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Critical oxygen demand in Plecoptera and Ephemeroptera nymphs as determined by two methods

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The positions of the critical points on oxygen consumption/oxygen concentration curves and the oxygen levels for 95% sustained survival were compared for water insect nymphs. The influence on the oxygen level for 95% sustained survival of internal changes close to emergence was also studied. Nymphs of *Diura nanseni, Taeniopteryx nebulosa* (Plecoptera) and *Cloëon dipterum* (Ephemeroptera) were used.

The critical point on the oxygen consumption/oxygen concentration curve and the oxygen level for 95% sustained survival corresponded well. The latter values were at 8.0°C for nymphs one month prior to emergence; *Diura* 3.1 ± 0.4, *Taeniopteryx* 5.1 ± 0.5, and *Cloëon* 2.2 ± 0.2 mg O_2 l^{-1}. Close to emergence the values were higher; *Diura* 5.3 ± 0.4, and *Taeniopteryx* 7.1 ± 0.2 mg O_2 l^{-1}. The main explanations proposed for this were an increase of the internal metabolism that was measured to about 30% and an increased diffusion resistance to oxygen of the nymphal cuticle.

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Положение критических точек на кривых потребления кислорода/концентрации кислорода и уровня содержания кислорода при выживаемости 95% сравнивали у водных личинок насекомых. Исследовали также связи внутренних изменений перед окукливанием с содержанием кислорода при 95% выживаемости. Использованы нимфы *Diura nanseni, Taeniopteryx nebulosa* (Plecoptera) и *Cloëon dipterum* (Ephemeroptera).

Критическая точка кривой потребления кислорода/концентрации кислерода и содержание кислорода при 95% выживаемости хорошо коррелирует. Содержание кислорода при 8°C у нимф за месяц до имагинальной линьки составляют: у *Diura* 3,1±0,4, *Taeniopteryx* – 5,1± 0,5, *Cloëon* –2,2±0,2мгO_2/1л. Перед имагиальной линькой содержание кислорода выше: у *Diura* 5,3±0,4 и *Taeniopteryx* – 7,1±0,2 мг O_2/1л.

Предложено объяснение этого явления: повышение уровня обмена, которое составляет около 30% и повышение диффузионной резистентности в кутикуле нимф к кислороду.

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1. Introduction

Low oxygen concentration in the water is a limiting factor in the distribution of many species. Several authors have tried to elucidate the ecological oxygen demand by respiratory curves, i.e., by measuring the oxygen consumption in relation to the oxygen concentration. Of special interest is the critical point of these curves, i.e. when the oxygen consumption is markedly reduced by the availability of oxygen (also called the incipient limiting level). This or closely related approaches have been used for invertebrates by among others Fox et al. (1937), Walshe (1948), Berg (1952), Mann (1956), Berg and Ockelmann (1959), Ambühl (1959), Berg et al. (1962), Knight and Gaufin (1963), Nagell (1973) and Van Howen (1975). Theoretical aspects of these curves have been treated by Fry (1947), Hughes (1964), Magnum and Van Winkle (1973) and Nagell (1973, 1974). From an ecological wiewpoint the use of the critical point includes the assumption that the oxygen concentration at this point is critical for the survival of the animal in the natural environment. In many cases this is probably so, although not always. For example anaerobic metabolism may be involved which means that the oxygen level for 95% sustained survival is lower than the critical point. Many species are facultative anaerobes and may switch to anaerobic metabolism at low oxygen levels (Magnum and Van Winkle 1973). Furthermore it is important that experimental conditions are comparable and not too unnatural thereby creating an abnormally high motor activity (Wautier and Pattée 1955, Eriksen 1963). Finally some respiratory curves are of the conformer type (Prosser and Brown 1961) with a poorly defined critical point.

No comparison of the critical point and actual survival which would elucidate the relevance of the respiratory curves obtained has been made. This is probably due to the technical difficulty of carrying out both types of measurements properly.

The ecological oxygen demand is thought to increase prior to emergence (Pleskot 1953). This hypothesis has never been experimentally tested. For certain species the increase might be of crucial importance for their distribution.

