THE EFFECT OF CHANGES IN BLOOD OXYGEN-CARRYING CAPACITY ON VENTILATION VOLUME IN THE RAINBOW TROUT (*SALMO GAIRDNERI*)

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SUMMARY

1. Changes in ventilation volume ($\dot{V}_g$) of rainbow trout caused by hypercapnia, hypoxia and anaemia were measured directly by collection of expired water.

2. Exposure to hypercapnic water (P_{CO_2} range 0·5–2 kPa) increased $\dot{V}_g$ (by up to four times) by augmenting ventilatory stroke volume; breathing frequency remained constant. O_2 added to the inspired water in maintained hypercapnia reduced $\dot{V}_g$ at all but the highest level of P_{CO_2}.

3. $\dot{V}_g$ increased when blood oxygen content was decreased by exposure to normoxic hypercapnia, but addition of O_2 to the water increased blood oxygen content and $\dot{V}_g$ decreased.

4. When blood oxygen-carrying capacity was depressed by hypoxia or anaemia, $\dot{V}_g$ increased as it did during normoxic hypercapnia.

5. We suggest that ventilatory responses to low levels of hypercapnia, to hyperoxic hypercapnia, to hypoxia, and to anaemia in trout are related to changes in levels of blood oxygen content under these conditions.

INTRODUCTION

Nearly all teleosts displaying respiratory sensitivity to hypercapnia increase ventilatory stroke volume with little or no change in breathing frequency (Dejours, 1973), but the reason for this response is unclear. Elasmobranchs initially respond to hypercapnia in a similar fashion to teleosts, with an increase in stroke volume; however, breathing returns to normal in these animals within several hours in maintained hypercapnia (Randall, Heisler & Drees, 1976). Ventilation in teleosts takes much longer, up to several days, to return to normal in maintained hypercapnia (Janssen & Randall, 1975).

Dejours (1973) noted that while normoxic hypercapnia stimulated breathing in trout, hyperoxic trout were insensitive to moderate increases in CO_2. Randall & Jones (1973) and Truchot, Toulmond & Dejours (1980) reported that ventilation was depressed in hyperoxic trout even though arterial P_{CO_2} (P_{a,CO_2}) levels increased, a condition indicated by decreased arterial pH (pH_a). Dejours (1973) concluded that CO_2 was not a strong ventilatory stimulant as long as there was sufficient oxygen in the blood. Thus, the ventilatory responses of trout to hypercapnia may be due to hypoxaemia rather than to direct effects of CO_2 on the ventilatory system. Hughes &

Shelton (1962), Randall & Jones (1973) and Janssen & Randall (1975) have suggested that the marked Bohr and Root effects observed in trout blood during hypercapnia result in reduced ability of the blood to carry oxygen, to which the animals respond by increasing their ventilatory convection. If, during hypercapnia, the oxygen content of the blood is raised by increasing the inspired oxygen tension (P_{I,O_2}), the hypoxaemia will be corrected and the ventilation volume should decrease. It follows that, if $\dot{V_g}$ is related to the level of oxygen carried in the blood, and not related intrinsically to blood CO_2 levels, $\dot{V_g}$ should be affected by any condition which alters blood oxygen content. In this study, our intentions were to examine the effects of various levels of inspired CO_2 on ventilation rate and volume, and to investigate the possible relationship of blood oxygen-carrying capacity to ventilation in the rainbow trout.

MATERIALS AND METHODS

Experiments were done on 42 hatchery-reared rainbow trout (*Salmo gairdneri*) ranging in mass from 0·15 to 0·38 kg. Fish were kept in outdoor holding tanks supplied with aerated dechlorinated tap water, and were fed trout pellets twice weekly. Water temperature in the holding tanks ranged from 6 to 11 °C (mean temperature $8·8 \pm 0·4$ (S.E.M.) °C). During all experiments water temperature was the same as that in the holding tanks.

