BLOOD GASES OF THE TENCH (*TINCA TINCA*) IN WELL AERATED AND OXYGEN-DEFICIENT WATERS

By F. B. EDDY*

*Research Unit for Comparative Animal Respiration,
University of Bristol, Bristol BS8 1UG*

INTRODUCTION

Detailed studies on the role of the blood in respiratory gas exchange have been conducted on the rainbow trout, dogfish and lungfish, and the results have been reviewed by Randall (1970) and Johansen (1970, 1971). Among the teleosts the less active species have received little attention in this respect. Garey (1970) measured the oxygen content of arterial and venous blood in the carp to determine the cardiac output and Itazawa (1967, 1970) determined the blood oxygen content of the same species under various environmental conditions. Other than these there have been few significant studies on blood-gas function in gas exchange of inactive teleost species.

The life and habits of the tench have been described by Day (1884) and by Kennedy & Fitzmaurice (1970); it is predominantly a bottom-living fish of slow and still waters. Although aspects of its respiratory physiology have been described in some detail (Hughes & Shelton, 1962; Hughes, 1964; Randall & Shelton, 1963; Randall, 1966) none of these studies involved the measurement of the blood gases. In the present study results are presented concerning breathing rate, ventilation volume, oxygen uptake and other respiratory parameters of the tench; however, the main object of the study was to examine the respiratory role of the blood of tench in both oxygen-rich and oxygen-deficient waters.

MATERIALS AND METHODS

Tench weighing between 150 and 400 g were obtained from suppliers at Great Stambridge, Essex. They were kept in de-chlorinated water contained in 50-gallon glass aquaria; the water temperature was maintained between 11–14 °C. They were fed twice weekly on chopped sheep's heart and maggots.

The fish selected for an experiment was anaesthetized with MS 222 (Sandoz), approximately 150 mg/l; it was then transferred to an operating table where a cannula was inserted into the dorsal aorta in a manner similar to that described for salmon by Smith & Bell (1964). A cannula for inspired water samples was implanted in the buccal cavity and a second cannula was placed in the operculum for the determination of the oxygen tension of expired water (Holeton & Randall, 1967). In the present study the design of the opercular cannula was slightly different from designs previously

---

* Present address: Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster.

described; a 1 mm thick rubber washer was placed over the cannula sleeve on both the inside and the outside of the operculum. These had the effect of holding the cannula firmly in place, making a watertight seal between the cannula sleeve and the operculum, and of sampling water a small distance from the inside surface of the operculum. For each fish the cannula was placed as nearly as possible in the same position in the operculum. Hughes & Knights (1968) suggested that water on the immediate inner surface of the operculum may have had least contact with the gills and may have an unrealistically high P_{O_2} value. In the present study, data obtained from cannulae suspected of being loose or of showing signs of leakage were discarded. The above procedures were carried out on fourteen tench.

A further three fish had only cardiac cannulae implanted. A 21-gauge needle attached to Portex PP 50 tubing was advanced into the heart from a ventral and anterior position; the point of entry to the body was a small distance anterior to the pectoral girdle and in the midline. The protruding end of the needle and the first part of the cannula tube were sutured on to the ventral surface of the lower jaw. The fish appeared to survive this operation with no ill effects. The blood cannulae were filled with heparinized saline (Wolf, 1963) containing approximately 100 I.U. of heparin/ml.

After implantation of cannulae the fish was placed in a respirometer through which de-chlorinated and aerated water flowed at a rate between 100–300 ml/min. The water was maintained at 13 ± 1 °C and the fish was left to recover for at least 24 h before the start of an experiment. The P_{O_2}, P_{CO_2} and pH of blood and water were measured using Eschweiler microelectrodes and microelectrode assemblies; these were maintained at the same temperature as the respirometer and after values had been recorded the blood was returned to the fish via the cannula. The oxygen uptake by the fish was calculated from the P_{O_2} difference between the water entering and leaving the respirometer, and the water flow.

Blood samples of 50–100 μl were also removed from the tench at intervals; the oxygen content of 10 μl subsamples was determined by the method of Tucker (1967). The stated accuracy of the method is 0·03 ml oxygen/100 ml blood, expressed as the mean difference from variations obtained by the van Slyke method (Tucker, 1967). In the present study determinations were made until values differing by less than 0·5 vol % had been obtained. A further part of the blood sample was placed in a 20 ml tonometer and equilibrated with air for a few minutes for the subsequent determination of the oxygen capacity. The remainder of the blood sample was analysed for lactate by an enzymic method (Hohorst, Kreutz & Bücher, 1959) using the reagents and the procedure supplied by Boehringer, Mannheim. The total amount of blood removed from a fish for these analyses rarely exceeded 0·4 ml.

The fish was studied in an unstressed state for the first 3–4 h of an experiment; then nitrogen was bubbled into the respirometer water supply so that the P_{O_2} value of the water steadily decreased 20–30 mmHg after about 4 h. Analyses of blood and water were made throughout this period and breathing rate was measured by counting the number of opercular closures per unit time.

The equations given by Holeton & Randall (1967) and by Randall, Holeton & Stevens (1967) were used to calculate percent utilization (%U), ventilation volume ($\dot{V}g$) and other respiratory parameters.

Symbols. The symbols used are similar to those employed by Rahn (1966) and by Jones, Randall & Jarman (1970).

