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SURVIVAL AND BEHAVIOUR OF AMMOCOETES AT LOW OXYGEN TENSIONS

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INTRODUCTION

There have been comparatively few studies on respiration in the ammocoete larva of lampreys. For this reason an investigation of the oxygen consumption of ammocoetes, living in burrows in a simulated natural substrate, was made to gain some knowledge of the oxygen requirements of these animals (Hill & Potter, 1970). In the present study we have attempted to relate aspects of the respiratory physiology of ammocoetes to their behaviour and survival in low oxygen tensions.

Ammocoetes are generally found in burrows in the silt beds at the margins of streams and rivers. They never appear to be found in stagnant areas, although the flow of water over their burrows is often slow. These observations, together with the fact that when aeration is turned off in laboratory aquaria ammocoetes emerge after a period from the substrate and eventually die, suggested that they might be sensitive to low oxygen tensions and unable to survive in very low concentrations. We therefore decided to examine this aspect at various low partial pressures of oxygen. An investigation was also made of the duration of survival of ammocoetes under deoxygenated conditions as Blažka (1958) has shown that another lower vertebrate, the Crucian carp, is able to tolerate anoxia for up to 2 months at a low temperature (5 °C). Since emergence may be dependent upon the amount of carbon dioxide in the water, a preliminary investigation was also made into the effects of this gas.

Lampreys and hagfishes, the only two extant groups of agnathan vertebrates, are the lowest chordates possessing haemoglobin. In both groups the haemoglobin is a monomer (Manwell, 1963; Briehl, 1963) in contrast to the tetramer found in higher vertebrates. Manwell (1963) examined the oxygen equilibria of erythrocyte suspensions and found that the haemoglobin of the ammocoetes of *Ichthyomyzon unicuspis* had a considerably higher affinity for oxygen than that of their adults, a finding which he feels may reflect the mud-dwelling habits of the larval stage. We therefore measured the oxygen equilibria of the blood of ammocoetes of *Ichthyomyzon hubbsi* to determine whether there might be a correlation between these and the results of our survival experiments.

Although there are haematological data for adult lampreys (Kisch, 1951; Thorson, 1959; Ivanova Berg & Sokolova, 1959; Korzhuev & Glazova, 1967), no similar information for ammocoetes has been found in the literature. It certainly cannot be

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assumed that the blood of ammocoetes will be the same as that of adults, as a great change occurs in the physiology of the animal at metamorphosis including an alteration in the haemoglobins (Adinolfi, Chieffi & Siniscalco, 1959; Manwell, 1963; Potter & Nicol, 1968). Since values for pH, haematocrit and the concentration of haemoglobin in the blood are relevant to a discussion of the significance of haemoglobin in this group, measurements were made of these parameters.

MATERIALS AND METHODS

Ammocoetes of *Ichthyomyzon hubbsi* Raney were collected with an electric fish-shocker (Smith-Root Electronics, Type V) from Bent Creek, a small tributary of the French Broad River in western North Carolina, during later March and early May 1969. Animals were maintained in aerated aquaria under constant light at one of three temperatures ($5 \pm 0{\cdot}2$ °C, $15{\cdot}5 \pm 0{\cdot}3$ °C, or $22{\cdot}5 \pm 0{\cdot}5$ °C) for at least a week before experimentation.

Survival experiments were carried out in glass aquaria ($30 \times 20 \times 20$ cm) containing fully aerated aged tap water and a substrate of washed sand. A plastic lid was placed over each aquarium and made airtight by sealing with petroleum jelly. A constant flow of the gas mixture (4–6 l/h) was kept bubbling into the aquarium water through an aerating stone whose lead passed through a stopper in the lid. A second hole, which accommodated another stopper containing an exhaust tube, was used for sampling the water to ascertain oxygen concentration. In each case 20 individuals were divided between two experimental aquaria and the rate of branchial beat was recorded at intervals on those animals which were visible. The animals were allowed to settle down for 16 h before the overlying water was siphoned off and replaced by water equilibrated with the gas mixture to be used in the experiment. The criterion for death was taken as the point at which no movement could be discerned in the branchial basket or velum, when the tail region of the animal was prodded with a rod. This process was facilitated by the fact that ammocoetes always emerged in lethal oxygen tensions and lay on the surface of the substrate during the period prior to death. Dead animals were removed through one of the holes in the lid by means of a rod fitted with a Nylon loop. Experiments were carried out at three temperatures (5, 15·5 and 22·5 °C) on animals ranging in length from 70 to 160 mm. Owing to a shortage of the smallest sized ammocoetes, it was only possible to experiment with these at one tension range (12–16 mmHg) and one temperature (15·5 °C).

The respirometer described by Hill & Potter (1970) was used to examine the behaviour of ammocoetes exposed to elevated carbon dioxide or lowered oxygen tensions at 15·5 °C, as well as to provide some additional data on survival. After the animals had been in the apparatus for 16 h, during which time fully aerated water was drawn through the chambers, the gas content of the water in the system was changed. Carbon dioxide (flow rate 100 ml/h), or pre-purified nitrogen (flow rate 1–2 l/h), was bubbled into the carboy supply reservoir to bring about a gradual increase in carbon dioxide in the first case and a slow drop in oxygen in the second. The experiment was terminated in each of the chambers at the time when the animal first emerged from the substrate in that chamber. The oxygen tension of water in the sampling bottles was calculated from the concentration determined by means of the Winkler method as

described by Mackereth (1963). An estimate of carbon dioxide was obtained in the first experiment from the pH (measured to the nearest 0·01 pH unit with a Corning Model 12 Research pH meter) and the total alkalinity (Mackereth, 1963). In another series of experiments the animals were suddenly exposed to different gas tensions by replacing the supply carboy with one in which the water was fully equilibrated with either pure nitrogen or a gas mixture. Gas mixtures employed contained a ratio of oxygen to nitrogen of either 1:99, 2:98 or 3:97 (volume:volume).

