

## THE EFFECT OF HYPOXIA UPON THE PARTIAL PRESSURE OF GASES IN THE BLOOD AND WATER AFFERENT AND EFFERENT TO THE GILLS OF RAINBOW TROUT

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### INTRODUCTION

Van Dam (1938), Itazawa (1957) and Saunders (1962) have recorded some of the effects of hypoxia on the gas tensions in blood and water afferent and efferent to the gills of fishes, but there has been no integrated study in which several of these parameters have been measured simultaneously in a single species of fish.

Rushmer & Smith (1959) have emphasized the importance of recording cardiovascular parameters in unrestrained, unanaesthetized, intact animals.

The object of this study was to record the effects of hypoxia in the environment on the  $P_{O_2}$ ,  $P_{CO_2}$  and pH of the blood and water afferent and efferent to the gills of unrestrained, unanaesthetized and intact fish.

### METHODS

The experiments were carried out on twenty-seven rainbow trout (*Salmo gairdneri*). Experimental procedures were identical to those previously described by Holeton & Randall (1967) except that the  $P_{O_2}$ ,  $P_{CO_2}$  and pH of the blood and water afferent and efferent to the gills were measured instead of pressure. Measurements were made using a Beckman model 160 Physiological Blood Gas Analyser. Samples of blood or water were admitted into a Beckman Modular Cuvette from the four implanted cannulae in the dorsal aorta, ventral aorta, buccal cavity and opercular cavity. Blood pressure was used to move blood into the cuvette, whereas slight suction was applied to the outlet of the cuvette to draw water into the system. Blood samples were returned to the fish after each analysis.

Partial pressures of oxygen were determined using a Beckman oxygen macro-electrode.  $P_{CO_2}$  determinations were made using a Beckman Severinghaus-type electrode. A glass electrode and a fibre junction reference electrode were used to measure pH. All electrodes were mounted in the modular cuvette, and maintained at the same temperature as the fish. Only two of the three electrodes were in operation during any single experiment, because of the slow response time of the electrode at 15° C.

The analysis time for blood  $P_{O_2}$  was from  $\frac{1}{2}$  to 2 min., for pH, 1-3 min., and for  $P_{CO_2}$  5-11 min.

The cuvettes were flushed, before admitting a blood sample, with Courtland saline (Wolf, 1963) containing a wetting agent (Tween-80, one drop per litre) and sodium

heparin (10 i.u./ml.) This procedure prevented clotting and haemolysis of the blood.

The  $P_{CO_2}$  and  $P_{O_2}$  electrodes were calibrated prior to each experiment with standardized gas mixtures, and the pH electrode was calibrated every two or three determinations using freshly prepared buffer solution.

Small quantities of blood were taken from the dorsal aorta at the beginning of each experiment in order to determine the haematocrit. In a separate series of experiments haematocrit and red blood cell count were determined throughout the experiment and no other parameters were measured. This was done to determine if there were any changes in red blood cell volume during the experiment.

Each experiment consisted of allowing the fish to utilize the oxygen dissolved in the water in the closed respirometer, so imposing a state of hypoxia on itself. The changes in  $P_{O_2}$ ,  $P_{CO_2}$  and pH in the blood and water during hypoxia were monitored. The rate of oxygen uptake was calculated from the rate of decrease of oxygen in the water.

Changes in the levels of blood lactate during hypoxia were also recorded. Blood samples of 0.5 ml. were removed from four fish before and after the fish were exposed to an hypoxic environment. Blood samples were placed in 10% trichloroacetic acid, filtered and stored in a refrigerator until analysed for lactate using the method of Barker & Summerson (1941).

The experiments were carried out in fresh water at  $15 \pm 1^\circ \text{C}$ .

#### RESULTS

The changes in oxygen uptake recorded with increasing hypoxia (Fig. 1) are not statistically significant.

Blood lactate increased by a factor of between two and three when the fish was exposed to low levels of oxygen in the water, indicating an increase in anaerobic metabolism under these circumstances (Table 1). The four fish used in these experiments weighed between 520 and 1015 g. The blood sample of 0.5 ml., removed before and after the fish was exposed to an hypoxic environment, represents a calculated maximum of 5% of the total blood volume. This removal of blood presumably did not affect the animal's ability to withstand an hypoxic environment.

