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OXYGEN CONSUMPTION IN AMMOCOETES OF THE LAMPREY *ICHTHYOMYZON HUBBSI* RANEY

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INTRODUCTION

Lampreys and hagfishes are the only extant members of the primitive group of vertebrates known as the Agnatha. Consequently their respiration is of phylogenetic interest as well as being particularly pertinent in a consideration of their general physiology and ecology. Yet there is virtually no data for respiration in the ammocoete larvae of lampreys, apart from the results of studies on *Ichthyomozon fossor* and *Petromyzon marinus* by Leach (1946). This author was, however, primarily interested in metamorphosis and did not investigate the effect on the rate of oxygen consumption of either temperature or the size of the animal. Furthermore, and very importantly, his experimental regime was far from ideal as he himself pointed out. This present investigation was undertaken to provide data on the rate of oxygen consumption of ammocoetes in conditions simulating that of their natural environment.

The lamprey used in this study, *Ichthyomyzon hubbsi* Raney, is a non-parasitic species that never undergoes an anadromous migration. Its ammocoetes are found in burrows in the margins of many of the streams in the mountains of western North Carolina. Feeding is carried out by filtering out micro-organisms from the water passing through the branchial basket, which is thus not used exclusively for respiration.

According to Horn & Bailey (1952), *I. hubbsi* undergoes metamorphosis in the autumn of the third or fourth year of larval life. Since samples of ammocoetes collected in the field thus contain individuals of different year classes, size must be taken into consideration when determining oxygen uptake. The temperature of the streams inhabited by *I. hubbsi* varies greatly, dropping to about 3 °C in winter and rising to at least 22 °C in summer. These wide temperature fluctuations must also influence the oxygen uptake of ammocoetes, and a determination of this effect is important in an investigation of their respiration.

Our observations on a number of different species indicate that healthy ammocoetes, recently removed from a substrate, undergo periodic swimming movements. Such behaviour undoubtedly raises the respiratory rate and thus it seemed essential that oxygen consumption be measured in an apparatus containing a medium into which the ammocoetes would burrow and remain quiescent. The continuous-flow respirometer which is described in this paper provides this substrate, and at the same time

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prevents either an oxygen deficit or an accumulation of carbon dioxide to build up in the interstitial water of the substrate. This apparatus should be suitable for respiration studies in a wide variety of burrowing animals.

MATERIALS AND METHODS

Capture and maintenance of animals

During February 1969, an electric fish-shocker (Smith-Root Electronics Type V) was used to collect ammocoetes of *I. hubbsi* from Bent Creek, a small mountain brook opening into the French Broad River in western North Carolina. After transportation back to the laboratory, the animals were kept in large aerated polyethylene aquaria supplied with a natural silt–sand substrate containing diatoms and other micro-organisms which are the main constituents of the diet of ammocoetes (Creaser & Hann, 1929). The animals were separated into three length categories, small (34–47 mm), medium (75–105 mm) and large (116–153 mm), and maintained under constant light at a temperature of $15{\cdot}5 \pm 0{\cdot}3$ °C.

Experiments were initiated after the ammocoetes had been maintained at 15·5 °C for 3 weeks. Measurements at $3{\cdot}5 \pm 0{\cdot}2$ °C and $22{\cdot}5 \pm 0{\cdot}5$ °C were made on animals which had been transferred in their aquaria from 15·5 °C to constant-temperature rooms at the experimental temperature for an additional 2 weeks. This method of transfer ensured that the animals were not subjected to a sudden change in temperature but to a change phased over several hours. Before each run the animals were placed in an aquarium with a substrate of washed sand for 24 h so that most of the food and silt in the gut would be eliminated. At the end of this period the animals were first stimulated to emerge by gentle stirring of the substrate and then transferred to the chambers of the respirometer. After each experiment the animals were anaesthetized in MS. 222 (Sandoz), dried by gentle rolling on a piece of absorbent paper to remove surface water, and weighed to the nearest milligram.

