RESPONSES OF THE RESPIRATORY PUMPS TO HYPOXIA IN THE RAINBOW TROUT (*SALMO GAIRDNERI*)

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INTRODUCTION

Recordings of the time course of pressure changes in the respiratory cavities of fishes have revealed a fairly consistent pattern for a number of different species (Hughes, 1960; Saunders, 1961). It is clear that some relationship is to be expected between the pressure changes and the flow of water through the system, but the situation is complicated because of variations in the gill resistance during the respiratory cycle (Hughes & Shelton, 1958). In spite of a number of recent measurements of ventilation volume and the effects of environmental changes on the activity of the pumps on the respiratory and cardiac sides of the exchanger (Holeton & Randall, 1967; Piiper & Schumann, 1967; Hughes & Umezawa, 1968), no detailed attempt has been made to correlate the pressure changes with the volume of water pumped across the gills. It was the purpose of the present study to measure the ventilation volume of a fish and to record simultaneously the pressure changes in the buccal and opercular cavities together with the differential pressure across the gills.

MATERIALS AND METHODS

Rainbow trout (400–600 g) obtained from a hatchery at Nailsworth, Gloucestershire, were kept in laboratory holding tanks for at least 1 week before use in the apparatus (Fig. 1). The fish were anaesthetized in MS 222 before cannulation of the buccal and opercular cavities by means of polyethylene tubing (Saunders, 1961). Darkened respiratory chambers were used with dimensions that were close to those of the fish in order that the fish should remain in more or less the same position but have sufficient room to move its opercula. The cannulae were brought out of the respirometer and could be used for recording pressures or for sampling the inspired and expired water. A Beckman O_2 electrode was kept in a water jacket maintained at the same temperature as the closed circulation. The flow of water through the respirometer was regulated and measured by means of a flowmeter. Samples of the inflow and outflow water could also be led over the O_2 electrode as shown in the diagram (Fig. 1). After cannulation and recovery from the anaesthetic, the fish was placed in the respirometer at least overnight before any measurements were made. The usual procedure was to sample the water from the four cannulae in rotation, and to record the buccal, opercular and differential pressure. This usually took about 12 min. In

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some of the later experiments, the inflow and outflow from the respirometer were sampled and P_{O_2} was measured using an Eschweiler micro-electrode, whose water-bath was adjusted to the same temperature as the water circulation. This reduced the time required to complete the set of measurements to about 8 min. The temperature of the circulating water was usually kept at about 13·5 °C.
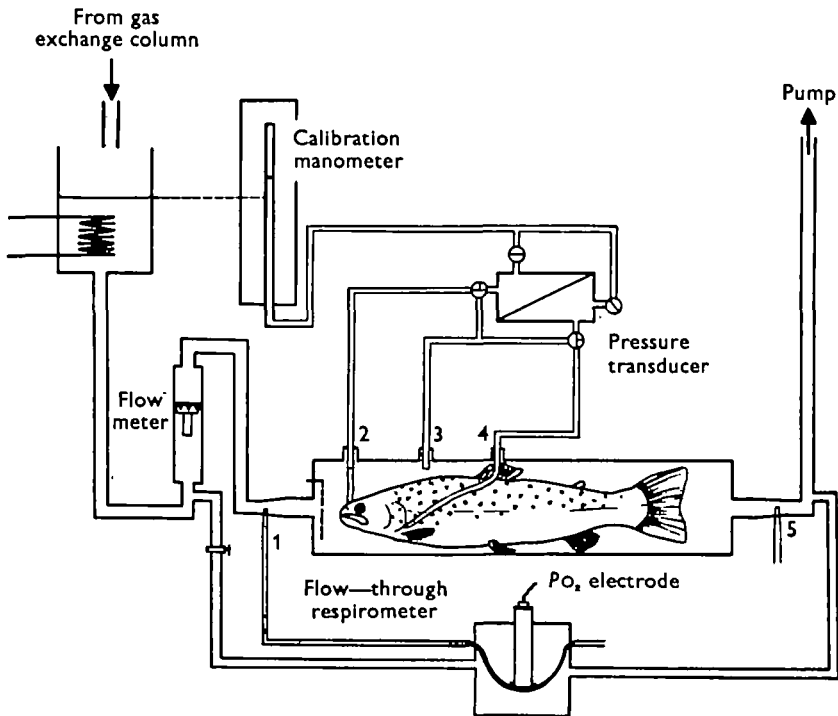

Fig. 1. Diagram of the apparatus used for recording pressure changes in the respiratory cavities and simultaneous measurements of minute volume and O_2 consumption. Pressures were recorded through buccal (2) and opercular (4) cannulae, relative to zero pressure in the respirometer (3). The taps of the Sanborn 268 manometer are shown in the position used for recording the differential pressure. P_{O_2} was measured with a Beckman macroelectrode which is shown in position for measurement of the inflow (through cannula 1); by means of cannulae 2, 4 and 5, the P_{O_2} of the inspired, expired and outflow waters were determined.

After initial determinations with air-saturated water the O_2 content was lowered by bubbling nitrogen through the gas–exchange column. Usually the P_{O_2} changes were relatively slow (about 20 mm Hg/20 min) and the fish was kept at a given P_{O_2} for approximately 30 min before recordings were made. The P_{O_2} was not lowered too far in order to prevent the fish from entering the struggling phase of the response to hypoxia. Even so it seems probable that the unrest resulting from hypoxia contributes to the increased O_2 consumption. The same specimens were used on several separate days and in some instances were maintained in the apparatus for 1 week. The pressures were recorded using a Sanborn differential pressure transducer (Model 268 B).