Accurate measurements of $\dot{V_g}$ are essential so we chose to measure $\dot{V_g}$ directly by collection of expired water, using a technique first described by van Dam (1938). This technique is more accurate than that of estimating $\dot{V_g}$ indirectly using the Fick principle, a method which has been widely used in the past. Direct measurement of $\dot{V_g}$ involves the separation of expired from inspired water by a rubber membrane fitted around the jaws of the fish. The major disadvantage of this method is that the membrane may restrict the breathing of the animal. Cameron & Davis (1970) described a membrane made from a surgeon's latex rubber glove which circumvents this problem, and their technique for measuring $\dot{V_g}$ was adopted for this study.

All surgical procedures were carried out under general anaesthesia. Fish were initially anaesthetized in water containing MS 222 (tricaine methane sulphonate, 1:10000 dilution), then transferred to an operating table similar to that described by Smith & Bell (1967). During surgery the gills were continuously irrigated with water containing a reduced level of MS 222 (1:20000 dilution).

Oral membranes were prepared from no. $8\frac{1}{2}$ surgeon's latex rubber gloves and fitted following the technique of Cameron & Davis (1970). A membrane was made from a section of glove cut to include the thumb and a surrounding circular skirt 12 cm in diameter. A V-notch was cut in the thumb to fit the shape of the jaws and the membrane was sutured to the skin around the mouth with no. 4–0 surgical silk while the gills were ventilated via the mouth tube.

A dorsal aortic cannula was implanted in fish used in experiments requiring blood samples as well as ventilatory measurements, before they were fitted with an oral membrane. The cannula consisted of a 15 cm length of polyethylene tubing (PE 50, Intramedic, Becton Dickinson and Co., Parisppany, N.J.) filled with heparinized saline (50 i.u. heparin per ml), and was implanted while the gills were ventilated

through the opercular openings. The cannula was inserted into the dorsal aorta through the roof of the mouth at the level of the second gill arch openings, with the aid of an 18 gauge Sherwood catheterization needle (Sherwood Medical Co., St Louis, MO.). A single stitch anchored the cannula to the roof of the mouth, and the cannula was passed through the snout via a sleeve of PE 190 tubing as described by Smith & Bell (1964). After placement, the cannula was checked for patency by withdrawing blood into the tubing, then flushing with heparinized saline before plugging it with a metal pin. After surgery, animals were transferred to a ventilation chamber and allowed to recover for 24 h.

The type of ventilation chamber used in this study was a modified van Dam chamber similar to that described by Cameron & Davis (1970). The chamber consisted of a rectangular perspex box divided by a partition into two compartments, a small anterior compartment, and a larger posterior compartment which had an opaque rectangular tube into which the fish was placed. The partition had a central hole 10 cm in diameter. The skirt of the oral membrane was clamped to the partition around the periphery of the hole by means of a perspex ring held by wing nuts. The anterior compartment, continuously supplied with fresh water, was separated from the posterior compartment by the membrane and partition. Water from the anterior compartment ('inspired water') could only enter the posterior compartment ('expired water') by passing through the gills and opercular openings. Each compartment had a stand-pipe drain which was used to set the water level, and during the period of recovery from anaesthesia the front drain was set 1–2 cm higher than the rear drain to create a positive pressure head across the gills to force-ventilate the fish. The fit of the membrane was checked during force-ventilation by injecting 1 ml of a dye solution into the anterior compartment and watching for dye leakage into the posterior compartment around the membrane seals. Any leaks were repaired before proceeding with the experiment.

About 1 h before an experiment was begun, the drains were set at the same level so that water overflowed continuously from the anterior drain while the posterior drain passed water expired by the fish. Water levels in the two compartments were taken to be identical when the membrane exhibited some slackness, indicating the lack of a pressure difference between the compartments. Ventilation volume was measured by collecting the outflow from the posterior drain for periods of 1 min. Ventilation frequency (V_f) was obtained during the same time periods by counting opercular movements.

