J. exp. Biol. (1979), **82**, 139–162
With 12 *figures*
Printed in Great Britain

RESPIRATORY, VENTILATORY, AND CARDIOVASCULAR RESPONSES TO EXPERIMENTAL ANAEMIA IN THE STARRY FLOUNDER, *PLATICHTHYS STELLATUS*

BY CHRIS M. WOOD,* B. R. MCMAHON AND D. G. MCDONALD*
Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4

(*Received* 31 *August* 1978)

SUMMARY

Unrestrained, quiescent starry flounder maintained approximately normal levels of O_2 uptake in the face of severe experimental anaemia. At haematocrits above about 5 %, the only major compensation was a reduction in venous O_2 tension which lowered venous saturation and thereby kept a constant difference between arterial and venous O_2 contents. Below a haematocrit of about 5 %, this difference decreased, and many additional compensations were invoked, including increases in ventilation, expired O_2 tension, arterial O_2 tension, and cardiac output, and decreases in systemic vascular resistance and blood pH. All changes could be reversed by restoration of haematocrit. Exercise performance and post-exercise changes in blood pH and lactate differed only slightly between anaemic and normal flounder. In wild flounder, anaemia commonly occurs and apparently only causes death at the haematocrit value (about 5 %) below which most major compensations are implemented. The respiratory strategy of the flounder during anaemia is compared with that of the rainbow trout.

INTRODUCTION

The ability of teleost fish to survive in the real or functional absence of most of their haemoglobin has been known for many years (e.g. Nicloux, 1923; Schlicher, 1926; Anthony, 1961). A few studies have examined specific aspects of compensation (Cameron & Wohlschlag, 1968; Cameron & Davis, 1970; Wood & Randall, 1971; Jones, 1971*a*; Holeton, 1971*a*, *b*, 1977; Haswell & Randall, 1978). In the most detailed of these investigations, Cameron & Davis (1970) showed that the rainbow trout (*Salmo gairdneri*) in freshwater maintained an approximately normal resting O_2 uptake, in the face of a 70 %+ reduction in haematocrit, by pumping a much greater volume of the reduced O_2 capacity blood through the gills. This was accomplished almost entirely by elevation of cardiac stroke volume and facilitated by a decrease in peripheral vascular resistance. The trout thereby sustained normal O_2 uptake despite a greatly reduced difference between arterial and venous O_2 contents. Ventilation and blood O_2 tensions remained unchanged.

Recently we have analysed respiratory gas exchange under resting conditions in

* Present address: Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1.

the starry flounder (*Platichthys stellatus*) in sea water (Wood, McMahon & McDonald, 1979). The flounder is a benthic, inactive fish whereas the trout is pelagic and active. Relative to *S. gairdneri*, *P. stellatus* exhibits low blood O_2 tensions, a low blood O_2 capacity, a high blood O_2 affinity, a low arterial-venous O_2 content difference, and an extremely high cardiac output. It appears that the flounder maintains a much higher cardiac output than the trout when at rest, thereby gaining cardiac efficiency but sacrificing the high O_2 transport capacity during exercise which can be achieved by the trout. The latter operates less efficiently at rest, but can call upon a considerable circulatory reserve during frequent exercise by raising cardiac output to more efficient levels. In view of these differences, especially in resting cardiac output, responses to haemoglobin deficiency might well be very different in the two species. Therefore in the present study we have examined the respiratory, ventilatory, and cardiovascular adaptations to experimental anaemia in *P. stellatus*.

SYMBOLS

Symbols employed for respiratory parameters follow the system of Dejours (1975) and are defined in the text when first employed.

MATERIALS AND METHODS

Starry flounder (*Platichthys stellatus* Pallas) were collected (from January to April) and acclimated for at least 10 days to running sea water (salinity = 26–28 ‰, temperature = 7·5–10·5 °C) at Friday Harbor Laboratories, University of Washington, as described previously (Wood *et al.* 1977, 1979).

The first series of experiments concentrated on respiration, ventilation, and cardiovascular function during progressive experimental anaemia. The fish ($N = 6$; 425–1128 g) were fitted with arterial and venous catheters, inspired water catheters, ventilation collection masks, and opercular impedance leads while under MS-222 anaesthesia. The operative procedures have been described in detail previously (Wood *et al.* 1979) and in general follow the methods developed by Watters & Smith (1973). After surgery, the animals were placed in individual chambers (60 × 35 cm × 15 cm deep) which were filled to a depth of 6 cm with fine beach sand and shielded from the investigators. During the whole experimental period (1–3 weeks), the fish remained buried in the sand with only the eyes and mouth exposed, a behaviour which appears to duplicate natural resting conditions (Wood *et al.* 1979). After at least 72 h recovery, a set of control measurements was taken at the unbled haematocrit (Ht = 10·7–20·3 %). Blood was then withdrawn via the caudal vein catheter, centrifuged at 3000 ***g*** for 5 min to separate the erythrocytes, and the homologous plasma plus 'buffy coat' re-infused with sufficient saline (see below) to replace the missing red cell volume. The flounder was allowed to stabilize for at least 24 h at the new lower Ht, and then the set of measurements was taken again. The procedure was repeated 3–6 times so as to progressively lower the Ht from the original level to approximately 1 % ('severe anaemia') in a series of steps over 4–14 days. At no time was more than 1 ml of blood/100 g removed from a fish, so multiple bleedings were necessary to achieve low Ht's.

For four of the flounder, the separated red cells were immediately resuspended to the original Ht in Cortland saline (Wolf, 1963) adjusted to 160 m-equiv. l^{-1} sodium concentration by addition of NaCl, and heparinized at 100 i.u./ml. The cells were stored at 4 °C and resuspended in fresh saline daily. Haemolysis was negligible. After measurements had been recorded from the flounder at a Ht of about 1 %, the accumulated erythrocytes in saline from that animal were slowly re-infused into the caudal vein over a period of about 30 min. A partial or complete set of measurements was then taken 24 h after re-infusion.

In the above experiments the complete set of measurements comprised direct determinations of Ht, inspired water O_2 tension (P_{I,O_2}), mixed expired water O_2 tension (P_{E,O_2}) ventilation volume ($\dot{V}_w$) ventilation rate (f_R) arterial O_2 tension (P_{a,O_2}) venous O_2 tension (P_{v,O_2}) arterial O_2 content (C_{a,O_2}) venous O_2 content (C_{v,O_2}) heart rate (f_H), dorsal aortic blood pressure (BP_a), caudal venous blood pressure (BP_v), arterial pH (pH_a), and venous pH (pH_v). For the original control samples and at selected other Ht's, total blood O_2 capacity ($C_{O_2}^{max}$) and plasma O_2 capacity ($C_{O_2\,diss}^{max}$) were also measured. The analytical techniques have been described in detail previously (Wood *et al.* 1977, 1979). At least three separate determinations of each parameter, at each Ht, were taken for each fish. From these direct measurements, the following derived parameters were calculated as in Wood *et al.* (1979): O_2 consumption ($\dot{V}_{O_2}$), ventilatory stroke volume ($\dot{V}_{s,R}$), haemoglobin-bound O_2 capacity per unit Ht ($C_{HbO_2}^{max}/Ht$), arterial O_2 saturation (S_{a,O_2}), venous O_2 saturation (S_{v,O_2}), cardiac output ($\dot{V}_b$), and cardiac stroke volume ($V_{s,H}$). Systemic vascular resistance (R_s) was calculated as $(BP_a - BP_v)/\dot{V}_b$.

For one of the six fish, some pH measurements were lost due to pH electrode failure, so an additional flounder was fitted with only arterial and venous catheters and subjected to the sequential bleeding procedure. In this animal, only pH_a, pH_v, and Ht were measured. P_{v,O_2} measurements during progressive anaemia were also recorded from three fish of the second experimental series (see below) which were fitted with only venous catheters. Data from these fish bearing only blood catheters were not significantly different from those taken from the six fish bearing ventilation masks, impedance leads, and blood and water catheters.