Respirometer

The respirometer (Fig. 1) was mounted on a frame in a constant-temperature room. The animal chambers were made from glass cylinders with a standard taper socket (19/38) at one end. Nylon mesh (0·5 mm bar) was stretched across the opposite end and glued in place. A glass funnel was pressed up against this end of the chamber and the joint was sealed with a thick layer of silicone rubber. Borosilicate glass beads (1 mm in diameter) were introduced into the chamber to simulate the animal's natural substrate. A large glass carboy, in which a thermometer and an aerating stone were suspended, was used as a water reservoir. From this container water passed into the seven animal chambers by way of a manifold, entry into each chamber being controlled by a three-way tap. The water passed out of the chambers into sampling bottles through a short piece of thick-walled latex tubing. A peristaltic pump was used to control the flow of water through the sytem. Displaced water from sampling bottles was led into graduated cylinders to enable an accurate measurement to be made of the flow rate in each of the seven channels.

Chambers and sampling bottles of two different volumes were used since there was a great difference in the size of the ammocoetes. In the case of animals of large and

of medium size the chambers were 6 cm in diameter, had a total volume of 225 ml and contained 100 ml of glass beads. The space above the beads was essential to allow the swimming movements necessary for the inititation of burrowing. The chambers used for small ammocoetes were 5 cm in diameter, had a capacity of 80 ml and contained 40 ml of beads. Sampling bottles of 38 ml capacity were used with the small chambers and also with the medium-size animals at 3·5 °C, while 60 ml capacity bottles were employed in all other cases.

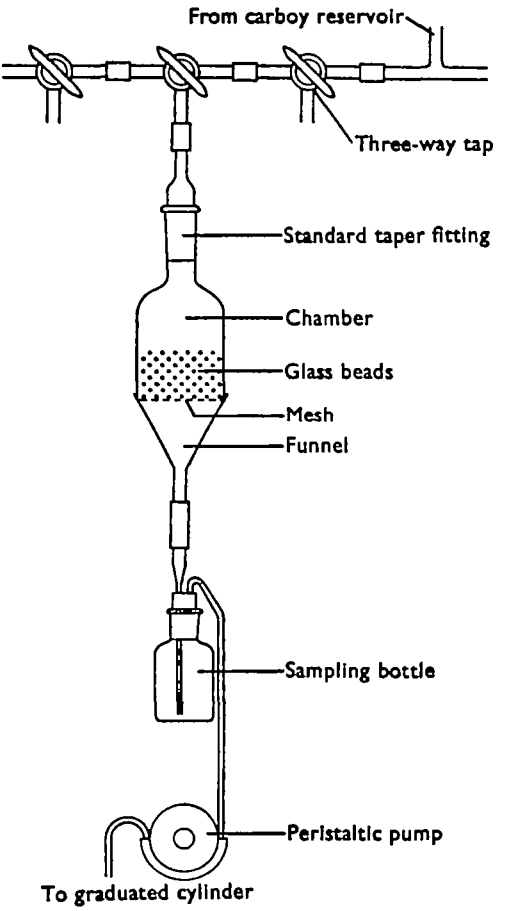

Fig. 1. Diagram of respirometer used for determining oxygen consumption in ammocoetes of *Ichthyomyzon hubbsi*, showing one of the seven channels.

Flow rates varied from 25 ml/h for medium-sized animals at 3·5 °C to 140 ml/h for large animals at 15·5 °C. These flow rates were chosen so as to give an oxygen tension in the experimental chambers of at least 70% of that in the control chamber since, as Hughes (1964) has pointed out, fishes exposed to low oxygen tensions have decreased oxygen consumption. Measurements were spaced at intervals of 2–4 h to provide for at least a three times displacement of the water in the sampling bottles.

All the components of the respirometer between the reservoir and the sampling bottles were autoclaved for 1 h at 120 °C before each experiment. The water was boiled and filtered before being aerated to saturation and introduced into the system.

Measurement of oxygen consumption

The Winkler method, as described by Mackereth (1963), was used for the determination of the amount of dissolved oxygen in the sampling bottles. Samples were titrated with a microburette of 10 ml capacity. The difference in oxygen concentration between water that had passed through a chamber containing an animal and water that had passed through a control chamber in which there was substrate but no animal was used as a measure of oxygen consumption in each case. The rate of oxygen consumption was obtained by substitution in the following formula:

$$R = \frac{(O_1 - O_2)f}{W},$$

where R = rate of oxygen consumption (μl/g/h),

O_1 = oxygen concentration (μl/ml) of sampling bottle corresponding to a chamber in which no animal was present, i.e. the control,

O_2 = oxygen concentration (μl/ml) of sampling bottle corresponding to a chamber in which an animal was present,

f = flow rate (ml/h),

W = wet weight of animal (g).

