlate (data not shown). In addition, the volume of hindgut contractions ( $\sim 0.0063 \mu\text{L}$ ) did not change during  $P_{O_2}$  variations.

#### Anaerobiosis

After 30 min of anoxia, L-lactate was detected neither in the tissues of *E. sylvicola* nor in those of *E. torrentis*.

#### Discussion

In single mayfly larvae (*Epeorus sylvicola*, *Ecdyonurus torrentis*), oxygen consumption rates and, simultaneously, the occurrence and rate of gill movements and heartbeats were measured to assess specific adaptations to their microhabitats. In *E. sylvicola*,  $\dot{M}O_2$  was higher than in *E. torrentis* (Table 1; Fig. 3). A higher  $\dot{M}O_2$  in lotic than in lenitic species was also reported previously (Fox and Simmonds 1932; Fox et al. 1934, 1936a, 1936b). In contrast to *E. torrentis*, the  $\dot{M}O_2$  of *E. sylvicola* was independent

of temperature. Computations with allometric equations (Schmidt-Nielsen 1984) exclude that differences in mass-specific oxygen consumption because of differences in body mass (Table 1) have caused this result. Constancy of metabolic rate during varying temperature was also reported for some invertebrates of intertidal zones, which was discussed as a result of biochemical adaptations of metabolic enzymes (Hochachka and Somero 1980; Vetter and Buchholz 1997). The range of oxygen consumption rates in *E. torrentis* and *E. sylvicola* during normoxia (between 5.8 and 13  $\mu\text{mol g}^{-1} \text{h}^{-1}$ , 11° and 15°C) is similar to results in other mayfly larvae and aquatic arthropods:  $\sim 12.5$ , 13.4, and 15.7  $\mu\text{mol g}^{-1} \text{h}^{-1}$  (at 8°C) in the mayflies *Drunella aculea*, *Ecdyonurus dracon*, and *Siphonurus lacustris* (Golubkov and Tiunova 1989);  $\sim 5.6$  and 11.2  $\mu\text{mol g}^{-1} \text{h}^{-1}$  (at 6°C) in the Trichoptera *Stenopsyche marmorata* and the Plecoptera *Skwala pusilla* (Golubkov et al. 1992); and  $\sim 7.8 \mu\text{mol g}^{-1} \text{h}^{-1}$  (at 12°C) in the Amphipode *Gammarus fossarum* (Franke 1977).

Easy access to oxygen in rapidly flowing and well-aerated water makes a permanently high oxygen consumption rate in *E. sylvicola* possible and makes oxyregulatory behavior superfluous (Fig. 3A). In *E. torrentis*, which also inhabits still-water regions, oxyregulation was observed as being particularly pronounced at 15°C (Fig. 3B). Obviously, these larvae are adapted to situations of limited oxygen supply. The  $P_C$  was approximately 5 kPa ( $\approx 1.5 \text{ mL O}_2 \text{ L}^{-1}$ ) at both temperatures. For other mayfly species, which also inhabit lenitic sites of fresh water, the  $P_C$  was either in a similar range (1.5–2  $\text{mL O}_2 \text{ L}^{-1}$ ; *Cloeon dipterum* and *Leptophlebia marginata*; Fox et al. 1936a, 1936b) or higher (3.5–4  $\text{mL O}_2 \text{ L}^{-1}$ ; *E. dracon* and *S. lacustris*; Golubkov and Tiunova 1989).

Oxyregulation in mayfly larvae is brought about by ventilatory convection (Wingfield 1939). In *E. torrentis*, gill movements started below 16 kPa in one individual until all of them ventilated at 6 kPa. When ventilation had begun, the gills beat rapidly at a maximum rate, which appeared to be an all-or-nothing reaction (Fig. 4A). During severe hypoxia (less than  $P_C$ ), the GBR decreased. Obviously, the ventilatory response is controlled by the ambient  $P_{O_2}$ . Similar ventilatory patterns were measured in two burrowing mayfly larvae (*Hexagenia limbata*

