

OXYGEN CONSUMPTION, VENTILATORY
FREQUENCY AND HEART RATE OF LAMPREYS
(*LAMPETRA FLUVIATILIS*) DURING THEIR
SPAWNING RUN

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SUMMARY

1. The standard rate of oxygen consumption, ventilatory frequency and heart rate of adult *Lampetra fluviatilis* were measured during the light phase of the photoperiod and at times corresponding to various stages in the upstream migration.

2. All three parameters increased during the spawning run but only in mature individuals were significant differences found between the sexes.

3. The regression coefficients for the logarithmic relationship between oxygen consumption and body weight of immature animals were 0.912 and 0.925 at 9.5 and 16 °C respectively.

4. Both the standard rate of oxygen consumption and the amount of oxygen taken up during activity increased greatly during the hours of darkness.

5. Oxygen consumption, ventilatory frequency and, to a lesser extent, heart rate increased significantly at 9.5 °C over the 100-20% range of saturation with air.

6. Below 20% saturation with air, lampreys no longer remained attached by their oral disc for prolonged periods and the ventilatory frequency rose even more rapidly to reach a maximum of 175 beats/min at 12.5%. Exposure to 7.5% resulted in death within 5-8 h.

INTRODUCTION

Recent studies on lampreys have shown that both ammocoetes and adults will settle down and exhibit little or no movement under certain experimental conditions (Potter, Hill & Gentleman, 1970; Claridge, Potter & Hughes, 1973), thus enabling standard oxygen consumption (see Beamish & Mookherjee, 1964) to be measured directly (Hill & Potter, 1970; Potter & Rogers, 1972; Beamish, 1973). Differences between the standard rate of oxygen consumption of different life cycle stages can generally be attributed to the marked behavioural, morphological and physiological changes that take place at metamorphosis. In the context of respiration, the reorganization of the branchial region is of special significance since it results in a switch from a unidirectional to a tidal respiratory water current. As Randall (1972) has pointed out, the

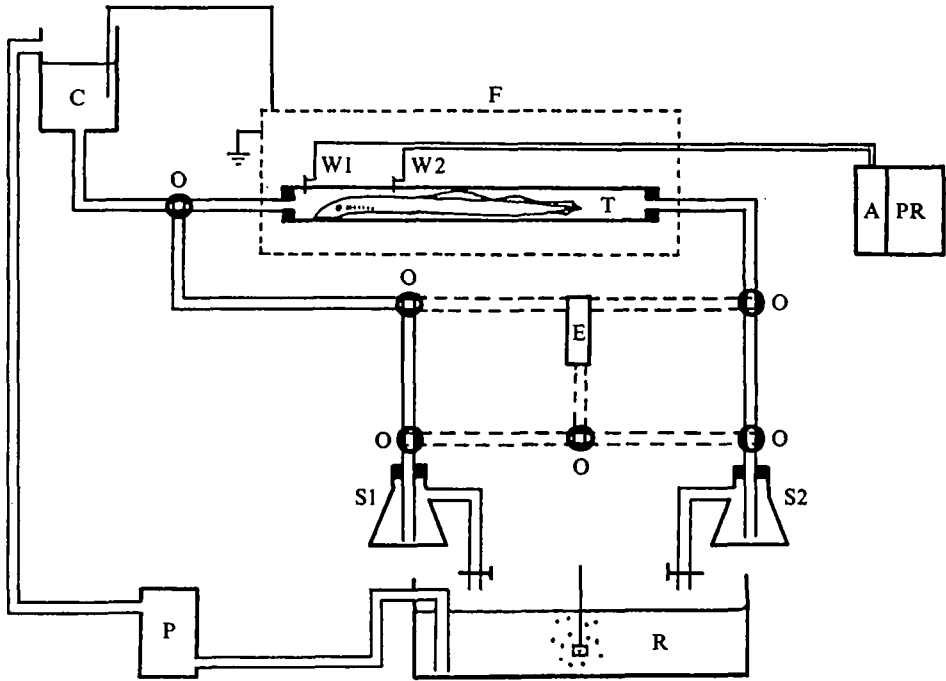


Fig. 1. Diagram of apparatus used to measure oxygen consumption, ventilatory frequency and heart rate. A, PR, Amplifier and pen recorder; C, constant head apparatus; E, oxygen electrode; F, shielded cage; O, three-way taps; P, filter pump; R, water reservoir and aerator; S₁ and S₂, sampling bottles for oxygen determinations; T, tube containing lamprey; W₁ and W₂, electrodes for detecting respiratory potentials and ECG.

latter is unique amongst aquatic vertebrates and is apparently an adaptation to feeding on host fishes by means of a suctional disc.

In the present investigation, the rate of oxygen consumption, and the concomitant ventilatory and cardiac frequencies, have been determined in the adult River lamprey, *Lampetra fluviatilis*, at various times during its spawning migration. These results, obtained during the light phase of the photoperiod when animals were at their least active, are contrasted with those measured during the hours of darkness. A study was also carried out on the physiological responses to hypoxia and of the level of reduced oxygen that can be tolerated. Comparisons have been made with previous work and with those on similar aspects of the physiology of larval lampreys.