The percentage utilization of oxygen in the water by the fish did not change markedly with increasing hypoxia except for an initial drop when the  $P_{O_2}$  in the water fell from 155 to 120 mm. Hg. Ventilation volume increased during hypoxia to offset the decreased oxygen content of the water (Fig. 3). This 13-fold increase in ventilation volume was calculated by the Fick principle using the following equation:

$$\dot{V}_g = \frac{\dot{V}_{O_2}}{(P_{i_{O_2}} - P_{e_{O_2}}) \alpha_{O_2}}$$

$\dot{V}_g$  ventilation volume (ml./min./kg.)

$\dot{V}_{O_2}$   $O_2$  uptake (ml./min./kg.)

$\alpha_{O_2}$  solubility coefficient of oxygen in water (ml./ml./mm. Hg.)

$P$  partial pressure of gas (mm. Hg)

*Subscripts*O<sub>2</sub> molecular species*i* referring to water in buccal cavity (inspired)*e* referring to water in opercular cavity (expired)

The  $P_{O_2}$  of the blood sampled from the dorsal aorta decreased from 122 to 19 mm. Hg when the environmental  $P_{O_2}$  was slowly reduced to 30 mm. Hg (Fig. 4). When the

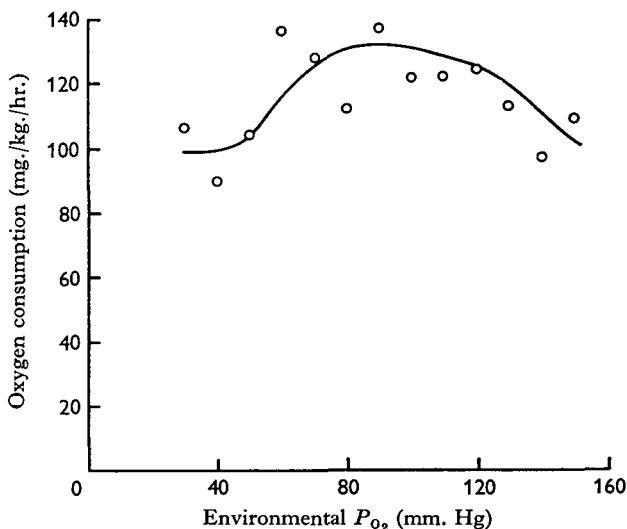


Fig. 1. The effect of hypoxia on the rate of oxygen uptake by rainbow trout. (Mean values from twenty-seven fish.)

Table 1. Changes in blood lactate in the rainbow trout before (aerated water) and after (hypoxic water) subjection to an hypoxic environment

Expt.	Blood lactate (mg./100 ml.)	
	Aerated water	Hypoxic water
1	22.52	50.66
2	10.60	30.46
3	9.27	36.75
4	8.69	21.86
Average	12.77	34.86

fish was in aerated water the  $P_{O_2}$  of the dorsal aortic blood (Fig. 4) was significantly higher than the  $P_{O_2}$  of the expired (opercular) water (Fig. 2), providing proof of a functional countercurrent flow arrangement between blood and water (van Dam, 1938; Krogh, 1951).

The  $P_{O_2}$  of blood sampled from the ventral aorta was between 30 and 35 mm. Hg in the resting fish, falling to 6 mm. Hg in the fish exposed to an environmental  $P_{O_2}$  of 30 mm. Hg (Fig. 4).

When the fish was in aerated water the ventral aortic  $P_{CO_2}$  was 2.5 mm. Hg, whereas the dorsal aortic  $P_{CO_2}$  was between 1 and 1.5 mm. Hg. These values increased to

4.5–5.00 mm. Hg and 3.5–4 mm. Hg in the ventral and dorsal aortae respectively when the fish was exposed to an hypoxic environment. This increase in  $P_{CO_2}$  in the blood is related to the experimental design rather than an inability of the fish to eliminate  $CO_2$ . The system is closed and the same water is continually recirculated past the fish. The decrease in  $O_2$  in the water is associated with an increase in  $CO_2$  in the water. The  $P_{CO_2}$  in the water rose to about 2 mm. Hg during the course of an

