The Oxygen Consumption of Mayfly (Ephemeroptera) and Stonefly (Plecoptera) larvae at Different Oxygen Concentration

by

Bjorn Nagell*

Institute of Zoophysiology, University of Uppsala, Sweden

ABSTRACT

1. The aim of this investigation was to elucidate how four acquatic insect larvae, from different habitats and having different respiratory organs or types of respiratory regulation, react to a lowered oxygen concentration, and how their oxygen consumption is affected. The species investigated were the stoneflies *Taeniopteryx nebulosa*, *Diura nanseni* and *Nemoura cinerea* and the mayfly *Cloëon dipterum*.
2. The measurements were performed in a respiratory apparatus of open, flowing-water type. Its design is shown in Fig. 1.
Water of known oxygen concentration was allowed to flow past the experimental larvae. The oxygen consumption of the larvae was calculated from the lowering of the oxygen concentration which ensued.
3. The water used in the experiments was standardized, so that the electrode had the necessary stability (conductivity 470 micromhos/cm). The calcium ion was excluded in order to prevent the precipitation of $CaCO_3$ in the electrode capillary.
4. A large variation in the values of oxygen consumption was found as seen in Fig. 2—5. The reason for that is a corresponding variation in the motor activity of the experimental animals.
5. The physiological reasons for the general form of the curves A and C in Fig. 2—5 are discussed. The curves A and C represent oxygen consumption of the larvae at different degrees of stimulation, entailing different levels of motor activity. Curve A represents intentionally activated animals, curve C non-activated, motionless animals. The curves A and C are boundary curves corresponding to a sort of scope for activity of the animals. Over this scope area a series of curves of the same form could in principal be construed, representing different degrees of stimulation.
6. Within a certain oxygen concentration interval a motor activation was observed caused by a reduced oxygen concentration. The result of that activation can be seen in Fig. 2—5 as a zone with no or very few oxygen consumption values between curve C and D. The more easily activated the species is, the broader the

zone will be. *Cloëon* has the most narrow zone and was observed to be less activated than the other species.

7. Small larvae of *Cloëon* (2—4 mm and 4—6 mm) and *Nemoura* (2—4 mm) showed clearly a greater ability to take up oxygen at low oxygen concentrations than full-grown larvae (see Fig. 8 and 9).

8. The critical point on the curve representing mean oxygen consumption as a function of oxygen concentration was found to be at 4—5 mg $O_2/1$ for *Taeniopteryx* and *Diura*, at 2.0—2.5 mg $O_2/1$ for *Cloëon*, and at about 5—7 mg $O_2/1$ for *Nemoura*. The values refer to 8°C. *Cloëon* is the species which is exposed to the greatest variations in oxygen concentration in its natural environment.

9. No influence on the oxygen consumption of starvation for 4 to 5 days was found. No difference between the oxygen consumption values obtained in the presence or in the absence of calcium ions could be observed during the experiments (Fig. 10, 11).

10. The basic picture obtained in this investigation is a set of oxygen consumption values scattered between a curve connecting highest values obtained and a curve of the standard metabolism together with a zone in which the larvae are activated by reduced oxygen concentrations. This picture is presumed to be general in aquatic animals with a well developed motor activity.

1. INTRODUCTION

The oxygen concentration is a well-known and important ecological factor in the water biota. Variations in the oxygen concentration will greatly influence the occurrence of animal species.

Water pollution often results in a reduced oxygen content of the water. In surveys of water pollution there is a need of biological indicators. The knowledge of indicators can also, in part, be used for the establishment of water pollution criteria in the aid of protection of aquatic life. It is urgent to improve the knowledge of the environmental needs of such indicators. One of the ways to do this is to study the species oxygen requirements by means of oxygen consumption studies and survival tests. If the species' needs and tolerances are known, the frequency and the distribution of the species in a water course will give information about the oxygen concentrations which have prevailed during the species' life-story. It is this property of indicating past environmental conditions that makes these biological indicators useful as indicators of pollution.

Many species of *Ephemeroptera* and *Plecoptera* form a quantitatively important part of the invertebrate fauna in running waters. Among them there are several species of interest as indicators of the oxygen condition. In many running waters they also play an important role as fish food. Thus their oxygen consumption should also be of interest in production biology, as a tool to evaluate the energy flow through such ecosystems.

In this investigation larvae of three stonefly and one mayfly species were studied. The species represent different biotopes and have diffe-

rent types of respiratory organs. The aim of the investigation was to study how the oxygen consumption and the behaviour of the larvae was influenced by different oxygen concentrations.

In order to carry out investigations of this kind, a respirometer is necessary which is capable of maintaining for a long period constant oxygen concentration, temperature and removal of excretion products. The type of apparatus which best satisfies these requirements is an open, flowing-water respirometer. In combination with a polarographic electrode such an apparatus has been used for example by MANN (1958, 1965), EDWARDS & LEARNER (1960), BIELAWSKI (1959, 1961) BERG & JONASSON (1965), BRAFIELD & CHAPMAN (1967) and KLEKOWSKI & KAMLER (1968). For the persent investigation an apparatus of this type was developed.

2. MATERIAL AND METHODS

2.1 Types of measurements

The larvae used in the experiments were *Diura nanseni* (KEMPNY), *Taeniopteryx nebulosa* (L.), *Nemoura cinerea* (RETZIUS), all stoneflies *(Plecoptera)*, and *Cloëon dipterum* (L.), a mayfly *(Ephemeroptera)*. Only larvae in the same stage of development and in good condition were used. The results presented are from larvae in the last instar (full-grown larvae), unless otherwise stated.

The oxygen consumption of these species was measured at 8.0°C at different oxygen concentrations between 0.5 to 16 mg O_2/l. The procedure of the experiments is described in part 2.4. During the experiments the behaviour of the animals was observed. The oxygen consumption was measured in the following experiments:

1. Standard experiments with all the species.
2. Experiments with intentionally irritated animals, performed with moderate irritation with *Nemoura, Cloëon* and *Taeniopteryx* and with very strong irritation with *Taeniopteryx*.
3. Experiments with anaesthetized animals of all the species.
4. Experiments with *Nemoura* and *Taeniopteryx* in water containing calcium ions.
5. A study of the influence of starvation with *Nemoura and Taeniopteryx*.
6. Experiments with larvae in an early stage of development of *Nemoura* (2—4 mm long) and *Cloëon* (2—4 mm long and 4—6 mm long. Cerci not included).
7. A study of the respiratory movements of *Diura* at different oxygen concentrations.

2.2 Morphological and ecological notes about the species investigated

All the four species have a closed tracheal system. They vary extensively as regards gills and respiratory movements. *Nemoura* and *Taeniopteryx* do not preform respiratory movements; the former has no gills and the latter is provided with six immobile filiform gills. *Diura*, having no gills, performs respiratory movements by moving the body up and down. *Cloëon* has seven pairs of leaf-shaped gills which can be moved with different frequencies thereby regulating the oxygen supply to the animal.

Some information about the environmental needs of these species is given in the literature. *Nemoura cinerea* has a very wide ecological amplitude (BRINCK, 1949). It occurs in the majority of localities in which other *Plecoptera* occur and also in ditches and fairly polluted watercourses, where the oxygen concentration may periodically be low. It is normally not exposed to rapid flows. *Taeniopteryx* occurs in deeper streams and rivers that have, at least partly, soft beds, not in small shallow streams with little or no water in summer (BRINCK, 1949). The supply of oxygen may be estimated as being better than in many of the habitats of *Nemoura*. Diura is typical for northern rivers and is often also found in *jockks* (BRINCK, 1949). It lives under or between stones, where the oxygen supply is good. This species prefers to stay in moderate to rapid rates of flow (ULFSTRAND, 1967). However, the rate of flow in the microhabitat of the species is considerably lower than in the free water. *Cloëon* lives mainly in ponds. The oxygen supply is good in summer but may be low in some ponds during the night. If the pond is covered with ice during winter very low oxygen concentrations or oxygen depletion can occur. (NAGELL, to be published).

2.3 The respirometer

A respirometer of the open flowing-water type with two respiratory chambers was developed (Fig. 1.).