The water used in these experiments was dechlorinated Vancouver City tap water, hardness 5·4 mg l^{-1} CaCO$_3$ (D. J. Randall, personal communication), $P_{CO_2} <$ 0·04 kPa (0·3 mmHg) and pH 7·4–7·5. The concentrations of gases in the water supplied to the fish could be adjusted by combining the outflow from reservoirs containing water equilibrated with air, N$_2$, CO$_2$, or O$_2$ gas mixtures. Air-saturated water (P_{O_2} 20·6 ± 0·6 kPa; mean ± 1 S.E.M.; 154·5 ± 4·5 mmHg) was mixed with water from a reservoir equilibrated with a 10 % CO$_2$ in air gas mixture to give normoxic hypercapnic water (the P_{CO_2} was varied from 0·5 ± 0·03 to 2·0 ± 0·09 kPa; 3·8 ± 0·2 to 15 ± 0·7 mmHg, depending on the experimental requirements). Hyperoxic hypercapnic water was produced by substituting hyperoxic water (P_{O_2} 60·4 ± 1·7 kPa;

453 ± 13 mmHg) for air-saturated water in the above mixture. Hypoxic water (P_{O_2} $12\cdot4\pm0\cdot6$ kPa; $93\pm4\cdot5$ mmHg) was obtained by combining water from the air-saturated reservoir with N_2-saturated water.

Anaemia was induced in three fish by withdrawing a quantity of blood through the dorsal aortic cannula, separating the cells from the plasma by centrifugation, and returning the plasma to the fish after adding the appropriate amount of Courtland's saline to compensate for the loss of the volume of the cells. This procedure resulted in the reduction of the mean haematocrit (measured in 50 μl blood samples centrifuged for 3 min at 5000 rev/min) from $22\cdot3\pm2\cdot4\%$ before bleeding to $14\cdot3\pm2\cdot3\%$ after bleeding.

Blood oxygen content (C_{a,O_2}) of 20 μl samples from the dorsal aorta was determined using either a Tucker chamber with a volume of 2700 μl (Tucker, 1967), or a Lex-O_2-Con TL oxygen content analyser (Lexington Instruments, Waltham, MA.) Oxygen contents of blood samples taken consecutively from the same animal agreed within $0\cdot2$ vol. % when measured using both techniques. Since this difference represented only 5 % of the lowest oxygen content measured in this study (4 vol. %), oxygen content data obtained from both methods of measurement were pooled.

Gas tensions in both blood and water were measured using Radiometer P_{O_2} and P_{CO_2} electrodes in conjunction with a Radiometer PHM 71 B Acid-Base Analyzer which included a P_{CO_2} module with a 'linearizing' circuit. The P_{O_2} electrode was maintained at the prevailing water temperature by a water-jacket connected to the freshwater supply, and was calibrated using N_2-saturated water (zero P_{O_2} standard) and air-saturated water.

Two steps were taken to increase the sensitivity of the P_{CO_2} measuring system, in order accurately to measure the low CO_2 tensions involved in this study. First, the sensitivity of the CO_2 electrode was enhanced by increasing the temperature of the water in the water-jacket surrounding the electrode to 41 °C. Second, the gain of the PHM 71 B was expanded to read 2 kPa at full scale. These steps made it necessary to recalibrate the meter scale in arbitrary units, using a CO_2-free solution to establish the minimum reading, and a precision mixture of CO_2 and N_2 gas dissolved in water to give a full-scale reading of 2 kPa. The P_{CO_2} electrode was calibrated before each determination. In order to check the linearity of this system, a calibration curve was constructed over the range of $0\cdot1$–$2\cdot0$ kPa using precision mixtures of CO_2 and N_2 dissolved in water. The scale was linear over this range.

The pH of arterial blood was measured using a Radiometer pH electrode in a water-jacketed cuvette maintained at the temperature of the fish.

Two series of experiments were done in this study. In the first series, the effects of hypercapnia and hyperoxic hypercapnia on ventilation were observed. $\dot{V}_g$, V_f and inspired water P_{O_2} and P_{CO_2} (P_{I,CO_2}) were recorded during a sequence of 20 min test periods. Fish were first exposed to normoxia, then to one of four levels of normoxic hypercapnia, then to hyperoxia at the same level of hypercapnia, and finally returned to normoxia. This sequence was repeated at least once for all fish in this series.

Three treatments were used in the second series of experiments. Ventilatory and blood variables were measured:

(a) during the above regime at a P_{I,CO_2} of $0\cdot8$ kPa (6 mmHg),

(b) during 20 min periods of hypoxia, and

(c) during 20 min periods starting 1 h after the induction of anaemia.