RESULTS

1. *Pressure changes in the respiratory cavities*

Recordings of the pressure changes in the buccal and opercular cavities of the trout (Hughes & Shelton, 1958) showed that, taking the external pressure to be zero, there were periods when the recorded pressures were greater and less than zero. In the buccal cavity a positive pressure of about 1 cm of water is the most apparent. Direct
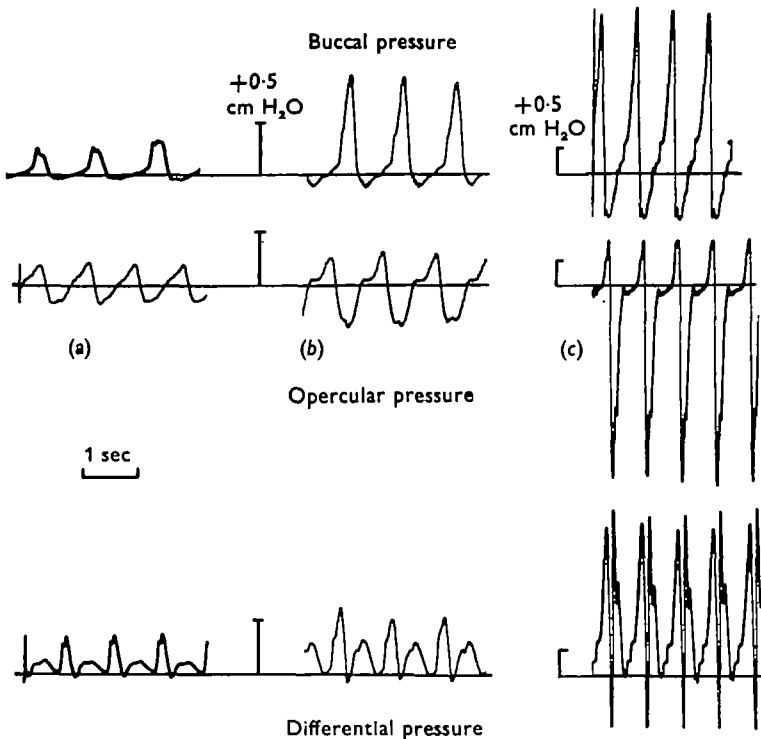

Fig. 2. Pressure recordings obtained from a single specimen during experiments in which the P_{O_2} of the inspired water was successively lowered. The buccal, opercular and differential pressures were recorded in succession at each P_{O_2}, (*a*) 157 mm Hg, (*b*) 80 mm Hg and (*c*), 42 mm Hg. Calibration pressures for (*a*) and (*b*) are the same, but the sensitivity for (*c*) was half that for (*a*) and (*b*). In the differential pressure, a positive pressure indicates a greater pressure in the buccal cavity.

recording of the differential pressure across the gills (Roberts & Hughes, 1967; Hughes & Roberts, 1969, 1970), confirmed the basic type of waveform derived by successive recording from the two cavities. In the present series of experiments similar waveforms have been recorded and changes in them studied when the fish was subjected to a lowering of the P_{O_2} in the inspired water. Changes in total amplitude of the pressure waveform (Fig. 2) were readily determined but for more detailed analyses the areas beneath the curve were measured, especially for the differential pressure across the gills.

(*a*) *Effect of hypoxia on the amplitude of the buccal and opercular pressures.* As the P_{O_2} in the water was lowered, the most obvious response was an increase in amplitude

of the recorded pressures. Changes in frequency were small except with extreme hypoxia. Increases in amplitude were relatively small until the P_{O_2} was lowered to about 80 mm Hg whereupon the amplitude increased markedly (Figs. 2 and 3a). When the P_{O_2} was returned to normal the amplitude of the buccal pressure fell back quite sharply to its normal level, there being a slight hysteresis in this response (Fig. 3b). The effect on the buccal and opercular pressures was mainly on their positive and negative phases respectively, although there were also increases in other phases of the pressure waveforms. Changes in shape of the recorded pressures resulted mainly from their increasing amplitude.
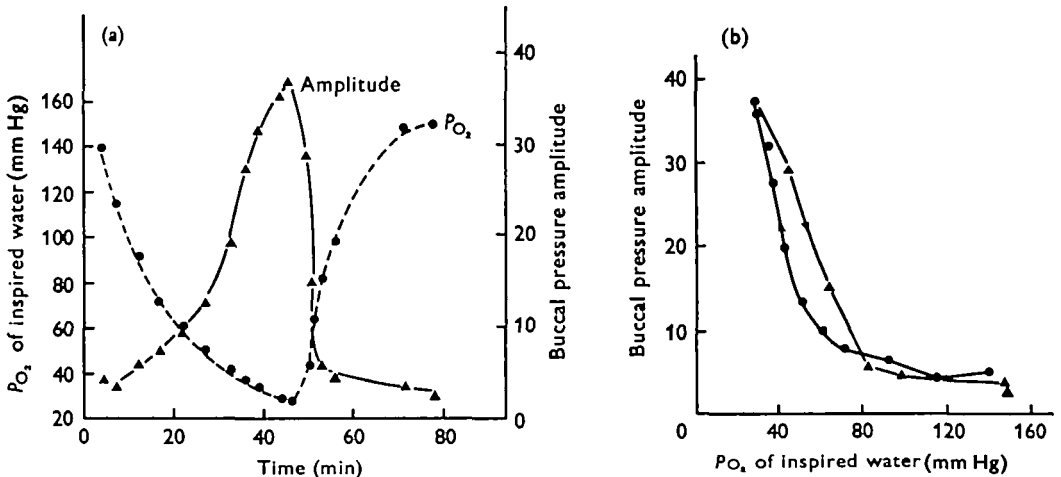

Fig. 3. Effect of lowering the P_{O_2} of the inspired water on the amplitude of the buccal pressure. (a) Changes in amplitude and P_{O_2} of inspired water are plotted against time, and show a gradual increase in amplitude, and a sudden return to normal levels when P_{O_2} was increased. This hysteresis is clear in (b) where the buccal pressure is plotted against P_{O_2}. Note the relatively small change in amplitude at P_{O_2} from 155 to nearly 80 mm, and the slight difference in time course between increasing and decreasing the P_{O_2}.

(b) *Effect of hypoxia on the differential pressure curve.* As a result of the increased amplitude of the buccal and opercular pressure, there is a general increase in mean amplitude of the differential cycle. Normally this pressure waveform is composed of three parts as indicated in Fig. 4. It has two main components corresponding to the opercular suction pump and buccal pressure pump phases, and these are separated by a transitional phase when there is a reversal of the pressure gradient across the gills. In general it is found that the area of the opercular phase (A) is smaller than that of the buccal phase (B), and that the reversal phase (C) is extremely small. In most of the fish studied the ratio between the opercular and buccal phases averaged 0·5–0·7 when the fish was at rest, but in one particular specimen used in the present series of experiments, the ratio was 1:2. These general observations on variation in the balance of the opercular and buccal pump fit in with other observations on this ratio in the rainbow trout (Hughes & Roberts, 1970).