The cylindrical vessel (K) contains the experimental water, the oxygen concentration of which can be adjusted to the desired value with the help of nitrogen, oxygen or air blown through the gas diffuser (F). During the experiments the water is continually forced from the container by means of an over-pressure created with the help of an air pump and the height of the water column in the water filled container (H). The water passes from the container through two capillaries (B), both of which give the same rate of flow into the respiratory chambers (C) containing the experimental animals. Thereafter the water from one of the chambers passes into the electrode chamber (E) where its oxygen concentration is analyzed. The water from the other respiratory chamber is shut off from the electrode chamber with the cock (D₁) and passes along the alternative route L to the outlet. The cock D₁ can be changed to a second position; the
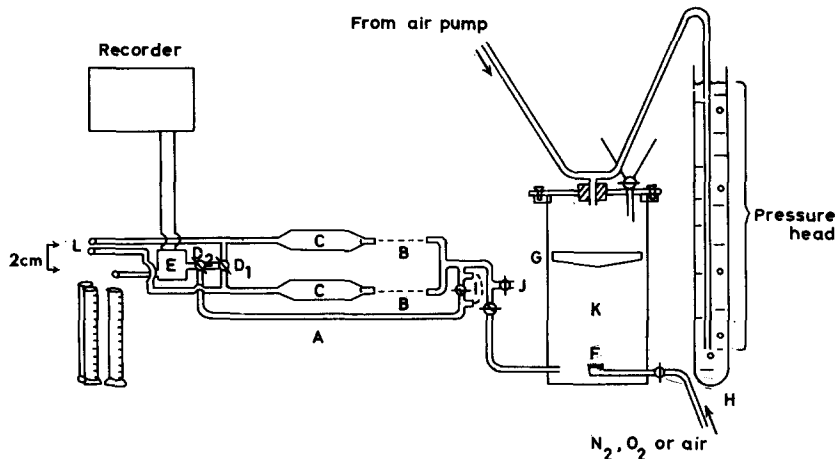
464

Fig. 1. The flowing-water respirometer. All parts of the figure are not on the same schale. A, pipe for transferring water from the container directly to the electrode chamber; B, capillary; C, respiratory chamber; B, capillary; C, respiratory chamber; D, two-way stopcock. For the numbering of the stopcocks, see the text; E, electrode chamber; F, gas diffuser; G, float; H, water-filled container for regulating the pressure over the capillaries B; I, capillary used in calibrating the electrode; J, tap, from which water samples for Winkler analysis can be taken; N, cylindrical water container (volume 10 litres); L, alternative outlet.

routes of the water from the respiratory chambers are then switched so that the water from the shut off respiratory chamber flows through the electrode chamber. During the measurements the position of the cock D_1 is switched regularly by an electric motor. The outlet from the alternative route L is situated 2 cm above the outlet from the electrode chamber. This arrangement has the effect that the water automatically takes the route through the electrode chamber when it is open.

With the help of the cock D_2 water can be admitted directly from the container (K) to the electrode chamber. The oxygen content of the water in the container (K) can thereby be analyzed whenever wished during an experiment.

The electrode used was a dropping mercury electrode of the ordinary vertical type (Radiometer B 400) working at the second plateau with a drop frequency of 15 drops per minute. As a reference electrode a naked Ag/AgCl electrode prepared by the method of Briggs & Knowles (1961) was used.

In the respiratory chamber the oxygen concentration of the water is reduced gradually to a certain level when it passes the animals. The animals at the inflow end thus have access to water with a slightly higher oxygen concentration than those at the outflow end. If this difference is large the oxygen consumption of the animals at the both ends cannot be accepted as referring to approximately the same oxygen concentration. The oxygen consumption/oxygen con-

465

centration curves might then be incorrectly influenced. In order to avoid this it is desirable to keep the difference in oxygen concentration between both ends of the respiratory chamber as low as possible; this difference however, or more precisely the difference in the oxygen concentration of the water flowing into respectively out from the respiratory chamber, is used in calculating the animals' oxygen consumption. Therefore it is not possible to let it become too small. It must be possible to measure the difference with a good precision.

A small difference naturally demands a high precision of the electrode. In order to meet the demands discussed above a difference around 10% was created in the experiments. This was regulated by means of the number of animals placed in the respiratory chamber and an approximate knowledge about the oxygen consumption per individual.

If the precision of the electrode is $\pm$ 2% and the difference between the inflowing respectively outflowing water of the respiratory chamber is 10%, the precision of the difference between the two values will be $\pm$ 27%. The precision of the calculated oxygen consumption will of course be the same, thus relatively high. The need for an electrode with a very high precision is under such circumstances easily understood.

The type of electrode with the best long-term stability and precision is the vertical dropping-mercury electrode. This type was therefore chosen although the membrane covered electrodes of the Clark type are much easier to handle.

The electrode used had a precision better than $\pm$ 1%. Four different electrodes were tested. The results were: Mean value $\pm$ S.D. = 6.29 $\pm$ 0.01 (n = 14); 8.46 $\pm$ 0.04 (n = 12); 11.10 $\pm$ 0.01 (n = 8); 8.12 $\pm$ 0.07 (n = 13). The precision, or the standard deviation expressed as percentages of the corresponding mean value were $\pm$ 0.2%, $\pm$ 0.5%, $\pm$ 0.1% and $\pm$ 0.9% respectively. The deflections of the electrode were measured under constant conditions at 30-min. intervals and are assumed to be independent of each other.

The motor that operates the cock D_1 switches its position every 7.5 minutes. During the last 4 min. of each 7.5-min. period the oxygen concentration is recorded. Thus about 60 oxygen concentrations values, one for each mercury drop from the electrode, are achieved for each 4-min. period. The mean value for the period is then used in the calculations of the oxygen consumption. Each value of the oxygen consumption in the diagrams represents such a 4-min. period. In the diagrams the oxygen concentration for the actual oxygen consumption is taken as the mean of the oxygen concentration of the in-flowing respectively out-flowing water to the

466

respiratory chamber. The measuring processes are regulated with an electric timer and the received values are automatically recorded.

In order to reduce the diffusion of oxygen into the water from the air in the container (K) there is a float made of Plexiglas (G). With the help of this float even a low oxygen concentration of the water in the container could be kept constant for as long a period as 15 hours. The whole apparatus is immersed in a water thermostat.

2.4 The experimental larvae and the experimental procedure

The experimental larvae were collected at the following localities and temperatures: *Nemoura* in a little brook in May, at a water temperature of 6—8°C, *Cloëon* in a small pond in May, at 10—15°C and *Taeniopteryx* in February in a stream at 0°C, all in the vicinity of Uppsala. *Diura* was collected in a rapid torrent in May, at 2°C at Abisko in northern Sweden.

Before use, the larvae acclimated during six days to the experimental temperature in tap-water (for its composition, see HÖGLUND (1961) p. 33) and to exposure to light from 8 a.m. to 8 p.m. However, *Diura nanseni* was acclimated for only one day, owing to the fact that last instar larvae emerged if acclimated to 8°C for so long a period as six days. The last 24 hours before an experiment the larvae were kept in standardized experimental water without food, so that their intestines were emptied. This was done in order to prevent the deposition of feacal pellets in the respiratory chamber, since their putrefaction could add to the oxygen consumption.

Thereafter the larvae were placed in both respiratory chambers in which cotton netting had been evenly spread. The number of animals is given in the figures. After that the animals were allowed to adapt during the night at an oxygen concentration of about 9 mg/l. Next morning the measurements were performed during two hours. Only the values from the second hour were accepted. Then a new oxygen concentration was chosen and measurements performed for two further hours. On the first day, measurements were usually carried out at four different oxygen concentrations. No one of these concentrations was so low that there was a risk of injury to the larvae. After this they spent the night at a concentration of about 9 mg O_2/l. The next day one measurement was performed at a level between the overnight concentration and the lowest concentration of the previous day. Then three measurements were made at levels lower than this. After the experiment the larvae were dried at 110°C to constant weight and weighed.

In some experiments the animals were anaesthetized. The anaesthetic used was ethyl urethane in a concentration of 0.06 molar for *Diura* and *Nemoura*. At this concentration *Cloëon* ceased to

creep around, but to stop the movements of the gills a concentration of 0.14 molar was required. Ethyl urethane interferes with many enzymes in the cell, including dehydrogenases. However, only a relatively high concentration (0.1—0.5 molar) reduces the respiration. (cf. GIESE 1963).

The experimental water was standardized at the following composition (in mg/l): Na^+ 59.5, Cl^- 45.3, Mg^{2+} 21.6, SO_4^{2-} 86.0, K^+ 15.0 and HCO_3^- 103.3. The calcuim ion was excluded because it disturbs the function of the electrode by predipitation of $CaCO_3$ (BRIGGS et al. 1958). However, for short periods of time (10—20 hours) it appeared possible to perform measurements also in the presence of Ca^{2+}. The conductivity of the water was 470 micromhos/cm at 20°C.

This high conductivity is required in order to give the electrode a high degree of stability. The pH of the experimental water was 7.8—8.3.

The flow rate in the respiratory chamber was around 0.1 mm/second, correspondig to a total flow through the chamber of 60 ml/h.

3. RESULTS

3.1 The oxygen consumption/oxygen concentration curves

The results of the measurements of full-grown larvae are presented in the Fig. 2—5, one figure for each species investigated. Each dot in the diagrams represents the mean value of oxygen consumption of the animals in one respiratory chamber during a four minute period at a certain oxygen concentration. The diagrams both include measurements from standard experiments and experiments with especially irritated or anaesthesized animals.

The results from *Taeniopteryx* in Fig. 2 are suitable to take as a starting point for the presentation of the results.

A striking feature is the large variation in the values of oxygen consumption. The diagram shows that the variation is smaller at low and larger at high oxygen concentrations.

In Fig. 2 two groups of dots are encircled. The high values encircled are measured on animals provoked into high activity by vibrations and flashes of light. The highest ones of those values are received during the first one and a half hour of such an irritation. The low values encircled are from animals observed to be very inactive or motionless. The other values marked with dots are from animals not especially irritated.