A second series of experiments examined the influence of severe anaemia on the responses to exhausting activity. All fish (286–850 g) in this series were fitted with venous catheters and allowed to recover for at least 72 h in darkened individual chambers (30 × 30 cm × 15 cm deep) filled to a depth of 6 cm with sand. The experimental fish ($N = 4$) were then progressively bled down to a Ht of about 1 % over a period of 4–6 days as described above. The control fish ($N = 6$) were not bled. Both groups were then subjected to exhausting activity by 10 min of manual chasing. Blood samples were drawn prior to exercise and at selected times up to 24 h post-exercise; these samples were analysed for Ht, pH_v, and blood lactate levels. The exercise, blood sampling, and analytical procedures are described in detail by Wood *et al.* (1977).

A third experimental series assessed the natural variability of Ht in starry flounder in the wild. Fish ($N = 97$) over a wide weight range (95–1656 g) were examined. (The animals were collected by otter trawl from East Sound of Orcas Island, Washington State, during April and May, 1976.) The flounder were held for at least

Table 1. *Ventilatory, respiratory, and cardiovascular parameters in resting starry flounder before and after severe experimental anaemia (means ± S.E.; N): a comparison with the rainbow trout*

	Platichthys stellatus (present study)			*Salmo gairdneri* (Cameron & Davis, 1970)	
	Control	Anaemia	*P*	Control	Anaemia
Weight (g)	580·8 ± 73·2 (10)	—	—	175 – 400	—
Temp. (°C)	7·5 – 10·5	—	—	8·0 – 13·5	—
Ht (%)	14·5 ± 1·3 (10)	0·8 ± 0·1 (10)	0·001	22·8 ± 1·5 (18)	3·8 ± 0·9 (16)
P_{I,O_2} (torr)	138·6 ± 2·3 (6)	141·6 ± 3·6 (6)	n.s.	160·3 ± 0·5 (18)	161·2 ± 0·6 (16)
P_{E,O_2} (torr)	44·2 ± 3·3 (6)	81·8 ± 9·9 (6)	0·01	86·1 ± 2·3 (18)	98·9 ± 4·1 (16)
U_{w,O_2} (%)	67·6 ± 3·3 (6)	42·3 ± 6·8 (6)	0·01	46·0 ± 1·5 (18)	39·1 ± 2·4 (16)
$\dot{V}_{O_2}$ (ml O_2.kg^{-1}.min^{-1})	0·462 ± 0·037 (6)	0·402 ± 0·055 (6)	n.s.	0·645 ± 0·025 (18)	0·568 ± 0·042 (16)
$\dot{V}_w$ (ml.kg^{-1}.min^{-1})	110·5 ± 9·1 (6)	156·9 ± 8·0 (6)	0·01	171·4 ± 7·6 (18)	180·5 ± 17·3 (16)
f_R (no. min^{-1})	41·7 ± 2·1 (6)	34·8 ± 1·1 (6)	0·05	73·8 ± 3·4 (18)	73·8 ± 3·8 (16)
$V_{s,R}$ (ml.kg^{-1}.stroke^{-1})	2·65 ± 0·14 (6)	4·50 ± 0·17 (6)	0·001	2·34 ± 0·09 (18)	2·46 ± 0·24 (16)
C_{a,O_2} (vol %)	4·72 ± 0·67 (6)	0·62 ± 0·16 (6)	0·01	—	—
C_{v,O_2} (vol %)	3·54 ± 0·60 (6)	0·11 ± 0·02 (6)	0·01	—	—
$C_{a,O_2} - C_{v,O_2}$ (vol %)	1·18 ± 0·10 (6)	0·50 ± 0·05 (6)	0·001	(3·52)*	(1·15)*
P_{a,O_2} (torr)	37·4 ± 3·2 (6)	85·3 ± 8·3 (6)	0·01	105·0 ± 4·0 (18)	124·1 ± 4·3 (16)
P_{v,O_2} (torr)	17·4 ± 2·2 (9)	7·7 ± 1·1 (9)	0·01	31·9 ± 2·5 (53)	25·0 ± 1·6 (25)
$C_{O_2}^{max}$ (vol %)	5·93 ± 0·67 (6)	(0·98 ± 0·11)† (6)	—	—	—
$C_{HbO_2}^{max}$/Ht (vol %.%$^{-1}$)	0·348 ± 0·009 (6)	—	—	—	—
$C_{O_2 diss}^{max}$ (vol %)	0·700 ± 0·034 (6)	—	—	—	—
S_{a,O_2} (%)	90·1 ± 4·6 (6)	(93·1 ± 10·1)† (6)	—	—	—
S_{v,O_2} (%)	67·0 ± 4·4 (6)	(32·2 ± 12·1)† (6)	—	—	—
% O_2 transport by physical solution	8·7 ± 0·6 (6)	72·3 ± 8·8 (6)	0·001	—	—
pH_a	7·889 + 0·040 − 0·037 (7)	7·750 + 0·026 − 0·024 (6)	0·001	7·82 ± 0·02‡ (11)	7·86 ± 0·02‡ (11)
pH_v	7·876 + 0·034 − 0·032 (7)	7·686 + 0·031 − 0·029 (6)	0·001	—	—
$\dot{V}_s$ (ml.kg^{-1}.min^{-1})	39·9 ± 2·7 (6)	79·4 ± 7·2 (6)	0·01	18·3 ± 1·9 (18)	49·3 ± 5·0 (16)
f_H (no. min^{-1})	33·9 ± 2·4 (6)	36·2 ± 2·8 (6)	n.s.	62·9 ± 3·4 (126)	65·0 ± 2·0 (42)
$V_{s,H}$ (ml.kg^{-1}.stroke^{-1})	1·19 ± 0·07 (6)	2·27 ± 0·28 (6)	0·02	0·28 (?)	0·96 ± 0·11 (25)

Table 1—*continued*

	Platichthus stellatus (present study)			*Salmo gairdneri* (Cameron & Davis, 1970)	
	Control	Anaemia	*P*	Control	Anaemia
BP_a (cm H_2O)	25·9 ± 1·9 (6)	14·1 ± 2·7 (6)	0·01	—§	—§
BP_v (cm H_2O)	2·82 ± 0·18 (6)	4·07 ± 0·57 (6)	0·05	—	—
R_v (cm H_2O . min . kg . ml^{-1})	0·599 ± 0·070	0·134 ± 0·036	0·01	—§	—§

Note: *P* determined by Students paired two-tailed *t*-test, using each animal as its own control.
* Values calculated from other data presented by Cameron & Davis (1970).
† Values calculated indirectly; see text for details.
‡ Values from Haswell & Randall (1978).
§ Actual means not given by Cameron & Davis (1970), but decreases during anaemia reported.

10 days after capture to negate the haemoconcentration reported to occur after trawling ('capture stress': Fletcher, 1975). Fish with evidence of trawl damage were not used. Blood samples were obtained by quickly removing the fish from the water and blindly puncturing (i.e. without incision) either the haemal arch or ventricle with a no. 22 needle attached to a heparinized syringe. Occasional haemolysed samples were rejected.

RESULTS

A linear relationship between Ht and $C_{O_2}^{max}$ *in vitro* was observed, described by the regression equation:

$$C_{O_2}^{max} = 0{\cdot}357\ \mathrm{Ht} + 0{\cdot}70 \quad (n = 18),$$

where $r = 0{\cdot}96$ ($P < 0{\cdot}001$). Since the intercept of this equation was identical to the oxygen content value determined on air-equilibrated plasma samples (i.e. $C_{O_2\,diss}^{max}$; Table 1), and since $C_{O_2}^{max} - C_{O_2\,diss}^{max} = C_{HbO_2}^{max}$, a strict proportionality between $C_{HbO_2}^{max}$ and Ht was apparent; i.e. $C_{HbO_2}^{max}/\mathrm{Ht}$ and $C_{O_2\,diss}^{max}$ are both constants. The experimental reduction in Ht from 14·5 ± 1·3% ($\bar{x}$ ± 1 S.E.) to 0·8 ± 0·1% in the first set of experiments caused an average 84% decline in $C_{O_2}^{max}$ (Table 1). Despite this severe impairment of blood O_2 carrying ability, the anaemic flounder were observed to survive at least 7 weeks in the laboratory as long as $P_{I,\,O_2}$ was kept normoxic.

The respiratory, ventilatory, and cardiovascular responses of individual fish to progressive experimental anaemia are shown in Figs. 1–9, while the control and severe anaemia (Ht about 1%) data are summarized in Table 1. As each fish was used as its own control, paired statistical comparisons (two-tailed Student's paired *t*-test) were employed. The data of Cameron & Davis (1970) on the rainbow trout are included for comparison in Table 1.