With an increase in pumping under hypoxia it was common for the relative contribution of the two pumps to remain fairly constant. In some cases, however, the ratio changed, usually due to a relatively greater increase in the opercular part of the

differential curve (Fig. 4). In some specimens under stress it was noted that the reversal phase tended to increase, and under extreme conditions there might be two phases of reversal as the two pumps became less closely coupled. Similar uncoupling and associated double reversals were quite common during temperature stresses in rainbow trout (Hughes & Roberts, 1970).
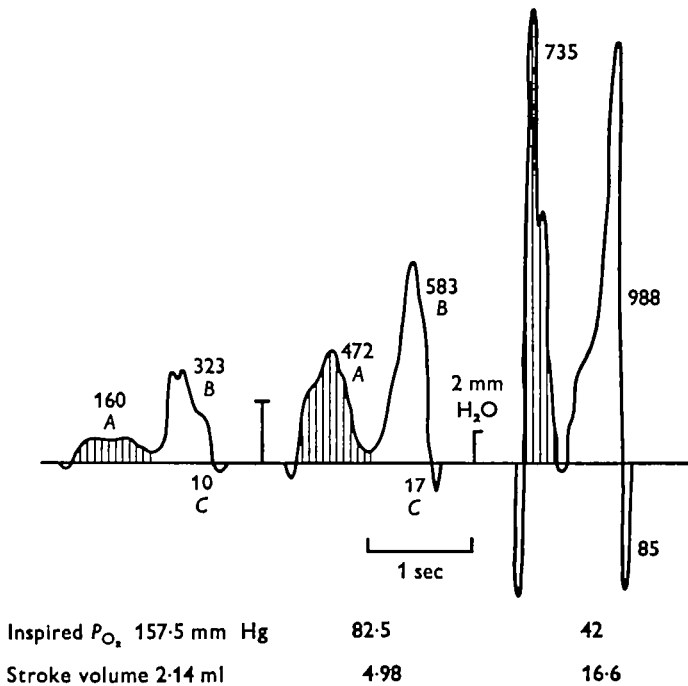

| Inspired P_{O_2} 157·5 mm Hg | 82·5 | 42 |
| Stroke volume 2·14 ml | 4·98 | 16·6 |

Fig. 4. Tracings of differential pressure curves recorded at three different levels of the P_{O_2} of the inspired water. Each curve has been subdivided into three areas (A), (B) and (C); (A) is shown cross-hatched and represents the opercular suction pump phase, whereas (B) represents the buccal pump phase, and (C) is the reversal phase. The numbers indicate the relative areas of the three phases. In this particular fish (A) has remained smaller than (B) throughout the experiment, but its increase in area is relatively greater.

2. *Effects of hypoxia on the respiratory frequency and volume output of the buccal and opercular pressure pumps*

The respiratory frequency was readily determined from the pressure recordings during the period when the minute volume was estimated. Minute volume was estimated from measurements of the P_{O_2} of the inflow and outflow of the respirometer and of the inspired and expired water of the fish.

$$\text{Minute volume} = \text{flow through respirometer} \times \frac{P_{O_2} \text{ inflow} - P_{O_2} \text{ outflow}}{P_{O_2} \text{ insp} - P_{O_2} \text{ exp}}.$$

The volume of water pumped per respiratory cycle (stroke volume) was calculated by dividing minute volume by respiratory frequency.

(a) *Changes in minute volume resulting from changes in the inspired P_{O_2}.* As the P_{O_2} of the inspired water was lowered there was a marked increase in ventilation. As can be seen from Fig. 5, the minute volume changed from a level of about 0·2 l/kg/min to
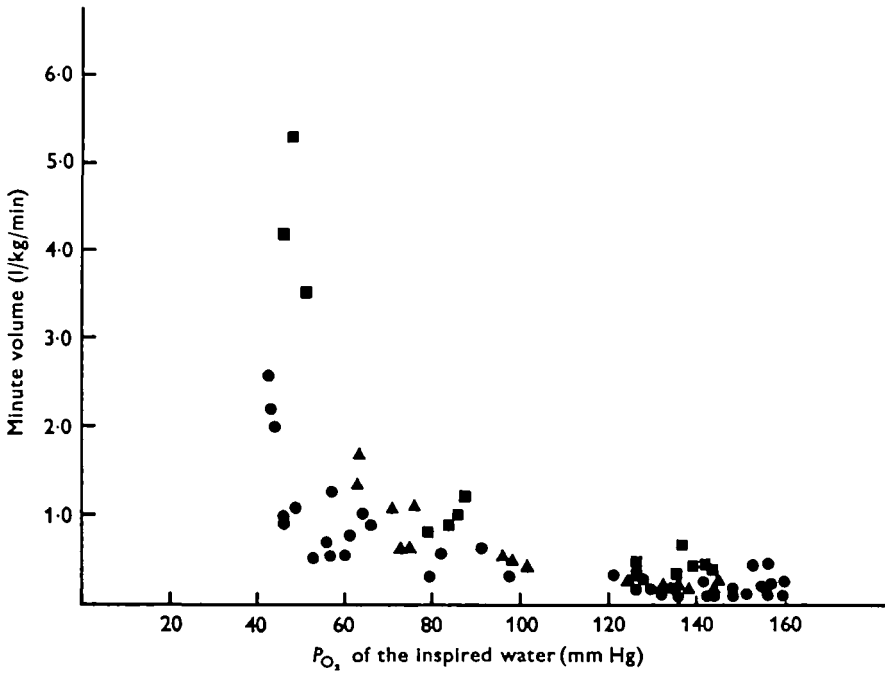
Fig. 5. Relationship between the P_{O_2} of the inspired water and minute volume. Data for three specimens used in several experiments are pooled. The marked increase in the minute volume when the P_{O_2} was below 80 mm Hg is clearly illustrated. Rainbow trout: ■, 402 g; ▲, 528 g; ●, 566 g.
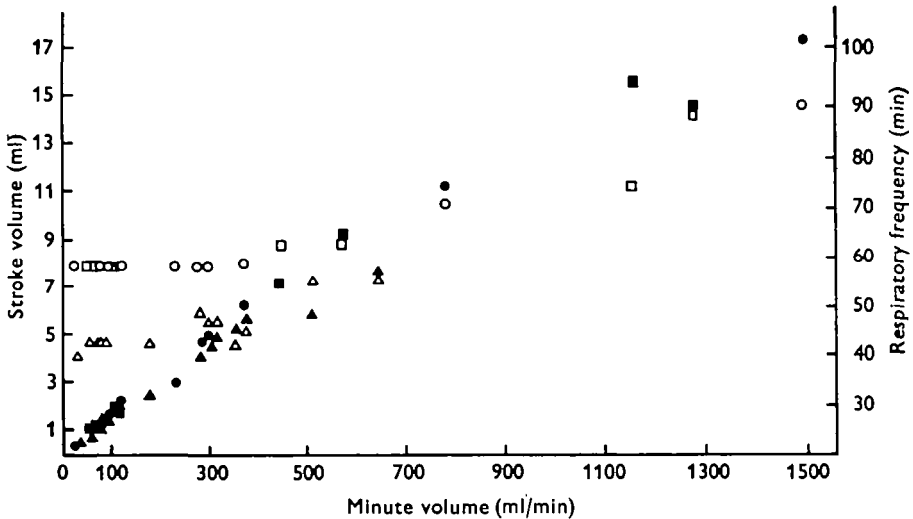