The aim of the present investigation was to carry out a comparison between critical points on the respiratory curves published by Nagell (1973) and oxygen levels for 95% sustained survival. Furthermore the aim was to experimentally and quantitatively test the hypothesis put forward by Pleskot. Plecopteran and ephemeropteran nymphs were used in the experiments.

2. Material and methods

2.1. Nymphs

Nymphs of *Diura nanseni* (Kempny), *Taeniopteryx nebulosa* (L.) (Plecoptera) and *Cloëon dipterum* (L.) (Ephemeroptera) were used. The collection sites were for *Diura* in River Tandån, Sälen in western Sweden, latitude 61° and for nymphs of the respiratory curves at Abisko, latitude 69.5°. *Taeniopteryx* was collected at two localities within ca. 30 km from that used for the collection of nymphs for the respiratory curves, 70 km N Uppsala, latitude 60.5°. Collection of *Cloëon* took place in the same pond outside Uppsala as used for collection of nymphs for the respiratory curves. After collection the nymphs were kept at 0.5°C and prior to experiments they were acclimated to 8.0°C for ca. one week. The developmental stages were the same as those used in the respiratory curves i.e. most *Cloëon* nymphs were in L VI (Cianciara 1979). *Diura* and *Taeniopteryx* were in the last instar. Two stages of last instar nymphs were tested in the two plecopterans; one about one month prior to emergence with pale wing-pads and one close to emergence with dark wing-pads. The darkening of the wing-pads was thus used as a measure of the internal changes during the month prior to emergence. The darkening was followed in 10 *Taeniopteryx* nymphs at 0.1°C during 4 wk up to emergence.

In order to study the length of the diffusion path through the body cuticle of *Taeniopteryx* one month prior to emergence and close to emergence microscopical transverse sections were made. The nymphs were preserved in Duboscq-Brasil, embedded in hard paraffin with some celloidin and the sections coloured by haematoxylin.

2.2. Methods of measurement

The respiratory curves used for comparison were those published by Nagell (1973). In order to determine the oxygen level for 95% sustained survival a new apparatus was developed (Fig. 1). It consists of two containers (A) with 80 l water in each. The oxygen concentration in the water was kept constant at two different optional levels by slowly bubbling through a mixture of air and nitrogen. Mixing was carried out in a manometric device connected to each container. Without bubbling the oxygen level of the water decreased due to bacterial respiration. From the containers the water was forced, by pressure created in the water-filled tube B, into a capillary system C. This system mixed the water into five different oxygen concentrations, those of the containers and in between mixtures 1:3, 1:1 and 3:1. A test chamber (D) with the larvae was connected to each outlet.

The two containers (A) (38 × 38 × 60 cm) were made of 1 cm thick toughened glass glued by silicon rubber. On the top there was a plexiglass lid. To counteract bending of the glass and the risk of breakage due to the pressure, each container was fixed in a metal frame. In each container a thermostat kept the water at 28.0°C. It was not practical to submerge these containers in water. Instead they were kept a few degrees above

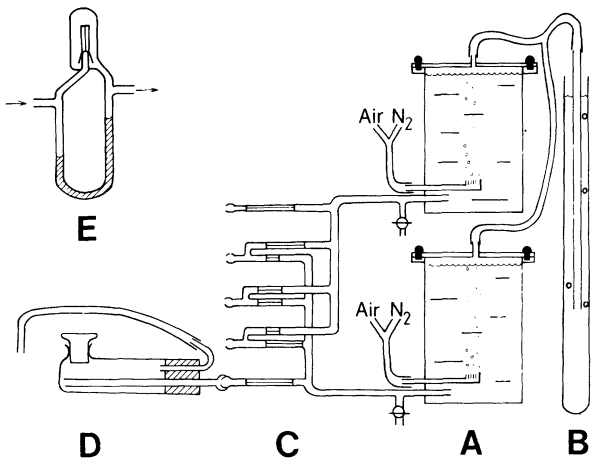

Fig. 1. Apparatus for determining the oxygen level for 95% sustained survival. A, water containers. B, pressure tube. C, capillary system. D, test chamber. E, gas manometer. Two manometers, one for air and one for nitrogen, are connected to each container. Note that the figure is not in the same scale throughout.

the maximum room temperature by an immersion heater. The capillary system was made of precision capillaries and was together with the test chambers submerged in water kept at 8.0°C.