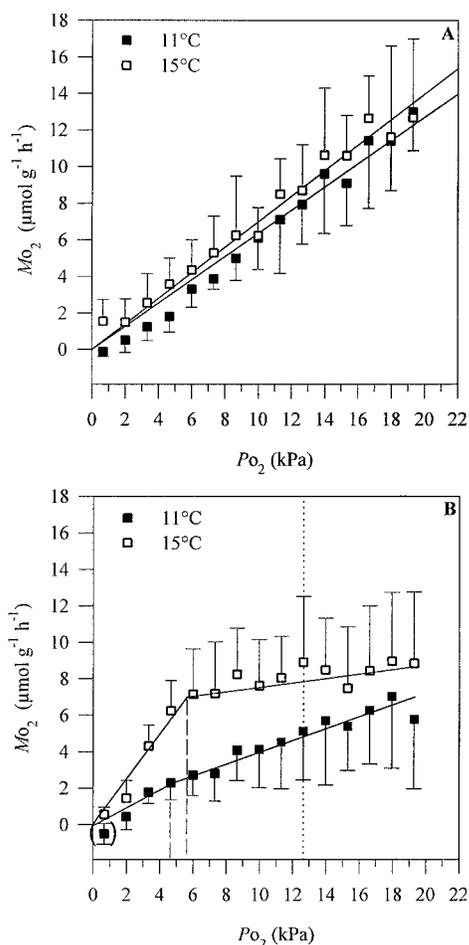


Figure 3. The oxygen consumption rate ( $\mu\text{mol g}^{-1} \text{wwt h}^{-1}$ ; mean values  $\pm$  SD) of *Epeorus sylvicola* (A) and *Ecdyonurus torrentis* (B) at varying ambient  $P_{O_2}$  (kPa) and at two different temperatures (11°C: closed squares; 15°C: open squares). In *E. sylvicola*, linear relationships were found (11°C:  $r^2 = 0.99$ ; 15°C:  $r^2 = 0.98$ ), which were congruent at both temperatures. In *E. torrentis* at 15°C, oxyregulatory behavior down to 5.6 kPa ( $P_C$ ; dashed line) was observed. At 11°C,  $M_{O_2}$  was reduced in this species, and oxyregulation was much less pronounced ( $P_C \approx 4.5$  kPa; dashed line). Below 12.6 kPa (dotted line),  $M_{O_2}$  differed significantly with temperature. The determination of the  $M_{O_2}$  at 0.7 kPa and 11°C (in brackets) was incorrect.

and *Ephemera simulans*; Eriksen 1963). However, their GBR was lower, a fact that could be explained by the enlarged respiratory surfaces of their pinniform tracheal gills compared with the gills of *E. torrentis* (Eastham 1936, 1938). In additional experiments, it was proved that *E. torrentis* larvae could maintain a constant GBR for a longer period at low ambient  $P_{O_2}$  (30 min, 1 kPa; Fig. 5). After the animals had been exposed to a  $P_{O_2}$  near to anoxia during the modulation experiments, GBR was reduced and ventilatory activity stopped at a lower  $P_{O_2}$  during hypoxia (Fig. 4B vs. 4A), which suggests the influ-

ence of additional factors on ventilatory response apart from ambient  $P_{O_2}$ . Another consequence of exposure to severe hypoxia (1 kPa) was the occurrence of gill movements during subsequent normoxia (Fig. 5), which may have to do with processes for repaying an oxygen debt.

A participation of peristaltic hindgut movements in respiratory control can be excluded because rate and volume were

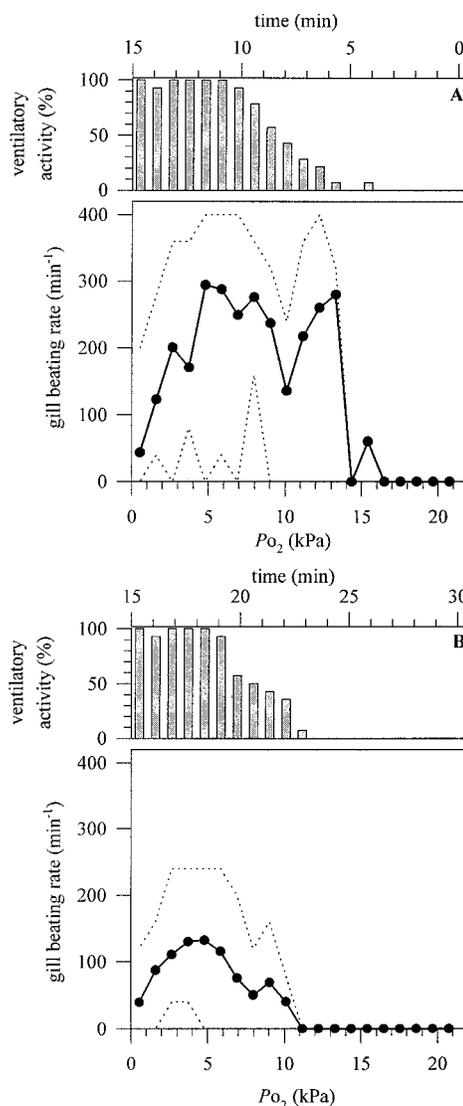


Figure 4. Ventilatory activity (% of ventilating individuals) and gill beating rate (mean values of two periods  $\pm$  variation range;  $\text{min}^{-1}$ ) in *Ecdyonurus torrentis* ( $N = 7$  individuals;  $T = 11^\circ\text{C}$ ) at declining (A) and increasing (B) ambient  $P_{O_2}$  (data from sinusoidal  $P_{O_2}$  modulation experiments: upper abscissa showing time course of experiments). During decreasing ambient  $P_{O_2}$ , a hypoxic ventilatory response (occurrence and rate of ventilatory movements) was measured (A). After exposure to  $P_{O_2}$  values near to anoxia, the ventilatory response was depressed during increasing ambient  $P_{O_2}$  (B).