MATERIAL AND METHODS

River lampreys were collected from just below the weir near Tewkesbury on the River Severn between October, when the animals were starting their entry into fresh water, and January of 1972/3 and 1973/4. They were acclimated in a constant temperature room for three or more weeks to a temperature of 9.5 ± 0.5 °C and to a light/dark cycle paralleling field conditions. Some of the animals were acclimated to a higher (16 ± 0.5 °C) or lower (5 ± 0.5 °C) temperature by changes upwards or downwards of 1 °C per day, and then left at these temperatures for at least a further two weeks before examination. A number of lampreys were also captured in April on their

spawning beds at Tenbury Wells on the River Temе, a tributary of the Severn, but these were maintained and studied at only a single temperature (9.5 °C).

River temperatures at the time when animals were first caught lay between 8 and 10 °C. The temperature decreased during the subsequent weeks to reach a minimum of approximately 4–5 °C before rising in the spring to 10–11 °C at which level spawning was observed.

Oxygen consumption, respiratory frequency and heart rate of lampreys at the three acclimation temperatures were recorded at intervals from the commencement of the spawning run. The respirometer consisted of a tubular animal chamber made of polythene, supplied with a continuous flow of dechlorinated, aerated water from a constant head reservoir (Fig. 1). A three-way tap in the supply line enabled an equal volume of water to flow through a sampling bottle, thereby providing a measure of the oxygen concentration of the water passing over the lamprey. The amount of oxygen taken up by the animal was then calculated from the difference between this value and that obtained from a bottle located just after the animal chamber and before the main 50 l reservoir. Oxygen concentrations were determined by Winkler analysis using the semi-micro syringe pipette method of Whitney (1938). The water circulation between the main reservoir and the constant head was completed by an Eheim filter pump, the flow rate through the animal chamber being maintained at 40–60 ml/min. By adjusting the position of the electrodes recording the respiratory potentials (Claridge *et al.* 1973) it was also possible to pick up the electrocardiogram. This enabled the calculation of both ventilatory frequency and heart rate from the same trace. No oxygen determinations or traces were made until the animal had been in the apparatus for at least 18 h. Subsequent measurements for standard values were carried out only at those times during the light phase when the lamprey was inactive and attached for long periods to the side of the tube.

The respirometer was also used in March at 9.5 °C to provide comparative rates of oxygen consumption of ten lampreys during the hours of darkness. The oxygen content of the water that had passed over the lamprey was recorded continuously on a Heath flat-bed recorder using a Radiometer oxygen electrode and meter. The deviations of this concentration from the base line, which reflected the oxygen content of the water flowing into the animal chamber, enabled estimates to be made of the standard and maximum rates of oxygen consumption. The area between the two traces on the pen recorder was measured for each hour on a Hayashi Denko Automatic Area Meter, and compared with an area equivalent to a known rate of oxygen uptake. In this way, the mean oxygen consumption of the animal per hour could be calculated.

Hypoxia experiments were performed in November and December on eight different lampreys acclimated to 9.5 °C. Reduced oxygen concentrations were achieved by bubbling differing flow rates of nitrogen into the constant head reservoir: this enabled the desired level of saturation to be reached and held constant within 10 min. Measurements of the oxygen consumption, ventilatory frequency and heart rate of each animal were made over a 10 min period, following a preliminary exposure of 30 min during which the experimental animals were subjected to water at 90% of air saturation. The water was then resaturated with air and the lamprey left for 30 min before repeating the above procedure for each of the subsequent 10% reductions in level down to 20% of full air saturation.

In January, studies on lethal oxygen concentration levels were carried out in a 32 l glass tank into which nitrogen and compressed air were bubbled, the mixture of these gases being controlled by flowmeters. The lid, which was tight fitting with only a small aperture in one corner, could be moved slightly to one side for rapid sampling of water for oxygen determinations. An animal was regarded as dead when no respiratory pumping had been detected for 10 min. This represented a reliable criterion of death as these animals did not recover on transfer to fully saturated water, whereas ventilatory movements were sometimes observed after being absent for periods of up to 5 min. Dead animals were removed immediately and, where there was complete or partial survival in each batch of five lampreys, the experiment continued for 96 h.

At the termination of all experiments, the total length, wet weight and sex of each lamprey was recorded.