Constant pressure in the containers was created by increasing the depth of the orifice of the tube submerged in tube B at the same rate as the water level in the containers decreased. An automatic device was also used in which water was added by a fine capillary to B so the water level there increased at approximately the same rate as it decreased in the containers. It was then necessary to adjust the pressure manually only every third day.

Air and nitrogen were obtained from cylinders and regulated by manometers and precision needle valves. The principle of the manometers is shown in E. (Fig. 1). At the top of the inlet part there is a capillary with known characteristics. With two such manometers connected to each container, optional and constant gas mixtures were obtained. However, when water is bubbled in this manner carbon dioxide is washed out and there is a pronounced increase in pH. This was counteracted by blowing carbon dioxide through in a 10 s pulse each 8 h. In that way pH was kept within the range 7.10–7.25. By changing the pulse frequency the pH level can easily be changed.

At the start of an experiment the containers were filled with dechlorinated tap water (main composition in mg l^{-1}: $Cl^- = 12$, $Ca^{2+} = 31$, $HCO_3^- = 58$ and conductivity 240 μS cm^{-1}). The air below the lid was replaced by nitrogen and the water bubbled for 24 h (10 l h^{-1}) with gas mixtures to obtain the required oxygen concentrations. The flow was then let through the capillary system and the test chambers were connected. The flow rate in the chambers was ca. 0.15 mm s^{-1} corresponding to 100 ml h^{-1}. The outlets were identical and placed at the same level. The flow was checked by measuring the time of 10 drops leaving the outlet of each chamber. This check is important as it reveals disturbances in the mixing function of the capillary system. It is important to clean the capillary system with alcohol between each experiment and at least once a month with concentrated sulphuric acid.

When the apparatus was properly managed with all pressure corrections made including those for variations in atmospheric pressure, the oxygen concentrations were kept within ± 0.1 mg l^{-1}.

Ten nymphs of *Diura* and *Taeniopteryx* and 20 of the smaller *Cloëon* were used in each test chamber. *Diura* was provided with terylene netting as substrate and fed once a day with tubificids.The other species were given pieces of partly decomposed apple leaves.

The results were evaluated as follows. Survival in each chamber was determined daily, converted to percent and then to probits, while the corresponding oxygen concentration was converted to logarithms. Using linear regression of these values a logarithm-probit curve for each exposure time (reading) was calculated. From this curve the oxygen concentration for 95% survival was calculated. The procedure is used frequently in toxicology when evaluating dosage-mortality curves. The present case however concerns survival instead of mortality and a substance increasing survival instead of increasing mortality. The principles of evaluating dosage-mortality curves have been given elsewhere (cf. Bliss 1935). The oxygen concentrations for 95% survival obtained were then plotted against the corresponding exposure times (see Figs 2–4). The level for 95% sustained survival is attained when the curve reaches a plateau, i.e., when survival is independent of oxygen concentration.

The respiratory curves used for comparison were obtained in an open flowing-water respirometer at 8.0°C (Nagell 1973, 1975). Water with known oxygen concentration was forced by pressure from a container and through a chamber containing the nymphs. The nymphal respiration was calculated from the difference between oxygen concentrations at the inlet and at the outlet, as measured by polarographic electrodes. The conditions in the chambers were similar to those in the survival test apparatus described above.