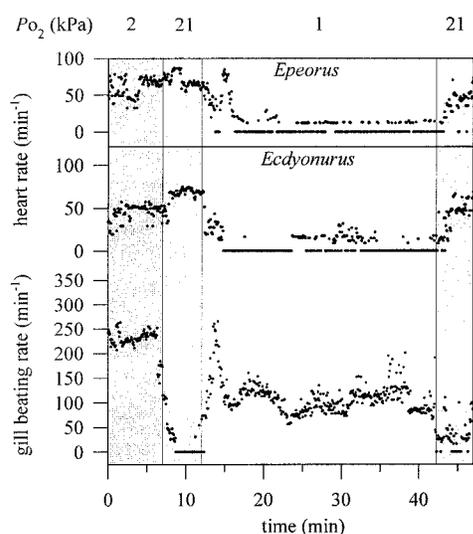


Figure 5. Effects of prolonged severe hypoxia (1 kPa; 30 min) on heart rate and gill beating rate ( $\text{min}^{-1}$ ) in *Epeorus sylvicola* ( $N = 5$  individuals) and in *Ecdyonurus torrentis* ( $N = 5$  individuals;  $T = 11^\circ\text{C}$ ). At the onset of severe hypoxia (1 kPa), the heart of *E. sylvicola* beat faster for a short period before it contracted at irregular intervals. The HR pattern of *E. torrentis* was similar except for the lack of the starting overshoot. After normoxia was applied again, the heart rate slowly increased in both species. A small overshoot of GBR was found in *E. torrentis* during the transition to severe hypoxia, followed by variations around  $100 \text{ min}^{-1}$ . After a more severe hypoxic phase (1 vs. 2 kPa), gill beating was measured even during normoxia (21 kPa).

not influenced by the ambient  $P_{O_2}$ . A simple calculation also shows that they do not play any role in gas exchange as suggested (Dewitz 1890). From rate and volume (*E. sylvicola*:  $160 \text{ h}^{-1} \times 6.3 \text{ nL}$ ; *E. torrentis*:  $70 \text{ h}^{-1} \times 6.3 \text{ nL}$ ), the flow rate computes to  $1 \mu\text{L h}^{-1}$  and  $0.44 \mu\text{L h}^{-1}$ , respectively. This amount of water contains  $\sim 0.4 \text{ nmol}$  and  $0.15 \text{ nmol}$  oxygen ( $T = 11^\circ\text{C}$ ;  $P_{O_2} = 19.3 \text{ kPa}$ ). The calculated amount of oxygen would be a contribution to the normoxic  $\dot{M}O_2$  of only  $1.5 \times 10^{-4}\%$  and  $1.9 \times 10^{-4}\%$ , respectively (Bäumer 1998).

HR in *E. sylvicola* was higher, and the heartbeats of individuals seemed to stop at a  $P_{O_2}$  that was a little lower than that in *E. torrentis* (Fig. 6). However, heart activity was independent of ambient  $P_{O_2}$  over a wide range of hypoxia in both species. During the normoxic-to-hypoxic or the inverse transitions, the heartbeat patterns were similar in both species. The higher HR in *E. sylvicola* corresponds to a higher metabolic rate (Table 1). This seems to be characteristic for lotic mayfly species:  $96 \text{ min}^{-1}$  in the lotic *Baetis rhodani* versus  $33 \text{ min}^{-1}$  in the lenitic *C. dipterum* (Fox and Simmonds 1932). Keeping up HR during hypoxia in an oxyconformer means using a higher percentage of oxygen consumption for heart supply, because an anaerobic energy supply of heart activity can be excluded. In a lotic species like *E. sylvicola*, the maintenance of circulatory convection at