(A rate of 1 μl/g/h is equivalent to 1 ml/kg/h or 1·429 mg/kg/h).

The rate of oxygen consumption of each individual animal is based on the mean value of the first four readings taken after the oxygen consumption of all animals had reached a plateau. Data are presented for the oxygen consumption of medium-size animals at 4 h intervals over a 24 h period to illustrate the pattern of oxygen consumption during the course of an experiment (Fig. 2).

Twelve medium-size and 12 large animals were used for measurements of oxygen consumption at 15·5 °C, and ten medium-size animals at 3·5 and 22·5 °C. In the case of small ammocoetes 33 animals were separated into 11 groups each containing three animals of similar length. Each group was then placed in a separate chamber and the total oxygen uptake was measured. These rates are plotted as a function of the mean weight of the three animals in each case in the graph showing the relationship between rate of oxygen consumption and weight of animal (Fig. 3).

After completion of the experiments and computation of the results it was apparent, in view of the large difference in rate of oxygen consumption between 3·5 and 15·5 °C, that it would be of interest to have a measurement within this temperature range. Accordingly, nine medium-size animals were transferred from 15·5 to 9·5 ± 0·5 °C for 8 days and their oxygen uptake was then determined.

RESULTS

Ammocoetes burrowed into the glass-bead substrate within 60 sec in all cases except at 3·5 °C. Occasional changes in position were seen in some animals during the initial part of the experiment, and in a few chambers all three animals of the smallest

size category emerged after a few hours and swam around intermittently before re-burrowing 2 or 3 h later. All animals eventually settled into a relatively constant position.

At a temperature of 22·5 °C the medium-size animals were extremely active during transfer and burrowed with great rapidity, while at 15·5 °C the burrowing speed and general activity were noticeably reduced. At 3·5 °C it was much more difficult to stimulate the animals to emerge from the sand substrate in the holding aquarium, and in a few cases it took as long as five minutes for them to burrow in the glass beads. A greatly reduced frequency of branchial beat was observed at this lower temperature.

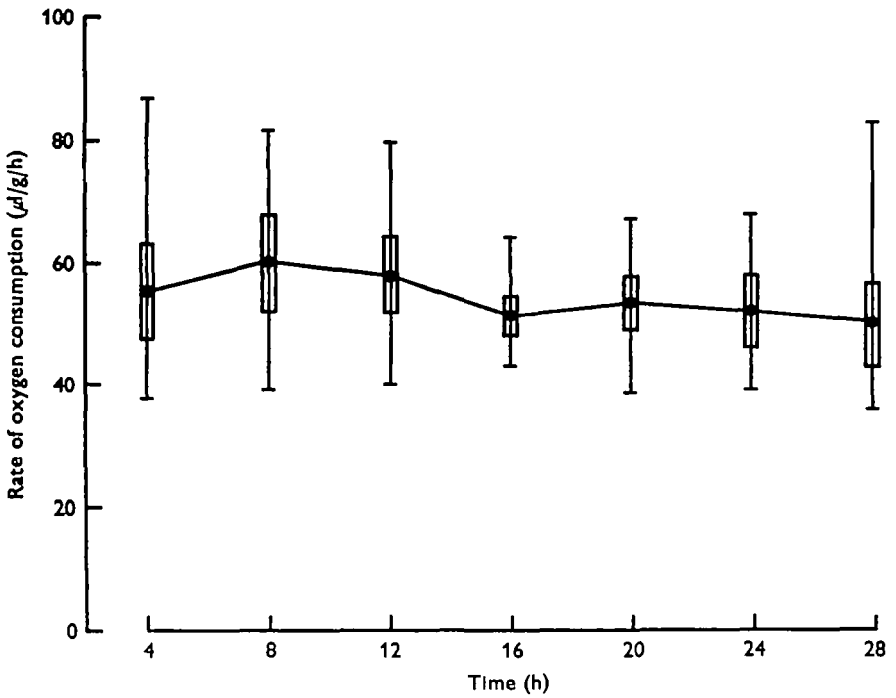

Fig. 2. Rate of oxygen consumption of 12 medium-size (0·74–1·64 g) ammocoetes of *Ichthyomyzon hubbsi* at 15·5 °C over a 24 h period commencing at 3·00 p.m. The mean (closed circle), two standard errors to either side of the mean (open rectangle) and the range (vertical line) are given for each reading.