All data are presented as arithmetic mean ± 1 S.E.M. Means were tested for significant differences using Student's *t*-test, with 5 % (*P* < 0·05) taken as the statistical limit of significance.

RESULTS

(1) *Effects of normoxic and hyperoxic hypercapnia on ventilation*

Ventilatory changes in response to altered inspired gas tensions were complete within the first 5 min, so the results expressed here represent means of values recorded at the 10, 15 and 20 min points in each test period. Fig. 1 shows ventilation volume and frequency data recorded from 20 trout exposed sequentially to normoxia, to hypercapnia (at one of four levels of CO_2), to hyperoxia at the same level of CO_2, then to normoxia again. Exposure to CO_2 at all but the lowest level caused $\dot{V}_g$ to increase significantly above normoxic (control) values. Addition of oxygen to the hypercapnic water caused $\dot{V}_g$ to decrease significantly from normoxic hypercapnic values at all but the highest level of CO_2. At this level of CO_2, additional oxygen in the water had no effect on ventilation. At a P_{I,CO_2} level of 0·9 kPa (6·8 mmHg) (group B in Fig. 1), $\dot{V}_g$ more than doubled, increasing to 391·5 ± 60 ml min^{-1} kg^{-1} from 155·5 ± 27·3 ml min^{-1} kg^{-1} in normoxia. Raising the oxygen level in the water then caused a decrease in $\dot{V}_g$ to a level not significantly different from the initial value in normoxia. Animals exposed to this level of CO_2 did not struggle, whereas those at higher levels of CO_2 became agitated towards the end of the 20 min hypercapnic periods.

Ventilation frequency did not change significantly from control values during any of the trials, indicating that V_f is not a dependable index of changes in ventilatory flow in trout under these conditions. Changes in $\dot{V}_g$ resulted solely from increased stroke volume, and there appears to be no interaction between amplitude and frequency of breathing in trout exposed to these levels of CO_2 and O_2.

(2) *Effects of changes in blood oxygen content on ventilatory and blood variables*

Results obtained from 18 fish in hypercapnia, hyperoxic hypercapnia, hypoxia and anaemia are shown in Table 1 along with control values (labelled 'air') recorded during normoxic periods before each treatment. Since $\dot{V}_g$ measurements during exposure to high levels of CO_2 were complicated by struggling, we limited the P_{CO_2} tension to 0·8 kPa (6 mmHg) in this series of experiments.

$\dot{V}_g$ is inversely related to C_{a,O_2}. This relationship can be seen clearly in Fig. 2, where C_{a,O_2} and $\dot{V}_g$ data from Table 1 are plotted in bar graph form. In hypercapnic and hypoxic animals and during anaemia, $\dot{V}_g$ increased as C_{a,O_2} decreased, while in hyperoxic hypercapnic animals $\dot{V}_g$ decreased as C_{a,O_2} increased, even though the level of CO_2 in the water remained unchanged.