RESULTS

Lampreys, kept at 9.5 °C in the laboratory from January developed secondary sexual characters (see Hardisty & Potter, 1971) in mid-April at approximately the same time as those found in the field. In the males, the testis showed a breakdown of the lobule wall and the release of sperm into the body cavity, while in females the diameter of the oocytes had reached the normal size at maturity, i.e. slightly greater than 1 mm. Animals acclimated to 16 °C from December onwards became very active in the daytime during March and although this behaviour is typical of spawning individuals, their secondary sexual characters were not fully developed. Furthermore, the testis was still intact and the oocytes measured only 800–900 µm in diameter. Animals displaying this condition at this temperature generally did not survive for much longer than a week. Lampreys held at 5 °C failed to reach maturity even by May, when they showed the same gonadal and somatic development as the March animals acclimated to 16 °C. No significant difference ($P > 0.05$) was found between either the length or the weight of males and females at the different times. The mean length and weight (± 1 S.E., and the range) for the River lampreys used in the experiments was 29.5 cm (± 0.22 , 23.7–35.4) and 46.8 g (± 0.99 , 21.4–78.1) respectively.

The oxygen consumption, respiratory frequency and heart rate of males did not differ significantly ($P > 0.05$) from the respective values obtained for females examined at the same time, with the exception of the sexually mature animals. In the latter, the oxygen consumption and respiratory frequency of both laboratory-held and field-caught individuals differed significantly between the sexes ($P < 0.001$). With respect to heart rate, a lower level of significance ($P < 0.05$) was observed in the case of animals taken from the spawning beds and no significant difference was found in those brought through to sexual maturity in the laboratory. For these reasons, the data have been pooled for the two sexes apart from those recorded for fully mature individuals.

At 9.5 °C the mean standard oxygen consumption increased during the time period equivalent to the spawning run in the field (Fig. 2). Although a similar pattern was found at 5 and 16 °C, the approach of sexual maturation was delayed or hastened by the lower and higher temperature respectively. The increase in the standard rate of oxygen consumption with time was paralleled at all three temperatures by similar changes in the respiratory and cardiac frequencies (Figs. 3, 4). The Q_{10} for the

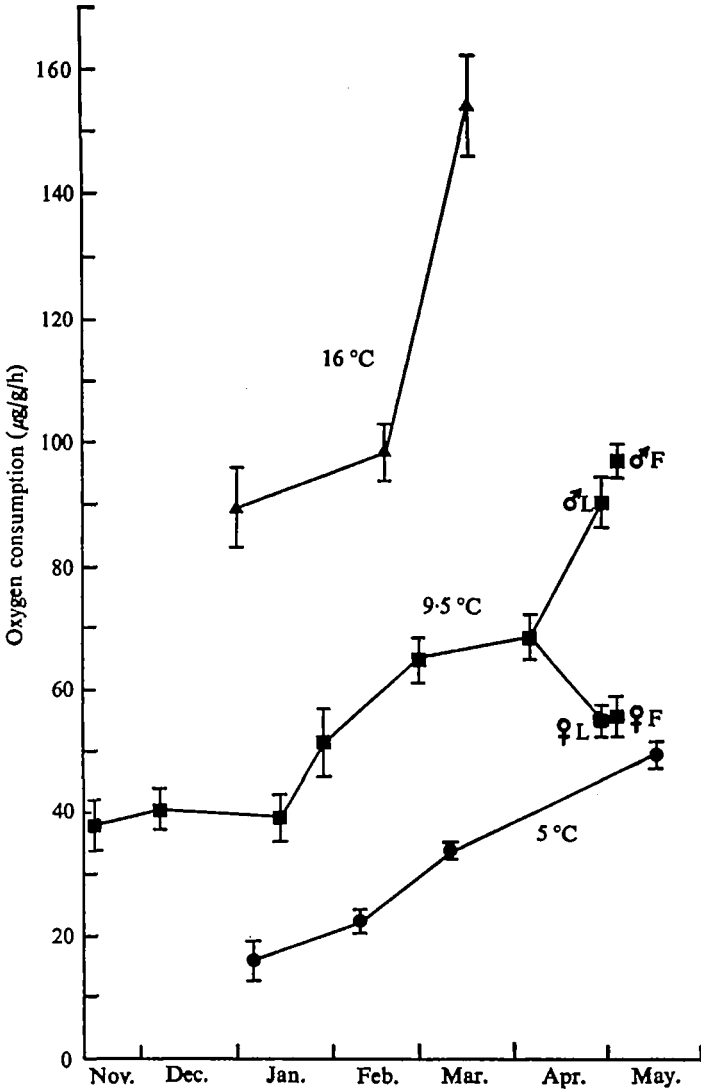


Fig. 2. Standard oxygen consumption (mean \pm 95% confidence limits) of adult *Lampetra fluviatilis* measured at 5, 9.5 and 16 °C. Data for both sexes in this and in Figs. 3 and 4 are pooled, apart from in the case of the sexually mature animals. ♂L and ♀L represent values for animals held in the laboratory since January while ♂F and ♀F correspond to those recently brought back from the spawning beds.

physiological measurements made on animals at the beginning of their spawning run was highest in the case of oxygen consumption and lowest for heart rate (Table 1).