The first two or three readings for each of the medium-size animals (Fig. 2) at 15·5 °C showed that *c.* 17 h were required for all animals to achieve a relatively steady rate of oxygen consumption following their introduction into the experimental chambers. These initial readings showed elevated values for oxygen consumption in a few animals. The high upper limit of the last reading in Fig. 2 is due to a greatly increased rate in one animal. No conspicuous circadian rhythm in oxygen consumption by ammocoetes was found in experiments which were allowed to run for up to 36 h. The time required for acclimation of all medium-size animals at 22·5 °C, and of all large animals, was also about 17 h. On the other hand, medium-size animals at 3·5 °C, and also the small animals, required 24–28 h to acclimate.

The respective mean weights, together with their standard deviations, standard errors and range, are shown in Table 1.

Table 1. *The number, mean weight together with standard deviation and two standard errors of the mean, and the weight range of animals used in experiments*

(In the case of small animals, measurements of oxygen consumption were based on groups of three animals.)

Temperature (°C)	Size category	Number of animals	Mean weight	Two standard errors	Standard deviation	Range
15·5	Small	33	0·14	0·014	0·023	0·11–0·17
15·5	Medium	12	1·24	0·168	0·29	0·74–1·64
15·5	Large	12	3·44	0·400	0·70	2·09–4·71
22·5	Medium	10	1·22	0·186	0·28	0·57–1·59
3·5	Medium	10	1·27	0·240	0·41	0·75–1·72
9·5	Medium	9	1·01	0·188	0·28	0·75–1·50

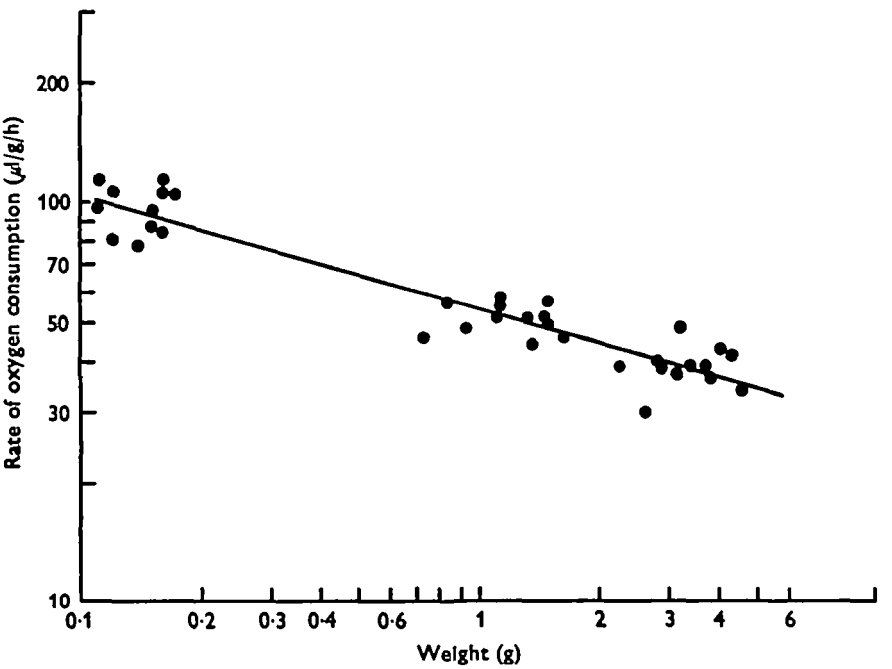

Fig. 3. The relationship between rate of oxygen consumption and wet weight of ammocoetes of *Ichthyomyzon hubbsi* at 15·5 °C. Regression line fitted by the method of least squares.

The rate of oxygen consumption of small, medium-size and large animals at 15·5 °C was plotted graphically as a function of the weight of the animal (Fig. 3). A line, fitted to the points by the method of linear least squares, shows the close relationship between rate of oxygen consumption and weight of animals. Small ammocoetes can be seen to have a much higher rate of oxygen consumption than medium-size or large animals.