Arterial blood oxygen tension (P_{a,O_2}) did not change significantly from the control value during CO_2 exposure, but more than doubled during hyperoxic hypercapnia. P_{a,O_2} also increased significantly during anaemia, but decreased significantly during hypoxia.
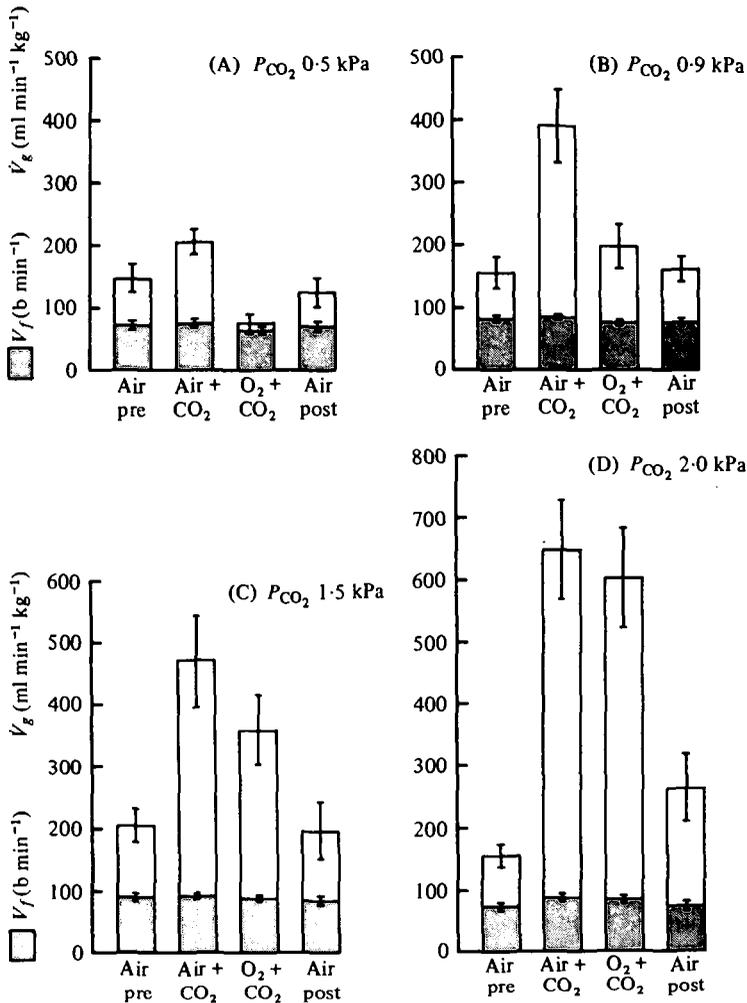
Fig. 1. Effects of CO_2 on ventilation of 20 rainbow trout. Ventilation volume ($\dot{V}_g$, unshaded) and ventilation frequency (V_f, shaded) were recorded during normoxia (air, pre: control value), normoxic hypercapnia (air + CO_2), hyperoxic hypercapnia (O_2 + CO_2), and post-hypercapnic normoxia (air, post) in each group of five fish. Each group was exposed to a different level of P_{I,CO_2}, ranging from 0·5 to 2·0 kPa (3·8–15 mmHg). Error bars indicate ± 1 S.E.M.

Measurements of $\dot{V}_g$, P_{I,O_2} and oxygen tensions of mixed expired water (P_{E,O_2}) were used to calculate oxygen uptake ($\dot{V}_{O_2}$) using the Fick equation:

$$\dot{V}_{O_2} \text{ (ml min}^{-1}\text{ kg}^{-1}) = (\alpha O_2).(\dot{V}_g).(P_{I,O_2} - P_{E,O_2})$$

where αO_2 is the solubility of oxygen in fresh water at the experimental temperature. In spite of the large changes in $\dot{V}_g$ observed during changes in blood oxygen carrying capacity, the animals maintained $\dot{V}_{O_2}$ at levels not significantly different from the control value throughout the experiments.

Arterial blood pH decreased significantly from control during both hypercapni

Table 1. *Effects of normoxic hypercapnia* (P_{I,CO_2} 0·8 *kPa; 6 mmHg), hyperoxic hypercapnia, hypoxia and anaemia on 18 rainbow trout*

(Data are expressed as mean ± 1 s.e.m. Asterisks indicate those values differing significantly
(< 0·05) from normoxic control values.)