Fig. 6. Plots showing the relative roles of stroke volume and respiratory frequency during increases in minute volume resulting from lowering of the P_{O_2} of the inspired water. Data for a single trout on 3 successive days are shown. Frequency is shown by open symbols and for any day remains constant up to about 500 ml/min. In contrast, the stroke volume (● ■ ▲) increases steadily as minute volume increases. The rate of increase declines above 750 ml/min as the frequency rises.

0·6 l/kg/min as the P_{O_2} was lowered from 140 to 80 mm Hg. With further lowering of the P_{O_2} of the inspired water to 50–70 mm Hg the minute volume increased very sharply to a level of 1–5 l/kg/min. This marked increase is closely associated with an increase in the area beneath the differential pressure curve (Fig. 4).

(b) *Relative roles of frequency and stroke volume in producing changes in minute volume.* During hyperventilation of different fishes increases in minute volume involve changes in frequency and stroke volume to varying extents. In the trout it was found that the respiratory frequency remained relatively constant for an individual fish although the minute volume increased quite markedly. In Fig. 6 the frequency and stroke volume are plotted for a range of minute volumes recorded in experiments on 3 different days with the same rainbow trout. It is apparent that the frequency level was variable in different experiments, but in all cases it remained relatively constant over a range of minute volumes from 0 to 500 ml/ min. Increases in the minute volume over this range were achieved by a more or less linear increase in stroke volume. When the minute volume increased from 500 to over 1500 ml/min, the frequency increased by as much as 50 %, but the stroke volume also continued to increase. These observations are in agreement with those of Stevens & Randall (1967) on the same species, where the increased ventilation resulted from increased exercise of the fish.

3. *Effects of hypoxia on pressure/flow relations*

One of the aims of these experiments was to study the relationship between differential pressure and the volume pumped across the gills of the fish. There is no doubt that in general an increase in the total area beneath the differential pressure curve is associated with an increase in stroke volume. The precise nature of this relationship, however, varies between individuals and even in the same individual at different times when subjected to identical changes in the P_{O_2} of the inspired water. No doubt these variations are related to changes in gill resistance which have been found to occur in the tench (Hughes & Shelton, 1958). Moreover, there may be variations owing to morphological changes in the respiratory pumps or to changes in co-ordination between these two pumps (Ballintijn, 1968).

(a) *Relationship between differential pressure and the P_{O_2} of the inspired water.* In all experiments a lowering of the P_{O_2} resulted in an increase in the differential pressure across the gills. Measurements of amplitudes were not satisfactory, but determinations of the area beneath the curve for a single cycle or of the equivalent mean differential pressure operating throughout a period during which the minute volume was measured were more appropriate. Changes in minute volume are shown in Fig. 5, where there is a gradual increase until the P_{O_2} falls below a level of 80 mm Hg. Below this level the differential curve shows a very marked increase (Fig. 4).

(b) *Relationship between differential pressure and minute volume or stroke volume.* In Fig. 7a is plotted the relationship between minute volume and mean differential pressure operating across the gills during the period when the minute volume was measured. In Fig. 7b the relationship between the average volume pumped during each respiratory cycle (stroke volume) is plotted against the area of the differential pressure curve during a single cycle. In both types of plot there is evidence of a region where there is a direct relationship between the pressure gradient and flow. The precise nature of this relationship varies from individual to individual and also for a