Overall, there was no significant change in resting $\dot{V}_{O_2}$ during severe anaemia (Fig. 1, Table 1), although minor decreases did occur in several animals at very low Ht's. However, below Ht's of approximately 5%, this maintenance of $\dot{V}_{O_2}$ occurred at the cost of a progressively increasing $\dot{V}_w$ (Fig. 2A). The rise in $\dot{V}_w$ was accompanied in an inverse fashion by a fall in the extraction of O_2 from the ventilatory flow

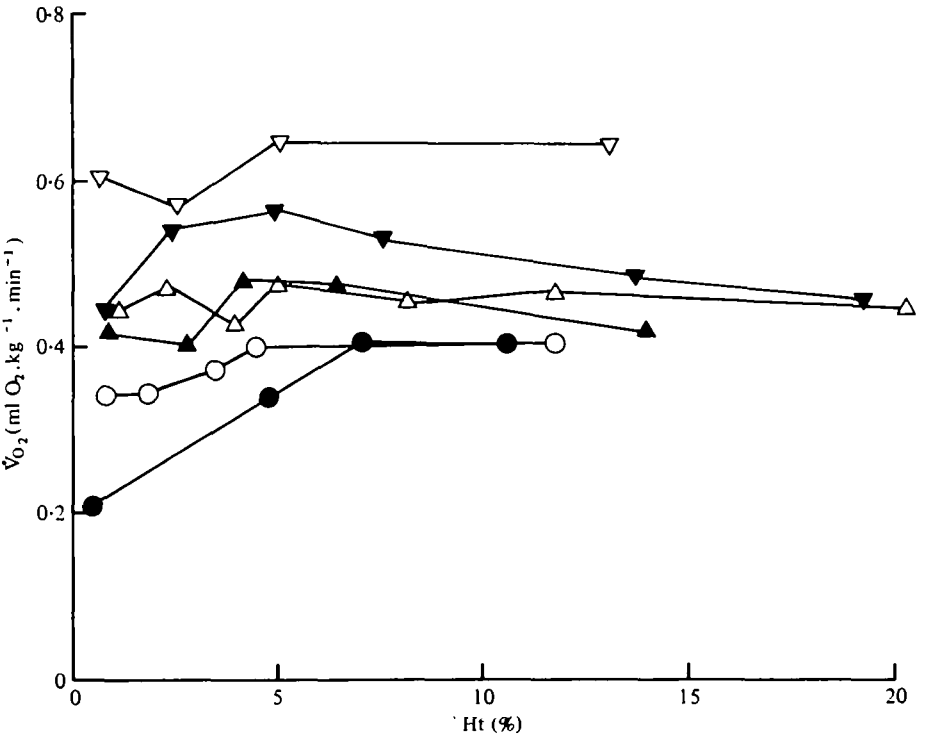

Fig. 1. The relationship between haematocrit (Ht) and resting O_2 uptake ($\dot{V}_{O_2}$) in starry flounder rendered progressively anaemic. Each symbol represents data from a single animal: ○, 620 g; ●, 642 g; △, 660 g; ▲, 1128 g; ▽, 425 g; ▼, 682 g; (◆, 870 g; ◇, 286 g; □, 416 g; ■, 482 g in other Figs.). Each point represents the mean of at least three determinations.

(i.e. increased P_{E,O_2}), which has been expressed as % utilization

$$\left(U_{w,O_2} = \frac{P_{I,O_2} - P_{E,O_2}}{P_{I,O_2}} \times 100\,\%\right)$$

in Fig. 2B. The overall changes in U_{w,O_2} and P_{E,O_2} were highly significant, while P_{I,O_2} remained constant (Table 1). The elevation of ventilation occurred entirely though a marked increase in $V_{s,R}$, again starting below a Ht of about 5% (Fig. 3B, Table 1). Rather curiously, f_R declined slightly but significantly as $V_{s,R}$ increased (Fig. 3A, Table 1). The ventilatory pump perhaps gains efficiency by functioning at lower rate but higher stroke volume during severe anaemia.

As expected, both C_{a,O_2} and C_{v,O_2} declined in approximate proportion to the Ht (Fig. 4A, B, Table 1). However for C_{v,O_2} there occurred a slight but definite deviation from linearity, C_{v,O_2} declining somewhat more steeply than Ht (Fig. 4B). P_{a,O_2} was relatively low over the range of control Ht's, but rose dramatically in severe anaemia (Fig. 5A, Table 1). Most of the increase occurred below Ht's of about 5%. On the other hand, P_{v,O_2} steadily declined over the whole range of Ht's examined (Fig. 5B), thereby explaining to some extent the slight non-linearity of the C_{v,O_2} versus Ht relationship.

In the control flounder, the measured values of S_{a,O_2} and S_{v,O_2} were 90·1 ± 4·6% and 67·0 ± 4·4% respectively (Table 1), very similar to the values (89% and 70

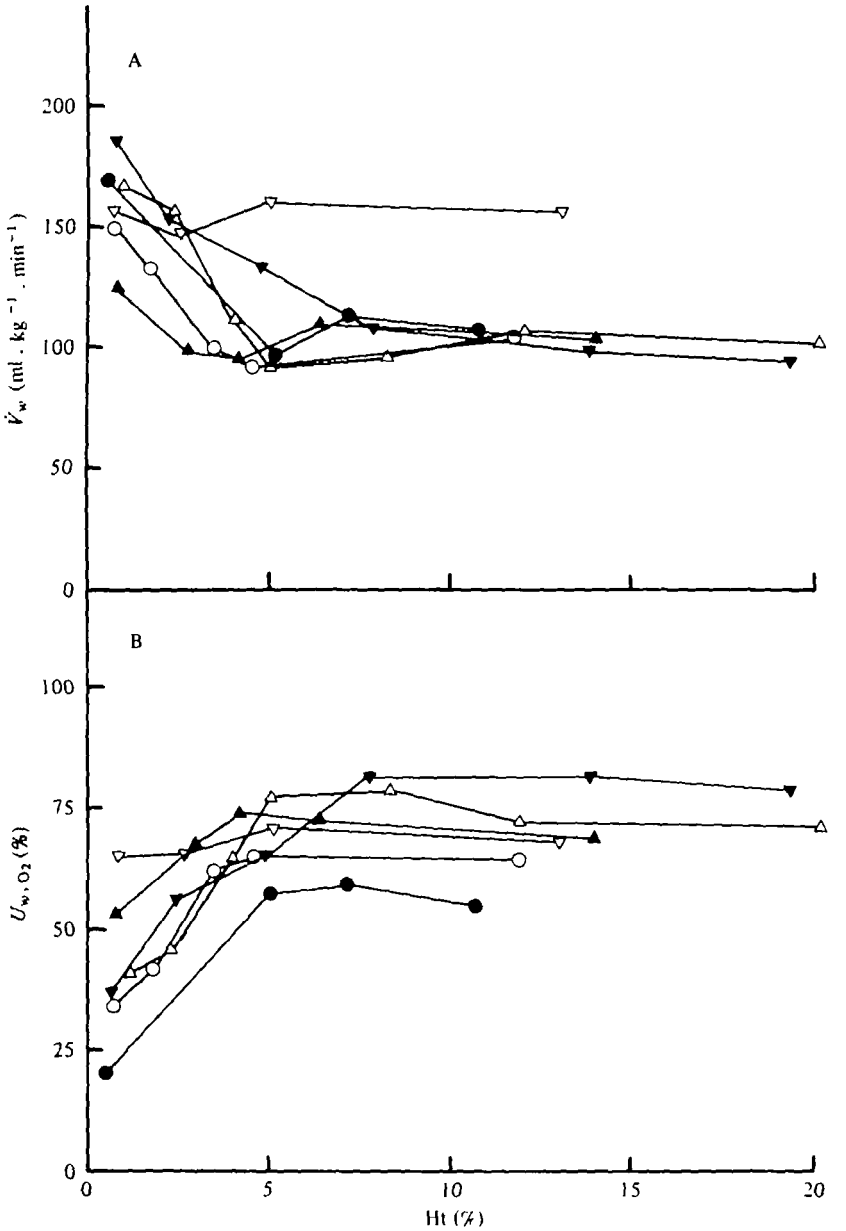

Fig. 2. The relationships between haematocrit (Ht) and: (A) ventilation volume ($\dot{V}_w$); (B) percent utilization of O_2 from the ventilatory flow ($U_{w,O_2} = P_{I,O_2} - P_{E,O_2})/P_{I,O_2} \cdot 100\%$) in starry flounder rendered progressively anaemic. Other details as in Fig. 1.