|				Experimental conditions		
	Variable	Air	Air + CO_2	$O_2 + CO_2$	Hypoxia	Anaemia
$\dot{V}_g$	ml min⁻¹ kg⁻¹	194·4±5·6	321·3±29·4*	116·7±8·5*	414·7±28·0*	309·5±17·2*
V_f	b min⁻¹	70·3±2·6	73·2± 2·8	66·4±3·8	70·7± 2·7	73·3± 1·2
V_{sv}	ml kg⁻¹	2·7±0·1	4·4± 0·4*	1·8±0·1*	5·9± 0·3*	4·2± 0·3*
P_{I,O_2}	kPa	20·0±0·6	19·8± 0·2	60·4±1·7*	12·4± 0·6*	20·2± 0·1
P_{E,O_2}	kPa	11·7±0·4	13·7± 0·7*	51·1±2·1*	8·6± 0·5*	13·9± 0·3*
P_{a,O_2}	kPa	14·6±0·4	15·0± 0·9	33·3±1·8*	9·4± 0·8*	17·2±0·3*
C_{a,O_2}	vol. %	7·5±0·5	6·0± 0·6*	8·5±0·5*	4·9± 0·3*	5·1± 0·4*
$\dot{V}_{O_2}$	ml min⁻¹ kg⁻¹	0·51±0·02	0·73± 0·10	0·42±0·06	0·49± 0·06	0·53±0·04
pH_a		7·99±0·10	7·79± 0·03*	7·77± 0·02*	8·01± 0·02	7·95± 0·02
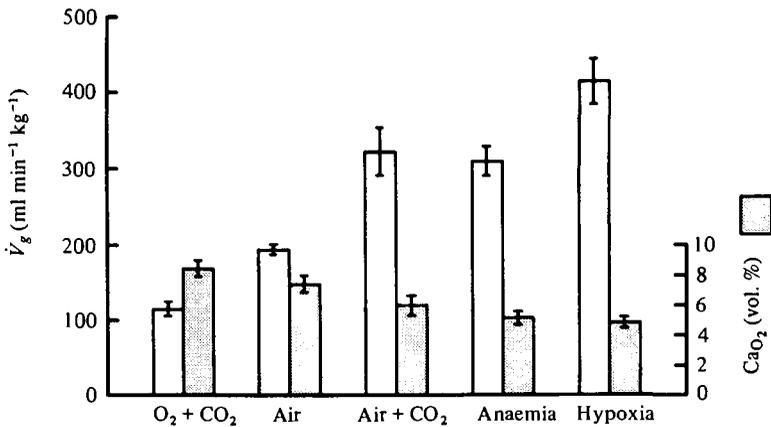
Fig. 2. Effects of hyperoxic hypercapnia, normoxic hypercapnia, anaemia, and hypoxia on blood oxygen content (C_{a,O_2}, shaded) and ventilation volume ($\dot{V}_g$, unshaded) in 18 rainbow trout (for inspired water gas tensions, see Table 1). Results are plotted in order of descending C_{a,O_2} and include control data in normoxia. Error bars indicate ± 1 s.e.m.

and hyperoxic hypercapnia, indicating that the increased CO_2 levels in the inspired water were responsible for increased blood CO_2. No significant change in pHa from the control value was observed in either hypoxia or anaemia, when P_{I,CO_2} was very low.

DISCUSSION

Blood oxygen content decreased and ventilation volume increased in trout exposed to elevated P_{I,CO_2} levels. These effects of hypercapnia were reversed by raising the oxygen level in the inspired water. Reducing blood oxygen content by hypoxia and anaemia also produced concomitant increases in ventilation volume.

The $P_{I,CO_2} - P_{a,CO_2}$ difference is maintained in hypercapnic fish (Cameron & Randall, 1972; Janssen & Randall, 1975; Truchot *et al.* 1980) so that as P_{I,CO_2} increased, P_{a,CO_2} must also have increased in our experimental animals. Evidence for this is provided by the decrease in pHa measured during hypercapnic periods. Increased P_{a,CO_2} and decreased pHa cause both decreased haemoglobin-oxygen affinity (Bohr effect) and a reduction in blood oxygen carrying capacity (Root effect) in trout (Randall, 1970). An increase in water flow past the gill filaments is required under these circumstances to maintain oxygen uptake in the face of CO_2-induced hypoxaemia. In this study the animals augmented ventilation volume by increasing stroke volume (frequency remained constant) to compensate for hypoxaemia (Fig. 1, Table 1), a response consistent with the observations of other workers (Dejours, 1973; Randall & Jones, 1973; Janssen & Randall, 1975).