The values marked with triangles and squares are the results of an experiment in order to investigate the effect of a very intense irrita-
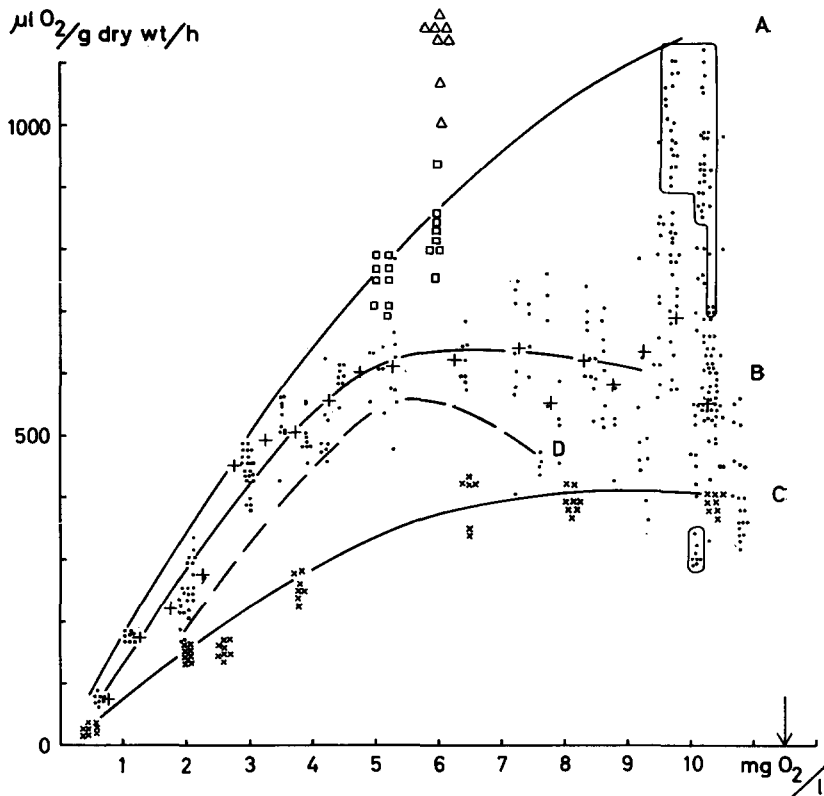
468

Fig. 2. The oxygen consumption of full-grown larvae of *Taeniopteryx nebulosa* at 8.0°C, plotted versus oxygen concentration. The diagram is based on experiments with altogether 170 individual larvae, divided into ten groups of about the same size. For explanation of the triangle and square signs, see the text (p. 470).

The oxygen consumption values from unanaesthetized animals are marked by dots, those from animals anaesthetized by ethyl urethane by crosses (curve C). The plus signs forming the curve B represent the mean values of the oxygen consumption within intervals of 0.5 mg O_2/l along the abscissa. Encircled values of high oxygen consumption are not included in the mean values because they refer to intentionally irritated animals. The low encircled values refer to occasions when the larvae were very inactive or motionless. For explanation of curve A, see the text. The dotted line, curve D, is the approximate upper limit of a zone void of oxygen consumption values. The arrow at the abscissa marks the oxygen concentration in equilibrium with air at 8.0°C.

tion. The animals were placed in the respiratory chamber without access to any cotton netting. In addition they were irritated mechanically by a metal rod, within the chamber, operated by a magnet from the outside. Under such conditions the animals climb on each other and strongly irritate each other.

469

The triangles mark the values from the first 30 minutes of such a treatment. The highest values were received in the beginning of the stimulation. In spite of a continued strong stimulation the values decreased to a lower level after that time. This lower level the animals kept for one more hour. The values from that period are marked with squares.

Four curves A, B, C and D are drawn by hand in the figure. The curve A represents the oxygen consumption at a high but not extremely high motor activity. The curve and its form is further discussed in section 4. The curve B is fitted to the mean values with the values of intentionally activated animals excluded. As seen the curve decreases somewhat above 6—7 mg O_2/l. The curve C represents anaesthetized animals. For reasons discussed later this curve is supposed to be a curve of the standard metabolism.

Between the dotted curve D and the curve C there is a zone with very few or no oxygen consumption values. The curve D is an approximate upper limit of that empty zone. The maximum width of the zone is at about 5 mg O_2/l. At lower and higher concentrations the zone diminishes gradually and tends to disappear. The same types of curves are drawn for the other three species in the Fig. 3, 4 and 5. The results of *Nemoura* and *Taeniopteryx* are in principle relatively alike. The span between the oxygen consumption values is larger for those species than for the other two investigated. This is the case, especially at higher oxygen concentrations.

The form of the curve A is smooth and convex for all species.

The curves B of *Cloëon* and *Diura* have sharper bends than those of the other two. For *Diura* the curve B declines somewhat at higher oxygen concentrations forming a maximum point. The same is the case for *Taeniopteryx*. For *Cloëon* the curve B after the bend seems to rise slightly. At higher oxygen concentrations than 5.5 mg O_2/l the curve of *Nemoura* is drawn dotted due to the large variation in the oxygen consumption values. It ought to be noticed that the principal form of the curve C is smooth and convex and very similar for all species. The empty zone between curve C and D of *Diura* and *Taeniopteryx* has a pronounced top at about 5 mg O_2/l. The zones of *Cloëon* and *Nemoura* have no such tops. The zone of *Cloëon* is much narrower than those of the other species.

3.2 Activity and oxygen consumption

The larvae were very restless immediately after the transfer into the apparatus. It took one to two hours for the animals to calm down In the experiments with calcium ions the measurements started immediately after the transfer. The result was, as seen in Fig. 11, a clearly higher oxygen consumption in the initial stage.
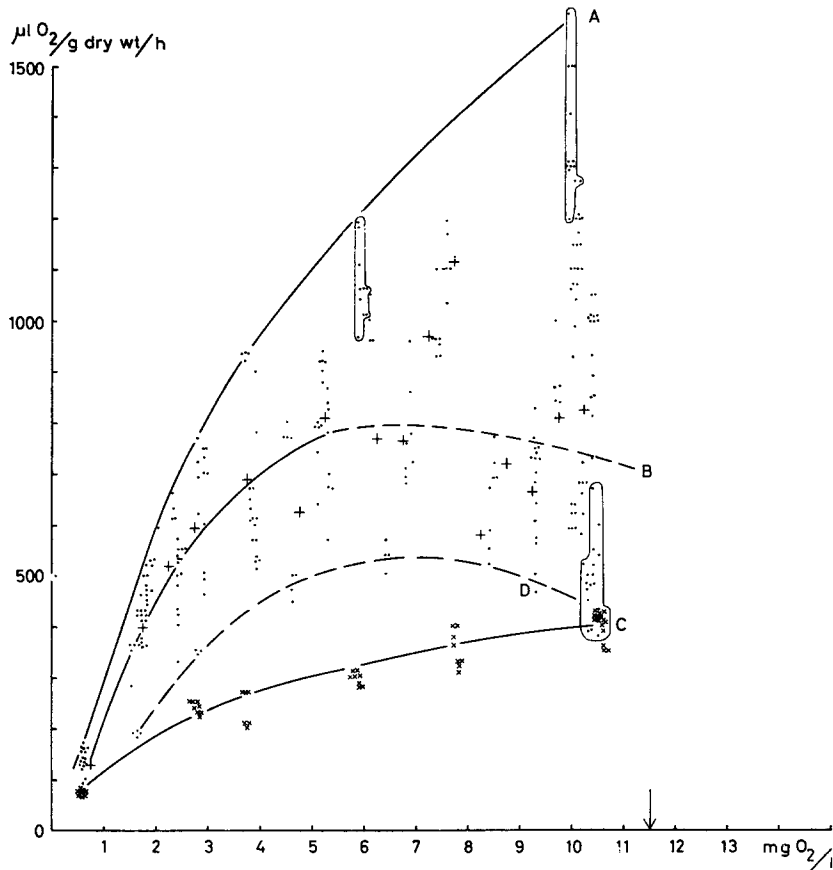
470

Fig. 3. The oxygen consumption of full-grown larvae of *Nemoura cinerea* at 8.0°C, plotted versus oxygen concentration. The diagram is based on experiments with altogether 984 individual larvae, divided into ten groups of about the same size.