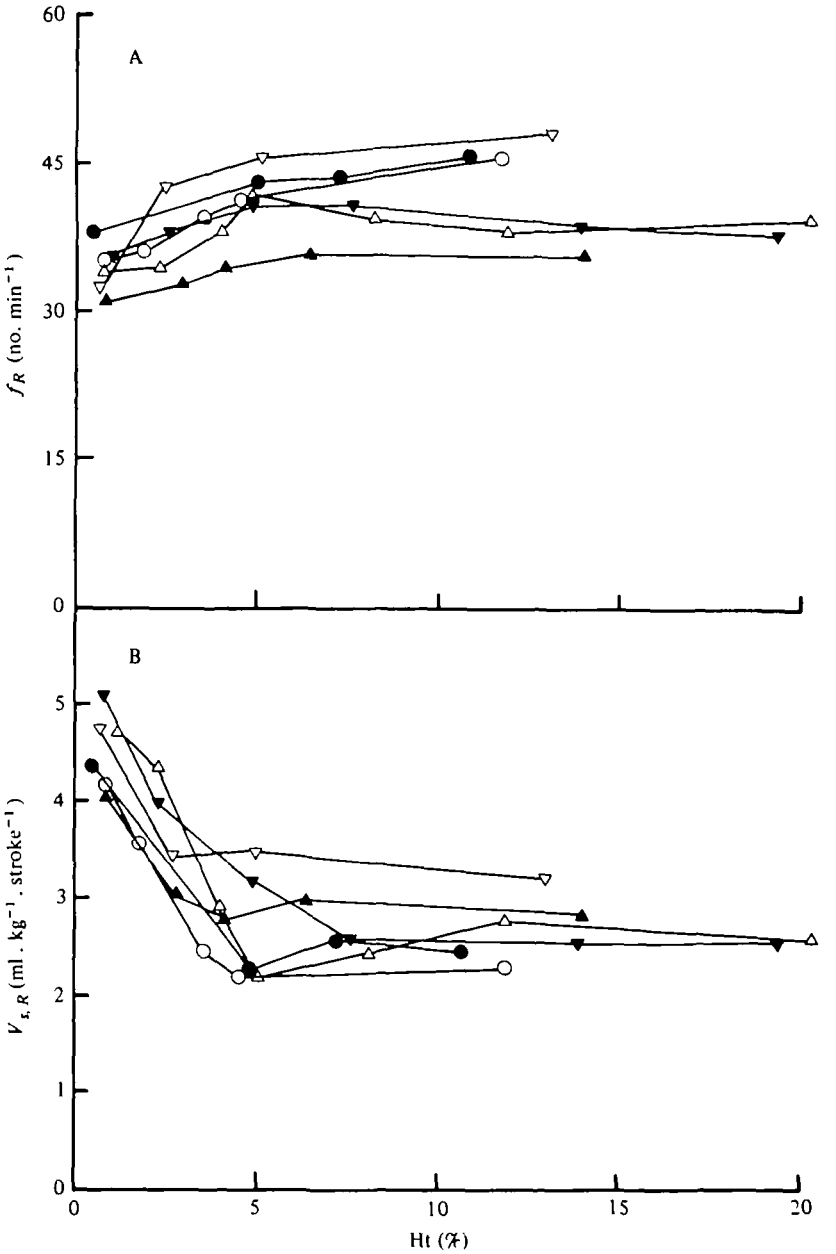

Fig. 3. The relationships between haematocrit (Ht) and: (A) ventilation rate (f_R); (B) ventilatory stroke volume ($V_{s,R}$) in starry flounder rendered progressively anaemic. Other details as in Fig. 1.

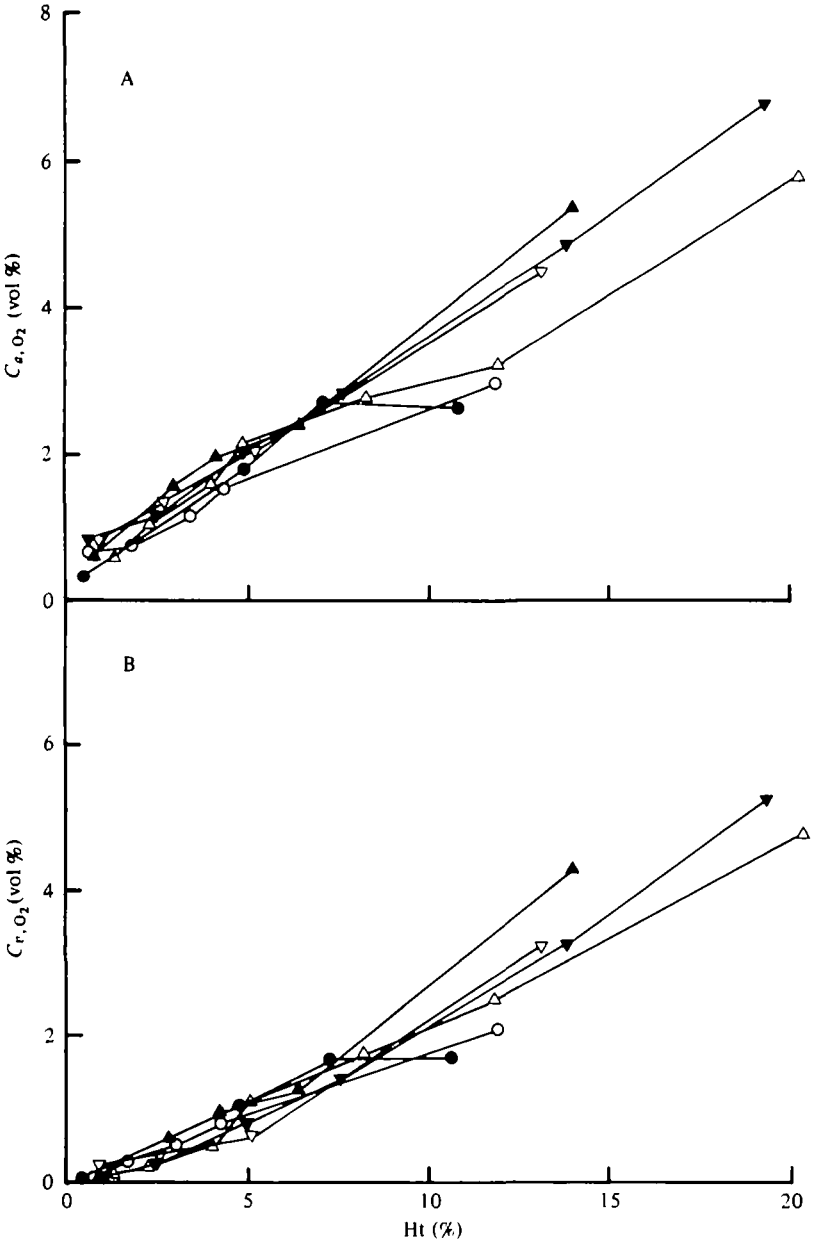

Fig. 4. The relationships between haematocrit (Ht) and: (A) arterial O_2 content (C_{a,O_2}); (B) venous O_2 content (C_{v,O_2}) in starry flounder rendered progressively anaemic. Other details as in Fig. 1.

respectively) which could be predicted from mean blood P_{O_2}'s (Table 1) and the *in vivo* blood O_2 dissociation curve presented previously for *P. stellatus* (see Fig. 2 of Wood *et al.* 1979). Values for $C^{max}_{HbO_2}$ were determined only for control haematocrits, so no direct assessment of saturation values during severe anaemia was possible. However since $C^{max}_{HbO_2}/Ht$ and $C^{max}_{O_2\,diss}$ apparently remain constant (see above), saturation values during anaemia could be calculated from measurements of P_{O_2}, Ht, and C_{O_2} (Wood *et al.* 1979), e.g.

$$S_{a,O_2} = \frac{1}{Ht}\cdot\left(C_{a,O_2} - P_{a,O_2}\,\frac{C^{max}_{O_2\,diss}}{155\ torr}\right)\cdot\frac{1}{C^{max}_{HbO_2}/Ht}\ 100\,\%.$$

The results of these calculations are extremely variable ($S_{a,O_2} = 93{\cdot}1 \pm 10{\cdot}0\,\%$, $S_{v,O_2} = 32{\cdot}3 \pm 12{\cdot}1\,\%$; Table 1) because they are critically dependent on the accuracy of the (very low) Ht measurement. Nevertheless, these figures are in reasonable agreement with values ($S_{a,O_2} = 96\,\%$, $S_{v,O_2} = 45\,\%$) predicted using the measured P_{O_2}'s in severe anaemia (Table 1) and the *in vivo* blood O_2 dissociation curve (Fig. 2 of Wood *et al.* 1979). On this basis, it appears that the progressive fall in P_{v,O_2} as anaemia develops causes a significant decline in S_{v,O_2} as the P_{v,O_2} changes span the 'knee' of the hyperbolic dissociation curve. However the large increases in P_{a,O_2} have negligible effect on S_{a,O_2} as they occur over the relatively flat upper region of the dissociation curve.