$\dot{V}g$ Ventilation volume (ml/min/kg).
$\dot{V}_{O_2}$ Oxygen uptake (ml/min/kg).
P Partial pressure of gas (mmHg).
C Concentration of gas (ml/100 ml blood).
$\dot{Q}$ Cardiac output (ml/min/kg).
T Transfer factor (ml/min./mmHg/kg).

Subscripts.

g referring to gills;
a arterial blood leaving gills;
v venous blood entering gills;
i referring to blood in the buccal cavity, i.e. inspired water;
e referring to blood in the opercular cavity, i.e. expired water.

RESULTS

The experimental results are shown in Fig. 1*a, b, c, d* and in Fig. 2*a, b, c*. The greatest number of determinations were carried out on unstressed fish in well-aerated water; for these the mean value, the standard error of the mean and the number of determinations are indicated. When the oxygen in the water was reduced the individual determinations are shown. The individual results for four unstressed tench are shown in Table 1, these indicating the range of results obtained.

Resting tench observed in the stock aquaria generally had a slow breathing rate; on some occasions opercular movements appeared to cease and the only indication of gill ventilation was the movement of the opercular valves which are well developed in the tench. When in the respirometer the fish frequently did not show this quiet behaviour making it impossible to record true resting values. Tench in well-aerated water in the respirometer will be referred to as unstressed tench. These problems are discussed by Heath (1972) who suggests that in this type of experiment there is always some disturbance to the fish unless extremely elaborate precautions are taken.

The respiratory parameters measured varied considerably between individual fish; this is illustrated in Fig. 1*a* where the effect of hypoxia on the breathing rate of 4 fish is shown. In most cases when the environmental P_{O_2} had dropped to around 30 mm-Hg the breathing rate had approximately doubled and the maximum rate had apparently been reached. The oxygen tensions of inspired and expired water are shown in Fig. 1*b*. The P_{O_2} of inspired water in the buccal cavity was always slightly lower than that of the water entering the respirometer; this was probably because of the nature of the water flow through the respirometer which permitted a small amount of expired water to be re-breathed. For unstressed fish the percent utilization of oxygen from the water was $54.9 \pm 2.1 \%$ (21); this decreased to a value of about 35% when the environmental P_{O_2} had fallen to approximately 25 mmHg.