Fig. 10 shows a large difference between the upper and lower curves in the left part of the diagram referring to the first two days for *Taeniopteryx*. The curves correspond to the two respiratory chambers examined in the same experiment. In the chamber with the highest oxygen consumption the motor activity was observed to be high. The reason is supposed to be the presence of an unusually restless specimen, raising the activity level of the whole group of larvae by crawling around energetically. After 42 hours this individual calmed down and before the measurements the next day it was dead. At that moment the oxygen consumption sunk to a distinctly lower and more ordinary level.
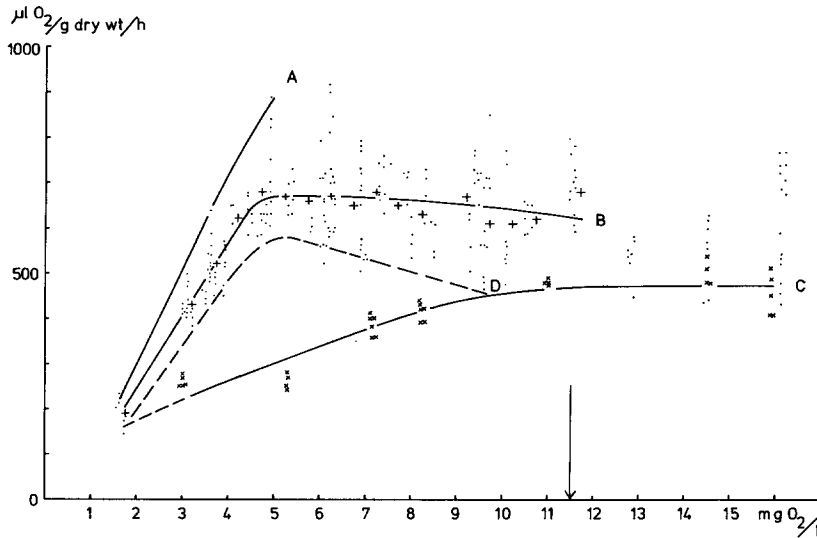
Fig. 4. The oxygen consumption of full-grown larvae of *Diura nanseni* at 8.0°C, plotted versus oxygen concentration. The diagram is based on experiments with altogether 101 individual larvae, divided into nine groups of about the same size.
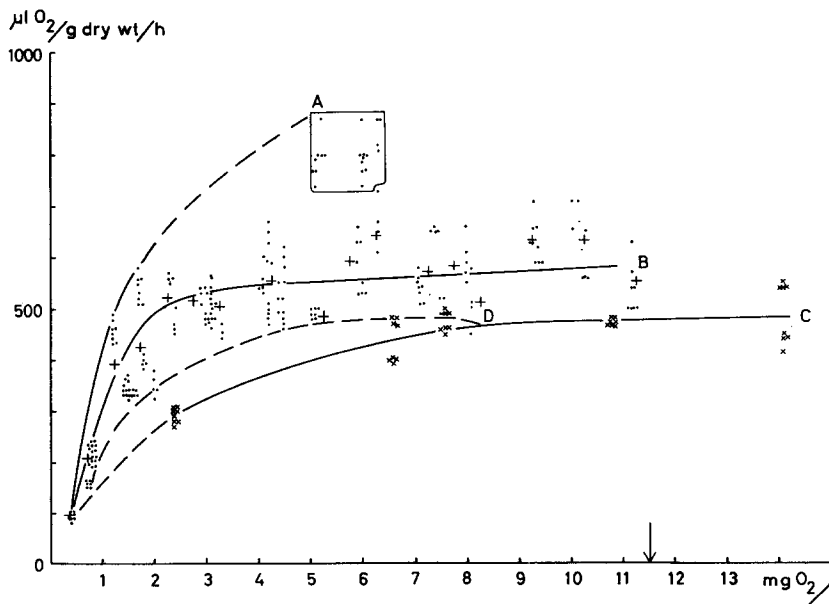

Fig. 5. The oxygen consumption of full-grown larvae of *Cloëon dipterum* at 8,0°C, plotted versus oxygen concentration. The diagram is based on experiments with altogether 418 individual larvae, divided into six groups of about the same size.

472

3.3 Observed activity and the zone without oxygen consumption values between the curve C and D

It was impossible to estimate the motor activity of *Cloëon* and *Nemoura* by direct observation because there were too many individuals in each respiratory chamber. However, in the case of *Taeniopteryx* and *Diura* it was possible to do so due to the much smaller number in each respiratory chamber, about 15 individuals.

At 8—10 mg O_2/l the individuals of *Taeniopteryx* could be quiet for as long periods as 15 to 30 minutes. A lowered oxygen concentration activated the larvae and shortened the quiet periods considerably. The activity of the animals rose to a maximum at about 5 mg O_2/l and declined again at still lower oxygen concentrations. These observa-
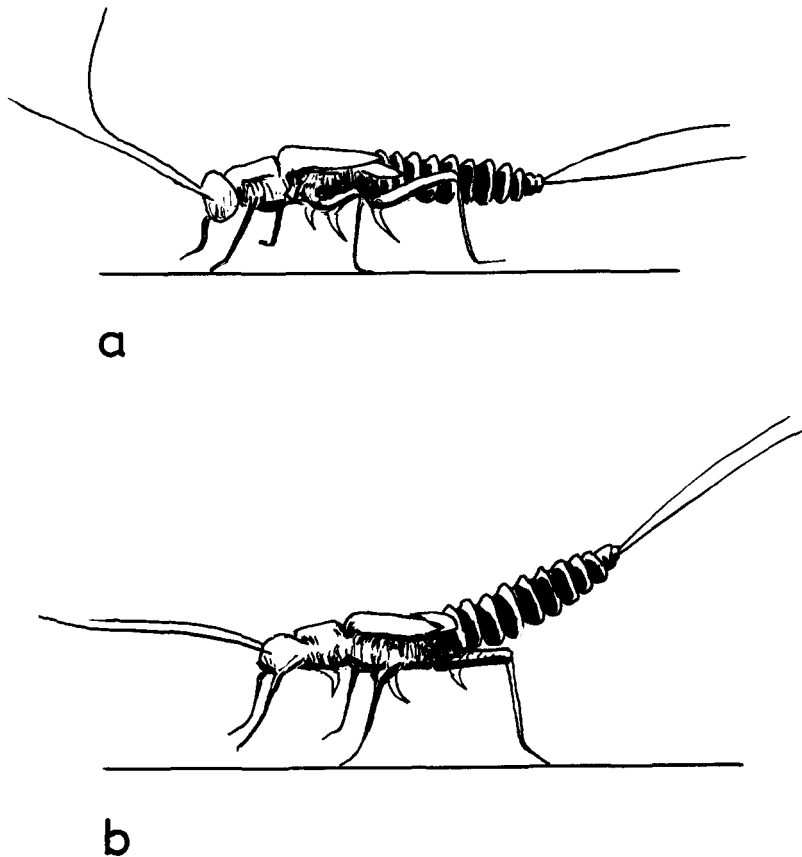

a

b

Fig. 6. *Taeniopteryx nebulosa*, showing normal position (a) and stilted position (b). Figures drawn from photos.

tions correspond, closely to the curve D, the highest point of which lies at about 5 mg O_2/l. (Fig. 2).

At oxygen concentrations lower than about 4 to 5 mg O_2/l some of the *Taeniopteryx* larvae raised their bodies from the underlying surface and stood with their legs straightened (see Fig. 6). This posture was observed by KNIGHT & GAUFIN (1964) and called by them 'the stilty position'. In that position the motor activity is low and so is the oxygen consumption. At about 2 mg O_2/l the larvae fell to the side and did not react when mechanically stimulated. At that stage they had lost their irritability. When given oxygen-rich water they quickly recovered to activity.

In each experiment with *Diura* the respiratory movements were recorded for some of the larvae. The results are presented in Fig. 7. The maximum of the curve is at 4.5 mg O_2/l. This coincides with the highest point of the zone void of oxygen consumption values between curve C and D. The respiratory movements were observed to be intermittent above 7 mg O_2/l.
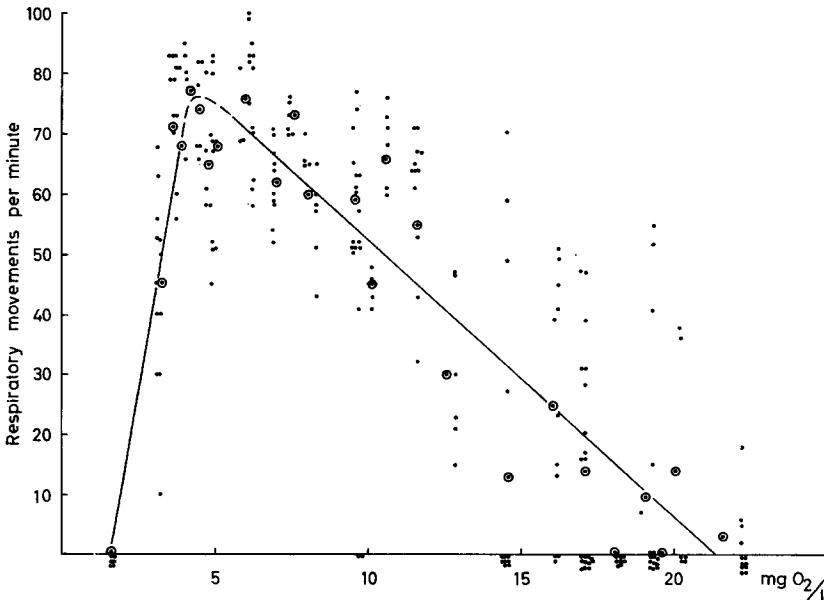

Fig. 7. Respiratory movements of *Diura nanseni* per minute at different oxygen concentrations at 8.0°C. The open circles indicate the mean values for intervals of oxygen concentration along the abscissa of 0.3 mg O_2/l up to 6.2 mg O_2/l and thereafter for intervals of 0.5 mg/l.

474

3.4 Young larvae and their oxygen consumption

The results are shown in Fig. 8 and 9. The curves A, B, C and D of full-grown larvae are inserted to facilitate comparison.