Oxygen consumption measurements were also carried out on nymphs in a similar stage of development to those used in the survival experiments. This was done using a closed bottle technique at 8.0°C in 130 ml flasks (cf. Tab. 2) The oxygen concentration was allowed to decrease only from ca. 11 to 8 mg l^{-1} to keep the oxygen consumption independent of oxygen concentration. The nymphs clung to terylene netting. Two small glass spheres were introduced and the bottles were gently shaken (40 strokes min^{-1}). Ten nymphs were used in each bottle. In some experiments the nymphs were anaesthethized in 0.06 M ethyl urethane and tied to a

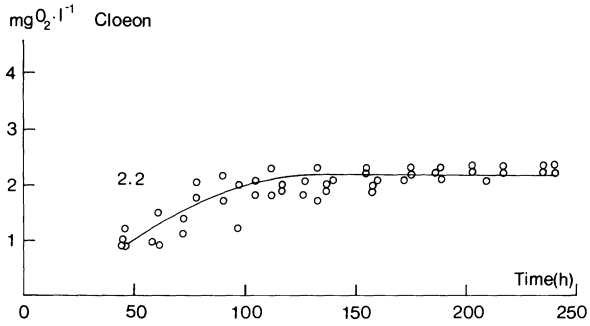

Fig. 2. Oxygen level for 95% survival of *Cloëon dipterum* nymphs (stages LV and LVI at 8.0°C). Six experiments with 100 nymphs each. Mean ±SD of the horizontal part i.e. level for 95% sustained survival is 2.2±0.2 mg O_2 l^{-1} (n = 20, beyond 150 h).

stiff nylon netting fixed diagonally in the flask in order to prevent them from accumulating on the bottom.

3. Results

The results of the survival experiments are given in Figs 2–4. Oxygen levels for 95% sustained survival were for *Cloëon* 2.2 ± 0.2 mg l^{-1}, for *Diura* one month prior to emergence 3.1 ± 0.4 and close to emergence 5.3 ± 0.4, and for *Taeniopteryx* 5.1 ± 0.5 and 7.1 ± 0.2 mg l^{-1}, respectively. Thus there is a pronounced increase in the critical oxygen demand close to emergence. The curves demonstrate that a rather long period is necessary to reach the plateau, usually about 100 h.

The mean respiratory curve of *Diura* is given in Fig. 5 in order to provide an illustratory example. The critical

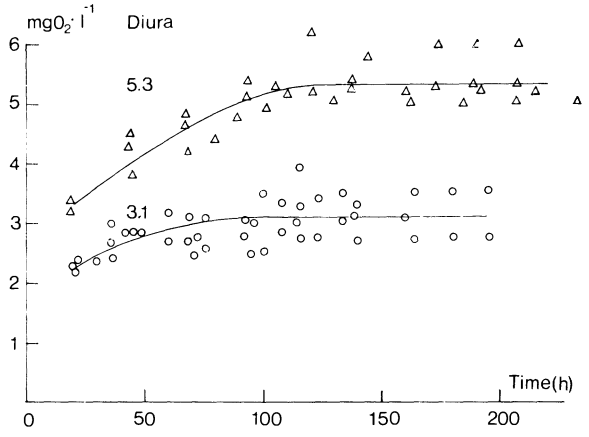

Fig. 3. Oxygen levels for 95% survival of *Diura nanseni* last instar nymphs at 8.0°C. Upper curve, nymphs close to emergence, four experiments with 50 nymphs each. Lower curve, nymphs ca. one month prior to emergence, five experiments with 50 nymphs each. Mean ±SD of horizontal parts 3.1 ± 0.4 (n = 20, beyond 105 h) and 5.3 ± 0.4 mg O_2 l^{-1} (n = 17, beyond 125 h).