The mean ventilation volume for unstressed tench was 132 ml/min/kg (Fig. 1*d*); and again there was variation between individuals, the values ranging between *ca.* ■0–200 ml/min/kg. It has already been mentioned that the design of the opercular
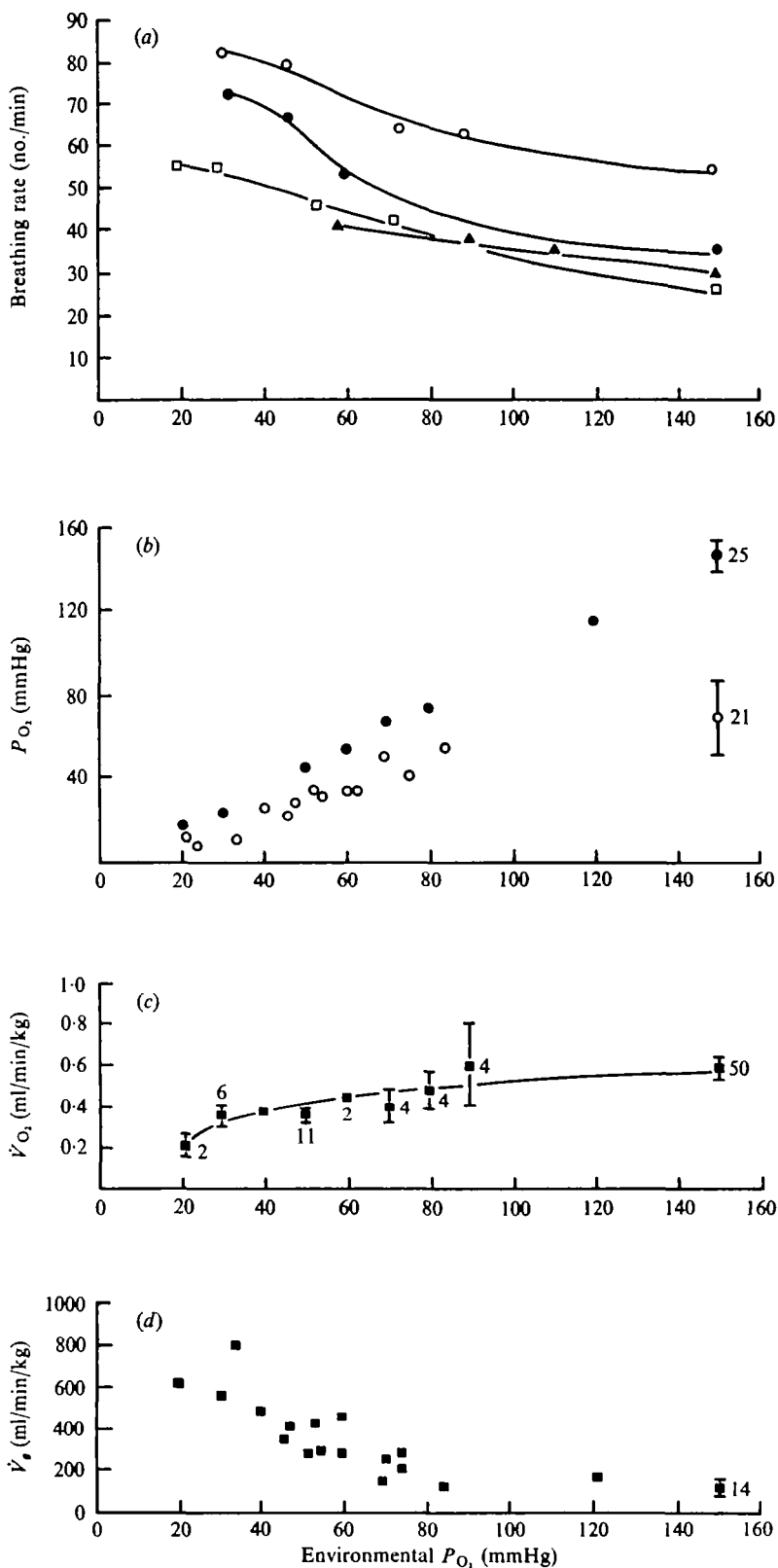
Fig. 1. The effect of decreasing environmental oxygen tension on the respiration of tench. (a) Breathing rate; results for four fish are shown. (b) P_{O_2} of inspired water, (●) and expired water (○). (c) Oxygen uptake, ml/min/kg. (d) Ventilation volume, ml/min/kg.
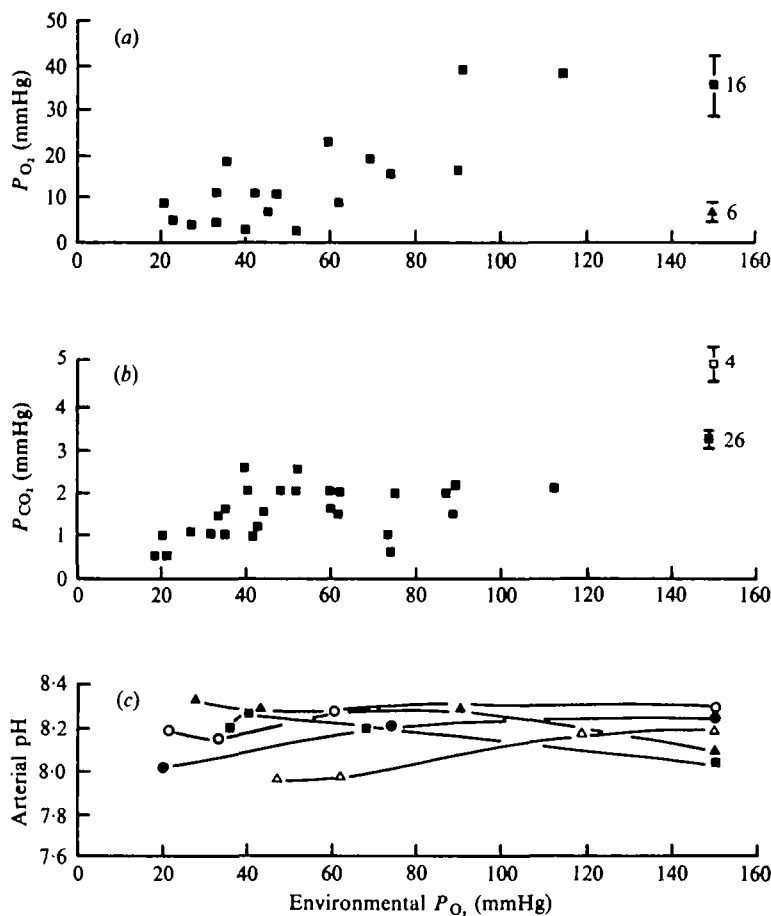
Fig. 2. The effect of decreasing environmental oxygen on P_{O_2}, P_{CO_2} and pH in tench blood. (*a*) Arterial oxygen tension (■), and venous oxygen tension (▲). (*b*) Arterial carbon dioxide tension (■), and venous carbon dioxide tension (□). (*c*) Arterial pH; individual results from 5 fish are shown.

Table 1. *Experimental results for four unstressed tench; temperature* 13 °C

Date	Pi_{O_2}	Pe_{O_2}	Pa_{O_2}	Ca_{O_2} (vol %)	Sat. (%)	Pa_{CO_2} (mean value)	pHa	$\dot{V}_{O_2}$	$\dot{V}g$	U (%)	Lactate mg/100 ml	Breathing rate (no./min)
23.2	142	75	12	6.3	66	3.7	8.13	0.55	106	47	8.2	60
2.3	145	52	30	9.5	96	3.2	8.05	0.13	20	65	21.1	34
9.3	148	72	60	5.5	80	3.0	8.25	0.46	118	53	—	34
12.3	148	60	29	1.8	67	4.0	8.23	0.51	131	60	9.2	37

Pi_{O_2}, Pe_{O_2}, Pa_{O_2} and Pa_{CO_2}: mmHg.
$\dot{V}_{O_2}$ and $\dot{V}g$: ml/min/kg.

cannula may give variable results for the P_{O_2} of expired water and thus cause variability in the calculated values for ventilation volume. However, the results in Fig. 1*d* indicate that there is little change in ventilation volume until the P_{O_2} of the water falls to about 80 mmHg, and with further decreases in the P_{O_2} of the water ventilation volume increased steadily up to approximately eightfold.

The mean oxygen uptake of unstressed tench was 0.5 ml/min/kg (Fig. $1c$). As the environmental P_{O_2} decreased the oxygen uptake tended to remain steady until the P_{O_2} of the water was reduced to 20–30 mmHg. Here the oxygen uptake fell significantly and the tench appeared to be in considerable respiratory distress.