Some differences can be seen. There are many values on the left side of curve A. This shows that young larvae are more efficient than
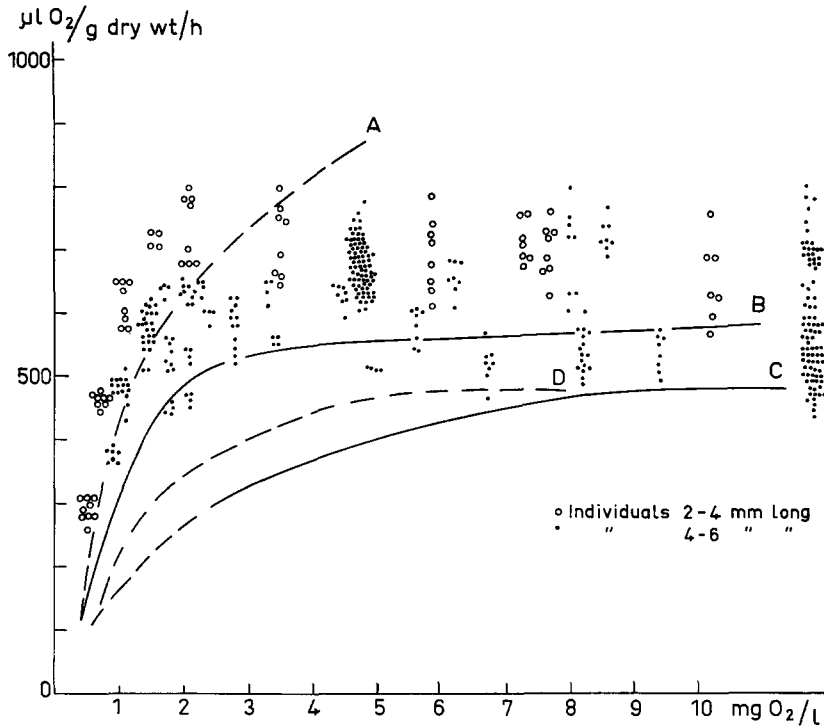

Fig. 8. The oxygen consumption of different size classes of young larvae of *Cloëon dipterum* at 8.0°C, plotted versus oxygen concentration. For the smallest class, the diagram is based on experiments with 468 individual larvae, divided into two groups, and for the other class, on experiments with 970 individual larvae, divided into six groups. Curves A, B and C relate to full-grown larvae (8—10 mm) and are inserted to facilitate a comparison.

the older and larger ones in taking up oxygen at low oxygen concentrations. This appears most clearly in the case of *Cloëon* in which 2—4 mm long larvae are more efficient than 4—6 mm long larvae, which in their turn excel the full-grown larvae. The mean values of the oxygen consumption of young *Cloëon* are clearly higher than those of full -grown larvae.
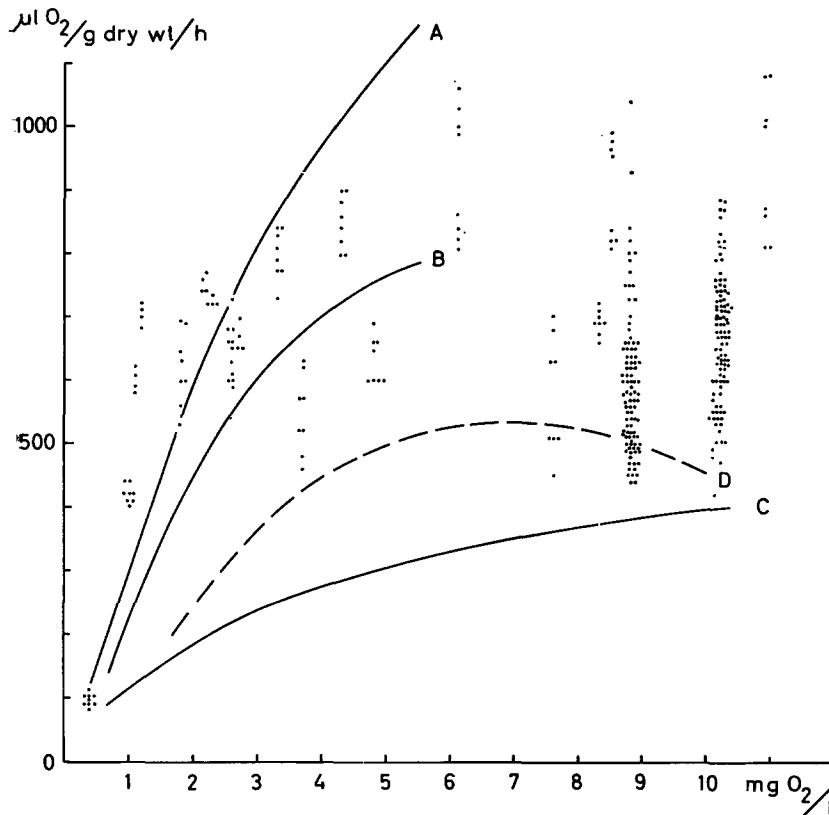
Fig. 9. The oxygen consumption of young larvae of *Nemoura cinerea*, 2—4 mm long, at 8.0°C, plotted versus oxygen concentration. The diagram is based on experiments with altogether 590 individuals, divided into four groups. Curves A, B and C relate to full-grown larvae (8—10 mm).

3.5 The critical point on the oxygen consumption/oxygen concentration curve

The turning point on the curve, when the oxygen consumption from being dependent alters to being independent of oxygen concentration is called the 'critical pressure' (LINDROTH, 1940), 'incipient limiting level' (FRY, 1947) or the 'critical point' (BERG, et al. 1962).

The critical point in this investigation refers to the mean value curve B (Figs. 2—5) and is found to be for *Cloëon*: 2.0—2.5 mg O_2/l for *Diura*: 4—5 mg O_2/l and for *Taeniopteryx*: 4—5 mg O_2/l. *Nemoura* has a poorly defined point at 5—7 mg O_2/l. The results are from experiments at 8.0°C.

3.6 Starvation and the oxygen consumption

In the standard experiments the animals starved for three days. In order to study the influence of such a period of starvation experiments were performed at a nearly constant oxygen concentration (Fig. 10 and 11). There are some fluctuations in the curves. It is not possible, however, to see any changes of the mean level of oxygen consumption during the experiments. That is most clearly seen in Fig. 11. The reasons for the high values in one of the curves of *Taeniopteryx* in Fig. 10 have already been discussed. The mortality in these experiments was very low. Of 190 specimens of *Nemoura*, six died and of 50 *Taeniopteryx* two.

3.7 Calcium content of the experimental water and oxygen consumption

For reasons already mentioned the experimental water in the standard experiments did not contain calcium ions. Some experi-
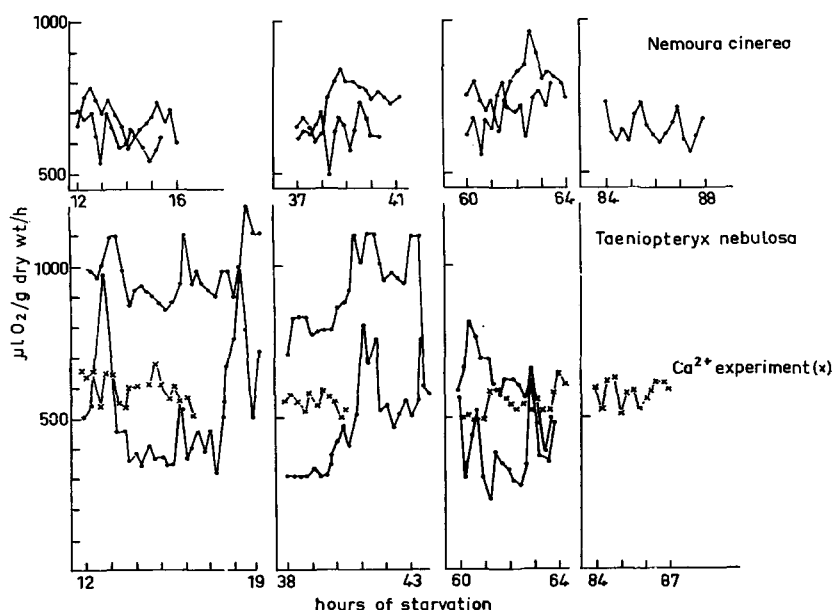

Fig. 10. The oxygen consumption of starving *Nemoura cinerea* and *Taeniopteryx nebulosa* at 8.0°C (full-grown larvae). The values for one and the same group of animals are connected. The number of larvae per group was about 95 in the case of *Nemoura* and about 16 in the case of *Taeniopteryx*. Standardized experimental water was used and the experimental conditions were the same for the different groups. An exception is the middle curve for *Taeniopteryx*, representing an experiment in which the water contained 10 mg Ca^{2+}/l. The oxygen concentration in the experiments with *Nemoura* was 10.1—10.5 mg/l and those with *Taenoipteryx* 9.7—10.9 mg/l.
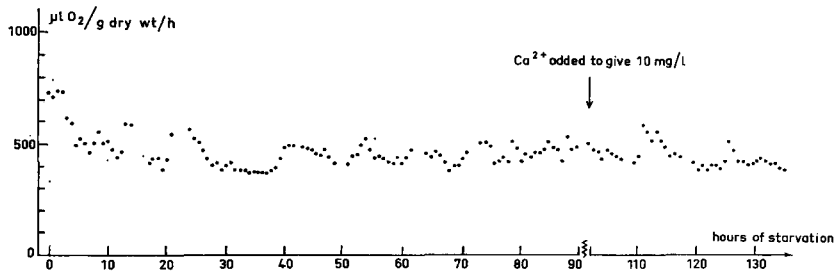
477

Fig. 11. Effect of addition of calcium ions on the oxygen consumption of starving young larvae of *Nemoura cinerea* (5—8 mm) at 8.0°C. The number of larvae was 100. The oxygen concentration was 10.7—11.3 mg/l. Each dot on the diagram corresponds to the mean value of three 4-minute measuring periods (for further information, see the text). The high values the first day were due to the fact that the measurements were started immediately after the larvae had been transferred to the apparatus.

ments with calcium in the water were carried out to ascertain whether any difference appeared between results obtained in the absence and in the presence of calcium ions. The results from experiments with a calcium content of 10 mg /l are shown in Fig. 10 and 11. In the same figure they can be compared with results from calcium free water. It is not possible to discover any clear difference.