A flow rate of 450 ml/h through the chambers was used in all experiments, causing a complete flushing of the system every 20 min. Frequent analyses were made of the gas-concentration of water entering the chambers. Fourteen animals were used in each experiment which was run for 10 h, except in the case of exposure to a partial pressure of 7–10 mmHg where it was continued until the animals died. The rate of branchial beat of each ammocoete was recorded at 15 min intervals, when the anterior part of the animal was visible. These experiments were performed on animals ranging in length from 70 to 130 mm.

Blood for determination of pH, haematocrit and haemoglobin concentration was taken from ten animals which had been kept in aquaria at 15·5° C for several days without disturbance. The animals were extracted from the substrate, quickly rolled in absorbent paper to remove surface water, and immediately cut in half in the post-cloacal region. Blood emerged instantaneously and the first drops were run into the corner of a Petri dish previously smeared with heparin. It was immediately sucked into a glass capillary and the pH was measured to the nearest 0·01 pH unit by means of an Astrup micro-electrode and a Radiometer Model 4 pH meter. Separate pH measurements were also made at 22·5 and 5·0 °C on blood obtained from each of eight and five animals respectively, which had been kept at that temperature. Fresh blood sucked into haematocrit tubes, internally coated with heparin, was centrifuged in an International Microcapillary Centrifuge Model M8 for 10 min. The haemoglobin concentration was measured by the cyanomethameoglobin method (Crosby, Munn & Furth, 1954), using Hemotrol (Preiser Scientific Inc. 70–4040) as a standard haemoglobin solution.

The oxygen equilibria of erythrocyte suspensions were determined by a method similar to that described by Manwell (1963). Blood from individual animals was suspended in a heparinized solution of 0·7% (w/v) NaCl in 0·02 M potassium phosphate buffer. Light scattering was reduced by addition of 2·5% bovine serum albumin. The blood was equilibrated against known gas mixtures and the oxygen equilibrium points were determined spectrophotometrically. Determinations were made at each of the three experimental temperatures on blood from eight animals held at that temperature, and at a lowered pH at 15·5 °C.

RESULTS

The oxygen partial pressure varied slightly during each of the survival experiments (Table 1) due mainly to slight differences in the degree of equilibration of the various gas mixtures with the water, and to the effect of removing the small plug from the aquaria to extract dead animals. It was never possible to obtain completely deoxygenated water by using 100% nitrogen, but the tensions obtained by this method were always very low.

The criterion for death at temperatures of 15·5 and 22·5 °C, namely the absence of a branchial or velar beat, indicates when the animal becomes moribund. This approximates to the time of death since animals rarely recover when placed in aerated water 30 min or more after cessation of beating. However, animals which exhibit very slow branchial beat can in most cases recover, and some animals are capable of reviving in the few minutes after the time when there has been no detectable branchial beat. This criterion is of little use at 5 °C in tensions below 2·5 mmHg, since animals that had exhibited neither branchial nor velar movement for several hours resumed branchial beating when placed in aerated water, and in several cases, re-burrowed within a further 24 h. To overcome this problem four sets of ten ammocoetes were exposed to deoxygenated water at 5 °C. One set was transferred to an aerated aquarium at each 12 h interval and observations made to determine whether or not the animals were capable of recovering.

Table 1. *Survival of ammocoetes of* Ichthyomyzon hubbsi *in water containing low oxygen tensions in (A) aquaria and (B) the respirometer*

(Twenty animals were used in aquaria experiments and run for 96 h, except in the case marked (†) which was terminated after 60 h. (*) Animals acclimated to oxygen tension of 12–16 mmHg for 4 days prior to experimentation. Fourteen animals were used in the respirometer and run for 10 h.)

	Temperature (°C)	Oxygen (mmHg)	Mean survival time (h)	S.E. of mean	Survival range (h)	Number alive at end of experiment
(A)	5·0	1·5–2·5	*c.* 36	—	24–48	0
	5·0	7–10	> 96	—	—	20
	15·5	2–4	4·1	0·29	3·0–5·3	0
	15·5	7–10	15·5	1·22	7·5–20	0
	15·5*	7–10	—	—	9·0– > 60	12†
	15·5	12–16	> 96	—	42– > 96	19
	22·5	13–16	—	—	16– > 96	6
	22·5	19–21	> 96	—	—	20
(B)	15·5	2–4	4·3	0·28	2·3–6·0	0
	15·5	7–10	9·6	1·41	4·3–24·0	0
	15·5	12–16	> 10	—	—	14

The results shown in Table 1 demonstrate that ammocoetes are capable of living in relatively low oxygen tensions and that there is an inverse relationship between lethal oxygen tension and temperature. However, ammocoetes are not able to survive anoxia even at 5 °C. These results also indicate that the difference between lethal oxygen tensions and the lowest oxygen tensions in which ammocoetes can survive is very fine. For example, whereas at 15·5 °C all animals died within a few hours at 7–10 mmHg, only one animal died in 12–16 mmHg over the 4 days of the experiment (Table 1A). Similar results were obtained in experiments using the respirometer. No animals died during the 10 h that the animals were exposed to 12–16 mmHg, whereas 12 of the 14 animals had died within this period in 7–10 mmHg (Table 1B). The more rapid death in the respirometer experiment is probably due to differences in the experimental regime. In the respirometer there is a rapid exchange of substrate water, whereas the interstitial water in the aquarium sand is oxygenated at the time when the water containing the gas mixture is introduced. However, the animals cannot

survive on the oxygen of the interstitial water for more than a short period of time, since they either emerge or raise their oral hoods to the surface of the substance thereby drawing in overlying water at the introduced gas tension.