P_{O_2}, P_{CO_2} and pH values in tench blood

In well aerated water the mean P_{O_2} value for arterial blood of unstressed tench was 35.8 mmHg the range being from 10 mm to over 60 mmHg. The oxygen dissociation curves of tench blood (Eddy 1973) indicate that at P_{CO_2} tensions of less than 5 mmHg the haemoglobin will be more than 80 % oxygen saturated when the P_{O_2} is about 10 mmHg. As the oxygen tension in the water was decreased the arterial P_{O_2} value decreased (Fig. $2a$), and when the environmental P_{O_2} was 20–40 mm Hg the oxygen tension in arterial blood had fallen to 5–20 mmHg. In several fish when the ambient P_{O_2} was as high as 40–50 mmHg the arterial P_{O_2} had dropped to around 5 mmHg; these fish also showed the lowest unstressed arterial oxygen tensions (10–15 mmHg), the highest arterial P_{CO_2} tensions and the lowest pH values. However, their ability to withstand hypoxia appeared to be about the same as the other experimental fish.

The oxygen tension in the ventral aorta of unstressed tench was about 7 mmHg.

In well aerated water the mean P_{CO_2} of unstressed tench was 3.3 mm Hg the range being approximately 2–4 mmHg. Decreasing the environmental oxygen tension caused a progressive decrease in arterial P_{CO_2} (Fig. $2b$), probably because increased gill ventilation caused CO_2 to be removed in increasing quantities from the blood perfusing the gills. When the environmental P_{O_2} had fallen to about 20 mmHg the arterial P_{CO_2} tension was reduced to less than 1 mmHg. The venous P_{CO_2} in unstressed tench was 5.0 ± 0.41 mmHg.

The mean arterial pH value of unstressed tench in well-aerated water was 8.18 ± 0.03 (11); as with blood P_{O_2} and P_{CO_2} there was variation between individual fish the range being pH 8.0–8.35. Decreasing the environmental P_{O_2} tended to have a variable effect on the arterial pH value and the pH response to hypoxia of 5 fish is shown in Fig. $2c$. In many experiments there was a tendency for arterial blood pH to increase as the degree of hypoxia increased; this is in keeping with a simultaneous decrease in arterial P_{CO_2} (Fig. $2c$) although the situation is complicated by the appearance of lactic acid in the blood.

The mean value for venous pH in unstressed tench was 8.08.

Oxygen content of tench blood

During the experiments blood was withdrawn from the tench for the simultaneous determination of P_{O_2}, oxygen content and oxygen capacity so that the relationship between the oxygen content and the oxygen tension of the blood *in vivo* could be derived. In Fig. 3 the percentage oxygen saturation of the blood is plotted against blood P_{O_2}; this represents the *in vivo* oxygen dissociation curve of tench blood at 13 °C. In the majority of resting tench the arterial blood was 75–95 % oxygen-saturated; however, in several individuals where the arterial P_{O_2} was low (*ca.* 10 mmHg) the percent oxygen saturation of the blood was less than 75 %. A few measurements of arterial blood oxygen content were made while the tench were under hypoxic conditions and these are also indicated in Fig. 3.
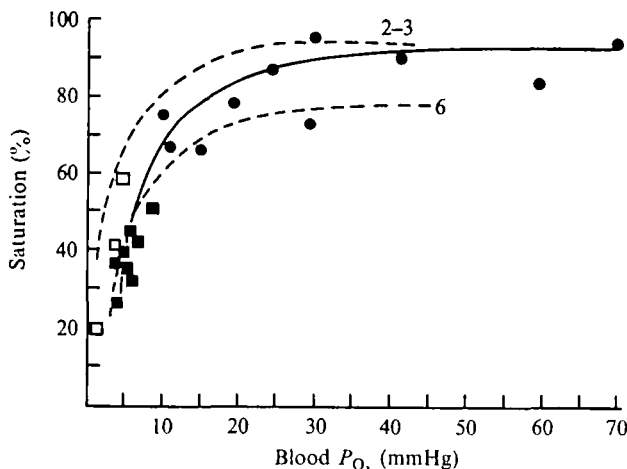
Fig. 3. The *in vivo* oxygen dissociation curve of tench blood at 13 °C. (solid curve). Arterial blood (●), and venous blood (■); oxygen content expressed as percentage saturation for fish in air saturated water. Arterial blood percentage saturation (□) when fish were in water 30–50 mmHg P_{O_2}. The broken lines are the *in vitro* determined oxygen dissociation curves at 13 °C. Upper line 2–3 mmHg P_{CO_2}, and lower line 6 mmHg P_{CO_2} (Eddy 1973).

It would seem from the above results that tench can accommodate wide variations in arterial blood oxygen content without ill effects.

The average value for venous oxygen saturation in unstressed tench was 40% ranging between 24–45%. The mean oxygen capacity of tench blood was found to be about 8 vol %, a value similar to that reported by Eddy (1973).

Lactate values in tench blood

The mean lactate concentration in unstressed tench blood was 14.03 ± 2.13 mg/100 ml (range 0.8–36.3 mg/100 ml, N = 19). This increased to 31.4 ± 6.14 mg/100 ml (range 12.9–97.3 mg/100 ml, N = 14) when the ambient P_{O_2} was 20–35 mmHg. The lactate values of blood from unstressed tench are similar to those found by Black *et al.* (1959) for rainbow trout. The increase in blood lactate indicates that the stress of hypoxia may cause some anaerobic metabolism in tench.