given individual when used in experiments on successive days. Thus in Fig. 7a most of the data show a fairly consistent relationship but the data obtained on 27 June (Expt 17) are rather different. In the latter experiment the flow at a given differential pressure was about half that measured on other days when the same fish was studied.
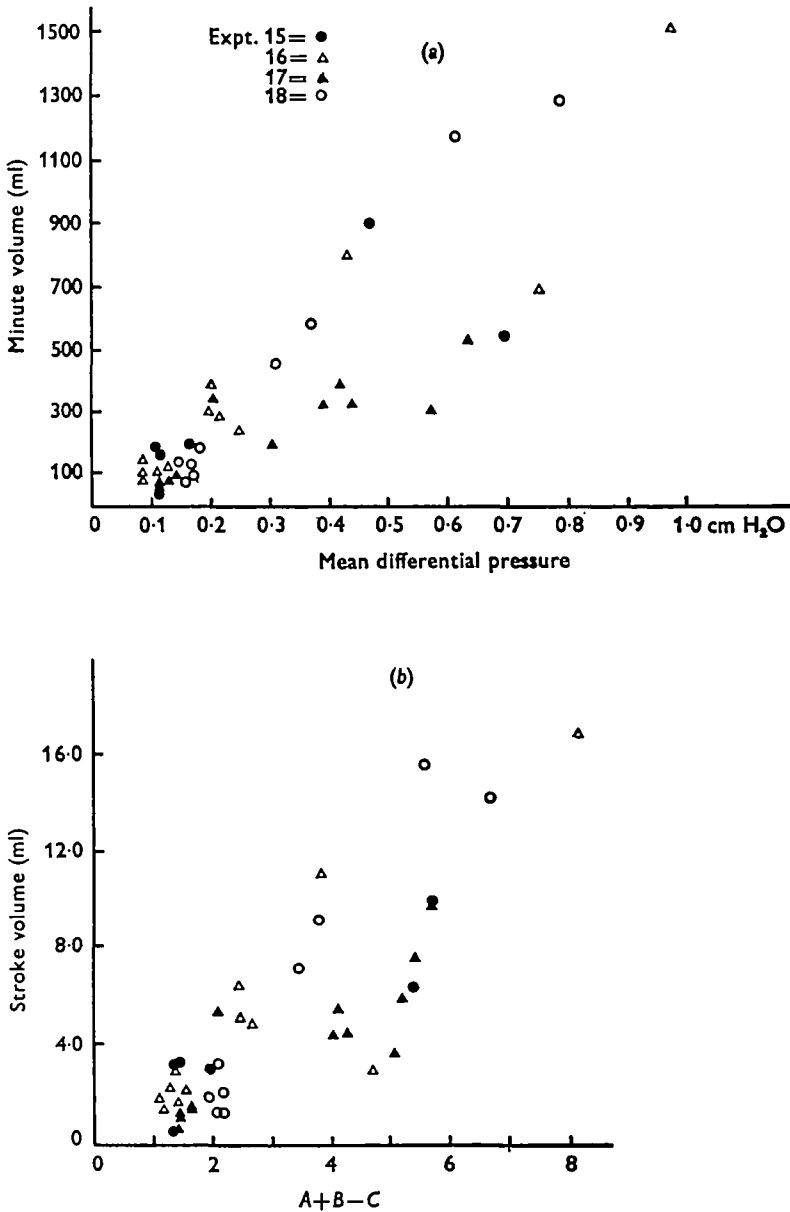

Fig. 7 (a) Relationship between minute volume and the mean differential pressure operating across the gills. Data for the same fish on four different days are indicated by different symbols.
   (b) Relationship between stroke volume and the area of the differential pressure curve during a single respiratory cycle. The components of the differential curve (A, B and C) are the same as shown in Fig. 4.

With increasing differential pressures the flow often increased more rapidly once it was above a certain level. This suggests that the resistance to flow of water through the gills had fallen. The ratio

$$\frac{\text{mean differential pressure}}{\text{minute volume}}$$

can be taken as a measure of the gill resistance (Hughes & Shelton, 1968).

(c) *Relationship between gill resistance and minute volume.* This relationship has not yet been worked out in detail, but results suggest that the resistance falls with an increase in minute volume (Fig. 8) as was found in the tench (Hughes & Shelton, 1958). The fall in resistance is probably due to an increased portion of the water flow
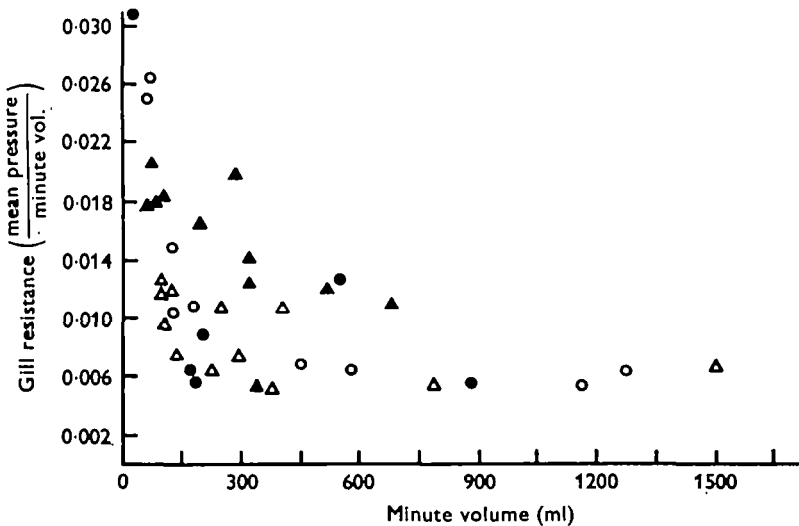

Fig. 8. Data from the same experiments as Fig. 7 are plotted to show the change in gill resistance (mean differential pressure/minute volume) with increasing minute volume. Notice that the resistance for 27 June (▲) is about twice that for the other days.

through the gills being shunted past the tips of the gill filaments. This could be a purely passive effect, as the increasing size of this slit has been observed directly during hyperventilation of the sucker, *Catostomus commensoni* (Saunders, 1961). As the level at which the resistance appears to fall markedly is also about the same level (300 ml/ min) as that at which the frequency of respiration increases, the lowered resistance to flow might be due to a significant decrease in coupling between different components of the pumping mechanism (Ballintijn, 1968). This might result, for example, if the normal highly co-ordinated changes in volume of the cavities with the active expansion and contraction of the gill sieve became disturbed. Unfortunately little is known of the phase relationship between the volume and associated pressure changes in the respiratory cavities.

4. Effect of hypoxia on consumption and percentage utilization of oxygen

(a) *Relationship between O_2 consumption and P_{O_2} of the inspired water.* During these experiments the environment and activity of the fish were kept as constant as possible, with the exception of the lowering of the P_{O_2}. It may be assumed, therefore, that all changes observed arose directly from the changes of P_{O_2}. As the P_{O_2} was lowered there was a gradual increase in the O_2 consumption of the fish (Fig. 9). This increase was
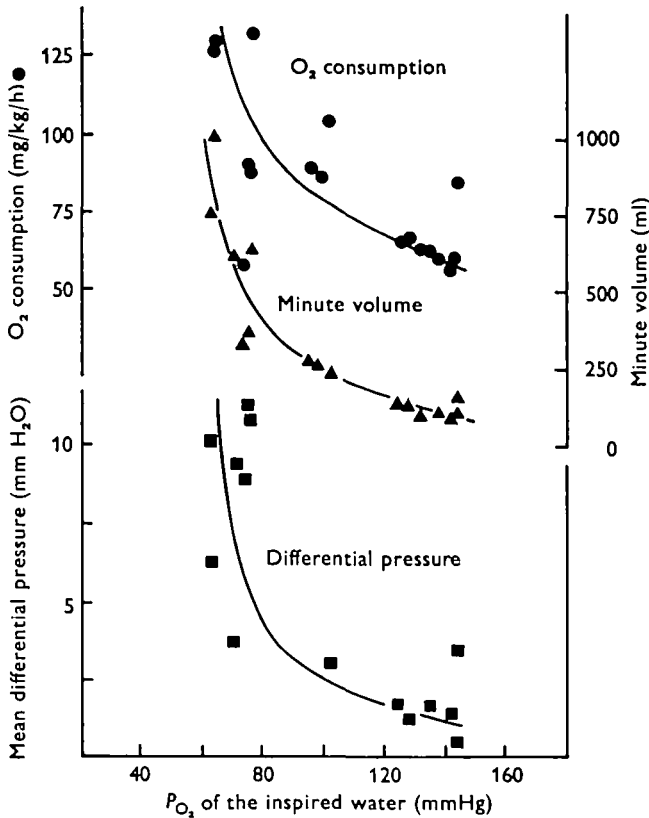