The following description of the behaviour of ammocoetes is based on observations of individuals within the respirometer at 15·5 °C and is apparently identical to that of ammocoetes in the natural substrate of aquaria. Ammocoetes which have burrowed and have been exposed to fully oxygenated water for 16 h generally lie along the mesh of the chamber in a horizontal position with the ventral surface downwards (Fig. 1).

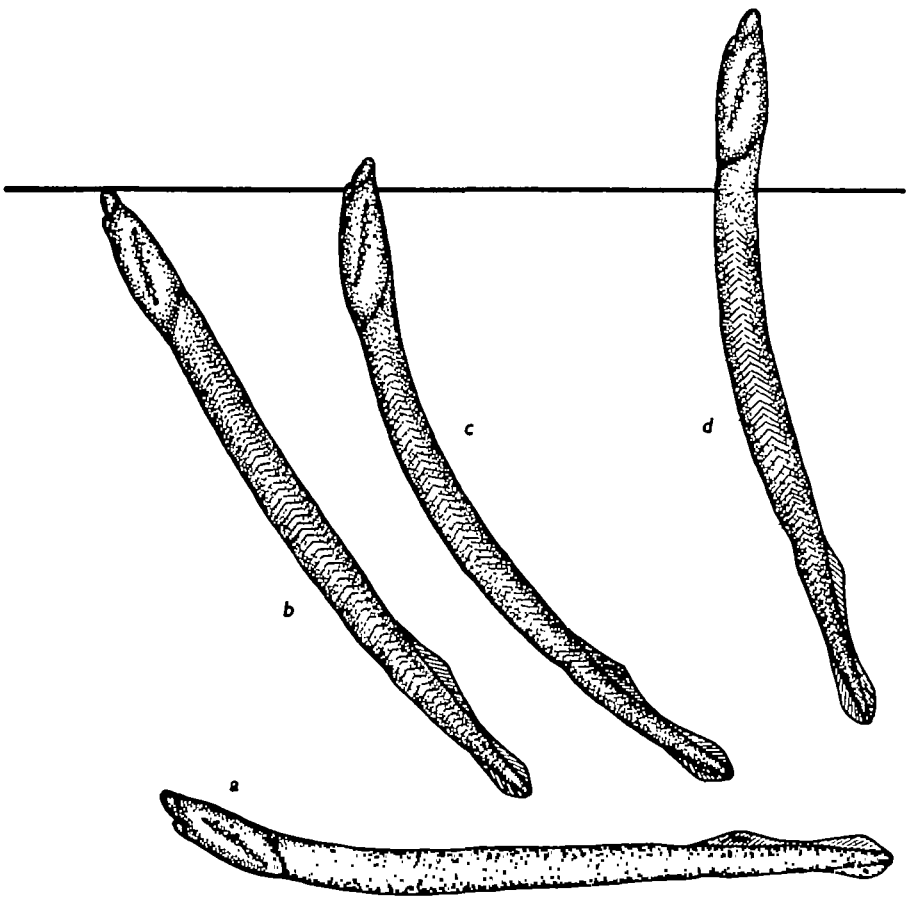

Fig. 1. Positions assumed by ammocoetes of *Ichthyomyzon hubbsi* when exposed to conditions of low oxygen.

In a few cases the oral hood is inclined slightly upwards. Under these conditions they were never seen to push their oral hoods out into the overlying water and they rarely moved.

Exposure to either high carbon dioxide or low oxygen tensions evoked a characteristic behaviour pattern. After a period of restlessness the oral hood was first pushed upwards at an angle in the substrate, eventually coming to lie just below the substrate surface (Fig. 1). The hood was then projected out into the overlying water. In some

cases this progressive series of movements was extended until the whole branchial basket was completely out of the substrate. The animal can retract back into the burrow from this position but occasionally emerges completely from the substrate and swims around. Ammocoetes may remain in any of the above stages for a few minutes to a much longer period.

Most ammocoetes remained in their burrows until the oxygen tension of the water approached a lethal level, as was shown by the experiments in which the oxygen tension of the water in the respirometer was slowly lowered over a few hours. Under these conditions ammocoetes emerged at a mean partial pressure of oxygen of 12·8 mmHg. The first animals to emerge did so at a partial pressure of 20 mmHg, after the experiment had been underway for 2½ h, and they continued to come out over the next 2 h, the last animal emerging at a partial pressure of 6·2 mmHg, by far the lowest oxygen value evoking emergence. The subsequent behaviour of ammocoetes which emerged depended on whether or not the oxygen tension of the water was lethal. This was clearly seen in the experiments in which animals were exposed to sudden changes in oxygen tension. In lethal tensions (Table 1), ammocoetes exhibited frequent swimming movements, which often involved swimming up into the tapered socket of the animal chamber. They then dropped to the surface of the substrate where they lay for a period before another period of swimming. Only rarely did they re-burrow in the substrate more than once. In an oxygen tension which was not lethal but did evoke emergence, such as 12–16 mmHg at 15·5 °C (Table 2, 3) ammocoetes generally showed repeated emergence, swimming and burrowing movements during the first few hours before finally remaining in the substrate with occasional exposure of their oral hoods or branchial baskets.

Table 2. *Time taken by ammocoetes of* Ichthyomyzon hubbsi *to emerge from the substrate in the respirometer at* 15·5 °C

Oxygen (mmHg)	Time of first emergence (h)	Mean emergence (h)	Number of animals out after 10 h
2–4	0·45	1·5	All dead
7–10	0·25	0·8	All dead
12–16	0·05	0·75	6

There is a marked difference in the partial pressure of oxygen that evokes emergence at 5 and 22·5 °C (Table 3). At 5 °C only three of the 20 ammocoetes emerged within 36 h in a partial pressure of oxygen of 7–10 mmHg and several others were seen with their oral hoods or entire branchial baskets above the substrate surface. All these latter animals re-burrowed. At 22·5 °C, nearly all the ammocoetes emerged in the higher partial pressure of 13–16 mmHg and a heavy mortality occurred (70%). Four animals emerged at the same temperature in 19–21 mmHg with no mortality.