DISCUSSION

Blood gas concentrations

In Table 2 some respiratory properties of tench blood are compared with those of other fish species. The most complete data exist for the trout and it is convenient to illustrate the respiratory properties of tench blood by comparing them with those of the trout. Blood oxygen affinity tends to influence the P_{O_2} of arterial blood; thus in trout the P_{50} value is 15 mmHg and the arterial P_{O_2} is about 100 mmHg while in tench P_{50} is about 2–3 mmHg resulting in an arterial P_{O_2} value of approximately 36 mmHg (Table 2). Carp appear to be similar to tench in this respect except that the arterial P_{O_2} is about 8 mmHg (Garey, 1970). In unstressed tench an increase in arterial P_{O_2} would result in a small increase in haemoglobin-bound oxygen and more ▮xygen dissolved in the blood plasma. If the arterial P_{O_2} of tench blood was increased

Table 2. *Mean values for respiratory properties of the blood of several fish species*

Species	Temperature (°C)	O₂ capacity (vol. %)	P_{O_2} Art.	P_{O_2} Ven.	P_{CO_2} Art.	P_{CO_2} Ven.	pH Art.	pH Ven.	C_{O_2} Art.	C_{O_2} Ven.	P_{50} (mmHg)
Trout *Salmo gairdneri* a	9	9 b	82–130 a	30	1–1·5 b (15 °C)	2–3	7·8 b (15 °C)	7·7	8·5 b (15 °C)	6·3	14 0·3 mmHg P_{CO_2} c
Dogfish *Scyliorhinus stellaris* d	15–17	4·2 e	49 d	10	1	2·6 d	7·78	7·71 d	4·1	1·1 d	21·5 2·2 mmHg P_{CO_2} f
Carp *Cyprinus carpio* h	10	7 h	8 h	−	−	−	−	−	5·2	2·5 h	3 1–2 mmHg P_{CO_2} i
Sting ray *Dasyatis sabina* g	20–26	2·6 g	90 g	14	−	−	−	−	−	−	26 g
Tench *Tinca tinca*	13	8	36	6	3·3	5	8·16	8·08	7·5	3·1	3 2–3 mm HgP$_{CO_2}$

Authors. (*a*) Davis & Cameron (1971). (*b*) Holeton & Randall (1967). (*c*) Eddy (1971). (*d*) Piiper & Baumgarten-Schumann (1968*a, b*). (*e*) Baumgarten-Schumann & Piiper (1968). (*f*) Pleschka, Albers & Spiach (1970) (*Scyliorhinus canicula*). (*g*) Cameron, Randall & Davis (1971). (*h*) Garey (1970). (*i*) Black & Irving (1937).

P_{O_2} and P_{CO_2}, mmHg. C_{O_2} (blood oxygen content), vol. %.

from 36 to 100 mmHg it is estimated that only an extra 3 % oxygen would be added to the blood.

Since the arterial P_{O_2} of tench blood is comparatively low it follows that the P_{O_2} of venous blood must also be low. The high affinity of the blood for oxygen ensures that venous blood contains 2–4 vol. % oxygen when the venous P_{O_2} is 5–7 mmHg (Fig. 3); also since the unloading tension of tench blood is low the tissues must be able to function at a lower P_{O_2} value than that for trout. However, it is possible that the actual quantitative transfer of oxygen to the tissues is similar in both trout and tench because the values for the Bohr effect are approximately the same. The Bohr effect ($\Delta \log P_{50}/\Delta$ pH) for rainbow trout is $-0·57$ at 15 °C, while the value for tench blood at 13 °C is $-0·64$ (Eddy, 1971, 1973). This assumes that other factors influencing the transfer of oxygen to the tissues such as blood buffering and the properties of the circulatory system are similar in the two species. Several unstressed tench were found to have arterial P_{O_2} values of about 10 mm-Hg which is considerably lower than the mean value; a similar situation was reported for carp by Garey (1970) where in many cases the arterial P_{O_2} tension was found to be about 10 mmHg. Steen & Kryusse (1964) found that arterial blood of resting eels was far from fully oxygen saturated and suggested that since the cost of ventilation in fish is high, it is more economical at rest to have a low rather than a high arterial saturation; they suggested that during exercise the arterial saturation would be elevated by increased ventilation and by the flow of blood from non-respiratory to respiratory pathways in the gills. It seems possible that similar mechanisms could exist in carp and tench although the arterial percent saturation would not normally fall as low as 50 % as it does in eels.

Using mean values from all the experiments, various respiratory parameters were calculated for tench. The Fick principle was employed to calculate cardiac output ($\dot{Q}$) which was found to be in the range 14–18 ml/min/kg, which compares with a value of 12 for carp (Garey, 1970) and 10·4 for rainbow trout (Davis & Cameron, 1971). The oxygen transfer factor was calculated from the equation of Randall, Holeton & Stevens (1967)

$$T_{O_2} = \frac{\dot{V}_{O_2}}{\frac{1}{2}(Pi_{O_2} + Pe_{O_2}) - \frac{1}{2}(Pa_{O_2} + P\bar{v}_{O_2})}$$