Fig. 9. Graph showing changes in O_2 consumption, mean differential pressure, and minute volume during experiments in which the P_{O_2} of the inspired water was lowered. At P_{O_2} levels below 80 mm, there was a marked rise in all three parameters plotted.

fairly gradual down to P_{O_2} of 90 mm Hg, but then the O_2 consumption increased quite markedly. The initial resting levels found were in the range 40–60 mg/kg/hr, which rose to levels of 80–90 mg at P_{O_2} of 90 mm and then to levels above 120 mg with further lowering of the P_{O_2}. During the present series of experiments the lowering of the P_{O_2} was fairly gradual and was not usually continued below 60 mm Hg. Thus the fish was never exposed to the conditions under which other workers have observed a decline in O_2 consumption at lowered P_{O_2} as the respiration became dependent.

(b) *Relationship between O_2 consumption and minute volume.* As shown in Fig. 10 an increase in O_2 consumption at lowered P_{O_2} is associated with a marked increase in ventilation. Up to minute volumes of about 300 ml/min there was a noticeable increase

in O_2 consumption, but above this level the O_2 consumption rose much more slowly. These changes in O_2 consumption were also related to well-defined changes in the proportion of the O_2 in the water that was extracted by the fish. Thus the percentage utilization fell from about 70% to below 50% when the ventilation volume exceeded 200 ml/min. With the higher minute volumes the utilization was less than 30–20%. It is thus apparent that, with increasing pumping during hypoxia, the fish is facing an increasing probem in obtaining sufficient oxygen; for not only is there a reduction in the O_2 content in the inspired water, but the fish is also extracting a smaller proportion of the O_2.
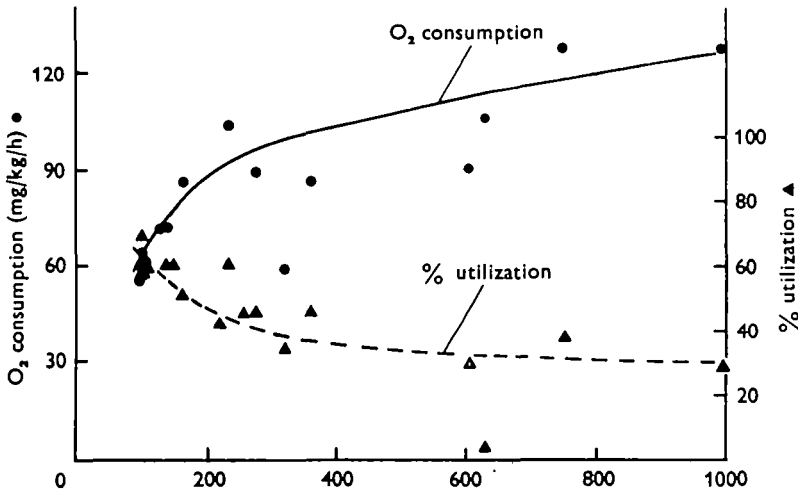

Fig. 10. Plots to show the increase in O_2 consumption and fall in percentage utilization as the minute volume increases during hypoxia. The fish weighed 528 g.

(c) *Relationship between the percentage utilization and minute volume.* Increases in the flow of water past the gills are associated with a reduction in the percentage of the oxygen that is extracted. Such a relationship has been found for a number of species (van Dam, 1938; Hughes & Shelton, 1962; Saunders, 1962). As with the decrease in gill resistance with increasing water flow, so the fall in utilization is partly related to the increasing proportion of the water being shunted past the tips of the gill filaments, which is perhaps analogous with the anatomical dead space of the mammal (Hughes, 1966). In addition, however, the more rapid flow of water between the secondary lamellae would also increase the volume of water which fails to approach equilibrium with the blood and as such constitutes a physiological or diffusion dead space. Analysis of the data using log/log co-ordinates (Fig. 11), showed that for the trout the relationship between percentage utilization and minute volume fell on a straight line with a slope of about −0·38. This relationship is comparable to the relationships obtained by Saunders (1962) with other freshwater fish such as the bullhead, *Ictalurus nebulosus*, where the slope was about −0·54.

(d) *The O_2 cost of breathing.* The increased O_2 consumption of a fish during increased pumping resulting from hypoxia has been observed by a number of workers. Van Dam (1938) found that for the rainbow trout, a 3-fold or 4-fold increase in minute

volume was accompanied by a 75 % increase in O_2 consumption. Assuming that the whole of this extra oxygen consumed was due to the increased muscular effort of the respiratory pumps it was estimated (Hughes & Shelton, 1962) that the respiratory muscles used approximately 20–25 % of the O_2 uptake of the fish during such hyper-
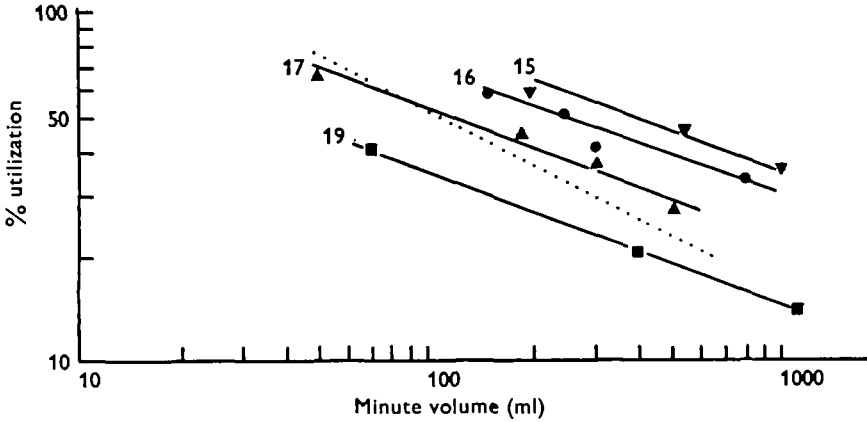