The pattern of emergence behaviour of ammocoetes in the aquaria at each of the three experimental temperatures was essentially the same as that just described for animals within the respirometer at 15·5 °C. Many ammocoetes did not emerge in oxygen partial pressures of 7–10 mmHg at 5 °C, 12–16 mmHg at 15·5 °C and 19–21 mmHg at 22·5 °C (Table 3) again demonstrating that these animals remain in their burrows until the oxygen tension approaches the lethal level. In a few cases a

difference could be seen in the degree of response of large, medium-size and small animals to certain oxygen tensions. For example, with aquaria at a partial pressure of 12–16 mmHg at 15·5 °C none of the medium-size (75–110 mm) but a few of the large (130–160 mm) and small (35–55 mm) ammocoetes emerged. Another difference lies in the relationship of the time to emerge, in the aquaria and in the respirometer, at 15·5 °C in oxygen partial pressures of 2–4, 7–10 and 12–16 mm (Tables 2, 3). Whereas ammocoetes emerge most rapidly in oxygen of 2–4 mmHg in the aquaria, their response is slowest at this tension in the respirometer.

Table 3. *Time taken by ammocoetes of* Ichthyomyzon hubbsi *to emerge from the substrate in aquaria*

Temperature (°C)	Oxygen (mmHg)	Time of first emergence (h)	Mean of first 3 emergences (h)	Number of animals out after 96 h
5·0	1·5–2·5	5·0	5·30	All dead
5·0	7–10	3·0	5·10	3
15·5	2–4	0·45	0·50	All dead
15·5	7–10	1·75	1·75	All dead
15·5*	7–10	1·25	1·90	5†
15·5	12–16	4·0	4·75	1
15·5	18–20	—	—	0
22·5	13–16	1·5	4·90	0
22·5	19–21	5·0	5·00	0

* Animals acclimated to an oxygen tension of 12–16 mmHg for 4 days prior to experimentation.
† Number which had emerged after 60 h.

The experiments in which the concentration of carbon dioxide was slowly increased over a period of time showed that carbon-dioxide concentrations of *c.* 10 mg/l in well-oxygenated water caused restlessness of the animals. Movement of the head upwards towards the surface of the substrate, with occasional protrusion of the oral hood, occurred at carbon-dioxide concentrations between 10 and 20 mg/l. The first emergence occurred at 20 mg/l. In many cases it was not possible to determine accurately the concentration necessary to bring about emergence, since nomograms for the determination of the concentration of carbon dioxide at the lowest pH values (5·32) were not available. However, it appears that in several cases concentrations of *c.* 140 mg/l were required to initiate complete emergence, at which time the oxygen tension was still over 70% of saturation.

The low pH associated with high carbon-dioxide concentrations may have been responsible for emergence. This possibility was tested by exposing animals in the respirometer to water which had been acidified with hydrochloric acid to a pH of 5·5. The early stages of emergence behaviour were elicited in all cases within 10 min of the introduction of acidified water into the respirometer, and actual emergence was recorded in 28% of the animals.

Two structures, the velum and the branchial basket, act to pass water over the gills. Close observations of the branchial basket of ammocoetes which had settled down in the chambers in fully oxygenated water were difficult because of the position the animal generally took up along the mesh. However, in some cases even when the whole of the anterior end of the animals could be clearly observed, no velar or branchial beat

was visible. Eventually ten counts were obtained, all of which lay between 72 and 96 beats/min with a mean value of 84·0. Four of these values were based on velar, rather than branchial beat, because the latter was not detectable. The only other occasion when branchial beat could not be seen was in some animals that had been emerged for some time in oxygen tensions of 1·5–2·5 mmHg at 5 °C and which could be shown to be capable of recovering by transference to oxygenated water. A beat was always conspicuous at other lowered oxygen tensions and elevated carbon-dioxide concentrations.

Ammocoetes in an oxygen tension of 12–16 mmHg at 15·5 °C maintained a greatly elevated rate of pumping for the 10 h of the experiment (Fig. 2). Animals in the survival experiments in aquaria showed that these rates could be maintained for long periods, as beats of 140–180/min were seen at the end of 4 days. The variation in the rate of pumping of the branchial apparatus, which is found in animals exposed to oxygen tensions of 12–16 mmHg at 15·5 °C, is partly a function of variation between measurements for the same animal at different times but is mainly due to differences between individual animals.