and was found to be 0·0061 ml/min/kg. mmHg which is similar to values reported for other fish species, e.g. 0·0056 ml/min/kg. mmHg for rainbow trout (Randall *et al.* 1967). This method of calculating the mean oxygen tension gradient between blood and water (ΔPg, the denominator of the equation) has been criticized on the grounds that the curved nature of the oxygen dissociation curves is not taken into account (Piiper & Baumgarten-Schumann, 1968 b). The value of ΔPg for tench calculated in this way was about 80 mmHg while the value calculated by a method similar to that of Piiper & Baumgarten-Schumann (1968 b) was about 88 mmHg. To arrive at more realistic values of ΔPg yet other factors have to be taken into account. These include the nature of the blood and water flows through the gills, and aspects of this subject have been discussed in detail by Hughes (1972) and by Scheid & Piiper (1972). In the present study some of the respiratory parameters calculated for tench can be considered only approximate and this applies particularly to values for ventilation volume, ΔPg and the O_2 transfer factor which are calculated via Pe_{O_2}. The measurement of Pe_{O_2} by the opercular catheterization technique has been criticized by Davis & Watters (1970) because using this method their results varied depending upon the location of the cannula in the operculum and they considered calculations based on these results likely to be misleading. In the present study the Pe_{O_2} values obtained for tench were reasonably uniform, and the values calculated for $\dot{V}g$ compare favourably with those obtained for trout by Davis & Cameron (1971), who determined the P_{O_2} of expired water using the oral membrane technique; moreover, the present results for $\dot{V}g$ are considerably lower than earlier values for tench cited by Davis & Cameron (1971). It is likely that the Pe_{O_2} values obtained for tench in the present study suffer from some inconsistencies, the chief one being that the value measured is not the true average P_{O_2} of expired water from the gill system. It is felt, however, that the results obtained are adequate to justify the comparisons of tench with other fish species in respect of respiration.

The *in vivo oxygen dissociation curve*

The *in vivo* relationship between blood P_{O_2} and blood oxygen content (expressed as percentage oxygen saturation) is shown in Fig. 3; this represents the *in vivo* oxygen dissociation curve at 13 °C for tench. Unlike the *in vitro* dissociation curve it is not constructed at any particular pH or P_{CO_2} value and its shape is determined by factors such as the acid-base status of the blood and the respiration rate of the tissues. Thus it represents the physiological blood oxygen dissociation curve which is most suited to the oxygen uptake system of the fish.

The *in vitro* oxygen dissociation curves for tench blood at 13 °C (Eddy, 1973) at P_{CO_2} tensions of 2–3 mmHg and 6 mmHg have been drawn in on Fig. 3 for compari-

son with the *in vivo* curve. There is a general similarity between the two types of curve but there are differences which deserve comment; these are mainly concerned with blood P_{CO_2} tensions. Determined with the P_{CO_2} electrode, the P_{CO_2} tension of arterial blood in unstressed tench was about 3 mmHg (Fig. 2b); this must also be the CO_2 tension of the arterial points in the *in vivo* oxygen dissociation curve (Fig. 3). However, from the *in vitro* dissociation curves shown in Fig. 3, the P_{CO_2} of most of these points should be more than 2–3 mmHg and in some cases greater than 6 mmHg. Similarly, according to the *in vitro* dissociation curves in Fig. 3, the *in vivo* venous points should have a P_{CO_2} tension in excess of 6 mmHg but in fact the measured tension was about 5 mmHg (Fig. 2b).

These discrepancies could arise from the difficulties involved in using the Severing-haus type P_{CO_2} electrode at relatively low temperatures and also using it to measure the low P_{CO_2} tensions found in fish blood; under these conditions the determinations are made uncertain by the difficulties of calibrating the electrode and its slow response time. In part they could arise from differences in blood buffering and this point has been discussed by Campbell (1968) in relation to the mammalian CO_2 dissociation curve. Compared to the situation *in vitro*, the buffering capacity of the haemoglobin *in vivo* is reduced because it buffers both the plasma and the interstitial fluid. Since the O_2 and CO_2 dissociation curves are functionally interdependent it follows that the greater buffering capacity *in vitro* ensures that when P_{CO_2} is increased the haemo-globin will remain more oxygen saturated than *in vivo*. Thus at similar O_2 and CO_2 tensions the blood *in vitro* should be more oxygen saturated than blood *in vivo* and in Fig. 3 this is seen to be the case.

If, therefore, measurements of *in vivo* P_{O_2} and P_{CO_2} for tench are made and the percentage oxygen saturations are read off from the *in vitro* oxygen dissociation curves, there will be a tendency for this amount to be overestimated by a small amount particularly at oxygen tensions of < 10 mmHg. The full significance of this cannot be fully appreciated until an accurate method for P_{CO_2} measurements in fish blood is available. It is not known whether this divergence occurs in other fish species as the necessary data are lacking.

Effect of hypoxia on tench respiration

The effect of hypoxia on fish respiration has been frequently investigated and the subject has been reviewed by Hughes (1964), Randall (1970), Shelton (1970) and by Johansen (1971). In tench the responses to hypoxia were similar to those previously described for teleost fish, there being a steady increase in breathing rate, amplitude and ventilation volume (Fig. 1a, d); the branchial pumps appeared to be fully extended when the P_{O_2} in the water was 20–30 mmHg. Working with the same species, Randall & Shelton (1963) found a similar pattern of events and noticed that the heart rate became increasingly reduced as the environmental P_{O_2} fell below about 30 mmHg, slowing even more as the P_{O_2} dropped further.