The general equation relating the total oxygen consumed (Y, μl O_2/h) by an animal, to weight (X, g) may be expressed as $\log Y = \log a + b \log X$, where a is a constant

and b the regression coefficient. Using the values for oxygen consumption of small, medium-size and large animals at 15·5 °C, the oxygen consumption/weight equation for ammocoetes of *I. hubbsi* is:

$$\log Y = 1{\cdot}735 + 0{\cdot}718 \log X.$$

The 95% confidence limits for b are $\pm 0{\cdot}0257$.

The mean rates of oxygen consumption of medium-size animals at various temperatures are shown in Fig. 4. Temperatures of 22·5 and 3·5 °C are probably close to the upper and lower limits experienced by these animals under natural conditions. The rate of oxygen consumption at 3·5 °C (8·1 μl/g/h) is very low and less than one-tenth the value recorded at 22·5 °C (90·1 μl/g/h). The mean rates at 9·5° and 15·5 °C were 22·4 μl/g/h and 51·2 μl/g/h respectively.

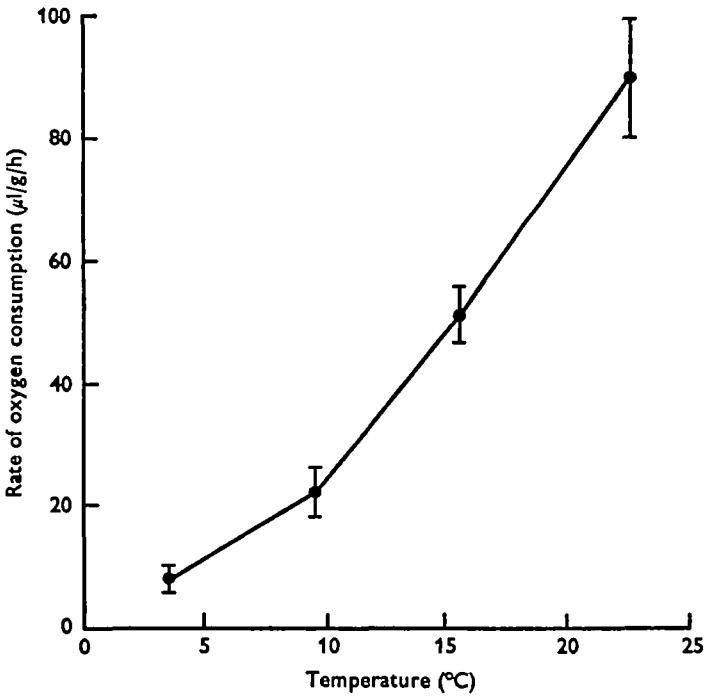

Fig. 4. Rate of oxygen consumption of medium-size (0·57–1·72 g) ammocoetes of *Ichthyomyzon hubbsi* at different temperatures. The mean (closed circle) and one standard deviation to either side of the mean (vertical line) are shown.

The Q_{10} values calculated from the mean rates of oxygen consumption shown in Fig. 4 are summarized below.

Temperature	3·5–9·5 °C	9·5–15·5 °C	15·5–22·5 °C	3·5–22·5 °C
Q_{10}	5·4	4·0	2·2	3·6

The Q_{10} can be seen to decrease with temperature and is always greater than two.

DISCUSSION

The main advantage of the respirometer used in this study is that it uses the principle of passing a constant, controlled flow of water through a substrate in which the animal has voluntarily burrowed. This avoids the problem of dead space which would arise if the water were drawn over the substrate, and it also makes unnecessary the use of anaesthetics or restraining agents.

Eriksen (1963) found that mayfly nymphs had a lower oxygen consumption when given a substrate into which they could burrow than when they were kept in bottles with no substrate. This difference he ascribed to the reduction in activity of the animals when in their burrows. He also found different rates of oxygen uptake with various particle-size compositions of the substrate. If the substrate was not particularly suitable, the nymphs were more active and spent a longer period establishing a burrow. In the case of ammocoetes of *I. hubbsi*, glass beads of 1 mm diameter provided a suitable substrate as ammocoetes burrowed into it immediately, and after a period of a few hours remained settled for an indefinite time. The accentuated activity of the smallest ammocoetes at the beginning of the experiment may be due to a reaction to the larger particle size of the substrate compared with the fine silt in which animals of this category are normally found. However, since small ammocoetes eventually remain quietly in their burrows, the glass beads also proved a suitable substrate in this case.