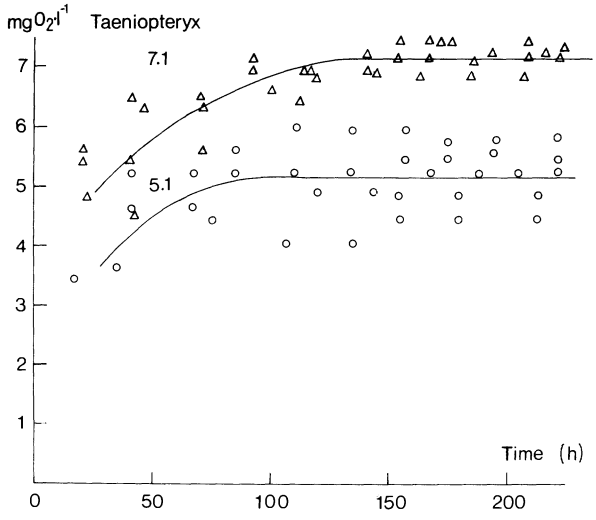

Fig. 4. Oxygen levels for 95% survival of *Taeniopteryx nebulosa* last instar nymphs at 8.0°C. Upper curve, nymphs close to emergence, four experiments with 50 nymphs each. Lower curve, nymphs ca. one month prior to emergence, five experiments with 50 nymphs each. Mean ±SD of horizontal parts 5.1 ± 0.5 (n = 22, beyond 130 h) and 7.1 ± 0.2 mg O_2 l^{-1} (n = 19, beyond 130 h).

point is marked by a circle. In Tab. 1. the oxygen levels for 95% sustained survival are compared with the critical points of the mean respiratory curves determined earlier (Nagell 1973). Where comparison is possible there is good correspondence.

There is a pronounced increase in the oxygen consumption of the nymphs close to emergence (Tab. 2). For unanaesthethized *Diura* of mixed sexes, the in-

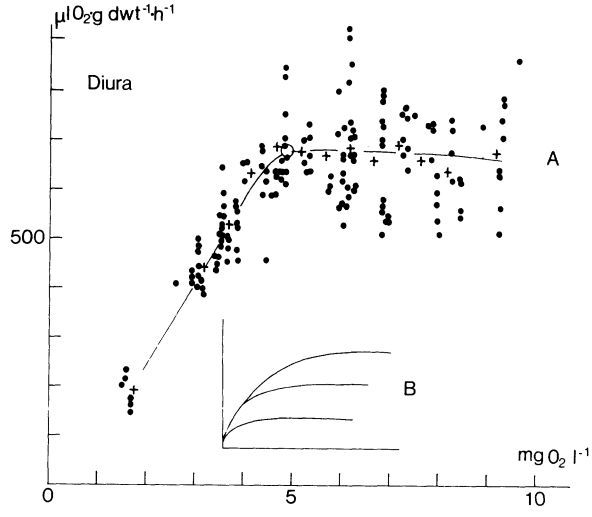

Fig. 5. A: mean respiratory curve at 8.0°C of *Diura nanseni* last instar nymphs close to emergence (Nagell 1973). The critical point is marked by a circle. The crosses are mean values within intervals of 0.5 mg O_2 l^{-1}. B: respiratory curves of baker's yeast at different temperatures (Wintzler 1941).

Tab. 1. Comparison of critical points (mg O_2 l^{-1}) of the mean respiratory curves (R) and the level for 95% sustained survival (S) at 8.0°C.

	Diura		*Taeniopteryx*		*Cloëon*	
	R	S	R	S	R	S
Close to emergence	4.9	5.3±0.4	–	7.1±0.2	–	–
Ca. 1 month prior to emergence	–	3.1±0.4	ca. 5.0	5.1±0.5	2.1	2.2±0.2

Tab. 2. The oxygen consumption (µl O_2 g d wt^{-1} h^{-1} ± SD) of nymphs of respiratory curves (R) and of nymphs of survival experiments (S) at 8.0°C (ana.: anaesthethized). The latter values determined by closed bottle technique.

	Diura		*Taeniopteryx*		
	R	S (n=5)	R	S (n= 3)	S.ana (n = 5)
Close to emergence	670	646±49	–	778±29	445±39 (♀) 542±43 (♂)
Ca. 1 month prior to emergence	–	493±44	620	–	334±31 (♀) 407±41 (♂)

Tab. 3. Distance (diffusion path) in µm between outside of the cuticle and its epithelium of *Taenipoteryx* nymphs. In the abdomen the measurements were made in the ventral proximal part. Each value is the mean of 20 measurements from one individual.