The logarithmic relationship (Fig. 5) between the oxygen uptake (Y) and the body weight (X) of lampreys at 9.5 and 16 °C at the commencement of their spawning run can be represented by the following equations.

$$\log Y = -3.987 + 0.912 \log X \quad (r = 0.847) \quad N = 21,$$

$$\log Y = -3.693 + 0.925 \log X \quad (r = 0.879) \quad N = 10.$$

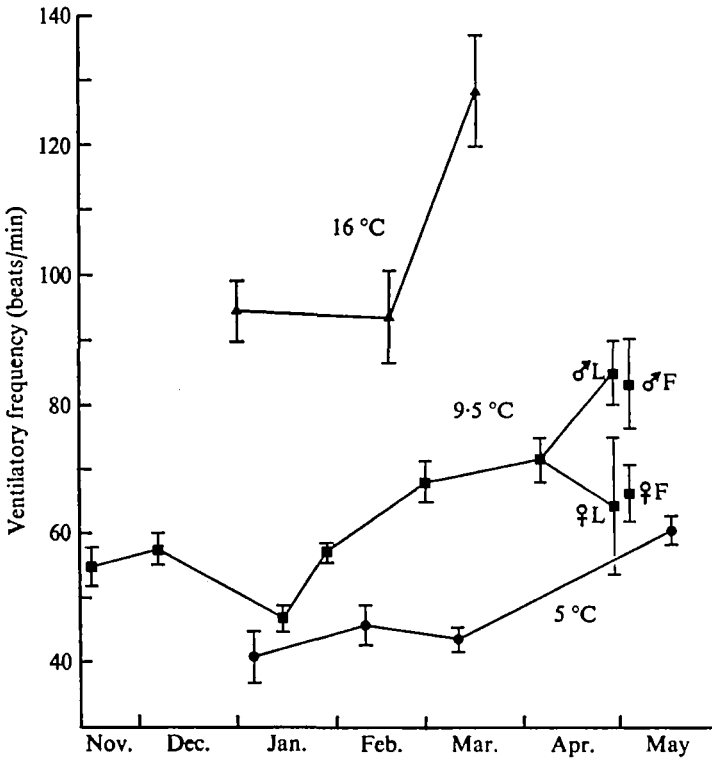


Fig. 3. Resting ventilatory frequency (mean \pm 95% confidence limits) of adult *Lampetra fluviatilis* recorded at 5, 9.5 and 16°C. For data on animals and captions see Fig. 2.

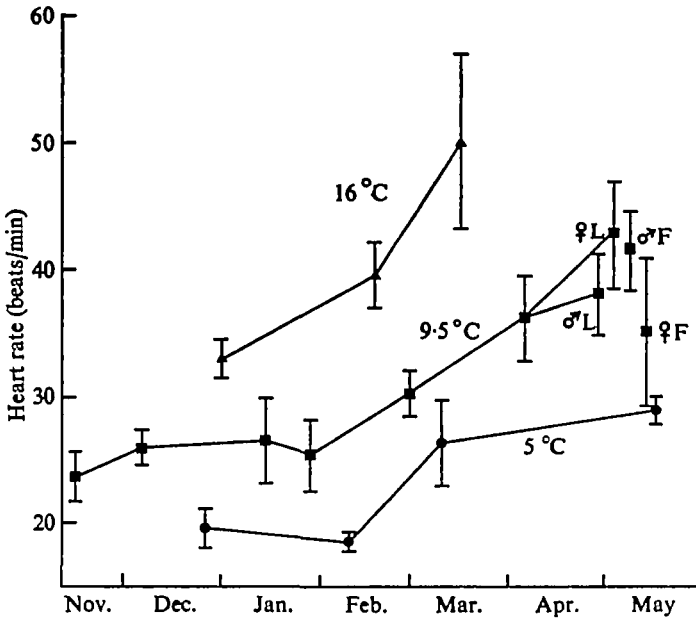


Fig. 4. Resting heart rate (mean \pm 95% confidence limits) of adult *Lampetra fluviatilis* recorded at 5, 9.5 and 16°C. For data on animals and captions see Fig. 2.

Table 1. The Q_{10} for physiological measurements on adult *Lampetra fluviatilis* at the commencement of their spawning run

Temperature range (°C)	Oxygen consumption	Ventilatory frequency	Heart rate
5.0-9.5	7.73	2.17	1.82
9.5-16.0	3.48	2.13	1.44
5.0-16.0	4.83	2.15	1.59