By using a continuous-flow system, in combination with measures to produce and maintain the apparatus and circulating water sterile, it was possible to keep the system running for a period of up to 3 days, despite the large surface area of the beads available for the growth of micro-organisms which might be introduced with the animals. Our measurements show that recordings should be made at intervals to ascertain when values for oxygen consumption reach a plateau, thus indicating that the animals have settled down. Even though transfer of the ammocoetes to the substrate in the chamber took less than 60 sec, this movement was sufficient to affect the rate in a few animals for several hours. Thus it is important to allow a period for settling down when estimating the oxygen consumption of ammocoetes. Some differential contamination of the chambers may occur after the system has been running for a time, particularly at higher temperatures, and therefore the initial records of oxygen consumption as the plateau is reached should be the values used for a measure of respiration.

Beamish & Mookherjii (1964) reviewed the use of the terms standard, routine and active oxygen consumption with respect to fishes. They defined the term routine oxygen consumption as 'the oxygen consumed by fish whose only movements are spontaneous'. No movements, apart from those of the velum and branchial basket, were seen in ammocoetes at either 3·5 or 15·5 °C, but by inference from slight changes in position some movement must occasionally occur. The ammocoetes were also relatively quiet at 22·5 °C, but the incidence of movement, although still rare, was higher. Thus the use of a substrate reduced spontaneous movements to a minimum and enabled us to be fairly certain that the values estimated for the rate of oxygen uptake approximate to the minimal oxygen requirements of the ammocoetes of *I. hubbsi* in a sedentary state. While these values obviously correspond to routine oxygen consumption as envisaged by Beamish & Mookherjii, they may also be close to their concept of standard oxygen consumption.

The only previous measurements of oxygen consumption recorded for the larval stage of lampreys are apparently those of Leach (1946). The life cycle of *I. fossor*, one of the species he studied, is similar to that of *I. hubbsi* since it is a non-parasitic lamprey which never undergoes an anadromous migration. The oxygen consumption of large ammocoetes measured in January and February at 15 °C, ranged from *c.* 100–140 μl/g/h. Measurements at higher temperatures on ammocoetes of *I. fossor*, and also on *P. marinus*, indicated that oxygen consumption was greater in *P. marinus* with a value as high as 450 μl/g/h being obtained for a 2·6 g animal examined in December at 22·5 °C. It appears that individuals of both species undergo a reduction in oxygen consumption during metamorphosis. On the other hand the adults of *I. fossor*, which are of similar weight to the largest ammocoetes (*c.* 2–5 g), show an increased rate (150–170 μl/g/h at 15 °C) just prior to reaching sexual maturity. Leach's work on ammocoetes was restricted to large animals at relatively high temperatures and was carried out under experimental conditions which had the following limitations. Measurements of oxygen consumption were made in a container without a substrate into which the animals could burrow, and no attempt was made to prevent a build-up in carbon dioxide or a drop in oxygen. The differences in technique between Leach's work and the experiments described in this paper probably account for the much lower rate of oxygen consumption recorded for *I. hubbsi* ammocoetes.

Leach pointed out that the very high values he obtained for 'winter' ammocoetes of *P. marinus* may have been due to the fact that measurements were made at much higher temperatures than are normally found under natural conditions at that time of the year. Even though our animals were acclimated for a lengthy period in the laboratory, measurements on 'summer' animals at 22·5 °C might be slightly lower than those recorded in this paper.

The very low oxygen consumption of ammocoetes may well be a major factor in enabling the animals to colonize the silt banks in slow-flowing areas where oxygen tensions must often be low. As an extension of this hypothesis an investigation has been carried out into their behaviour and ability to survive in water with very reduced oxygen concentrations (Potter, Hill & Gentleman, 1970).