	Abdomen	Coxa
One month prior to emergence	11.2±2.3 8.6±1.5	13.6±1.6 16.0±1.9
Close to emergence	18.3±4.7 23.5±5.6	25.7±3.7 29.2±4.8

crease is 31% and for anaesthethized *Taeniopteryx* it is 33% for both sexes.

The study of the darkening of the wing-pads of *Taeniopteryx* showed that they are distended but pale 3 to 4 wk before emergence. The first signs of darkening were seen ca. 15 d before emergence. The darkening increased during the next few days and was completed ca. 6–8 d before emergence. The course of events were similar in *Diura* nymphs.

The measurements on the microscopical sections of *Taeniopteryx* (Tab. 3) showed that the diffusion path from the cuticular epithelium to the outside of the cuticle increased 1.5 to 2 fold close to emergence.

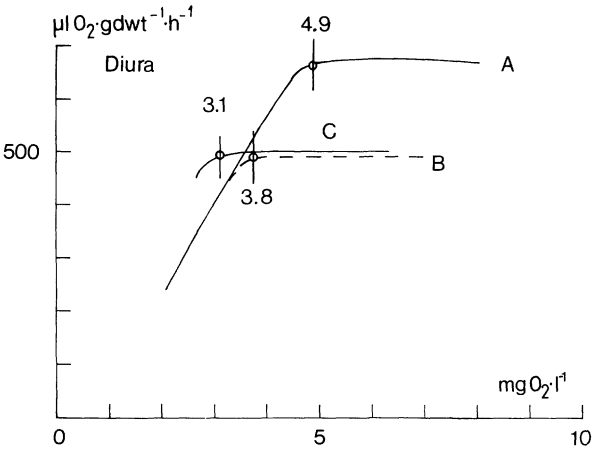

Fig. 6. The shift of mean respiratory curves between two oxygen consumption levels of *Diura*. A: the measured curve of nymphs close to emergence. B: hypothetical curve (broken) at the oxygen consumption level found for nymphs one month prior to emergence assuming the same diffusion resistance to oxygen in the cuticle as in nymphs close to emergence. C: the probable course of the curve of nymphs one month prior to emergence, taken in account the smaller diffusion resistance at this stage.

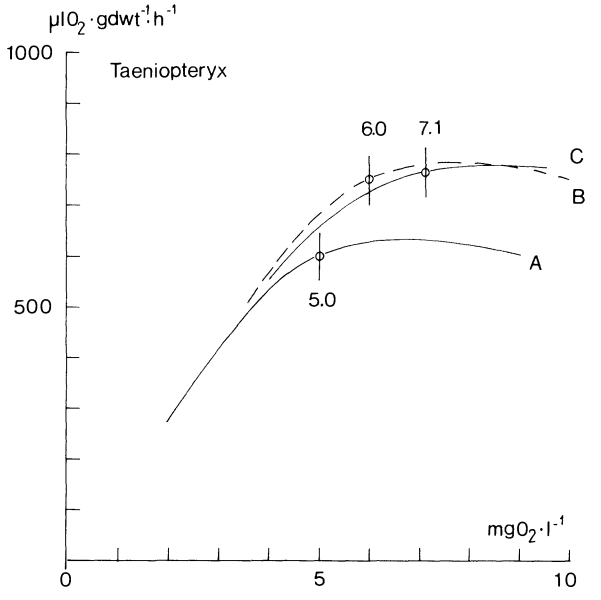

Fig. 7. The shift of mean respiratory curves between two oxygen consumption levels of *Taeniopteryx*. A: measured curve of nymphs one month prior to emergence. B: hypothetical curve of nymphs close to emergence assuming unaltered diffusion resistance. C: probable course of the curve close to emergence.

4. Discussion

In the three cases where comparison was possible (Tab. 1) the oxygen levels for 95% sustained survival fit very well with the critical points of the mean respiratory curves. There is probably a similar correspondence in the missing values for *Taeniopteryx* and *Diura*. This would mean that the critical point on the mean respiratory curve of *Taeniopteryx* close to emergence is ca. 7.1 mg O_2 l^{-1} and of *Diura* one month prior to emergence

ca. 3.1 mg O_2 l^{-1}. Those values are used in the following discussion to estimate the course of the corresponding mean respiratory curves. This is done as shown in Figs 6 and 7 by shifting the intact mean respiratory curves already determined from their oxygen consumption levels to the levels of the survival experiments (cf. Tab. 2). That means for *Diura* from a higher to a lower level and opposite for *Taeniopteryx*. In the beginning of the discussion it is assumed that the diffusion characteristics of the larval cuticle does not change during the month up to emergence. The only change assumed is that of the oxygen consumption level.