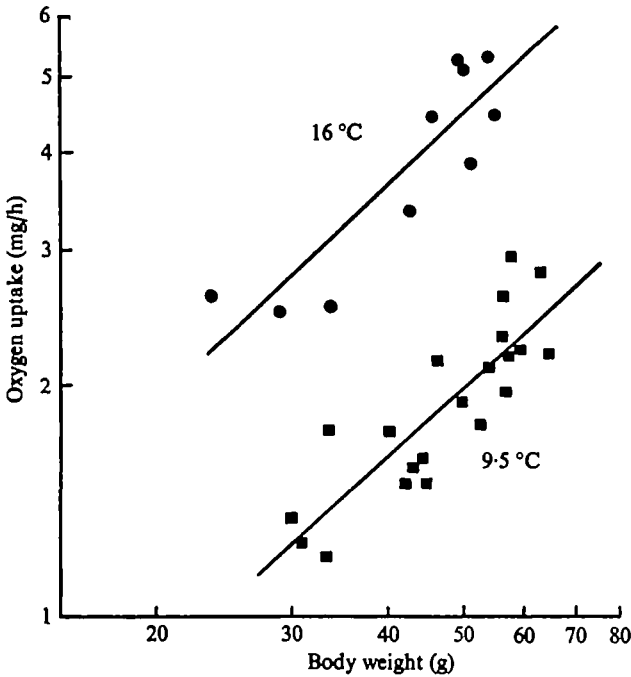


Fig. 5. The relationship between oxygen consumption and body weight of adult *Lampetra fluviatilis* measured at 9.5 and 16 °C during the early stages of their upstream migration, i.e. between October and January.

At 5 °C the relationship, based on only five animals, could be expressed as

$$\log Y = -4.833 + 1.002 \log X \quad (r = 0.612).$$

Although the rate of oxygen consumption remained at almost the same level throughout the day, examination of individuals in March over a 24 h period revealed an increase during the hours of darkness (Fig. 6). This elevation in metabolic rate occurred both in the standard oxygen consumption and in the amount of oxygen taken up during activity. After the cessation of activity, the rate rapidly returned to a level similar to that just prior to the initiation of movement.

Measurements of oxygen uptake were obtained at 9.5 °C for several sexually mature lampreys at times when they were showing vigorous movement during the daytime as is typical of spawning individuals. The rates, which ranged between 117.4 and 585.3 $\mu\text{g/g/h}$, encompass the mean value at 10 °C of 475.5 $\mu\text{g/g/h}$ given by Beamish

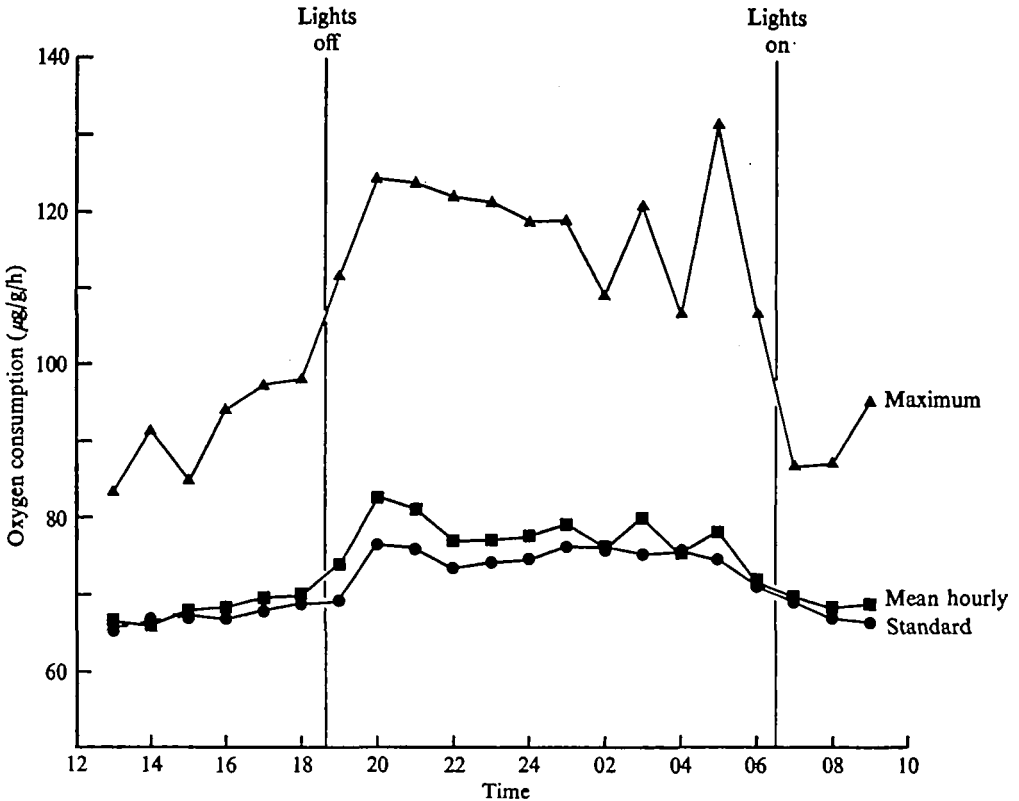


Fig. 6. The maximum, mean hourly and standard rate of oxygen consumption during the dark phase of a 24 h photoperiod in March. Values are also given for the immediately preceding and following hours when the animals were exposed to light.

(1973) for active trophic stages of the landlocked Sea lamprey, *Petromyzon marinus*, and are comparable to that of active salmonids.