Fig. 11. Relationship between percentage utilization and minute volume plotted on log/log co-ordinates. The data for the same trout used in four separate experiments are numbered 15, 16, 17 and 19. The thickest line is drawn through the data which overlaps that obtained for the bullhead by Saunders (1962), shown in dotted line.
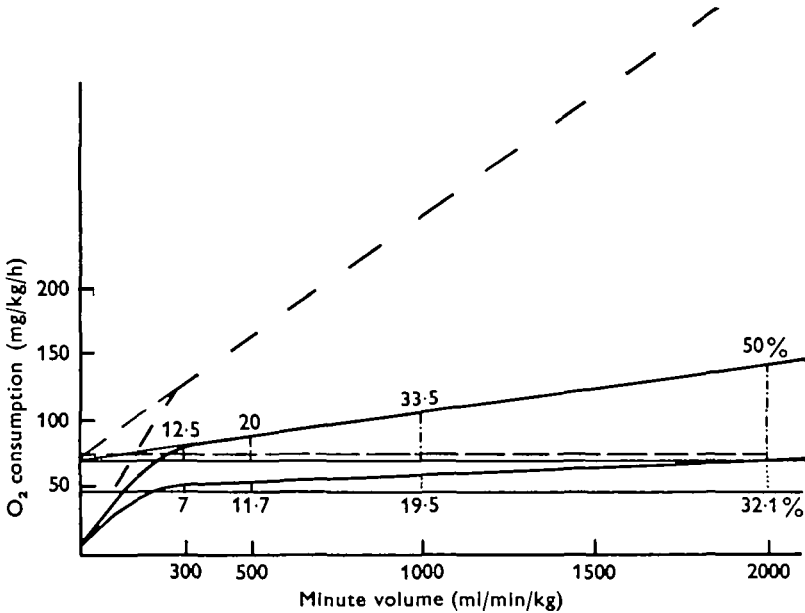

Fig. 12. Diagram to show the effect of increased ventilation on the total O_2 consumption of trout and tench, and how it may be used to estimate the O_2 cost of breathing. Two sets of data from the same trout are plotted (full lines) together with one set of data derived from similar plots by Schumann & Piiper (1966) for tench. In each case the upper part of the curve is extrapolated to zero ventilation and gives a base line for the O_2 cost of non-ventilatory activities of the fish. The O_2 cost of breathing is given by the increments above this line at 300, 500, 1000 and 2000 ml/min/kg and have been inserted for the two trout curves as a percentage of total O_2 consumption at these levels. These figures together with comparable estimates for tench are given in Table 1.

ventilation. Schumann & Piiper (1966) studied this problem in the tench and observed changes in O_2 consumption in relation to spontaneous changes in ventilation volume. They showed a marked increase in consumption with increase in pumping. Using a method of estimating the O_2 cost of ventilation similar to that described above, they obtained values as high as 40% at normal levels of ventilation.

From the present experiments plots can be made showing the relationship between minute volume and O_2 consumption of the fish (Fig. 12). These curves, like those of Schumann & Piiper (1966), show a sharp increase in O_2 consumption up to about 300 ml/min/kg fish, but above this the curve flattens out and rises much less steeply. Taking the slope of this upper section of the curve and extrapolating back to zero ventilation, a value is obtained for the portion of the O_2 uptake which is used by non-ventilatory activities of the fish. Assuming that this remains constant at all levels of ventilation, one can estimate the O_2 consumption due to activity of the respiratory muscles as that portion which is above this level. In Fig. 12 two curves are given to show the range for an individual trout. Values obtained for the percentage of the O_2 consumption used in ventilation of three individual trout are set out in Table 1, where it is apparent that a marked increase takes places at higher ventilation where levels of up to 40% may be obtained but at normal levels values are closer to 10%. It should be noted that these figures are somewhat lower, but of the same order, as those obtained by Schumann & Piiper (1966) for the tench.

DISCUSSION

These experiments have shown that as the P_{O_2} of the inspired water is reduced the effects on the respiratory pumps are slight until levels of 70 and 80 mm Hg are reached. Similar observations have been made for other cardio-respiratory parameters by other workers using trout (Randall, Holeton & Stevens, 1967). Of particular interest in the present context is the significant rise in O_2 consumption. Similar increases can be observed in the data of Marvin & Heath (1968) and of Holeton & Randall (1967), although in the latter case the authors suggest that the increase is not statistically significant. Some of the differences observed by different workers are related to the rate at which the O_2 levels are lowered, and there is a great need for a systematic study of this aspect of the problem. From the present point of view, the effect of increasing ventilation has enabled analysis to be made of the mechanisms whereby this is produced, especially the relationship between the differential pressure and the volume pumped.

Initial increases in minute volume are mainly achieved as a result of a rise in stroke volume, and only at higher levels of ventilation does the frequency markedly increase. The relative importance of these two components during changes in ventilation volume vary in different species. Van Dam (1938) also observed a relatively constant frequency in trout, but in other fish (e.g. bluegill, *Lepomis machrochirus*) changes in frequency seem more important (Marvin & Heath, 1968). In the tench both frequency and stroke volume were found to increase, the stroke volume being the more important (Hughes & Shelton, 1962). In the eel van Dam (1938) also observed the dual role of both stroke volume and frequency. In other fish such as *Callionymus*, hypoxia produces a lowering of the respiratory frequency but an increased depth of ventilation

so that the slight rise in minute volume is mainly achieved as a result of this marked increase in stroke volume (Hughes & Umezawa, 1968).