The shift is justified as follows. The plateau of mean respiratory curves (cf. Fig. 5) is ultimately caused by the fact that the oxygen need is met. This is reflected at the subcellular level by the oxidized state of most of the enzymes of the respiratory chain within the mitochondria. In this state the relative affinity to oxygen of the respiratory chain is low. The increased availibility of oxygen at increased oxygen concentrations does not therefore increase the oxygen uptake. The mean respiratory curves of the nymphs can be compared to principally similar curves obtained at different oxygen consumption levels of baker's yeast (Wintzler 1941). A set of these curves are given in Fig. 5B. The theoretical background to the shape of the curves is discussed further by Jöbsis (1964), Nagell (1973) and Wilson et al. (1979). Wintzler increased the oxygen consumption level by elevating the temperature. All other conditions remained unaltered and the influence of temperature on the diffusion coefficient is small and therefore neglected. The curves reach plateaux at different levels and their shapes are very similar in the section of the plateau and a part of the bend. There are no reasons for the shape to change considerably if the change in the oxygen consumption level is not very large. Most probably this is also valid for the curves of *Diura* and *Taeniopteryx*.

Diura performs undulation movements which extend the plateau and sharpen the bend in comparison with the curves for yeast. This probably implies that the curve shape changes less in *Diura* than in yeast. Such an effect is demonstrated by a set of respiratory curves on goldfish at different oxygen consumption levels (Fry and Hart 1948), whose shape alters only slightly between adjacent levels.

Under these conditions the oxygen consumption/oxygen concentration curve of *Diura* in Fig. 6 is shifted from position A close to emergence to position B one month prior to emergence. The critical point of curve B is then situated at 3.8 mg O_2 l^{-1}. However, the critical point found experimentally was at 3.1 mg O_2 l^{-1}. The difference is too large to be neglected. There must be an additional factor besides the change in oxygen consumption level influencing the position of the point. A plausible explanation is that the diffusion resistance to oxygen of the cuticle has changed. This resistance is probably smaller one month prior to emergence than close to emergence. This means that nymphs one month prior to emergence extract oxygen more easily than those close to emergence and this also results in the critical point being shifted to the left. The probable course of this curve is drawn at C. It can then be concluded that the change in oxygen consumption level and in diffusion resistance are responsible for a shift of the critical point of 1.1 and 0.7 mg $O_2 \cdot l^{-1}$ respectively.

The same procedure is used in Fig. 6 for *Taeniopteryx*. Here the increase in the oxygen consumption and the change in the diffusion resistance mean a shift to the right of the critical point of 1.0 and 1.1 mg O_2 l^{-1} respectively. *Taeniopteryx* does not perform compensatory undulation movements and therefore the bend of the curve is not as distinct as in *Diura*. This makes the evaluation somewhat uncertain. To fit curve C to the measured values it was necessary to change its form to some degree.