This does not mean that these increases in P_{a,O_2} were unimportant in blood O_2 transport. S_{O_2} figures refer only to haemoglobin-bound O_2, while at low Ht's, O_2 physically dissolved in the blood becomes increasingly important. Indeed, O_2 delivery to the tissues by physical solution rose from $8{\cdot}7 \pm 0{\cdot}6\,\%$ of the total at control Ht's to $72{\cdot}3 \pm 8{\cdot}8\,\%$ under severe anaemia (Table 1). If P_{a,O_2} had not risen during severe anaemia, overall O_2 delivery would have dropped about one third from the control values. The fall in P_{v,O_2} also made a slight contribution to the increased transport by physical solution. However, the real significance of the P_{v,O_2} decline is that it represents an approximate doubling of the unloading of haemoglobin-bound O_2, based on the preceding saturation calculations.

Another important factor in maintaining O_2 delivery may have been blood pH. In all animals, there were highly significant decreases in both pH_a and pH_v during progressive anaemia (Fig. 6A, B; Table 1), the latter effect being slightly greater. The decreases were most rapid below a Ht of about 5 %. This has been seen more clearly in a similar experiment on a different batch of flounder (C. M. Wood, B. R. McMahon, and D. G. McDonald, unpublished results). In view of the Bohr shift known to occur in the blood of this species (Watters & Smith, 1973), the fall in pH_v probably contributed to the fall in S_{v,O_2}, and therefore helped to maintain O_2 transport. On the other hand, the fall in pH*a* probably had little or no effect on S_{a,O_2} because of the accompanying rise in P_{a,O_2}.

Fig. 7A shows that the combined effect of all these factors was to maintain $C_{a,O_2}-C_{v,O_2}$ constant down to a Ht of about 5 %. Below this point, $C_{a,O_2}-C_{v,O_2}$ declined significantly (Fig. 7A, Table 1), but far less markedly than if adjustments in P_{a,O_2}, P_{v,O_2} and pH_v had not occurred. As resting $\dot{V}_{O_2}$ remained essentially constant during severe anaemia (Fig. 1), $\dot{V}_b$ varied inversely with $C_{a,O_2}-C_{v,O_2}$, increasing significantly below a Ht of about 5 % (Fig. 7B, Table 1). The rise in $\dot{V}_b$ was accomplished entir

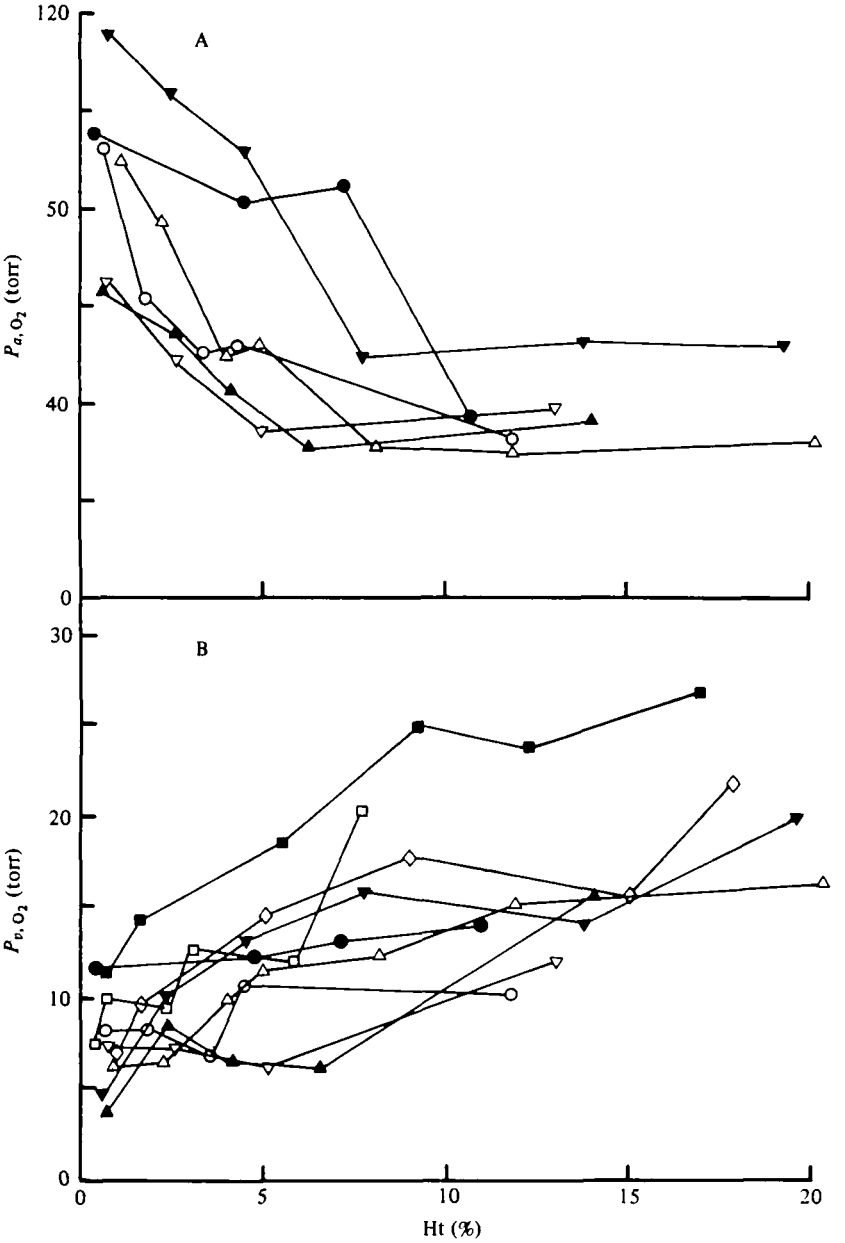

Fig. 5. The relationships between haematocrit (Ht) and: (A) arterial O_2 tension (P_{a,O_2}); (B) venous O_2 tension (P_{v,O_2}) in starry flounder rendered progressively anaemic. Other details as in Fig. 1.