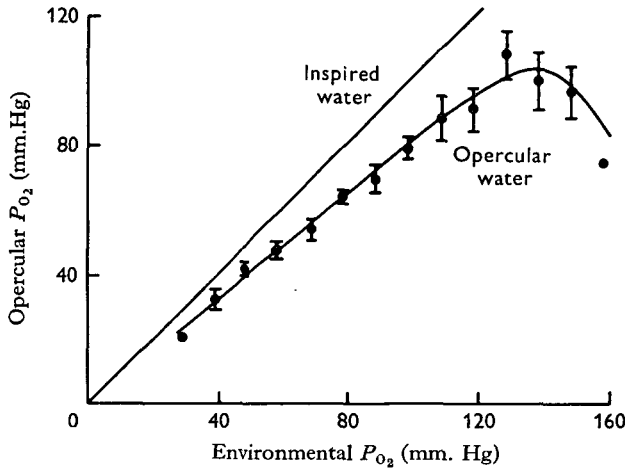


Fig. 2. The effect of hypoxia on the partial pressure of oxygen in the expired (opercular) water. Vertical bars =  $\pm 2$  S.E.

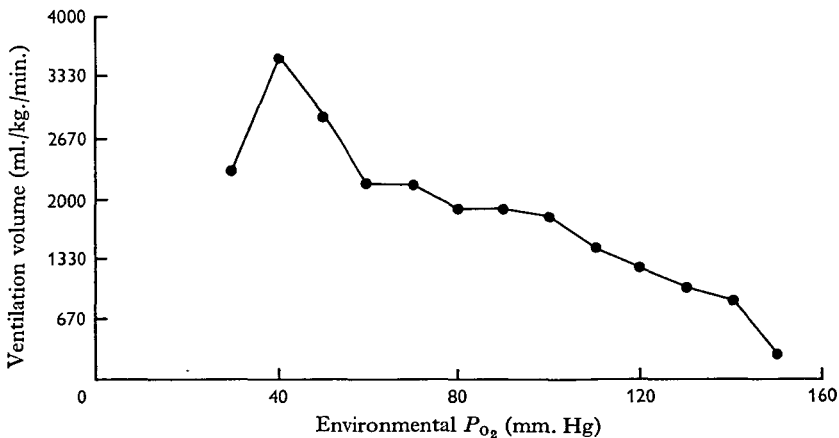


Fig. 3. The effect of hypoxia on the volume of water pumped over the gills by the fish.

experiment. It is not surprising therefore that the  $P_{CO_2}$  in the dorsal aorta rose to 3.5–4.0 mm. Hg under these conditions. This increased level of  $P_{CO_2}$  in the blood and water may have impaired the ability of the fish to withstand hypoxia and have resulted in increased levels of anaerobic metabolism. Increasing levels of carbon dioxide decrease the oxygen-carrying capacity of the blood (Root effect) and the data indicated a positive correlation between high oxygen capacity of the blood and the ability of the fish to withstand hypoxia. Those fish with a low oxygen capacity showed signs of respiratory collapse at higher environmental  $P_{O_2}$  than those fish with a high oxygen capacity

The pH of the blood was close to 7.7 (Fig. 5) and remained fairly constant, falling to 7.4 only when the environmental  $P_{O_2}$  dropped to 30 mm. Hg. Environmental pH dropped from 7.4 to 6.8 during the course of each experiment (Fig. 5). This is probably related to the accumulation of  $CO_2$  in the water as the experiment proceeded,

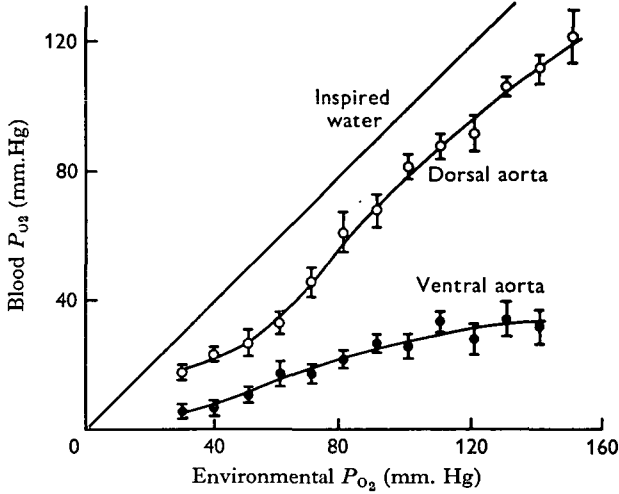


Fig. 4. The effect of hypoxia on the partial pressure of oxygen in the blood afferent (ventral aorta) and efferent (dorsal aorta) to the gills. Vertical bars =  $\pm 2$  S.E.