4. DISCUSSION

4.1 Oxygen consumption and motor activity
The form of the curves A and C

The results presented in parts 3.1—3.3, from intentionally activated animals, non-activated animals and animals newly transferred into the apparatus, clearly show a strong interrelation between the animals' oxygen consumption and their motor activity. The variation in oxygen consumption at a certain oxygen concentration, which is seen in Fig. 2—5, is explained by a variation in motor activity. No quantitative relationship between activity and oxygen consumption has been calculated, however, due to the difficulty of recording simultaneously the motor activity of 15 to 100 larvae.

The form of the curves A and C in Fig. 2—5 is smoothly convex. It conforms to some very interesting findings by WINZLER (1941). WINZLER measured the oxygen consumption of yeast cells at different oxygen concentrations. He received curves like curve A with steadily decreasing steepness and a convex form ending in a plateau as seen in Fig. 12.
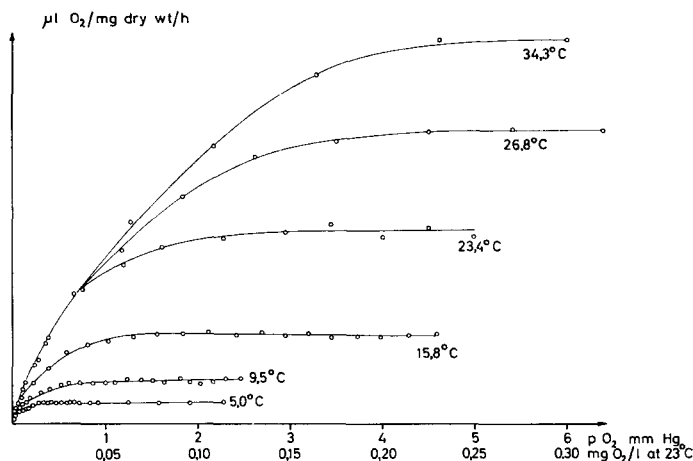
478

Fig. 12. The effect of temperature on the oxygen consumption/oxygen concentration curves of bakers' yeast. The ordinate; oxygen consumption in μl per mg dry weight and hour, the abscissa; oxygen expressed as pressure (mm Hg) or mg O_2/l. (After WINZLER 1941)

WINZLER did not himself explain the form of his curves. By means of cellphysiological knowledge, however, today it is possible to explain the form of the Winzler curves. Today the factors that regulate the oxygen consumption of the yeast cell are better known. The most important factors are: inside the cell, the internal metabolic processes including the chain of respiratory enzymes: outside the cell, the supply of metabolic substrate, the supply of oxygen and the temperature.

Winzler's curves imply that the transport of electrons by the chain of respiratory enzymes limits the oxygen consumption up to a certain point only. The respiratory chain consists of a chain of several enzymes, localized to the mitochondria. Along this chain electrons are transported from the metabolic substrate at one end of the chain to the oxygen at the other end of the chain. The enzyme molecules oscillate between a reduced state and oxidized state, but only up to a certain point. The oxygen consumption curve in Fig. 12 flattens to a plateau at a certain oxygen concentration at the same time as practically all the enzyme molecules of the respiratory chain remain in an oxidized state. At increasing oxygen concentration from that point the oxygen consumption depends only on the supply of metabolic substrate, that is the amount of metabolic substrate activated per unit time. At the plateau the yeast cell's oxygen consumption is no longer dependent on the oxygen concentration outside the cell.

At a somewhat lower oxygen concentration the oxygen supply to

479

the respiratory chain is reduced owing to reduced diffusion and the oxygen consumption is dependent on the oxygen concentration outside the cell. The oxygen consumption curve then bends downwards. At that point a fraction of the enzyme at the substrate end of the respiratory chain is less oxidized than at the plateau, due to lack of electron-accepting oxygen at the other end. At still lower oxygen concentrations the reduced fraction is greater and also fractions of enzymes closer to the oxygen end of the chain are reduced. A decreasing oxygen concentration progressively changes the fractions of oxidized and reduced enzymes in the succession of enzymes in the chain. These kinetic relationships imply an increasing dependence of the oxygen consumption with decreasing oxygen concentration and thus an increasing slope of the curve. The resulting oxygen consumption curve will be smoothly convex as received by WINZLER. There are even other reasons which influence the form of the curve. They are, however, beyond the aim of this article and will not be discussed here. They have been discussed by JÖBSIS (1964).

An elevation of the temperature results in a higher rate of the substrate activation and other metabolic reactions, without a corresponding increase in the diffusion rate of oxygen. The result is a higher oxygen consumption in spite of unaltered oxygen concentration and supply of metabolic substrate. This is reflected, as can be seen in Fig. 12, by an elevation of the whole curve, especially the plateau part, while the point at which the oxygen consumption becomes independent of oxygen concentration is displaced towards higher oxygen concentrations. Figure 12 shows six curves of different height, corresponding to six different temperatures, entailing different metabolic rates. However, the principal form of the curve is unaltered, since the same internal equilibria and dynamic processes are involved which regulate the oxygen consumption over the whole temperature range.

Now an interesting parallel can be drawn between the oxygen consumption of yeast and that of the investigated larvae. The same arrangement of enzyme systems and the oxygen diffusion regulate the oxygen consumption of the two kinds of organisms. There are strong reasons to assume that the oxygen consumption/oxygen concentration relationship for the investigated larvae should take on the same form as the curves of Winzler.

However, it is evident that the dots in Fig. 2—5 do not form so simple curves as those of Winzler. In the case of yeast cells the curve of oxygen consumption/oxygen concentration shows the relationship between oxygen consumption and oxygen supply only, at a certain temperature and a certain supply of metabolic substrate. Since other factors are not involved the values all lie neatly along the curve.

480

In the case of the investigated larvae, on the contrary, there are also other factors involved, namely the variable motor activity and the presence of an active ventilatory mechanism. On that account, there is a range of possible oxygen consumption values, at a certain oxygen concentration, corresponding to variations in motor activity. Since the motor activity of the animals has not been held constant in the experiments the resulting oxygen consumption values lie scattered, corresponding to differences in motor activity.

The six different oxygen consumption curves in the Winzler diagram were due to the different temperatures entailing different metabolic rates in the yeast cells. In the experiments with the larvae the temperature was held constant. However, an increasing stimulation of the larvae had the effect of increasing oxygen consumption in a way corresponding to the influence of increased temperature on the oxygen consumption of the yeast cells. Thus it should in principle be possible to obtain a series of convex oxygen consumption curves of different height for the investigated larvae similar to the curves of Winzler but due to different motor activity, corresponding to different degrees of stimulation. There are great practical problems connected with such a study and it has not been carried through in these experiments. It should be noted that the motor activity is not constant along such a curve. Each of the curves reflects the oxygen consumption at a changing motor activity but at a constant degree of stimulation. The highest values of the oxygen consumption of a certain degree of stimulation is received at the plateau part of the curve, at lower oxygen concentrations the motor activity and thereby the oxygen consumption is restricted due to the reduced oxygen supply.

Curve A in Fig. 2—5 represents one of the highest curves that would appear in such a series, corresponding to an intense, but not extreme stimulation. The curve C in Fig. 2—5 would be the lowest one of the curves, representing the oxygen consumption at no motor activity. Between these two curves other curves of the same form may be drawn.

The values marked by triangles in Fig. 2 of *Taeniopteryx* would then belong to curves higher than the curve A. The triangles correspond to a level of motor activity of the animals maintainable less than 30 minutes. On the other hand the squares in the middel part of the curve A in the same figure correspond to a degree of motor activity maintained by the animals for one or two hours but no longer.

In the Winzler curves the convex part was obtained within a concentration range of 0.00—0.12 mg O_2/l (23.4°C). In the case of an insect larvae, however, the actual curved interval is extended over a much wider range of oxygen concentrations, in the present case up

to 10 mg O_2/l or more. The reason for this is that the influx of oxygen is proportional to the concentration gradient of oxygen from the outside of the larva's cuticle over several membranes to the mitochondrion and the respiratory chain within it. In order to give the same flux of oxygen to the respiratory chain as that in the yeast cell a long diffusion path as in the insect larva demands a higher outside oxygen concentration.