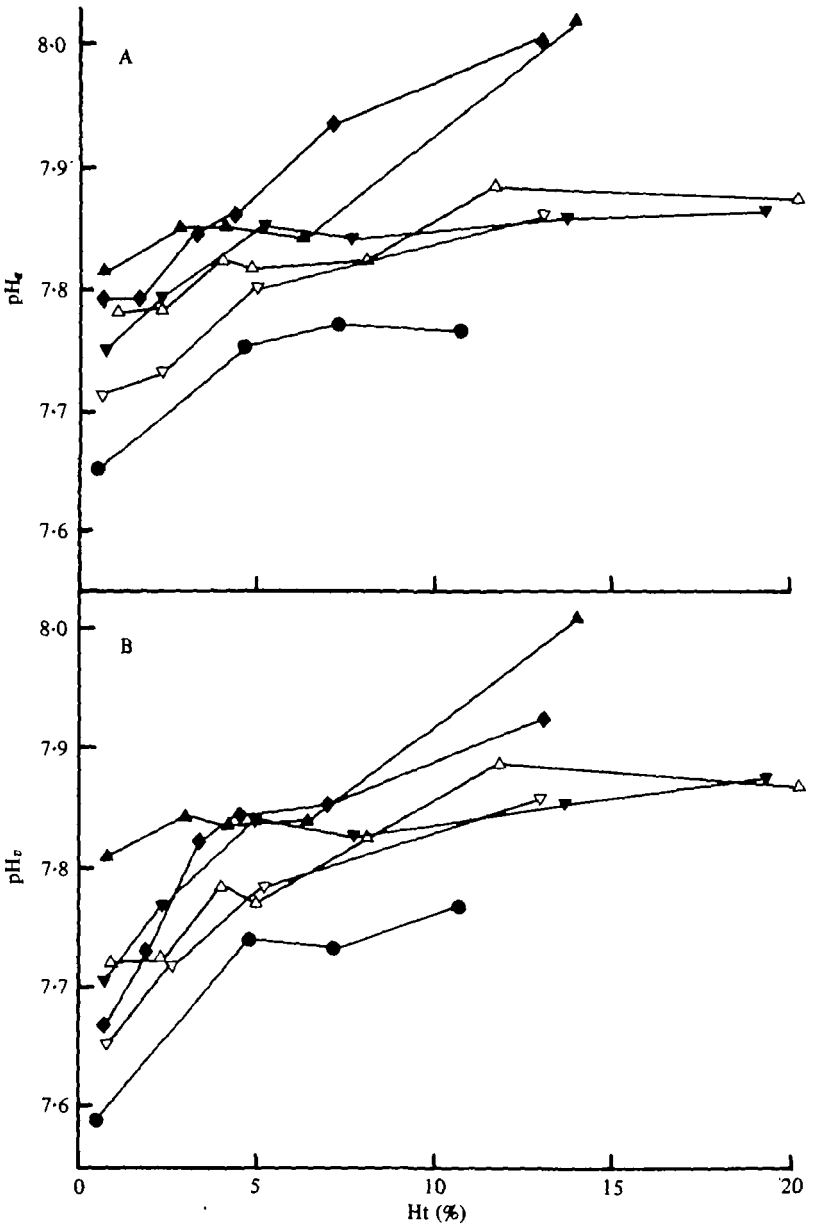

Fig. 6. The relationships between haematocrit (Ht) and: (A) arterial pH (pH_a); (B) venous pH (pH_v) in starry flounder rendered progressively anaemic. Other details as in Fig. 1.

through an elevation of $V_{s,H}$; there was no significant change in f_H (Fig. 8A, B, Table 1).

The increased $\dot{V}_b$ was not accompanied by an increased BP_a. Indeed, below a Ht of about 5%, BP_a fell significantly in all fish (Fig. 9A, Table 1) and BP_v increased slightly (Table 1). Consequently, the blood pressure differential across the systemic vascular bed declined while blood flow ($\dot{V}_b$) increased, indicating a profound drop in systemic vascular resistance (R_s) at low Ht's (Fig. 9B, Table 1). Calculations based on viscosity versus Ht data for trout blood (Cameron & Davis, 1970; Wood, 1974) suggest that the observed decrease in R_s was too large to result directly from the reduced viscosity of low Ht. A real systemic dilation must also have occurred.

Re-infusion of the homologous red blood cells was performed in four fish; the procedure proved fatal to one animal (erythrocytes stored up to 13 days) but was tolerated by the other three (storage = 4–11 days). Accounting for estimated losses of erythrocytes in blood sampling and handling, the Ht after re-infusion should have been about 70% of the control value, but the actual figures 24 h after re-infusion were only 38%, 40%, and 42% of the control Ht's. The data from one fish are summarized in Table 2; the results from the other two were qualitatively similar but C_{O_2} values *in vivo* and *in vitro* after re-infusion were not measured. Because of the 'loss' of erythrocytes in the re-infusion procedure, in Table 2, the data after re-infusion (column 4) have been compared with data at both the original control Ht (column 1), and at the closest haematocrit before severe anaemia (moderate anaemia, column 2) as well as at the severe anaemia Ht (column 3). The results clearly show that all the pronounced changes observed under severe anaemia (increases in $\dot{V}_w$, $V_{s,R}$, P_{a,O_2}, $\dot{V}_b$, and $V_{s,H}$; decreases in U_{w,O_2}, P_{v,O_2}, C_{a,O_2}–C_{v,O_2}, BP_a, R_s, pH_a, and pH_v) could be largely reversed by the increase in Ht. Therefore these changes resulted specifically from alterations in the red blood cell concentration and not from any non-specific effect of the experimental procedure. (For unknown reasons, BP_v remained elevated after re-infusion in this one flounder, but did not in the other two fish.)

C_{a,O_2} and C_{v,O_2}, unlike P_{a,O_2} and P_{v,O_2}, did not return to the levels expected on the basis of the Ht value after re-infusion (compare column 4 with column 2 in Table 2). *In vitro* equilibrations showed that this discrepancy was due to a substantial reduction in $C^{max}_{HbO_2}$/Ht with little or no change in $C^{max}_{O_2\,diss}$ 24 h after re-infusion (Table 2). This observation, together with the 'loss' of erythrocytes after re-infusion and the death of one fish, indicates some deterioration of the red cells during storage. Although haemolysis was negligible in the storage tubes, substantial haemolysis and 'loss' of erythrocytes probably occurred after the cells were returned to the flounder.

The second series of experiments was designed to assess the possible influence of severe anaemia on the acid-base responses to exhausting activity, responses which have previously been analysed in control *P. stellatus* (Wood *et al.* 1977). In this series, mean Ht was $16{\cdot}1 \pm 1{\cdot}2$% (6) in the control group and $0{\cdot}7 \pm 0{\cdot}1$% (4) in the anaemic group. In agreement with the preceding results (Fig. 6B, Table 1), initial pre-exercise pH_v was significantly lower in the anaemic fish (Fig. 10A), although there was no difference in mean venous lactate level (Fig. 10B). Ten minutes of manual chasing completely exhausted both groups, with no noticeable difference in swimming behaviour. Up to 4 h after exercise, changes in pH_v and venous lactate concentrations were