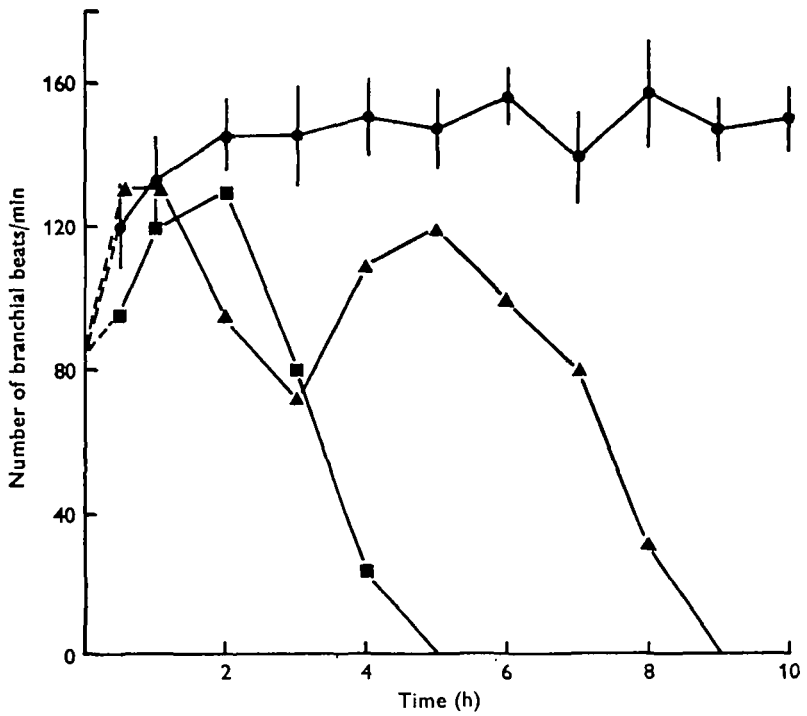

Fig. 2. The rate of branchial beat of ammocoetes of *Ichthyomyzon hubbsi* in low oxygen tensions at 15·5 °C. ●—●, 12–16 mmHg; ▲—▲, 7–10 mmHg; ■—■, 2–4 mmHg. Vertical line represents two standard errors to either side of the mean. Fourteen animals are represented at 12–16 mmHg, whereas a single animal is shown at 7–10 and 2–4 mmHg.

The branchial beat of each ammocoete at 15·5 °C, exposed to an oxygen tension of either 2–4 or 7–10 mmHg in the respirometer, was plotted on a separate graph as a function of the percentage time-to-death, and the branchial beat was extrapolated

from this for each successive 10% increment in time-to-death. Values obtained in this manner for all 14 animals were used to calculate means and two standard errors at each point and plotted as in Fig. 3. The initial increase in the rate and amplitude of branchial beat was only maintained for a short time, after which it dropped over the rest of the experiment. In an oxygen tension of 7–10 mmHg the beat of many animals showed a secondary increase prior to death. This feature is illustrated by the very gradual slope of the curve between 30 and 70% time-to-death in Fig. 3, and by the curve for the individual animal shown in Fig. 2. The pattern of rate of branchial beat in 2–4 mmHg is totally different (Figs. 2, 3). The initial increase in the rate of beat does not occur quite as rapidly, and there is never any marked secondary increase. The rate of beat in 2–4 mmHg and 7–10 mmHg never reached the values seen in 12–16 mmHg.

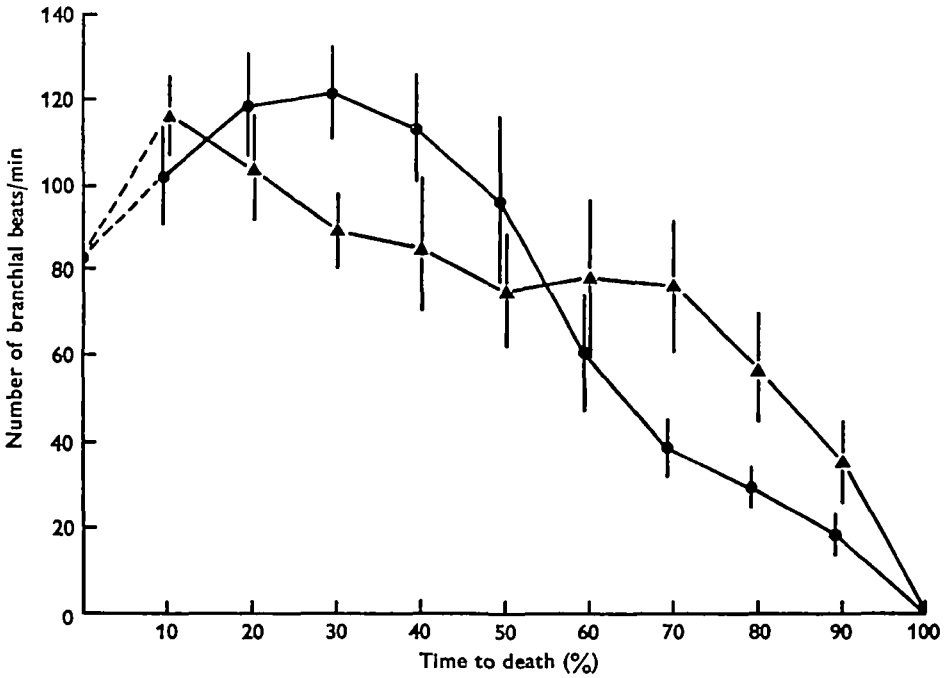

Fig. 3. The rate of branchial beat of ammocoetes of *Ichthyomyzon hubbsi* in oxygen tensions of 2–4 mmHg (●) and 7–10 mmHg (▲) at 15·5 °C. Vertical line represents two standard errors to either side of the mean.

Counts of rate of branchial beat from animals which either exposed their branchial basket or emerged completely in low but non-lethal oxygen tensions were obtained on several occasions at 5 and 22·5 °C. Branchial beats of 200/min were common in 12–16 mmHg at 22·5 °C, whereas a mean of 76 beats/min was recorded from animals in 7–10 mmHg at 5 °C.

An increase in carbon dioxide also brought an increase in the amplitude and rate of branchial beat, with rates of 120–170 beats/min being common at carbon-dioxide concentrations of 20 mg/l and above. No such increase was, however, found in a low pH when the concentration of carbon dioxide was low and the oxygen tension approached saturation.