Nymphs of Plecoptera and Ephemeroptera have closed tracheal systems. There are several reasons to suspect an increase in the diffusion resistance of the cuticle of moulting nymphs. Most important for *Diura* with no gills and probably also important for *Taeniopteryx* with only six small filamentous retractile coxal gills is the cuticular oxygen uptake at the body surface. It takes place in numerous tracheoles situated in the epidermal epithelium close to the cuticle. At moulting the epidermal cells separate somewhat from the old body cuticle and from the cuticle lining of the tracheae. The secretion from epidermis of a new cuticle begins. In later stages of moulting the space between the two cuticles is filled by an abundant moulting fluid whose chief function is to digest the inner layers of the old cuticle (Wigglesworth 1954, 1965). Thus during the moulting process there are two cuticles, a new one increasing in thickness and an old one decreasing, with the moulting fluid in between. Thus, during part of the moulting process the diffusion path between the outer surface of the cuticle and the tracheoles of the epidermal epithelium is longer than in a non-moulting nymph. In *Rhodnius* nymphs the distance between the outer body survace and the epithelium increased about two-fold close to moulting (Zwicky and Wigglesworth 1956). In *Taeniopteryx* the increase was of similar size. In accordance with Fick's law of diffusion this will mean a reduction of the flux of oxygen to the epidermal tracheoles. The same concerns the walls of the tracheae. However, a reduction of the flux through the tracheae is probably somewhat less important. The main oxygen uptake is in the tracheoles deeper in the body and they are not shed during moulting.

Although small, the gills of *Taeniopteryx* are not unimportant (Wichard 1974). They were observed to be connected to the body of the imago and not to the nymphal skin except for a very thin outer cuticular layer. Immediately after emergence they were visible on the imago, but retracted into each coxa within some minutes. The transverse sections showed that the

tracheae of the gills are connected to the tracheal system of the imago and, furthermore, the tracheae of the gills do not have double walls. Thus the gills are obviously fully operative during the whole moulting period.

With regard to the other main reason for the shift of the critical point, the change in oxygen consumption, the experiments with anaesthethized *Taeniopteryx* (Tab. 2) were designed to exclude the possibility that the increased metabolism close to emergence was due to increased motor activity. The experiments revealed that the reason was an increase of the cellular metabolism, which in turn probably is related to an intensified protein synthesis. It has long been known that during the development of the insect pupa the oxygen consumption follows a U-shaped curve being high at the outset, then falling and rising again before emergence (Wigglesworth 1957). Zwicky and Wigglesworth (1956) found a similar cycle for *Rhodnius* nymphs and were able to correlate the increase of consumption to increased protein synthesis.

The question arises if the increase of the oxygen demand close to emergence implies a serious disadvantange for *Diura* and *Taeniopteryx* in the natural environment. This is probably not the case, since at emergence the environmental oxygen concentrations are high for both species. This, however, might be a problem for other species emerging in water with poorer oxygen conditions and higher temperatures (Pleskot 1953).

The oxygen consumption values not taken from the respiratory curves were measured by means of the closed bottle technique. This usually gives higher values than the flowing-water system used for the respiratory curves (Kamler 1969), but this was not the case for *Diura* (Tab. 2). During the measurements it was not possible to observe any increased motor activity, probably since the nymphs did not irritate each other. There was plenty of space for them on the substrate. The same probably applied to *Taeniopteryx*. Furthermore the experiments continued for as long as 10 to 12 h which meant that the initial increase in activity due to the introduction into the flasks only slightly influenced the final value.

From an ecological viewpoint, the determination of the oxygen level for 95% sustained survival is generally preferable. Critical points on respiratory curves give a more indirect information with the inherent doubt that the critical point may not be critical for sustained survival. Furthermore, the type of respirometry suitable is more technically demanding than the survival test method (Klekowski and Kamler 1968, Nagell 1975).

There are several areas in which this new survival test method can be useful, such as in studies of oxygen uptake mechanisms (physiological, anatomical and behavioural), survival strategy of facultative anaerobic macroinvertebrates and some other areas in respiratory physiology. It would also be useful for studies on combined effects of low oxygen and environmental stress, such as the influence of toxicants, osmotic stress, temperature etc. This combination is often present in polluted waters.

In the more applied field it can be used to improve the knowledge of the critical oxygen demand of indicator species used for water quality assessment. At the moment practically all the knowledge in this field is based on field experience usually including the unknown influence of several not quantitatively known factors.

However this does not mean that the critical oxygen levels obtained by the present method are exactly the same as in the natural environment. Presumably they are somewhat lower. The influence of other stress factors in nature will tend to increase the critical oxygen level. The obvious value of the method is that it offers relative and comparable oxygen levels for 95% sustained survival obtained under controlled and reproducible conditions.

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