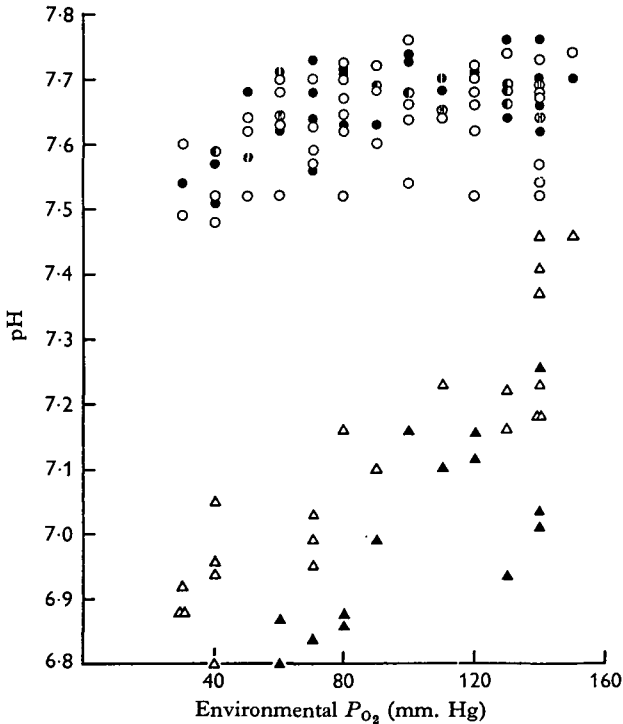


Fig. 5. The effect of hypoxia on the pH of blood in the dorsal and ventral aortae, and on the pH of the environmental and opercular water.  $\circ$  Dorsal aortic blood;  $\bullet$  ventral aortic blood;  $\Delta$  environmental;  $\blacktriangle$  opercular.

which was further indicated by the difference of about 0.2 pH units between water in the buccal and opercular chambers (Fig. 5). The eventual drop in pH in the blood during hypoxia must be related at least in part to the accumulation of lactic acid and  $\text{CO}_2$  in the blood. That an increase in  $P_{\text{CO}_2}$  of 3 mm. Hg should precipitate such a change indicates a comparatively weak buffering capacity of the blood compared with that of terrestrial vertebrates.

The haematocrit of the blood increased with the onset of hypoxia. The red blood cell count did not alter, indicating that the increased haematocrit resulted from cellular swelling rather than an increase in cell number. Ferguson & Black (1941) have shown *in vitro* that red blood cells of the rainbow trout swell with small increases in blood  $P_{\text{CO}_2}$ .

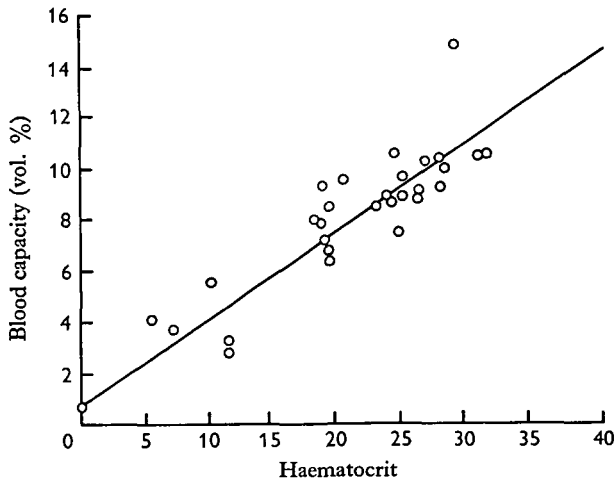


Fig. 6. The relationship between blood oxygen capacity and haematocrit in the rainbow trout.

The relationship between oxygen capacity as determined using a Van Slyke apparatus, and haematocrit for the rainbow trout is described by the linear regression equation; capacity =  $0.311$  haematocrit +  $0.7$  (Fig. 6). As the haematocrit of each fish was recorded during each experiment, it was possible to calculate the oxygen capacity of the blood of each fish. A series of oxygen dissociation curves, covering ranges between 0 to 1 and 10 mm. Hg  $P_{\text{CO}_2}$  (Randall, Beaumont and Holeyton, unpublished) showed that the blood of rainbow trout is similar to that of brook trout (Irving, Black & Safford, 1941; Black, Kirkpatrick & Tucker, 1966).