Hypercapnic trout responded to an increase in P_{I,O_2} by decreasing $\dot{V}_g$. As P_{I,O_2} was raised, C_{a,O_2} increased and hypercapnic hypoxaemia was rectified by the increased availability of oxygen in the ventilatory stream. Consequently, the convection requirement at the gill surface was reduced and $\dot{V}_g$ decreased. However, pH did not change in the transition from normoxic to hyperoxic hypercapnia, so that haemoglobin-oxygen affinity was presumably still impaired. The increase in C_{a,O_2} observed during hyperoxic hypercapnia must therefore have been due to the increased amount of oxygen in physical solution in the plasma, as indicated by the increased P_{a,O_2} during this period (Table 1). There have been few studies of teleost ventilation in hyperoxia, but published results clearly indicate that the ventilatory requirement is reduced during this treatment (Randall & Jones, 1973; Dejours, Toulmond & Truchot, 1977) and P_{a,CO_2} increases slightly due to decreased gill water flow (Dejours 1973). Indeed, hyperoxic trout have been shown to have no ventilatory response to CO_2 at levels near 1 kPa (Dejours, 1973), an observation confirmed by our data. Randall & Jones (1973), however, reported a greater proportionate reduction of $\dot{V}_g$ in animals exposed to hyperoxia alone than we observed during hyperoxic hypercapnia.

Reduction of blood oxygen content by hypoxia stimulated ventilation (Fig. 2, Table 1), while returning the animals to normoxia restored $\dot{V}_g$ to the control value. Randall & Jones (1973) found increases in $\dot{V}_g$ (measured directly) similar to those of this study, in trout subjected to the same degree of hypoxia; while Holeton & Randall (1967) reported that $\dot{V}_g$ increased by a factor of 6 at levels of hypoxia comparable to those used in our experiments. The latter authors, however, used the Fick equation to calculate $\dot{V}_g$, a technique for which the experimental errors are inherently greater than in direct measurement, and which may have led to an over-estimation of $\dot{V}_g$. Marvin & Heath (1968) recorded little change in ventilation rate in hypoxic bluegill, trout, and sunfish until the P_{I,O_2} was reduced below 6 kPa, but were not able to obtain reliable data on ventilation volume. In trout, Hughes & Saunders (1970) reported an increase in the amplitude of breathing with advancing hypoxia, leading to an approximate doubling of minute volume over the same range of P_{I,O_2} as used in our experiments.

In anaemic fish, the blood oxygen content dropped to the same level as that recorded in hypoxia, while $\dot{V}_g$ increased by a factor of 1·6 over the control value (Fig. 2, Table 1). Our results represent only short-term adaptations to anaemia; data

|were recorded 1–2 h after bleeding. Cameron & Davis (1970) found that the primary compensation for reduced blood oxygen-carrying capacity in trout made severely anaemic by either injections of phenylhydrazine or bleeding, was an increase in cardiac output with no change in $\dot{V}_g$. These workers' results, however, represent long-term adaptation to anaemia, as they allowed a 24 h recovery period after inducing anaemia, then made observations over the next 1–5 days.

During both hypoxia and anaemia, pH_a did not change significantly from the control value, indicating that the acid-base status of the blood was maintained during these treatments. Hypoxia, however, had a greater effect than anaemia on ventilation, for the same decrease in blood oxygen content. There must therefore be other factors in addition to C_{a, O_2} that contribute to the increase in $\dot{V}_g$ during hypoxia.

It is apparent from these experiments that ventilation volume is related to the level of blood oxygen during hypercapnia, hyperoxic hypercapnia, hypoxia and anaemia. Elevated ventilation volume is thus an appropriate response to hypercapnia in water-breathers, since it serves to maintain the oxygen supply to the tissues by means of increasing the amount of oxygen dissolved in the plasma. Oxygen uptake measurements indicate that increasing $\dot{V}_g$ is successful in helping trout adapt to adverse conditions that impair the blood oxygen transport system: $\dot{V}_{O_2}$ was maintained during all experimental treatments (Table 1).

Trout exposed to levels of CO_2 greater than 1 kPa show ventilation increases which are not offset by increasing the P_{I, O_2}. Carbon dioxide at high levels presumably has a direct effect on some component of the ventilatory system, an effect which is not observed at lower CO_2 levels. Struggling is also evident at high CO_2 tensions; this effect is probably not related to blood oxygen levels, but may be a generalized effect of CO_2 on the whole animal. Further investigations are needed to clarify the mechanism responsible for the responses to high CO_2 levels in trout.

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