At the conclusion of the studies so far described an additional experiment was performed to determine whether ammocoetes can acclimate to low partial pressures of oxygen, and if so what their behaviour was under these conditions. Twenty animals,

Table 4. *The pH at three temperatures, the haematocrit and the haemoglobin concentration of the blood of ammocoetes of* Ichthyomyzon hubbsi

	pH			Haematocrit	Hb. conc. g (%)
	5 °C	15·5 °C	22·5 °C		
Number of readings	5	10	8	10	10
Mean ± S.D.	7·91 ± 0·05	7·73 ± 0·09	7·44 ± 0·14	24·7 ± 6·1	7·42 ± 1·05
Range	7·60 – 7·99	7·60 – 7·92	7·24 – 7·59	17·1 – 33·7	6·20 – 8·80

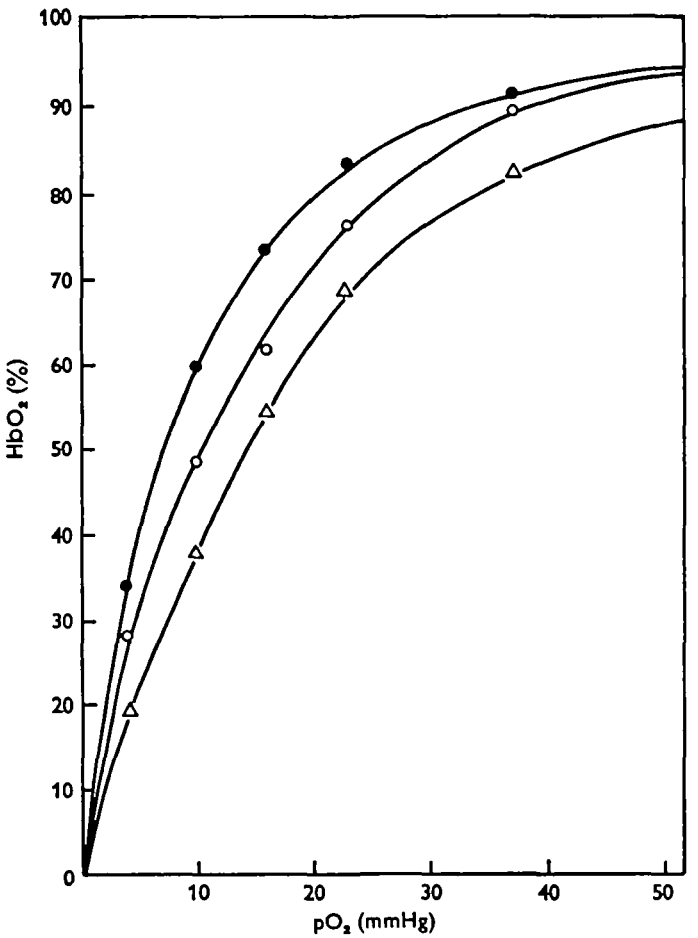

Fig. 4. Oxygen equilibrium curves of ammocoete erythrocyte suspensions. ●—●, pH 7·80, 5·0 °C; ○—○, pH 7·68, 15·5 °C; △—△, pH 7·40, 22·5 °C.

60–120 mm in length, were placed at 15·5 °C in two aquaria containing water with an oxygen tension of 12–16 mmHg. After 4 days, during which time they all remained in their burrows, the overlying water was drawn off and replaced by water with an

oxygen partial pressure of 7–10 mmHg. Mortality occurred in less than half the sample in the 60 h of the experiment, compared with the total mortality that occurs within 20 h in non-acclimated animals at this low oxygen tension (Table 1). Acclimated animals appeared incapable of re-burrowing once they had emerged. Counts of the branchial beat of emerged animals showed that they had a higher rate (130–160) than non-acclimated animals and that they were able to maintain this rate for several hours. This high rate, which was accompanied by a greater than normal amplitude, is similar to the beat exhibited by animals in an oxygen tension of 12–16 mmHg at 15·5 °C.

An investigation of the pH of the blood showed that higher values are found at lower temperatures (Table 4), a feature of some other poikilotherms (Rahn, 1967). Although variation occurred in the values for the haemotocrits, ammocoetes are characterized by having a relatively high haemoglobin concentration (Table 4).

The oxygen equilibria of erythrocyte suspensions were determined at pH values similar to that of the blood of animals at each experimental temperature (Fig. 4). These show the high affinity of ammocoete blood for oxygen. Additional measurements at 15·5 °C over a pH range of 7·68–6·70 indicated the absence of a significant Bohr effect, but a change did occur in the n value from 1·9 to 1·5.

DISCUSSION

The survival experiments showed that ammocoetes cannot survive sudden exposure to anoxic conditions for more than a short time. However, they are able to tolerate, for at least 4 days, oxygen tensions as low as 7–10 mmHg at 5 °C, 12–16 mmHg at 15·5 °C, and between 19–21 and 13–16 mmHg at 22·5 °C. The difference in the ability to survive low partial pressures of oxygen at different temperatures can be partially attributed to the considerable differences in the oxygen requirements of the animals at these temperatures (Hill & Potter, 1970). A second feature that probably contributes towards their ability to survive at low temperatures is the slightly greater affinity of their haemoglobin for oxygen at low temperatures.

In contrast to the ammocoete of *Petromyzon marinus* illustrated by Applegate (1950), the animals in our experiments always had the ventral region downwards after burrowing into the substrate. Furthermore, they always showed ventral arching of the body, i.e. contraction of the dorsal surface, as they moved up through the substrate or burrow. Although Applegate does not relate the various positions of his ammocoetes to the possible effects of low oxygen in the substrate, our results suggest that they might be envisaged in this context. The response to lowered oxygen of raising the head upwards, so that it lies just below the surface, enables the animal to sample the water above the substrate. This will amost certainly have a far greater oxygen concentration than the interstitial water, where recycling of water by the animal, together with the action of micro-organisms, can be expected to reduce the oxygen tension. After a period in this position the animal may draw back into the burrow, an action which should result in some flushing of the burrow water. Animals have been observed to follow a behaviour pattern of this nature in aquaria when exposed to lowered oxygen tensions at non-lethal levels. If, however, the water above the substrate is at an oxygen level approaching lethality, the animal emerges and swims around. Such a pattern of behaviour enables the ammocoetes to remain in their burrows until

oxygen tensions reach a dangerously low level, at which point emergence and subsequent activity may result in the animal finding a more favourable environment.