The percentage saturation of the ventral aortic blood was determined from these oxygen dissociation curves using the recorded  $P_{\text{O}_2}$  and  $P_{\text{CO}_2}$  data. The dorsal aortic blood was 95–100% saturated in the resting fish, whereas the venous blood in the ventral aorta was between 67 and 73% saturated. At an environmental oxygen tension of 30 mm Hg the ventral aortic blood was 3% saturated, the arterial (dorsal aorta) blood was 37% saturated. This drop in percentage saturation was due not only to the decreased  $P_{\text{O}_2}$  but also to the increased  $P_{\text{CO}_2}$  of the blood.

The oxygen content of the blood was calculated from the previously determined percentage saturation and the estimated oxygen capacity, which in general was about 9–10 vol. % in the group of fish used in these experiments. The oxygen capacity of the blood varied between fish, as did haematocrit.

Thus the oxygen content of the blood afferent and efferent to the gills was determined, and as oxygen uptake by the fish was measured, cardiac output was calculated, using the Fick principle.

No significant changes in cardiac output were observed when the fish was subjected to an hypoxic environment (Fig. 7). Stroke volume, estimated by dividing cardiac output by heart rate (Holeton & Randall, 1967), increased markedly as heart rate decreased during hypoxia.

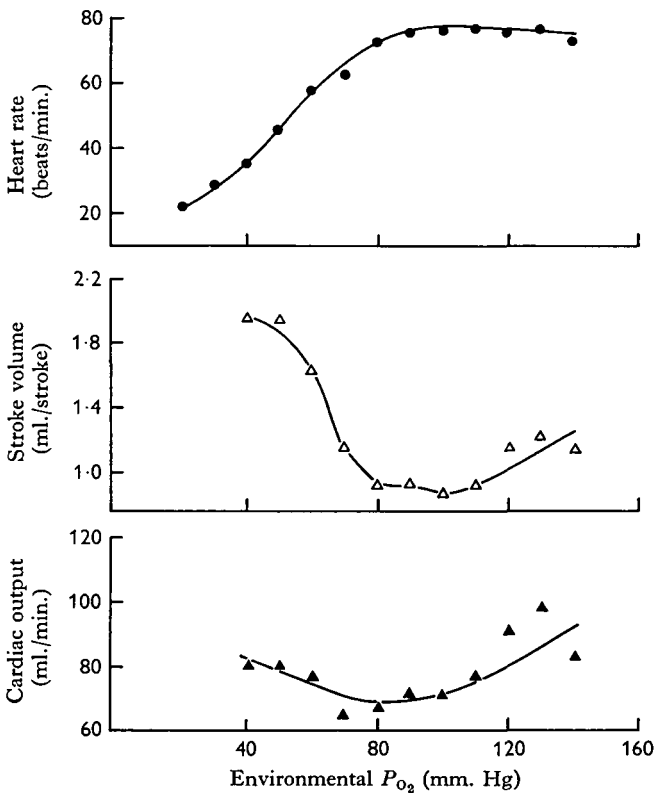


Fig. 7. The effect of hypoxia on cardiac output, stroke volume and heart rate in the rainbow trout.

#### DISCUSSION

The average rate of oxygen consumption reported here is only slightly higher than that recorded by Beamish (1964) for the brook trout of the same size and at the same temperature. This would support the conclusion that activity of fish in the respirometer was generally quite low.

The blood lactate levels for fish in aerated water are similar to those reported by Black (1955) and Black *et al.* (1962) for the same species of fish. The increase in lactate observed during hypoxia is presumably due to an increase in anaerobic metabolism, indicating an inability of the circulatory system to deliver sufficient oxygen to some tissues during hypoxia. The branchial muscles increase their demand for oxygen during hypoxia, and represent a possible source of lactate.