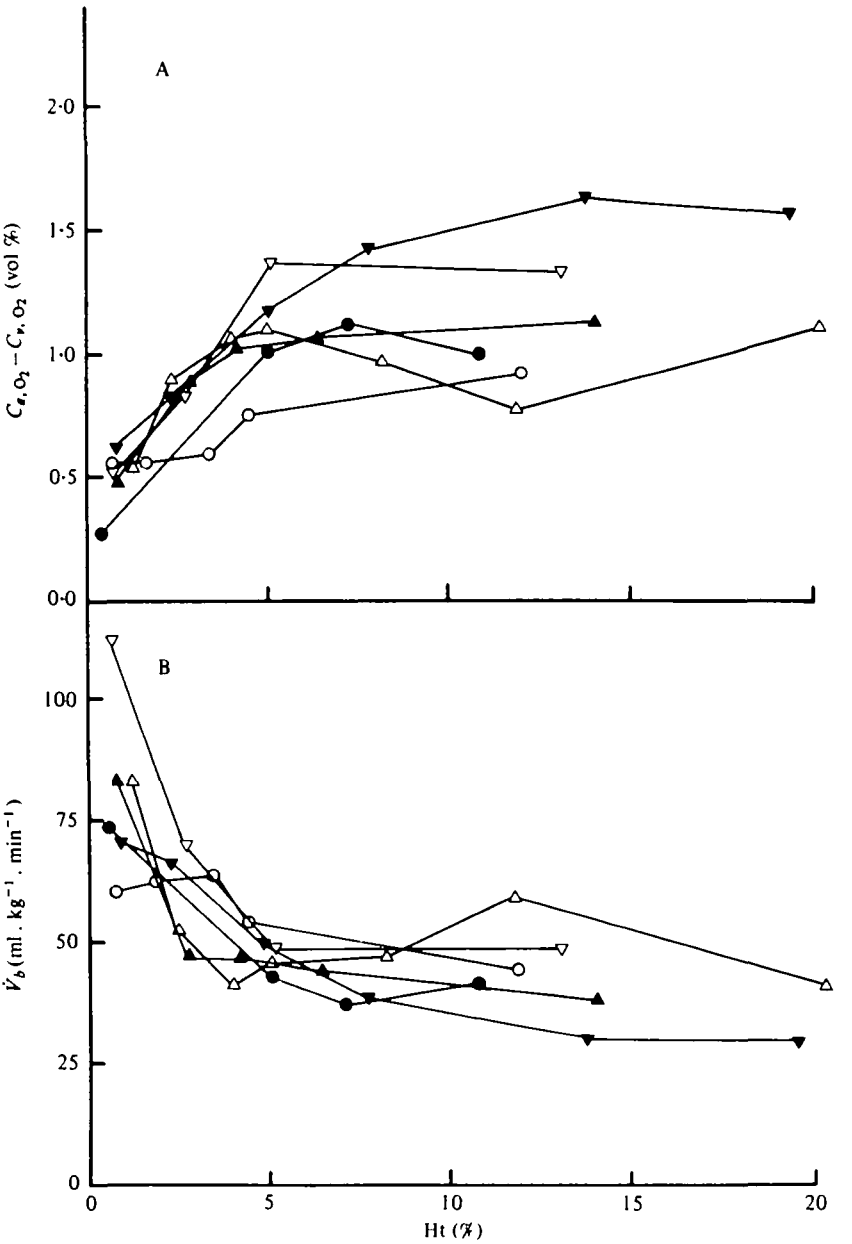

Fig. 7. The relationships between haematocrit (Ht) and: (A) arterial-venous blood O_2 content difference ($C_{a,O_2}-C_{v,O_2}$); (B) cardiac output ($\dot{V}_b$) in starry flounder rendered progressively anaemic. Other details as in Fig. 1.

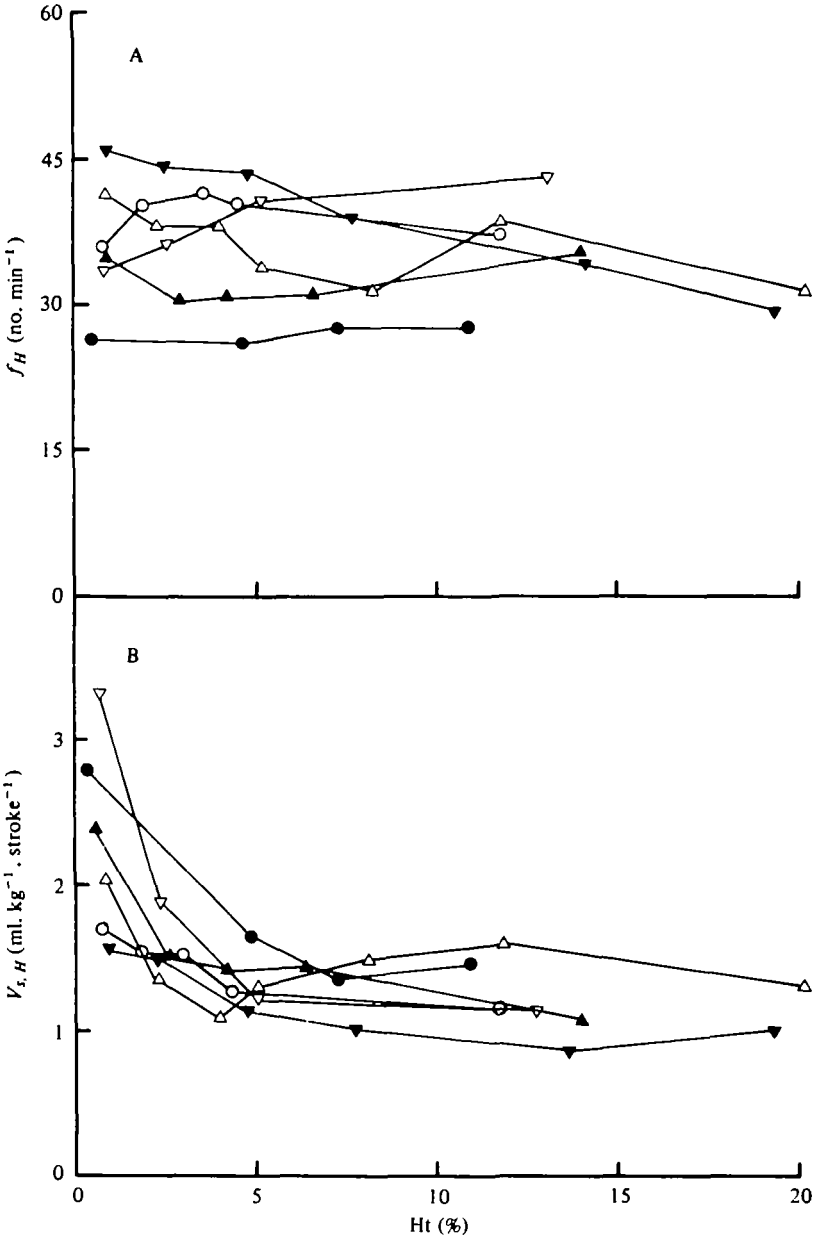

Fig. 8. The relationships between haematocrit (Ht) and: (A) heart rate (f_H); (B) cardiac stroke volume ($V_{s,H}$) in starry flounder rendered progressively anaemic. Other details as in Fig. 1.

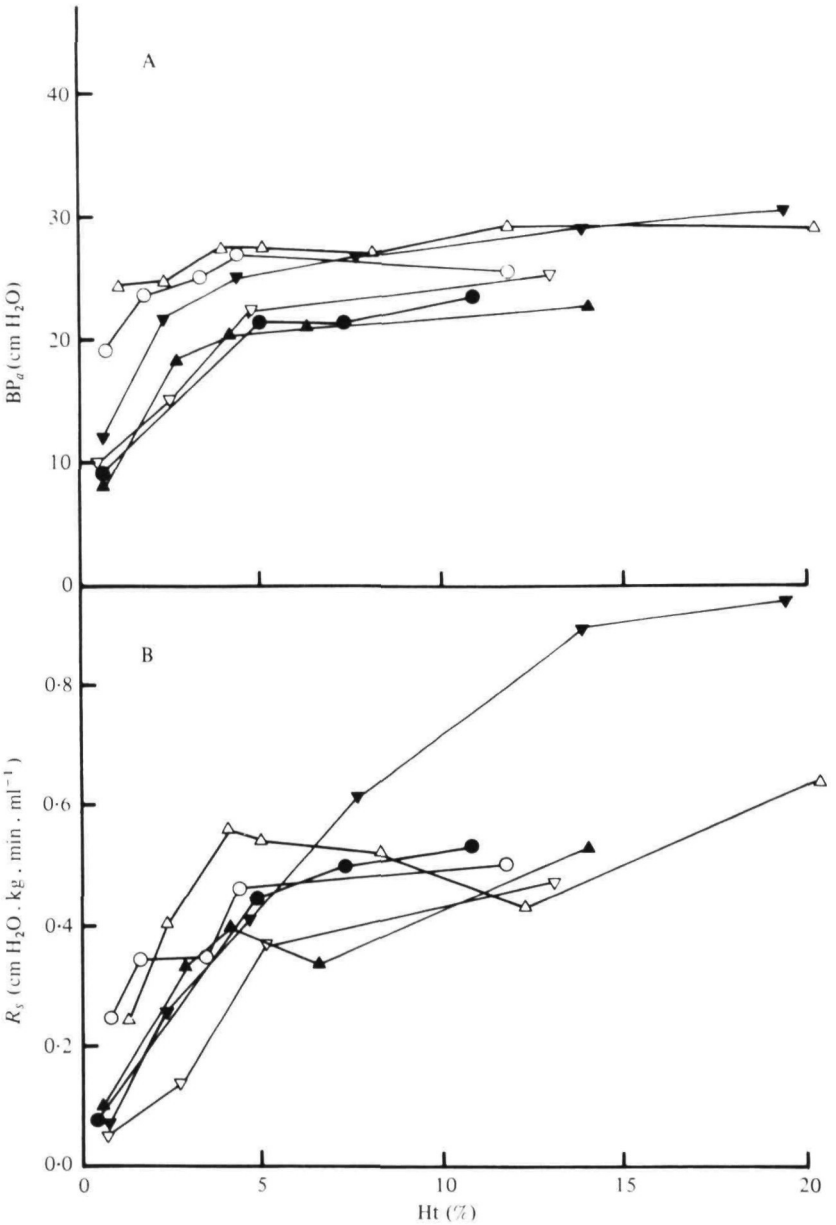

Fig. 9. The relationships between haematocrit (Ht) and: (A) mean dorsal aortic blood pressure (BP_a); (B) systemic vascular resistance (R_s) in starry flounder rendered progressively anaemic. Other details as in Fig. 1.