The only really conspicuous difference between the behaviour of ammocoetes in aquaria and in the respirometer is the more rapid emergence of ammocoetes in oxygen tensions of 2–4 mmHg in the aquaria, whereas their response is slowest at this tension in the respirometer. In this respect it is interesting to note that the animals also take longer to show an increase in the rate of branchial beating in nitrogen-equilibrated water in the respirometer than when in water of low oxygen tensions. The faster emergence in the aquaria can be explained by assuming that animals in sand at the beginning of an experiment are in oxygenated water. However, when nitrogen-equilibrated water is introduced, the animals are exposed to a decreasing oxygen tension. The emergence behaviour thus initiated is comparable to that seen in the respirometer experiment in which the oxygen tension of the water is gradually dropped. Thus it appears that the response of ammocoetes to water of low oxygen tension is different from that in virtually anoxic conditions.

It appears unlikely that carbon dioxide causes emergence under natural conditions when water is well oxygenated, since the concentration of carbon dioxide required to induce this behaviour is high. In fact, it is difficult to establish conclusively that emergence is due to carbon dioxide, since low pH values induce a similar response. In contrast to the similar emergence response evoked by high carbon dioxide and low pH, it should be noted that an accelerated rate of branchial beat was found only in the former of these experiments. The difference in response suggests that, although the emergence of animals from water of high carbon-dioxide concentration may be due to low pH, there is an independent respiratory response to carbon dioxide. High carbon-dioxide tensions in the water would probably cause an alteration in the acid-base relationship in the blood, a reaction that would not necessarily occur in water of low pH without accompanying increased carbon dioxide.

The reduced branchial pumping that takes place when the animal has settled down in fully aerated water in the substrate may well provide the reason for the significantly lower values that were obtained for oxygen consumption in ammocoetes of *I. hubbsi* (Hill & Potter, 1970) than were recorded by Leach (1946) for other species. In Leach's experiments the animals were not provided with a substrate, nor were they apparently given a reasonable length of time to settle down. Under these circumstances ammocoetes exhibit a conspicuous branchial beat which most probably results in the pumping of more water and the extraction of a greater amount of oxygen. The marked increase in the rate and amplitude of branchial beat of ammocoetes in low-oxygen conditions should also lead to a greatly increased flow of water over the gills. This is essentially the same response as that described for the eel (van Dam, 1938) and the tench (Randall & Shelton, 1963) under similar circumstances. The long survival time of animals in oxygen tensions of 12–16 mmHg at 15·5 °C may well be related to this ability to maintain a high rate of water flow over the gills. This view would certainly appear to be borne out by the different response of the branchial apparatus to reduced oxygen. In oxygen partial pressures of 7–10 mmHg at 15·5 °C, a lethal tension, there is only a short period of increased pumping, while in 12–16 mmHg at the same temperature, a non-lethal value, an increased rate is maintained for the whole of the experiment. Presumably the oxygen tension of the water is high enough under these

latter conditions to bring about sufficient saturation of the blood so that this rate of pumping fulfils the normal oxygen requirements of the animal, as well as the increased demands of the respiratory pump.

The high affinity of the blood of ammocoetes for oxygen, as shown by the equilibria curves, is likely to be of considerable importance in enabling the animals to survive low oxygen tensions which are probably often present in burrows under natural conditions. The difference at 15·5 °C in the percentage saturation of haemoglobin between the non-lethal oxygen partial pressures of 12–16 mmHg and the lethal 7–10 mmHg is *c.* 20%. The degree of saturation of the blood may be adequate for the animal's needs in the higher tension but is just insufficient at the lower. Furthermore, the steepness of the curve in these low oxygen tensions would result in a high degree of saturation at 18–20 mmHg. It may be for this reason that ammocoetes remained in their burrows at this latter tension and never showed emergence behaviour. The position of the oxygen equilibria curve would seem to give considerable selective advantage to an animal that may be occasionally exposed to conditions of low oxygen. It should also be noted that the concentration of haemoglobin is higher than in many other lower vertebrates, and therefore will be likely to contribute significantly to the oxygen-transport system.

Manwell (1963) and Antonini *et al.* (1964) recorded a large Bohr effect for the ammocoetes of both *Ichthyomyzon unicuspis* and *Petromyzon marinus*, although in both cases this effect was far less marked over the pH range (6·70–7·68) which was used in the present study. In *I. hubbsi* an insignificant Bohr effect was found between 6·70 and 7·58, but there is no reason to suppose that a Bohr effect similar to that found in *I. unicuspis* and *P. marinus* does not occur at lower pH. The significant haem-haem interactions ($n = 1{\cdot}5 - 1{\cdot}9$) which are found in the blood of ammocoetes of *I. hubbsi*, are very similar to those described by Manwell (1963) for ammocoetes of *P. marinus* and *I. unicuspis*. As Manwell has pointed out, the increased sigmoidal nature of the oxygen equilibrium curve is of considerable physiological advantage to a mud-dwelling animal.

The physiological mechanism involved in the limited ability to acclimate to low oxygen found in some animals is worthy of further investigation. It may well involve cardiovascular changes, such as selective ischaemia, and may also include a change in metabolism. Benesch and Benesch (1969) pointed out that acclimation to low oxygen could occur through alteration in the organic phosphate content of erythrocytes. It is not certain whether this method of acclimation is available to lampreys as their haemoglobin is not tetrameric, and Benesch found that diglycerophosphate (D.P.G.) only binds to molecules which are tetrameric in the deoxygenated condition. Whatever the mechanism, the ability to acclimate to low oxygen tensions may be of advantage to an animal which lives in an environment which must occasionally suffer lowered oxygen, enabling the animal to await more favourable conditions. However, it should be remembered that the animals emerge under these conditions and appear incapable of re-burrowing and are thus vulnerable to changes in the environment.