The oxygen tension of tench arterial blood tended to remain steady until the ambient P_{O_2} fell to about 80 mmHg when it began to decrease (Fig. 2b). When the environmental P_{O_2} was 20–30 mmHg the arterial P_{O_2} averaged about 5 mmHg; under these conditions the oxygen saturation of the blood was low but was prevented from being even lower by the fact that the arterial P_{O_2} had dropped from the unstressed arterial

value of about 3 mmHg to around 0·5 mmHg (Fig. 2b). Haemoglobin has a greater oxygen affinity at lower P_{CO_2} tensions and it is estimated this decrease in P_{CO_2} could increase the oxygen content of the blood by 10–20 %. A decrease in blood P_{CO_2} affects the blood pH value and removal of CO_2 from the blood by hyperventilation results in a decrease in the hydrogen ion concentration. However, hydrogen ions are returned to the blood in the form of lactic acid whose rate of appearance in the blood seems to be accelerated by increasing hypoxia. In unstressed fish the greatest concentrations of lactic acid were usually found in those fish which initially had the lowest arterial P_{O_2} tensions. As the level of hypoxia increased more lactate was formed and this was probably the cause of a decrease in arterial pH in some fish as shown in Fig. 2c, where the two fish which show a steady decrease in arterial pH also had the highest lactate values. Other fish which tended to show an increase in arterial pH tended to have the lowest lactate concentrations. Thus it appears that in most cases the blood pH tends to remain fairly steady under moderate hypoxic conditions despite the removal of hydrogen ions as carbonic acid and their addition as lactic acid.

Calculations for cardiac output ($\dot{Q}$), ΔPg and the oxygen transfer factor (T_{O_2}) were carried out for tench under hypoxic conditions; in making these calculations it was necessary to assume that the unstressed value for oxygen tension and oxygen content of venous blood decreased linearly to zero with decreasing environmental P_{O_2}. Although these calculations yield only approximate results they indicate that in tench, hypoxic conditions (20–30 mmHg in the environmental water) caused a small increase in $\dot{Q}$, a linear decrease in ΔPg to a value of about 20 mmHg and a fourfold increase in T_{O_2}. Thus the hypoxic response of tench is in principle similar to that of the trout as described by Randall *et al.* (1967) and Jones *et al.* (1970). The main difference in the hypoxic response of tench and trout can be related to the respective blood oxygen affinities; because of the high oxygen affinity, the blood of the tench averages more than 75 % oxygen saturated at low P_{O_2} values and this degree of saturation can be achieved with relatively low values of the ventilation to perfusion ratio ($\dot{V}g/\dot{Q}$). The low P_{50} value of tench blood ensures that the arterio-venous oxygen content remains reasonably constant even at quite low environmental oxygen tensions. Thus the tench is able to inhabit water with an oxygen level too low for an active species such as the trout.

As well as being dependent upon blood with a high oxygen affinity, the tench's resistance to hypoxia must in part depend on the ability to maintain a stable acid-base status; under hypoxic conditions the blood pH remains remarkably constant in spite of the addition of lactic acid. This suggests that the blood and body buffering systems of the fish are adapted to maintain a stable internal environment for oxygen transport under adverse respiratory conditions.

SUMMARY

1. The respiration of tench at 13 °C was investigated, particular attention being given to the role of the blood in uptake and transport of oxygen.

2. In well aerated water the mean value for arterial blood was 36 mmHg, for P_{CO_2} 3·3 mmHg and for pH 8·16; the respective venous values were 7 mmHg, 5 mmHg and 8·08. Arterial blood averaged about 75 % and venous blood about 40 % oxygen saturation. The mean value for oxygen uptake was 0·5 ml/min/kg and for ventilation volume 132/ml/min/kg.

3. The oxygen tension and the percentage saturation of the blood determined *in vivo* are discussed in terms of the oxygen dissociation curve determined *in vitro*.

4. When the environmental P_{O_2} was decreased, tench responded by increasing breathing rate and ventilation volume. Arterial P_{O_2} and P_{CO_2} decreased but arterial pH tended to remain steady. There was also a significant increase in blood lactate.

5. That tench can withstand severe hypoxic conditions is attributed to blood of high oxygen affinity and the ability to maintain a favourable acid-base status in the blood for oxygen transport.

6. Respiration in tench is compared with that in other fish species.

REFERENCES

BAUMGARTEN-SCHUMANN, D. & PIIPER, J. (1968). Gas exchange in the gills of resting unanesthetized dogfish *Scyliorhinus*. *Resp. Physiol.* **5**, 317–25.

BLACK, E. C. & IRVING, L. (1937). The effect of carbon dioxide upon the oxygen capacity of the carp (*Cyprinus carpio*). *Trans. R. Soc. Can.* Sec. V, 29–32.

BLACK, E. C., CHUI, W., FORBES, F. D. & HANSLIP, A. (1959). Changes in pH, carbonate and lactate of yearling Kamloops trout (*Salmo gairdneri*) during and following severe muscular exercise. *J. Fish Res. Bd Can.* **16**, 391–402.

CAMERON, J. N., RANDALL, D. J. & DAVIS, J. C. (1971). Regulation of the ventilation-perfusion ratio in the gills of *Dasyatis sabina* and *Squalus suckleyi*. *Comp. Biochem. Physiol.* **39**, 503–19.

CAMPBELL, E. J. M. (1968). Respiration. *A. Rev. Physiol.* **29**, 105–132.

DAVIS, J. C. & WATTERS, K. (1970). Evaluation of opercular catheterisation as a method of sampling expired water from fish. *J. Fish. Res. Bd Can.* **27**, 1627–35.