Since the lampreys exhibited little or no movement in the respirometer down to 20% saturation with air, the apparatus proved ideal for the study of the physiological responses of the animal to a wide range of hypoxic conditions. At lower concentrations, however, the animal became so active that electrical recording of the ventilatory and heart frequencies became impossible and oxygen uptakes could no longer be taken as corresponding to standard. It was thus necessary to use the tank system where the animal could be kept under constant examination and the respiratory frequency determined visually.

Recordings of ventilatory frequencies at very low concentrations were restricted to lampreys attached to the side wall of the tank, but these became difficult to make because of the increase in the number of detachments and periods of violent swimming. At concentrations of 9.5% of air saturation the behaviour pattern changed again, with animals lying on their sides for long periods and only occasionally showing swimming movements. The lamprey was capable of surviving concentrations of 9.5% for at least 96 h, but died within 5–8 h at approximately 7.5%. At this latter concentration the respiratory pumping was erratic and often ceased for up to five minutes at a time just prior to death.

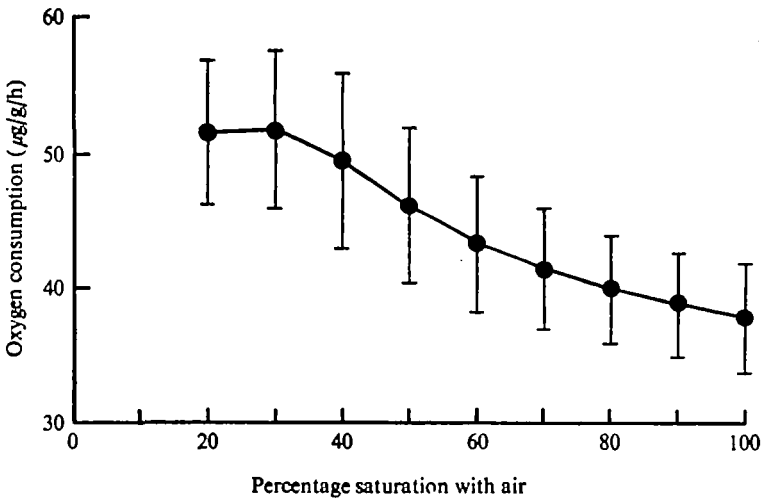


Fig. 7. The rate of oxygen consumption (mean \pm 95 % confidence limits) of adult *Lampetra fluviatilis* exposed in January at 9.5 °C to oxygen concentrations varying from 100 to 20 % saturation with air.

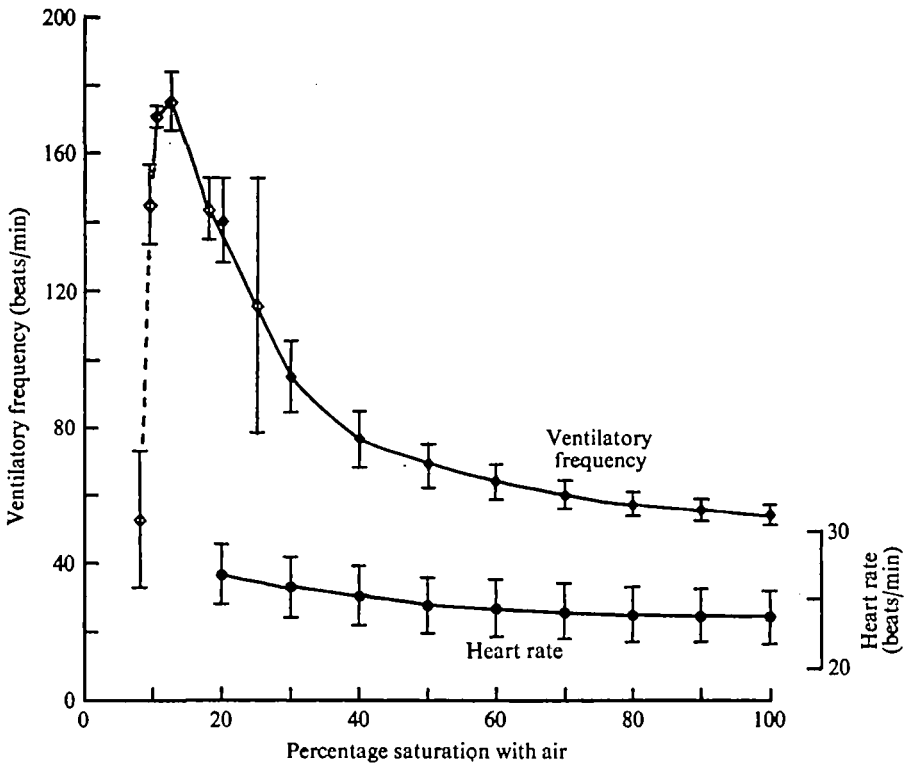


Fig. 8. The ventilatory frequency and heart rate (means \pm 95 % confidence limits) of adult *Lampetra fluviatilis* exposed in January at 9.5 °C to a range of saturation with air. The value at 7.5 %, a lethal concentration, represents the mean taken 1 h after the start of the experiment. \blacklozenge , Ventilatory frequency recorded in respirometer; \diamond , ventilatory frequency recorded in tank system; \bullet , heart rate recorded in respirometer.