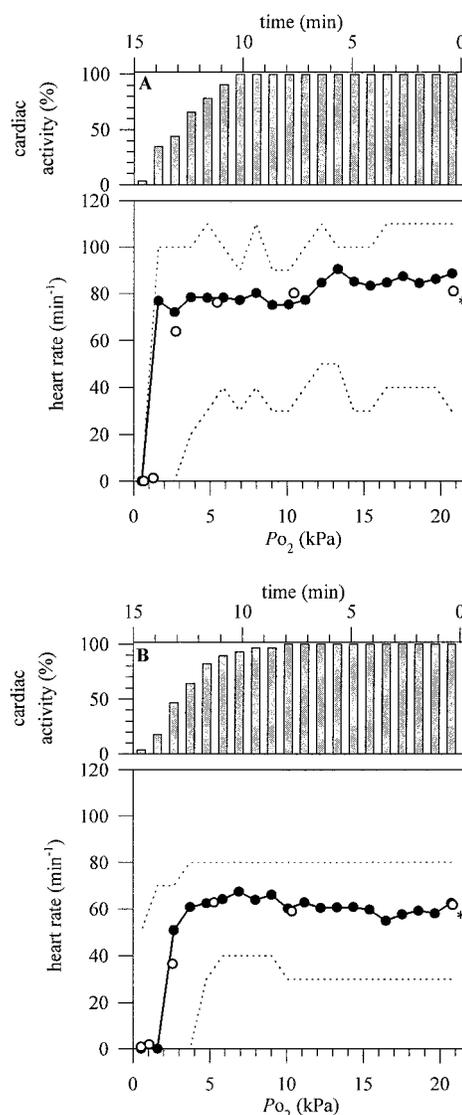


Figure 6. Cardiac activity (% of individuals showing heartbeats) and heart rate (mean values of two periods  $\pm$  variation range;  $\text{min}^{-1}$ ) in *Epeorus sylvicola* (A,  $N = 7$  individuals) and in *Ecdyonurus torrentis* (B,  $N = 7$  individuals;  $T = 11^\circ\text{C}$ ), resulting from sinusoidal  $P_{O_2}$  modulation experiments (closed circles; upper abscissa showing time course of experiments); both cardiac parameters were simultaneously measured with ventilatory parameters in *E. torrentis* (see Fig. 4). HR was significantly higher in *E. sylvicola* than in *E. torrentis*, and it did not change over a wide range of ambient  $P_{O_2}$  in both species. Bradycardia occurred at  $\sim 1.5 \text{ kPa}$  in *E. sylvicola* and at  $\sim 2.5 \text{ kPa}$  in *E. torrentis* independently of ambient  $P_{O_2}$  decreasing or increasing. When a  $P_{O_2}$  profile of 5 min of normoxia followed by 10 min of hypoxia was applied (open circles), bradycardia in *E. sylvicola* started at a  $P_{O_2}$  that was a little higher than that in the modulation experiments.

reduced oxygen supply is surprising. Under natural conditions, however, the internal  $\text{Po}_2$  may not only be influenced by ambient  $\text{Po}_2$  changes but also by higher animal activities, which may explain the capacity to keep up circulation during hypoxia.

Severe hypoxia (30 min, 1 kPa) seems to exclude a sufficient oxygen supply of the heart, which is reflected in heart arrests in both species (Fig. 5). In contrast to other freshwater animals (*Daphnia magna*; Paul et al. 1998), these mayfly larvae are not able to keep up heartbeats by anaerobic energy supply. The capacity for an anaerobic energy supply by lactate fermentation seems to be generally absent in these species because even after 30 min of anoxia lactate was not detected in their tissues. However, prolonged heart arrest is not lethal, because all individuals survived this phase of severe hypoxia.

In *E. torrentis*, the simultaneous consideration of (1) HR and the number of individuals showing cardiac activity (summarized as heart activity) and of (2) GBR and the number of individuals showing ventilatory activity (summarized as gill activity) showed that the heart activity was constant down to 6.7 kPa (Fig. 6B). The gill activity was maximum at 4.7 kPa in the case of normoxic-to-hypoxic transitions and at 3.3–4.7 kPa during hypoxic-to-normoxic transitions (Fig. 4). Thus, hypoxia led first to a reduced heart activity and then a reduced gill activity, which suggests a first priority for ventilation and oxygen supply and a second priority for cardiac activity and transport of metabolites.

Although *E. sylvicola* and *E. torrentis* inhabit the same brook and frequently inhabit similar sites in running water, they show specific physiological adaptations to the physical characteristics of their slightly different microhabitats. The oxyconformer *E. sylvicola* exclusively inhabits lotic sites of running water with a high ambient  $\text{Po}_2$ , conditions that allow the larvae to have a higher metabolic rate. Although marked oxygen deficiencies are unlikely in these zones, *E. sylvicola* is able to survive prolonged severe hypoxia (30 min, 1 kPa). *Ecdyonurus torrentis*, which partly lives in microhabitats with slightly flowing water and reduced oxygen availability, is able to regulate oxygen uptake during hypoxia with the aid of ventilatory gill movements and proved also to be hypoxia tolerant.

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