Delivery of oxygen to the gills during hypoxia was maintained by a large increase in ventilation volume from a resting rate of 274 ml./min./kg. to a maximum hypoxic rate of 3560 ml./min./kg. (Fig. 3). This 13-fold increase was associated with an increase in breathing rate from 80 to 120/min. (Holeton & Randall, 1967), and an increase in the depth of each breath from 3.43 ml. to a maximum of 29.5 ml. The ventilation volume for the resting trout reported here is less than that reported by Ogden (1945) for the dogfish, but higher than that reported by van Dam (1938) for a single trout. Van Dam's fish had a higher breathing rate than the fish used in this study, and it is possible that the rubber membrane used by Van Dam may have restricted the depth of breathing, reducing the ventilation volume, but resulting in a compensatory increase in breathing rate.

The percentage utilization of oxygen by Van Dam's trout was greater than the average value of 55 % for the resting trout obtained in this study on twenty-seven fish. Van Dam (1938) reported that a fourfold increase in ventilation volume produced a drop in percentage utilization from 80 to 60 %. Our data contained values from a trout similar in weight to that used by Van Dam, and in this fish a fourfold increase in ventilation volume resulted in a decrease in percentage utilization from 78 to 34 %. In general the highest percentage utilization was observed in large exceptionally quiet fish, and van Dam's fish appears to fit into this category. Activity of any sort resulted in rapid changes in the percentage utilization of oxygen by the fish.

Many factors appear to affect percentage utilization. In these experiments the fish maintained an upstream position in the tube, facing into a water velocity of 2 cm./sec. Thus, if the mouth of the fish was 2 cm.<sup>2</sup> and the gills offered negligible resistance to flow, 240 ml. of water would pass over the gills per minute without any effort on the part of the fish. The upstream position of the fish may have produced a higher ventilation volume and a lower percentage utilization than would have occurred if the fish was in still water.

Saunders (1962) reported an 82-fold increase in ventilation volume for a sucker in response to hypoxia. This was a maximum value based on a calculated increase in ventilation volume in a single fish, from 1.8 to 143 ml./sec. Thus this fish, weighing 250 g. was able to pump more than its own weight in water over the gills every 2 sec. The increases in maximal ventilation volume seen in the sucker, carp and bullhead (Saunders, 1962) are higher than the average values reported here. A 13-fold increase in ventilation volume occurred in the trout in response to hypoxia. The data reported here and that reported by Saunders (1962) and Van Dam (1938) indicate a large capacity of the breathing apparatus to increase ventilation volume.

There is considerable variation in ventilation volume between fish, and within a single fish under a variety of conditions. The data are expressed per kilogram of fish and do not take into account variability associated with size.

Arterial blood in the resting trout is 95 % saturated and has a very low  $P_{CO_2}$ . The venous  $P_{O_2}$  (35 mm. Hg) is somewhat higher than that estimated by Mott (1957) and Saunders (1952) or that recorded by Itazawa (1957) for the carp. The  $P_{CO_2}$  of the venous blood was lower than that reported previously for the trout and carp (Garey & Rahn, 1964), and the percentage saturation of the venous blood was 70 %. Thus, if one assumes an oxygen capacity of 9 vol. %, 6-7 % of the oxygen entering the tissues is carried in physical solution. The venous blood is 70 % saturated in the resting fish.



This is in sharp contrast with the assumption that venous blood in fish is almost completely deoxygenated (Mott, 1957; Saunders, 1962). Thus, haemoglobin acts as an oxygen store, as well as an oxygen carrier, which may be drawn upon when the oxygen requirements of the tissues increase, or the availability of oxygen is reduced.

Cardiac output did not alter even though hypoxia was associated with a marked bradycardia. It would appear therefore that hypoxia is associated with an increase in stroke volume to offset the decrease in heart rate (Holeton & Randall, 1967).

Table 2

Animal	Temp. (°C)	Cardiac output (ml./kg./min.)	Stroke volume (ml./kg.)	Method used	Reference
Bowfin, carp	(10°)	—	0.44, 0.36,	Ligation and subsequent weighing of heart chambers	Hart, 1943
Sucker, catfish	—	—	0.22, 0.52		
<i>Squalus</i> sp.	(11–17°)	9.0–33.0	0.25–0.93	Ligations, natural flow from cut ventral aorta	Burger & Bradley, 1951
<i>Opsanus</i> sp.	—	10.1	—	Fick principle, minimum theoretical values	Mott, 1957
<i>Tetraodon</i> sp.	—	15.5	—		
<i>Stenotomus</i>	—	15.7	—		
<i>Gadus morhua</i>	—	9.3	0.31 (can double)	Electromagnetic flowmeter	Johansen, 1962
<i>Amphiuma</i> sp.	—	40.0	0.96	—	Johansen, 1963
Sculpin	(15–18°)	21.4–34.2	—	Fick principle	Goldstein <i>et al.</i> 1964
Octopus	(7–9°)	5.0–32.2	0.41–2.0	Fick principle	Johansen, 1965
Frog	(20°)	57	1.0 (approx.)	Ventricular displacement	Shelton & Jones, 1964
Human	—	60–100	—	—	Prosser & Brown, 1961
Cat	—	69.0	—	—	1961
Trout	(12–18°)	65–100	0.85–2.0	Fick principle	Present study