The oxygen consumption at 9.5 °C rose gradually from 37.9 to 51.7 µg/g/h ($P < 0.001$) over the 100–30% saturation range, but no further increase occurred between 30 and 20% (Fig. 7). Although there was a highly significant difference ($P < 0.001$) between the ventilatory frequencies of 54.8 and 140.8/min recorded at the extremes of the percentage saturation range (100–20%), the heart rate showed a relatively smaller increase ($P < 0.05$) with respective means of 23.7 and 26.7/min (Fig. 8). Under normoxia, only the first two pairs of gill pouches could be observed pumping but, as the oxygen concentration in the water was lowered, both the amplitude of contraction and the number of pouches involved increased until, at 60% of air saturation, all seven pairs appeared to be exhibiting maximum irrigation.

The respiratory frequencies of animals in the tank at 20% corresponded closely with those recorded in the respirometer at the same concentration and continued to rise as the saturation was lowered, reaching a maximum of 175.4/min at 12.5% (Fig. 8). However, with the animal exhibiting reduced activity and using its sucker less frequently for attachment, the values dropped to 145.4/min at 9.5%. Lampreys displaying brachial pumping an hour after the commencement of exposure to a lethal oxygen concentration (7.5%) showed a mean rate of 53.0/min, this declining over the ensuing period to death.

DISCUSSION

This investigation shows the importance in physiological studies with lampreys of stating both the stage in the life cycle and the temperature regime to which the animals have been subjected. For example, the standard rate of oxygen consumption of adult *L. fluviatilis*, although remaining constant during the first half of the upstream migration, undergoes an approximately twofold increase during the final three months to sexual maturity. This elevation may be connected with the increase in sex hormone levels that, at least in males, takes place between February and the onset of spawning in April (Barnes & Hardisty, 1972), thus apparently paralleling a similar correlation between metabolism and endocrine function in the Brook trout, *Salvelinus fontinalis* (Beamish, 1964).

The effect of the temperature regime on maturing lampreys can be seen by the differences in gonadal and somatic development of animals held in the laboratory at 5 and 16 °C until May and March respectively. Thus, the oocytes of both groups at these times were only 80% of their normal mature size, even though rudimentary secondary sexual characters were present and, at 16 °C, the animals were displaying the daytime activity characteristic of spawning individuals. These data parallel the observations of Larsen (1973) who recorded delayed or hastened sexual maturation in *L. fluviatilis* acclimated to 6 and 11 °C respectively. On the other hand, 9.5 °C apparently proved to be a satisfactory holding temperature as the lampreys reached maturity at the same time as those in the field. Furthermore, values for the various physiological parameters were similar in both laboratory-held and field-caught sexually mature individuals. The markedly different rates of oxygen consumption in males and females at spawning may reflect the uptake of water by females at this stage which brings about a relative increase in body weight. Since this weight increase is unlikely, however, to have been entirely responsible for the differences between the sexes, the situation

In lampreys apparently parallels that found by Hughes & Knights (1968) in the Dragonet, *Callionymus lyra*, where in the mature stages a higher metabolic rate was recorded for males.

Previous studies on oxygen consumption in adult *L. fluviatilis* (Scherbakov, 1937; Wikgren, 1953; Korolewa, 1964) have given variable but consistently higher values than those we have recorded for similar temperatures, a feature which can probably be attributed to the following factors. In our study, measurements were made on animals maintained at high oxygen levels, a process facilitated by the use of continuous flow rather than closed system respirometry, and could be restricted to those periods when the animal was known to be inactive. The former of these two points is of importance since oxygen consumption in *L. fluviatilis* has been shown to increase with decreasing ambient oxygen levels, even at relatively high percentage saturations (Fig. 7).

In his study on the trophic phase of the landlocked Sea lamprey, *Petromyzon marinus*, Beamish (1973), using similar acclimation and experimental procedures to ourselves, obtained regression coefficients of 0.966 and 0.949 at 10 and 15 °C respectively for the logarithmic relationship between oxygen uptake and body weight. These are close to our values of 0.912 and 0.925 for adult *L. fluviatilis* at similar temperatures, but are appreciably higher than the 0.718 and 0.780 recorded for ammocoetes of *Ichthyomyzon hubbsi* and *Lampetra planeri* at 15 °C (Hill & Potter, 1970; Potter & Rogers, 1972). This difference may be attributable to the fact that in the larval studies several different year classes were represented.