DAVIS, J. C. & CAMERON, J. N. (1971). Water flow and gas exchange at the gills of rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **54**, 1–18.

DAY, F. (1884). *The Fishes of Great Britain and Ireland*. vol. II, p. 189. London: Williams and Norgate.

EDDY, F. B. (1971). Blood-gas relationships in the rainbow trout *Salmo gairdneri*. *J. exp. Biol.* **55**, 695–711.

EDDY, F. B. (1973). Oxygen dissociation curves of the blood of the tench *Tinca tinca*. *J. exp. Biol.* **58**, 281–93.

GAREY, W. F. (1970). Cardiac output of the carp (*Cyprinus carpio*). *Comp. Biochem. Physiol.* **33**, 181–89.

HEATH, A. G. (1972). A critical comparison of methods for measuring fish respiratory movements. *Wat. Res.* **6**, 1–7.

HOHORST, H. J., KREUTZ, F. H. & BÜCHER, T. (1959). Über Metabolitgehalte und Metabolit-Konzentrationen in der Leber der Ratte. *Biochem. Z.* **332**, 18–46.

HOLETON, G. F. & RANDALL, D. J. (1967). The effect of hypoxia on the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. *J. exp. Biol.* **46**, 317–27.

HUGHES, G. M. (1964). Fish respiratory homeostasis. *Symp. Soc. exp. Biol.* **18**, 81–107.

HUGHES, G. M. (1972). Morphometrics of fish gills. *Resp. Physiol.* **14**, 1–25.

HUGHES, G. M. & SHELTON, G. A. (1962). Respiratory mechanisms and their control in fishes. In *Advances in Comparative Physiology and Biochemistry* (ed. O. Lowenstein), vol. I, pp. 275–364. London: Academic Press.

HUGHES, G. M. & KNIGHTS, B. (1968). The effect of loading the respiratory pumps on the oxygen consumption of *Callionymus lyra*. *J. exp. Biol.* **49**, 603–15.

ITAZAWA, Y. (1957). Gas content of the blood to that of the medium in fish. *Bull. Jap. Soc. scient. Fish.* **23**, 71–80.

ITAZAWA, Y. (1970). Characteristics of respiration of fish considered from the arterio-venous difference in oxygen content. *Bull. Jap. Soc. scient. Fish.* **36**, 571–77.

JOHANSEN, K. (1970). Airbreathing in fishes. In *Fish Physiology* (ed. W. S. Hoar and D. J. Randall), vol. IV. New York: Academic Press.

JOHANSEN, K. (1971). Comparative physiology; gas exchange and circulation in fishes. *A. Rev. Physiol.* **33**, 596–612.

JONES, D. R., RANDALL, D. J. & JARMAN, G. M. (1970). A graphical analysis of oxygen transfer in fish. *Resp. Physiol.* **10**, 285–98.

KENNEDY, M. & FITZMAURICE, P. (1970). The biology of the tench *Tinca tinca* (L.) in Irish waters. *Proc. R. Ir. Acad.* Sec. B **69**, 31–82.

PIIPER, J. & BAUMGARTEN-SCHUMANN, D. (1968a). Transport of O_2 and CO_2 by blood and water in gas exchange of the dogfish (*Scyliorhinus stellaris*). *Resp. Physiol.* **5**, 326–37.

PIIPER, J. & BAUMGARTEN-SCHUMANN, D. (1968b). Effectiveness of O_2 and CO_2 exchange in the gills of the dogfish (*Scyliorhinus stellaris*). *Resp. Physiol.* **5**, 338–49.

PLESCHKA, K., ALBERS, C. & SPIACH, P. (1970). Interaction between CO_2 transport in the blood of the dogfish *Scyliorhinus canicula. Resp. Physiol.* **9**, 118–26.

RANDALL, D. J. (1966). The nervous control of cardiac activity in the tench (*Tinca tinca*) and in the goldfish (*Carassius auratus*). *Physiol. Zool.* **39**, 185–92.

RANDALL, D. J. (1970). Gas exchange in fishes. In *Fish Physiology* (ed. W. S. Hoar and D. J. Randall), vol. IV, pp. 253–92. New York and London: Academic Press.

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

RANDALL, D. J., HOLETON, G. F. & STEVENS, E. D. (1967). The exchange of oxygen and carbon dioxide across the gills of rainbow trout. *J. exp. Biol.* **46**, 329–48.

SCHEID, P. & PIIPER, J. (1972). Theoretical analysis of respiratory gas equilibrium in water passing through fish gills. *Resp. Physiol.* **13**, 305–18.

SHELTON, G. (1970). The regulation of breathing. In *Fish Physiology* (eds. W. S. Hoar and D. J. Randall), vol. IV, pp. 293–359. New York and London: Academic Press.

SMITH, L. S. & BELL, G. R. (1964). A technique for prolonged blood sampling in free swimming salmon. *J. Fish. Res. Bd Can.* **21**, 711–17.

STEEN, J. B. & KRYUSSE, A. (1964). The respiratory function of teleostean gills. *Comp. Biochem. Physiol.* **12**, 127–42.

TUCKER, V. A. (1967). Method for O_2 content and dissociation curves for microlitre blood samples. *J. appl. Physiol.* **23**, 410–14.

WOLF, K. (1963). Physiological salines for freshwater teleosts. *Progve Fish Cult.* **25**, 135–40.