In insects of the investigated types with a closed tracheal system the oxygen diffuses through the outside membrane, through the trachea, and the tracheole and at least two membranes, that of the traecheole and that of the cell, and a layer of cytoplasm before reaching the mitochondrion. This diffusion path is of course much longer than that in a yeast cell. Further physical aspects of insect respiration are discussed by BUCK (1962).

An oxygen consumption/oxygen concentration curve of the same principal form as that discussed above has been reported by FRY (1957) for fish forced to swim.

4.2 The standard metabolism and the scope for activity

The highest oxygen consumption level which is to be found for an animal during a certain not too-short time is called 'active metabolism' by FRY (1957) and 'activity metabolism' by PROSSER et al. (1961). If not defined in relation to the experimental conditions and the length of the expected time of activity the term is of limited value.

'Standard metabolism' can be measured as the oxygen consumption at minimal motor activity. In all species (Fig. 2—5) the lowest oxygen consumption values for unanaesthetized larvae coincide well with those for anaesthetized larvae at the beginning of curve C, at 1 or 2 mg O_2/l, and at the end of the curve, at 8 to 10 mg O_2/l. This fact demonstrates that the concentration of ethyl urethan used does not reduce the standard oxygen consumption. Thus the curves C seem to represent standard metabolism curves. The 'zone without oxygen consumption values' depends on the fact that here the ordinary oxygen consumption values are higher than the values of the standard metabolism. The reason for this is discussed in part 4.3. The results further demonstrate that the standard experimental conditions in the respiratory chamber did not induce an abnormal level of motor activity. Therefore it is probable that the oxygen consumption values obtained in the experiments are representative for larvae in their natural environment. The great influence of the experimental conditions on the oxygen consumption, e.g. the type of substratum, is clearly shown by WAUTIER & PATTÉ (1955) and ERIKSEN (1963).

The difference between 'active metabolism' and 'standard metabolism' forms 'the scope for activity' Fry, (1947), which is a measure of that part of the metabolism which can be transformed into motor activity, physiological regulation etc. In the present investigation the difference between the curves A and C can be regarded as a kind of 'scope for activity', the highest values of which, at high oxygen concentrations, appear in *Nemoura* and *Taeniopteryx*. In the experiments these species were observed to be more easily activated than the other two.

4.3 Motor activation correlated to oxygen concentration

What could be the reason for the existence of a zone without or with very few oxygen consumption values between curve C and D in Fig. 2—5? For *Taeniopteryx* the maximum of the zone is at about the same oxygen concentration as the described maximum of the motor activity (cf. p. 473). The zone diminishes when the activity goes down, when the larvae take the stilty position at lower oxygen concentrations or when the larvae are calm at higher oxygen concentrations. At 8—10 mg O_2/l the larvae could be very quiet for as long periods as half an hour. The same is found for *Diura* referring to the frequency of the respiratory movements (Fig. 7).

The observations indicate that the empty zone is the result of a motor activation appearing at a certain oxygen concentration interval. This explains why no values of the standard metabolism for unanaesthetized larvae are received within the zone.

The zone of *Cloëon* is the most narrow one. This species is found to have a strikingly lower motor activity, gill movements disregarded, than the other three species. The more easily activated the larvae are, the broader is the zone.

4.4 The oxygen consumption of young larvae

A comparison between the oxygen consumption data of a species and its microhabitat ought to include an investigation also of younger stages, as the characteristics of such stages may influence the natural occurence of the species.

The experiments showed that small larvae of *Cloëon* and *Nemoura* are more efficient in taking up oxygen at low concentrations than larger ones (Fig. 8, 9). The essential reasons for this may be thinner cuticles, shorter diffusion paths in the body and a body area larger in relation to the volume than in fully grown larvae.

The younger and thereby smaller stages of *Cloëon* have a higher oxygen consumption than the older ones. No difference in motor activity between the size classes that could explain this higher level was observed. Therefore the results are supposed to reflect the

general trend demonstrated by Zeuthen (1947) – that the oxygen consumption per unit weight of small organisms is more intense than that of larger ones.

4.5 The critical point on the oxygen consumption/oxygen concentration curve

The respiratory response of an animal to reduced oxygen concentration may be one of two different kinds. In one case the animal will retain its oxygen consumption relatively unchanged by compensatory respiratory mechanisms. In the other case the oxygen consumption will decrease with the falling oxygen concentration. Animals of the first type are called regulators and animals of the second type conformers (PROSSER & BROWN 1961). 'Regulators' and 'conformers' are ideal type concepts, however. Animals never fully belong to one or the other type. At a sufficiently low oxygen concentration, at the critical point, a regulator becomes a conformer. In many regulators the oxygen consumption shows a slight dependence on the oxygen concentration even above the critical point.

Of the investigated species *Taeniopteryx*, *Diura* and *Cloëon* are roughly seen regulators and *Nemoura* a conformer.

Diura, at decreasing oxygen concentration regulates its oxygen consumption by performing accelerated respiratory movements down to the critical point. The connection between the critical point and the maximum frequency of respiratory movements is clear for *Diura* (Fig. 7). Also for *Taeniopteryx* a pronounced critical point was found. This was surprising for a species that does not perform any respiratory movements. However, as already discussed, greatly increasing motor activity with decreasing oxygen concentration was observed for this species. The larvae are activated at reduced oxygen concentration, but at a certain value their activity is again subdued on account of the too meagre supply of oxygen and the oxygen consumption decreases. This value corresponds to the critical point.

Oxygen consumption measurements have previously been carried out on *Cloëon dipterum* by Fox et al. (1937). They present a mean-value curve with a critical point at 2.0 mg O_2/l at 10°C. They also tabulate the individual values of their measurements. When these values are plotted a diagram is achieved that permits a more precise determination of the critical point.

The position of the critical point in the individual value diagram of Fox is between 2.5 and 3.0 mg O_2/l. In my diagram (Fig. 5) the critical point lies between 2.0 and 2.5 mg O_2/l. Thus the critical point is shifted towards a higher oxygen concentration in Fox's experiments than in my experiments. The reason for this is the higher general oxygen consumption level in Fox's experiments. At 3 mg

484

O_2/l the mean oxygen consumption is about 1200 μl O_2 per g dry weight per hour in Fox's experiments and about 600 μl in mine. The last value is roughly compensated for the expected elevation of the metabolism from 8°C to 10°C (Q^{10} around 2).

The difference between the two values may be explained by the fact that Fox et al. performed their experiments in closed bottles without substratum. In order to mix the water in the bottles they were turned over every fifth minute. This arrangement probably activated the larvae and forced them to swim In my experiments, larvae were observed swimming only occasionally. Thus, the motor activity in the experiments of Fox et al. must be regarded as very high. A similar difference between the oxygen consumption in closed bottles and in a flowing-water respirometer is described by KAMLER (1969).

4.6 The oxygen consumption curves from an ecological viewpoint

To what extent do the oxygen consumption/oxygen concentration curves and the position of the critical point give usable information about the oxygen requirements of the species in nature.

The species *Diura* and *Taeniopteryx* have the highest oxygen requirements of the investigated species. Their critical points fall within the same oxygen concentration range. The two species both lose irritability at about the same oxygen concentration (2 mg O_2/l at 8°C). From the oxygen consumption results it is thus difficult to say which species has the highest oxygen requirements in nature. One difference of possible ecological importance is, however, that *Diura* remains at the same spot at the critical point, using the greater part of the energy for respiratory movements, while *Taeniopteryx* uses the energy for crawling about. This crawling brings new water to the gills. This improves the oxygen uptake; more important, it can move the individual away from an area with an unfavourable oxygen concentration. The lowest oxygen concentrations at the localities of *Diura* are clearly higher than those at many places inhabited by *Taeniopteryx*.

Cloëon has a lower critical point (2.0—2.5 mg O_2/l at 8°C) than any one of the other species studied and is occasionally exposed to low oxygen concentrations in nature. Values about 2 mg/l were regularly recorded at night time in July in a small pond inhabited by *Cloëon*. *Nemoura* which is not exposed to such low oxygen concentrations in nature, has a critical point at much higher values (5—7 mg O_2/l at 8°C) and an oxygen consumption curve more like a conformer. This may suggest the conclusion that *Nemoura* is particularly sensitive to low oxygen concentrations. However the following results show that this is not the case.

In one experiment *Cloëon* and *Nemoura* larvae (100 of each species) acclimated to 10°C for a month, were exposed simultaneously to an oxygen concentration held constant at 0.9 mg O_2/l at 8.0°C. After two hours about 20% of the *Nemoura* specimens and about 2% of the *Cloëon* specimens had lost irritability. In other experiments the oxygen concentration was determined at which 50% of the larvae died at 10°C after 20 hours' exposure to five different constant oxygen concentrations (totally 400 larvae of each species tested). The 50% lethal concentrations, extrapolated from the mortality curves, were 1.9 mg O_2/l for *Cloëon* and 0.4 mg O_2/l for *Nemoura* (NAGELL, to be published.)