Adult lampreys are characterized by both a very high concentration of haemoglobin and a large volume of red cells, according to the results obtained by Thorson (1959). Manwell (1963) and Korzhuev & Glazova (1967); but a significantly lower value was given by Kisch (1951) on the basis of one specimen of *Petromyzon marinus*. However,

it should be noted that very considerable changes occur in these parameters during the spawning run (Ivanova Berg & Sokolova, 1959). Our values for the ammocoetes of *I. hubbsi* are lower than those generally obtained for adult lampreys. However, before generalizations can be made about differences between larval and adult stages, data are required from ammocoetes of other species and from the adults of non-parasitic species. It is also important to obtain some information on relative blood volumes and oxygen-transport capacity. Data for hagfishes appear to be restricted to measurements on three animals of *Myxine glutinosa* by Wintrobe (1933). Although haematocrits are within the range of our values, the haemoglobin concentration is significantly lower. The much greater concentration of haemoglobin in adult lampreys compared with hagfishes may well be correlated with the greater activity of the former.

SUMMARY

1. Survival and behaviour studies were made on ammocoetes subjected to water of various tensions of oxygen and carbon dioxide.

2. Ammocoetes can tolerate, for at least 4 days, oxygen tensions as low as 7–10 mmHg at 5 °C, 12–16 mmHg at 15·5 °C and between 13–16 and 19–21 mmHg at 22·5 °C. A limited ability to acclimate to low oxygen tensions was found in some animals.

3. A characteristic emergence behaviour is evoked by low partial pressures of oxygen that approach the lethal level and by high concentrations of carbon dioxide.

4. Ammocoetes respond to low oxygen and high carbon dioxide by an increase in the rate and amplitude of beating of the branchial basket. This increase is maintained in animals able to survive at low oxygen tensions.

5. A high affinity of the blood for oxygen is evident from oxygen equilibrium curves determined on erythrocytes suspension. There was an insignificant Bohr effect at 15·5 °C in the pH range 7·68–6·70, although a change occurred in the *n* value.

6. Haemoglobin concentration, haematocrit and oxygen equilibria suggest that the characteristics of the blood contribute significantly to the ability of ammocoetes to survive in low oxygen conditions.

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REFERENCES

ANTONINI, E., WYMAN, J., RUMEN, N. & SINISCALCO, M. (1964). The oxygen equilibria of some lamprey haemoglobins. *Archs. Biochem. Biophys.* **105**, 404–8.

APPLEGATE, V. C. (1950). Natural history of the sea lamprey, *Petromyzon marinus*, in Michigan. *U.S. Fish Wildl. Serv.* (*Spec. Rep. on Fish*), no. 55, 1–237.

ADINOLFI, M., CHIEFFI, G. & SINISCALCO, M (1959). Haemoglobin pattern of the cyclostome *Petromyzon planeri* during development. *Nature, Lond.* **184**, 1325–6.

BENESCH, R. & BENESCH, R. E. (1969). Intracellular organic phosphates as regulators of oxygen release by haemoglobin. *Nature, Lond.* **221**, 618–22.

BLAŽKA, P. (1958). The anaerobic metabolism of fish. *Physiol. Zoöl.* **31**, 117–28.

BRIEHL, R. W. (1963). The relation between the oxygen equilibrium and aggregation of subunits in Lamprey haemoglobin. *J. biol. Chem.* **238** (7), 2361–6.

CROSBY, W. H., MUNN, J. I. & FURTH, F. W. (1954). Standardizing a method for clinical haemoglobinometry. *U.S. arm. Forces med. J.* **5**, 693–703.

VAN DAM, L. (1938). On the utilisation of oxygen and regulation of breathing in some aquatic animals. Thesis. Groningen.

HILL, B. J. & POTTER, I. C. (1970). Oxygen consumption in ammocoetes of the Lamprey *Ichthyomyzon hubbsi* Raney. *J. exp. Biol.* **53**, 47–57.

IVANOVA BERG, M. M. & SOKOLOVA, M. M. (1959). Seasonal changes in the composition of the blood of river lamprey *Lampreta fluviatilis* L. (In Russian.) *Vop. Ikhtiol.* **13**, 156–62.

KISCH, B. (1951). Erythrocytes in fishes. *Expl Med. Surg.* **9**, 125–37.

KORZHUEV, P. A. & GLAZOVA, T. N. (1967). Ecological and physiological adaptations of the blood in cyclostomata. (English translation). *Akad Nauk USSR* **172**, 102–3.

LEACH, W. J. R. (1946). Oxygen consumption of lampreys, with special reference to metamorphosis and phylogenetic position. *Physiol. Zoöl.* **19**, 365–74.

MACKERETH, F. J. H. (1963). Water analysis for limnologists. *Freshwat. Biol. Ass.* Scientific Publication no. 21, 1–70.

MANWELL, C. (1963). The blood proteins of cyclostomes. A study in phylogenetic and ontogenetic biochemistry. In *The Biology of Myxine*. Eds. A. Brodal and R. Fänge, pp. 372–455. Universitetsforlaget, Oslo, Norway.

POTTER, I. C. & NICOL, P. I. (1968). Electrophoretic studies on the haemoglobins of Australian lampreys. *Aust. J. exp. Biol. med. Sci.* **46**, 639–41.

RAHN, H. (1967). Gas transport from the external environment to the cell. In *Development of the Lung*. A CIBA Foundation Symposium. Ed. A. V. S. De Rueck and Ruth Porter. London: Churchill.

RANDALL, D. J. & SHELTON, G. (1963). The effects of changes in environmental gas concentrations on the breathing and heart rate of a teleost fish. *Comp. Biochem. Physiol.* **9**, 229–39.

THORSON, T. B. (1959). Partitioning of body in sea lamprey. *Science, N.Y.* **130**, 99–100.

WINTROBE, M. M. (1933). Variations in the size and haemoglobin content of erythrocytes in the blood of various vertebrates. *Folia haemat. Lpz.* **51**, 32–49.