This study has indicated parallel effects of hypoxia on differential pressure, minute volume and O_2 consumption. The correlation suggests that the increased O_2 consumption is due to the added work of breathing and has enabled estimates to be made of the proportion of the O_2 uptake used by the ventilatory musculature (i.e. the O_2 cost of breathing). No attempt was made to utilize the method introduced by Schumann & Piiper (1966) for estimating the non-ventilatory O_2 uptake at comparable flow rates by using fish artificially ventilated, following paralysis of the respiratory muscles by succinyl choline. Fig. 12 summarizes the data showing the relationship between O_2

Table 1. *Changes in the cost of breathing as a percentage of O_2 consumption of the fish at different levels of ventilation. Estimates for three specimens of trout and mean figures for tench*

Fish	Body weight (g)	Ventilation volume (ml/min/kg) 300	500	1000	2000
Trout	402		4·5	7·3	13·7
	528		12	20·4	34
	565 (a)	7	11·7	19·5	32·1
	(b)	12·5	20	33·3	50
Tench*					
(i) maximum	Method as above	41	53	70	82
(ii) minimum	Using succinyl-choline line	19	30·8	45	55·8

* Based on Schumann & Piiper (1966).

uptake and minute volume for an individual trout. Extrapolation of the upper part of these curves to zero ventilation gives a value for the portion of the O_2 uptake used in non-ventilatory activities. The added O_2 uptake at minute volumes of 300, 500, 1000 and 2000 ml/min/kg fish can therefore be calculated (Fig. 12, Table 1). Thus at a minute volume of 500 ml it is estimated that between 11·7% and 20% of the oxygen consumed by this particular trout is used by the respiratory muscles. The data of Schumann & Piiper gave a figure of about 53% using a similar method, but if their succinyl choline line was taken to indicate the level of nonventilatory O_2 uptake, then the proportion of the total uptake used by the ventilatory muscles is 30·8%. Over the range given in Table 1 and plotted in Fig. 12 it can be seen that as the ventilation volume increases so the percentage of oxygen required by these muscles increases very rapidly to figures as high as 82% in the tench and 50% in the trout. The level of ventilation (2000 ml/min/kg) is of course very great.

From the pressure and volume measurements obtained in the present experiments it is also possible to estimate the work of breathing, as distinct from the O_2 cost of breathing discussed above. The work of breathing can be considered as being made of three components, that required to pump water through resistances provided by (a) the gape of the mouth, (b) the gills and (c) the opercular slits. The main resistance is provided by the gills, and the differential pressure curve indicates the mean pressure drop here. Pressure gradients across the mouth and opercular openings occur during

half the cycle and together amount to about 40% of that across the gills. Hence the total work of breathing is given by:

$$\text{Min.vol} \times (\Delta_p \text{ gills} + 0.4 \; \Delta_p \text{ gills}),$$
$$(\text{ml/min} \times \text{dynes/cm}^2 = \text{ergs/min}),$$
$$(10^7 \text{ ergs} = 0.239 \text{ cal}).$$

The calculated work done can then be converted to the O_2 equivalent ($1 \text{ cal} \equiv 0.2 \text{ ml } O_2$). At low ventilation volumes of 100 ml/min estimates for different trout of about 500 gm weight gave values of about 0.0005 ml O_2/min. With increases in ventilation volumes the O_2 equivalent increases to varying extents as shown in Table 2. By comparing these estimates of the O_2 equivalent of the work of breathing with the known increase in O_2 uptake between two particular values of ventilation volume, some estimate of the efficiency can be obtained (Table 2). Estimates obtained for three fish averaged 9.8%.

Table 2. *Calculation of overall efficiency of the mechanism pumping water across the gills of a rainbow trout (566 g)*

Ventilation volume	100 ml/min	1200 ml/min
O_2 equivalent of work done/min	0.00005 ml	0.01452 ml
O_2 uptake/min	0.42 ml	0.70 ml

Hence increase from 100 to 1200 ml/min involves equivalent of 0.01447 ml O_2 extra work and costs 0.28 ml increase in O_2 uptake

Hence overall efficiency is $\dfrac{0.01447}{0.28} \times 100 = 5.17\%$.

This 'efficiency' includes a large number of factors which are involved as the O_2 uptake increases during hyperventilation. Clearly there is the increased work in pumping the water across the respiratory system, the efficiency of the conversion of chemical energy into mechanical work, the efficiency of this mechanical work which, in addition to overcoming the resistance to water flow, must also involve losses due to friction within the muscles. Finally, there must also be some efficiency related to the cardiac part of the system. However, it seems that this changes relatively little during hypoxia as deduced from the relatively constant product of systolic pressure and cardiac stroke volume (Randall, Holeton & Stevens, 1967).

The general conclusion from these observations is therefore that at low levels of ventilation the proportion of the O_2 uptake used by the ventilatory muscles is of the same order as the higher estimates obtained for mammals (Otis, 1965). However, because of the high density and viscosity of water and its relatively low O_2 content, the O_2 consumption by the respiratory muscles must increase very rapidly at higher ventilation volumes. The situation is aggravated by the lowering in percentage utilization of oxygen as flow increases. Consequently a limitation on O_2 uptake would be reached at ventilation volumes of perhaps 10 times the normal resting level. The efficiency with which the respiratory muscles produce ventilation of the gills is of the same order as that shown by the locomotory muscles (P. W. Webb, personal communication).

SUMMARY

1. Unanaesthetized rainbow trout, when subjected to a lowered P_{O_2} of the inspired water, show an increase in amplitude of pressures recorded from the buccal and opercular cavities. Pressure amplitudes were commonly found to be 0·5 cm of water in resting trout and increased 4- or 5-fold as inspired P_{O_2} was reduced. Differential pressures across the gills also increased with hypoxia.

2. Typically the minute volume in a 400–600 g trout increased from about 0·2 to 0·6 l/kg/min when the inspired P_{O_2} was lowered from 150 to 80 mm Hg, but rose to 1–5 l/kg/min at lower P_{O_2}. Increased minute volumes are mainly due to increases in stroke volume; respiratory frequency remains fairly constant at P_{O_2}'s above about 80 mm Hg.

3. The relation between differential pressure and minute volume is fairly linear over much of the range, but minute volume increases more rapidly than differential pressure.

4. Oxygen consumption of the non-swimming fish increases during hypoxia and is related to the increased ventilation and differential pressure across the gills.

5. Estimates of the oxygen cost of breathing were made from the increased oxygen consumption during hyperventilation. Comparison of these estimates with estimates of the work done, using the pressure and volume measurements, gave figures for the overall efficiency of the pumping mechanism of about 10%.

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