Thus despite the absence of respiratory regulation mechanism, *Nemoura* survives low oxygen concentrations better than *Cloëon*. On the other hand *Nemoura* loses irritability after a shorter time than *Cloëon*, which is active up to the very last minute before death. This ought to give *Cloëon* a better chance to leave areas poor in oxygen.

The oxygen consumption curves are usable from the ecological viewpoint. But as shown they sometimes can lead to misleading conclusions if not integrated with other facts about the animals.

4.7 The influence on the oxygen consumption of starvation, the lack of calcium ions and the osmotic pressure of the experimental water

A possible effect of starvation can be reduced oxygen consumption. However, no such reduction can be traced in Fig. 10, 11. The same is found by BERG et al. (1962).

The effect of starvation is obviously so small that the oxygen consumption values from the first and second days of the standard experiments may be regarded as being fully comparable. This applies to *Nemoura* and *Taeniopteryx*, probably also to *Diura* and *Cloëon*.

The effect of calcium ions on the permeability of cell membranes is well known. This ion reduces the permeability to water, to other ions and to many other compounds (GARY-BOBO 1970). Perhaps this could influence the oxygen uptake by the experimental larvae? In the experiments made (Fig. 10, 11) there are, however, no detectable differences between the results in the presence or in the absence of calcium.

The reason for this can of course be that there is no effect of calcium ion on the oxygen consumption at all. Another possible explanation is that the losses of calcium ions in the standard experiments were so small during the relatively short experimental period that effects, if they occur, did not have time to arise. During the acclimation period the larvae stayed in calcium-rich tap water.

The conductivity of the experimental water was for technical

reasons already mentioned, kept high (470 micromoh per cm at 20°C). Could this high conductivity and the osmotic pressure connected with it influence the oxygen consumption of the animals?

The difference in osmotic pressure between the experimental water and a water of 'standard composition' (RODHE, 1949) with a conductivity of 140 micromoh/cm is equal to the osmotic pressure of a 2.3 m molal NaCl solution.

The osmotic pressure in the haemolymph of larvae of *Hydropsyche* sp. and *Limnophilus vittatus* (Tricoptera) and *Ecdyonurus venosus* (Ephemeroptera) was determined by Fox & BALDES (1935) as osmotically equivalent to solutions of NaCl with concentrations of 132, 135 and 159 m molal/l, respectively. The values are probably about the same for the experimental animals.

The above mentioned osmotic difference of 2.3 m molal NaCl corresponds only to 1.5% of the osmotic pressure of a haemolymph osmotically equivalent to a 150 m molal NaCl solution. Compared with the osmotic pressure inside the animal a variation of 1.5% outside ought to have a very small – if any – osmotic effect on the animal. The effect on the oxygen consumption will probably be still smaller. Thus the osmotic effect of the used experimental water on the oxygen consumption ought to be negligible.

4.8 Concluding remarks

Oxygen consumption/oxygen concentration curves are usually presented only with the mean values obtained. This provides more easily surveyable results but involves a limitation of the information offered by the material. If all the oxygen consumption values are shown, the reader is given an insight into the limits within which the oxygen consumption varies and a better idea of how well defined the critical point is.

The basic picture obtained in this investigation is a set of oxygen consumption values scattered between a curve connecting the highest values obtained and a curve of the standard metabolism, together with a zone in which the larvae are activated by reduced oxygen concentrations. This picture is presumed to be general in aquatic animals with a well developed motor activity. In the majority of such cases, a diagram of the above mentioned type would be necessary in order to describe the oxygen consumption characteristics of the investigated species.

REFERENCES

BERG, K., JONASSON, M. & OCKELMANN, K. W. - 1962 - The respiration of some animals from the profundal zone of a lake. *Hydrobiologia* 19: 1—39.

BERG, K. & JONASSON, M.P. - 1965 - Oxygen consumption of profundal lake animals at low oxygen content of the water. *Hydrobiologia* 26: 131—143.

BIELAWSKI, J. - 1959 - A polarographic method of measuring the intensity of respiration in aquatic animals. *Acta Biol. Exper.* 19: 41—48.

BIELAWSKI, J. - 1961 - The use of a solid platium electrode for continuous recording of the rate of respiration. *Comp. Biochem. Physiol.* 3: 261—266.

BRAFIELD, A. E. & CHAPMAN, G. - 1967 - The respiration of Pteroides griseum (Bohadsch) a pennatulid coelenterate. *J. exp. Biol.* 46: 97—104.

BRETSCHKO, G. - 1965 - Zur Larvalentwicklung von Cloëon dipterum. Cloëon simile, Centroptilum luteolum and Baetis rhodani. *Z. wiss. Zool.* 172: 17—36.

BRIGGS, R., DYKE, G. V. & KNOWLES, G. - 1958 - Use of the wide-bore dropping-mercury electrode for long-period recording of concentration of dissolved oxygen. *Analyst* 83: 304—311.

BRIGGS, R. & KNOWLES, G. - 1961 - Developments in the use of the widebore dropping-mercury electrode for determining dissolved oyxgen and oxygen in gases. *Analyst* 86: 603—608.

BRINCK, P. - 1949 - Studies on Swedish stoneflies (Plecoptera). *Opusc. Ent. Suppl.* 11: 1—250.

BUCK, J. - 1962 - Some physical aspects of insect respiration. *Ann. Rev. Entomol.* 7: 27—56.

EDWARDS, R. W. & LEARNER, M. A. - 1960 - Some factors affecting the oxygen consumption of Asellus. *J. exp. Biol.* 37: 706—718.

ERIKSEN, C. H. - 1963 - The relation of oxygen consumption to substrate particle size in two burrowing mayflies. *J. exp. Biol.* 40: 447—453.

FOX, H. M. & BALDES, E. J. - 1935 - The vapour pressure of the blood of arthropodes from swift and still fresh waters. *J. exp. Biol.* 12: 174—178.

FOX, H. M., WINGFIELD, C. A. & SIMMONDS, B. G. - 1937 - The oxygen consumption of ephemerid nymphs from flowing and from still waters in relation to the concentration of oxygen in the water. *J. exp. Biol.* 14: 210—218.

FRY, F. E. J. - 1947 - Effects of the environment on animal activity. *Publ. Ont. Fish. Res. Lab.* 68: 1—62.

FRY, F. E. J. - 1957 - The aquatic respiration of fish: in Brown, M. E., The Physiology of fishes. Academic Press Inc. 447 pp.

GARY-BOBO, C. M. - 1970 - Effect of Ca^{2+} on the water and non-electrolyte permeability of phosfolipid membranes. *Nature.* 228: 1101—1102.

GIESE, A. C. - 1963 - Cell Physiology, p. 377. W. B. Saunders Company. 592 pp.

HÖGLUND, L. B. - 1961 - The reactions of fish in concentration gradients. Ann. Rep. Inst. Freshw. Res. Drottningholm. 43. 147 pp.

JÖBSIS, F. F. - 1964 - Basic processes in cellular respiration. Handbook of Physiology, Section 3: Respiration, Vol. 1. Amer. Physiol. Soc. 1056 pp.

KAMLER, E. - 1969 - A comparison of the closed-bottle and flowing-water methods for measurement of respiration in aquatic invertebrates. *Pol. Arch. Hydrobiol.* 16: 31—49.

KLEKOWSKI, R. Z. & KAMLER, E. - 1968 - Flowing-water polarographic respirometer for aquatic animals. *Pol. Arch. Hydrobiol.* 15: 121—144.

KNIGHT, A. W. & GAUFIN, A. R. - 1964 - Relative importance of varying oxygen concentration, temperature and water flow on the mechanical activity and survival of the plecoptera nymph, *Pteronarcys californica*, Newport. *Utah Acad. Sci.* 41: 14—28.

LINDROTH, A. - 1940 - Sauerstoffverbrauch der Fische bei verschiedenem Sauerstoffdruck und verschiedenem Sauerstoffbedarf. *Z. vergleich. Physiol.* 28: 142—152.

MANN. K. H. - 1958 - Seasonal variation in the respiratory acclimatization of the leech *(Erpobdella testacea)*. *J. exp. Biol.* 35: 314—323.

MANN, K. H. - 1965 - Energy transformations by a population of fish in the river Thames. *J. Anim. Ecol.* 34: 253—275.

PROSSER, C. L. & BROWN, F. A., JR. - 1961 - Comparative Animal Physiology. 2nd ed. W. B. Saunders Company. 688 pp. Philadelphia and London.

RODHE, W. - 1949 - The ionic composition of lake waters. *Verh. int. Ver. limnol.* 10: 377—386.

SVENSSON, P. O. - 1966 - Growth of nymphs of streamliving stoneflies in northern Sweden. *Oikos* 17: 197—206.

ULFSTRAND, S. - 1967 - Microdistribution of bentic species (Ephemeroptera, Plecoptera, Tricoptera, Diptera: Simuliidae) in Lapland streams. *Oikos* 18: 293—310.

WAUTIER, J. & PATTÉ, E. - 1955 - Expérience physiologique et expérience écologique. L'influence du substrat sur la consommation d'oxygène chez les larves d'éphéméroptères. *Bull. Mens. Soc. Linn. Lyon* 7: 178—183.

WINTZLER, R. J. - 1941 - The respiration of baker's yeast at low oxygen tension. *J. Cell. Comp. Physiol.* 17: 263—276.