The values for cardiac output and stroke volume reported in the literature are generally lower than those of the present study (Table 2). The minimum theoretical values for cardiac output estimated by Mott (1957) for three marine fishes are similar to that which would be expected in rainbow trout if the venous blood were completely deoxygenated. The assumption made by Mott (1957), that the venous blood is completely deoxygenated, is not valid, for in the resting trout the venous blood was 70% saturated. Because of the high percentage saturation of the venous blood returning to the heart one would expect a much higher calculated cardiac output than that estimated by Mott (1957). The cardiac output recorded by Hart (1943), Burger & Bradley (1951), Johanesen (1962) and Goldstein, Forster & Fanelli (1964) were obtained from fish that were either anaesthetized, restrained or not intact, and it is very possible that such treatment could have a marked effect on cardiac output.

The values for cardiac output and stroke volume were estimated from measurements of a number of parameters, many of which showed considerable variability, and were obtained using techniques pushed to the limits of their accuracy. Such estimates of cardiac output and stroke volume are indicative of an order of magnitude rather than an exact quantitative measurement of these parameters. Pooling data from a large number of fish removes much of the individual variability inherent in many of

the parameters, but at the same time reveals only general trends in a response, i.e. an increase in stroke volume with hypoxia, rather than illustrating any of the finer oscillations that may occur in stroke volume or cardiac output with the onset of hypoxia.

It was suggested (Randall & Shelton, 1963) that the bradycardia associated with hypoxia produces a decrease in cardiac output, slowing blood flow through the gills to facilitate oxygen uptake by the blood in the face of reduced oxygen levels in the water. There is no change in cardiac output during hypoxia, and it is difficult to explain why the trout should change from a high heart-rate, low stroke-volume to a low heart-rate, high stroke-volume condition with the onset of hypoxia. The velocity profile of blood flow through the gills would change under these two conditions. Rate and amplitude of breathing increase during hypoxia, and there is a synchronization of heart rate and breathing rate (Randall & Smith, 1967). It is possible that the changing pattern of blood flow may augment gas exchange during hypoxia, permitting a longer residence time for blood at the respiratory surface.

#### SUMMARY

1. The rate of oxygen uptake by rainbow trout does not alter during progressive deoxygenation of the environment. Blood lactate, however, shows a significant increase, indicating an increase in anaerobic metabolism during hypoxia.

2. The percentage utilization of oxygen from the water decreased from 55% to approximately 20% during hypoxia and was associated with a 13-fold increase in ventilation volume.

3. The arterial blood of a trout resting in aerated water was 95–100% saturated: the  $P_{O_2}$  was 122 mm. Hg and the  $P_{CO_2}$  was 1–1.5 mm. Hg.

4. The venous blood of a resting trout was 70% saturated, had a  $P_{O_2}$  of 35 mm. Hg and a  $P_{CO_2}$  of 2.5 mm. Hg.

5. During hypoxia the percentage saturation of the arterial blood decreased to 37% and that of the venous blood to 3%. The  $P_{O_2}$  in the arterial blood was 10 mm. Hg; in the venous blood, 6 mm. Hg. The  $P_{CO_2}$  in the arterial blood was 3.5–4 mm. Hg; in the venous blood, 4.5–5.00 mm. Hg respectively.

6. The ability of the fish to withstand hypoxia was related to the oxygen capacity of the blood, which was on average 9 vol. %. The red blood cells swelled during hypoxia, the hematocrit increased, but the red cell count did not alter.

7. Blood pH was 7.7, falling to 7.4 during hypoxia.

8. Cardiac output did not change during progressive hypoxia in the water, stroke volume increased to offset the decrease in heart rate.

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