The rates of oxygen consumption recorded for *P. marinus* and *L. fluviatilis*, together with those for the Pacific lamprey, *Lampetra tridentata*, quoted by Johansen, Lenfant & Hanson (1973) show considerable variation. For example, the rates at 5 °C were 30.3 µg/g/h in *L. tridentata*, 52.7 µg/g/h in *P. marinus* and 15.8 µg/g/h in *L. fluviatilis*, although the rate in the latter species did increase to 49.5 µg/g/h at a time corresponding to much later in the spawning run. At 15–16 °C, the respective values for the same three species were 49.4, 114.3 and 89.4 µg/g/h. Such differences are probably not due so much to interspecific variation as to differences either in the trophic status of the animal or in the regime to which it was subjected prior to experimentation. Despite the variations, however, it is clear that adult lampreys have a relatively high metabolic rate, comparable with many elasmobranchs and teleosts (see Hughes & Knights, 1968; Prosser, 1973), and appreciably higher than the value of 11.4–14.3 µg/g/h recorded at 10 °C for the hagfish, *Eptatretus stoutii* (Munz & Morris, 1965). Comparisons between the ammocoete and adult stages in the lamprey life cycle are made difficult by the marked difference in body weight. Burrowed ammocoetes of *I. hubbsi* and *L. planeri* weighing just over 1 g, however, exhibited oxygen uptake rates of between 55 and 75 µg/g/h at 15 °C (Hill & Potter, 1970; Potter & Rogers, 1972) compared with values of 114.3 and 89.4 µg/g/h in adult *P. marinus* and *L. fluviatilis* that were many times heavier. The increased rate in the adult lamprey is even more marked when the regression slope of less than one for the logarithmic relationship between oxygen uptake and body weight is taken into account.

The increase in both standard and maximum oxygen consumption in adult *L. fluviatilis* during the hours of darkness, parallels the nocturnal elevations in activity, ventilatory frequency and heart rate in the same species (Claridge *et al.* 1973). The

rhythmicity in the standard metabolism of lampreys is almost certainly related to the marked increase in movement that takes place at night, particularly in post-metamorphic stages (Hardisty & Potter, 1971; Potter & Huggins, 1973), a feature which may be associated with the presence of a well developed pineal gland (Eddy, 1972; Claridge *et al.* 1973).

Although the rate of oxygen uptake in *L. tridentata* remained constant down to 10 mmHg at 5 and 15 °C and 35–40 mmHg at 20 °C (Johansen *et al.* 1973), the metabolic rate in the River lamprey increased over the range 100–30% air saturation. Since there was no apparent change in the activity level of *L. fluviatilis* at saturations above 20%, this rise was almost certainly attributable to the increased metabolic cost of ventilation, a situation similar to that found in the trout (Hughes & Saunders, 1970). The dramatic change in the animal's behaviour at lower concentrations is clearly an avoidance reaction to unfavourable conditions, paralleling the response of teleosts and elasmobranchs to reduced oxygen levels just above those known to be lethal (Whitmore, Warren & Doudoroff, 1960; Randall, 1970).

The response of the ventilatory system to hypoxia in *L. fluviatilis* was similar to the patterns observed for adult *L. tridentata* and larval *I. hubbsi*, the maximum frequency being approximately two to three times greater than the resting value at full saturation (Potter *et al.* 1970; Johansen *et al.* 1973). The ventilation volume in adult lampreys undoubtedly rises more than this, however, as the number of pouches used and the amplitude of contraction of the branchial region also increased during hypoxia. The marked elevation in the ventilatory frequency of *L. fluviatilis* during periods of attachment at concentrations below 20% of full saturation is presumably attributable not only to the response of the respiratory pump to severe depletion but also to a feedback from the additional metabolic cost of the frequent swimming movements. The reduced frequency at 9.5%, compared with high rates at 12.5%, undoubtedly reflects the reduced activity level of the animal just above the lethal oxygen concentration. The tendency for apnoea to occur at the lethal saturation (7.5%) is possibly a forerunner of total respiratory failure and parallels the situation found in catfish at extremely low oxygen levels (Marvin & Heath, 1968).

A reduction in the ambient oxygen concentration below certain levels is accompanied in both elasmobranchs and teleosts by a marked bradycardia (Marvin & Heath, 1968) due to increased vagal tone (Shelton, 1970). There is, at least in the trout, however, an increase in stroke volume to maintain a relatively constant cardiac output (Holeton & Randall, 1967). The absence of hypoxic bradycardia has been found, however, by Saunders & Sutterlin (1971) in the Sea raven, *Hemitripterus americanus*, where at most only a transitory change was observed in heart rate down to oxygen tensions as low as 10 mmHg. In contrast to these data, the lamprey heart rate increased gradually over the range 100–20% saturation with air, although it is possible that bradycardia occurs at lower concentrations.

The results of this study show the relative ease by which standard oxygen consumption can be monitored in adult lampreys without having to extrapolate from data obtained for swimming animals. This is particularly important when one considers that lampreys, although capable of intense activity for short periods, cannot always be made to swim continuously (Beamish, 1973). Furthermore a comparison with ammocoetes can be made as they too will settle down and exhibit little or no

movement when they are provided with a suitable substrate (Hill & Potter, 1970; Potter & Rogers, 1972). This facility for comparisons may be especially useful in the future for studying aspects of metamorphosis during which marked changes take place in the respiratory, endocrine and other systems.

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