The greatly reduced oxygen consumption of *I. hubbsi* at 3·5 °C can be related to the physiology and ecology of the animals. Our field studies indicate that ammocoetes of this species show little or no increase in length during the winter months when temperatures are low. Culture of cells from ammocoetes of *Mordacia mordax* has shown that in this species growth is dependent on temperature (Stephenson & Potter, 1967), while Hardisty (1961) has correlated increases in growth and fat storage in ammocoetes of *Lampetra planeri* with increases in the density of phytoplankton in spring. Thus it may well be selectively advantageous for an ammocoete to reduce its respiration greatly during the winter until the following spring when temperatures rise and more food is available. It is interesting to note in connexion with this hypothesis that the copepod *Calanus hyperboreus*, which is also a herbivorous filter-feeder, has a reduced metabolism during the winter and increased growth and fat storage during the spring and summer when there is a greater amount of phytoplankton present (Conover, 1968).

The value of 0·718 for b in the equation relating the oxygen consumption and weight of medium-size animals at 15·5 °C is well within the range of values drawn up by

Winberg (1956) for a number of fresh water and marine fishes which had a mean of 0·8. This indicates a normal correlation between these parameters in ammocoetes of *I. hubbsi*, in contrast to the lack of relationship between weight and rate of oxygen consumption recorded for the hagfish *E. stoutii* by Munz & Morris (1965). These authors also reported a remarkably low rate of oxygen consumption (8–10 μl/g/h at 10 °C) in this species.

Kleerekoper, Taylor & Wilton (1961) found that the circadian rhythm of activity of transforming and adult *Petromyzon marinus* was lost when the animals were kept under constant dim light. The constant light and temperature to which our ammocoetes were exposed for a lengthy period before experimentation may have led to the elimination of a circadian rhythm of oxygen uptake that might be present under natural conditions.

The rate of oxygen consumption in the ammocoetes of *I. hubbsi* is lower than the values given by Winberg (1956) for several teleosts of similar weight. The results of Edwards, Finlayson & Steele (1968) indicate that even the relatively inactive plaice, *Pleuronectes platessa*, has a rate several times higher than that of an ammocoete of similar weight measured at the same temperature.

Due to differences in weight it is difficult to get an exact comparison between the rate of oxygen consumption of ammocoetes of *I. hubbsi* and that of the hagfish *E. stoutii*, but it is apparent that the rates are low in both cases. The adults of two anadromous parasitic species of lamprey have been studied. Scherbakov (see Winberg, 1956) gave a value for *Lampetra fluviatilis* of 0·14 mg/g/h (98 μl/g/h) at 16·6 °C for animals of 37 g mean weight, while Wikgren (1953) found the rate in *Petromyzon marinus* of *c.* 40 g weight, to lie between 65 and 168 μl/g/h in a temperature range of 7–18·5 °C. It is clear that the rate of oxygen consumption of the large adult lampreys which have been studied is markedly higher than that of *E. stoutii* which is of comparable weight. Furthermore, the values for adult lampreys are relatively greater than those we recorded for the ammocoetes of *I. hubbsi* when one takes into account the differences in weight, as can be seen by extrapolation of the relationship shown in Fig. 3 between rate of oxygen consumption and weight of animals. In view of the great changes undergone at metamorphosis, which includes the development of a definitive thyroid, it is hardly surprising that there would appear to be change in the relationship between oxygen consumption and weight at transformation. While there is now good evidence that ammocoetes and hagfishes are characterized by low rates of oxygen consumption, further data for ammocoetes of other species, and also for the adults of non-parasitic species, are necessary before one can generalize with complete confidence about this aspect of the physiology of the living representatives of the Agnatha.

SUMMARY

1. A continuous flow respirometer suitable for measuring oxygen consumption in some small burrowing aquatic animals is described.

2. Rates of oxygen consumption in ammocoetes of the lamprey *Ichthyomyzon hubbsi* are low, with mean values at 15·5 °C ranging from 38·8 to 97·1 μl/g/h for large (3·44 g) and small (0·14 g) animals respectively.

3. A Q_{10} of 3·6 was found for medium-size animals (1·18 g) between 3·5 and 22·5 °C.

4. The slope of the logarithmic linear regression relating weight and oxygen consumption was 0·718 at 15·5 °C.

5. The rates of oxygen consumption are discussed with respect to the ecology of ammocoetes and compared with those obtained for other lower vertebrates.

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