Table 2. *Ventilatory, respiratory, and cardiovascular parameters in a single starry flounder before, during, and after correction of severe anaemia by re-infusion of stored erythrocytes (weight = 660 g)*

	Control	Moderate anaemia	Severe anaemia	After re-infusion
Day	4	9	13	14
Ht (%)	20·3	8·2	1·2	7·8
U_{w,O_2} (%)	71·4	77·9	41·0	69·2
$\dot{V}_{O_2}$ (ml O_2.kg^{-1}.min^{-1})	0·447	0·456	0·443	0·463
$\dot{V}_w$ (ml.kg^{-1}.min^{-1})	101·1	95·5	163·0	102·6
f_R (no.min^{-1})	39·1	39·5	34·9	36·8
$\dot{V}_{s,R}$ (ml.kg^{-1}.stroke^{-1})	2·59	2·42	4·67	2·79
C_{a,O_2} (vol %)	5·88	2·78	0·62	1·97
C_{v,O_2} (vol %)	4·78	1·81	0·08	0·91
$C_{a,O_2}-C_{v,O_2}$ (vol %)	1·10	0·97	0·54	1·06
P_{a,O_2} (torr)	30·5	30·6	89·4	37·4
P_{v,O_2} (torr)	15·9	11·9	6·8	10·8
$C_{O_2}^{max}$ (vol %)	8·11	3·72	(1·17)*	2·37
$C_{HbO_2}^{max}$/Ht (vol %.%$^{-1}$)	0·363	0·365	(0·363)*	0·226
$C_{O_2\,diss}^{max}$ (vol %)	0·733	0·730	(0·733)*	0·610
pH_a	7·876	7·824	7·786	7·821
pH_v	7·866	7·816	7·724	7·818
$\dot{V}_s$ (ml.kg^{-1}.min^{-1})	40·7	47·0	82·6	43·7
f_H (no.min^{-1})	31·5	31·7	41·1	32·2
$\dot{V}_{s,H}$ (ml.kg^{-1}.stroke^{-1})	1·29	1·48	2·01	1·36
BP_a (cm H_2O)	28·4	27·4	24·6	28·5
BP_v (cm H_2O)	2·31	3·30	4·50	4·82
R_s (cm H_2O.min.kg.ml^{-1})	0·642	0·510	0·240	0·545

Note: All values represent means of at least three determinations.
* Values estimated assuming $C_{HbO_2}^{max}$/Ht, and $C_{O_2\,diss}^{max}$ unchanged from day 4.

very similar in the two groups (Fig. 10A, B). At 6 h, lactate levels were slightly higher and pH_v significantly lower in the anaemic fish. By 24 h, Ph_v had returned to normal in both groups; lactate had similarly returned to resting levels in the control fish but remained slightly elevated ($P = 0{\cdot}05$) in the anaemic flounder. Anaemia therefore seemed to have only a small influence on the response pattern late in the recovery period.

The frequency distribution of Ht's occurring in 97 flounder collected from the wild is shown in Fig. 11. The mean Ht was 19·9 %, but there was considerable variability (range 4·3–34·6 %). The data were not normally distributed but skewed strongly to the left with a mode (25·5 %) considerably higher than the mean (19·9 %). Only one Ht below 6 % was observed. There was no significant relationship between Ht and body weight. However, virtually all animals were parasitized by gill copepods, and casual observation indicated that Ht was negatively correlated with the degree of infestation.

DISCUSSION

Down to a Ht of about 5 %, the starry flounder was able to sustain resting $\dot{V}_{O_2}$ with little or no disturbance of any parameter except P_{v,O_2}. By gradually reducing P_{v,O_2} as Ht fell (Fig. 5 B), the animal increased the relative unloading of O_2 from the blood (i.e. decreased S_{v,O_2}). Consequently $C_{a,O_2}-C_{v,O_2}$ was maintained constant

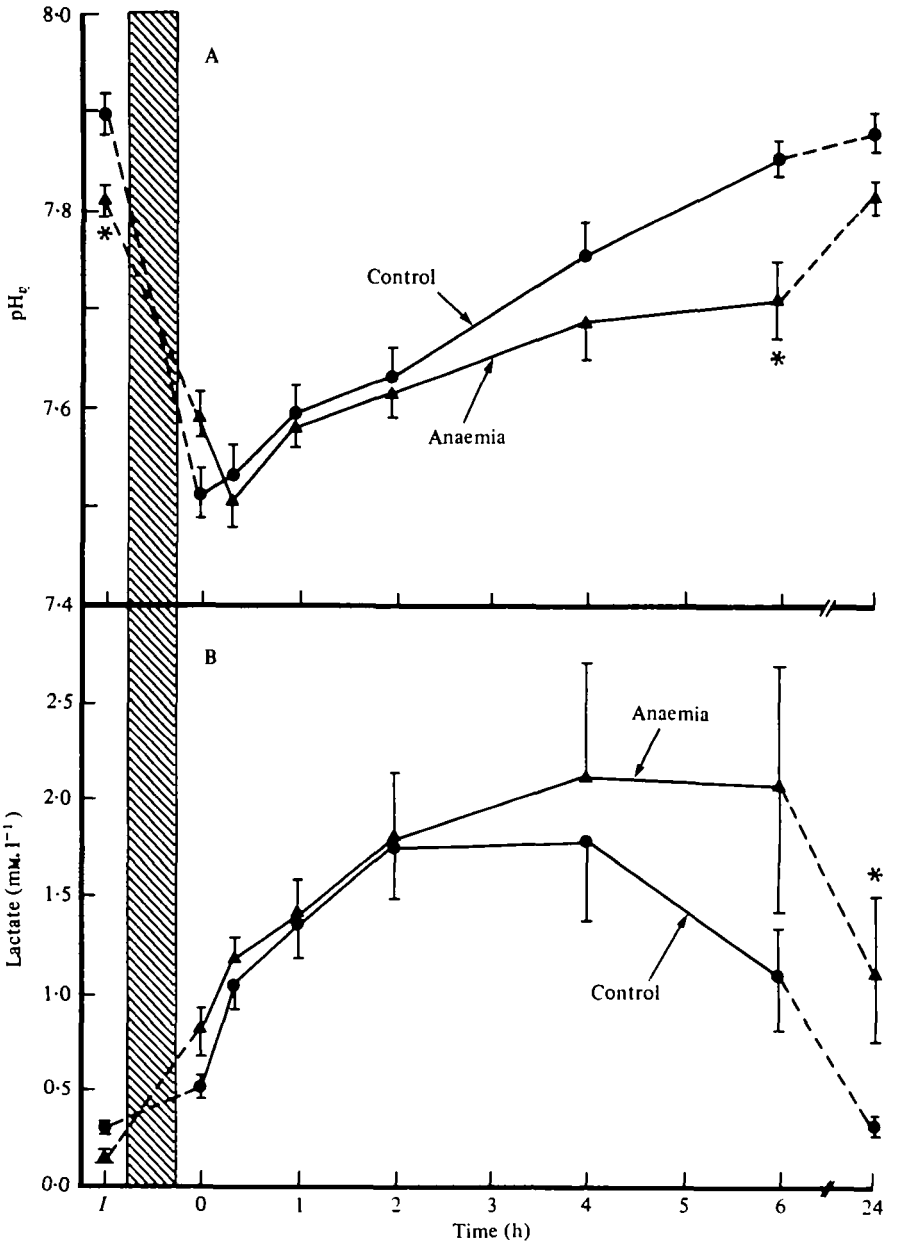

Fig. 10. (A) Venous pH (pH_v) and (B) venous lactate concentrations before and after 10 min of exhausting activity in control (●; $N = 6$) and severely anaemic (▲; $N = 4$) starry flounder. Means ± 1 S.E. Activity = bar. I = initial resting sample. Time 0 = immediately post-exercise. * = means significantly different between the two groups ($P \leq 0{\cdot}05$).

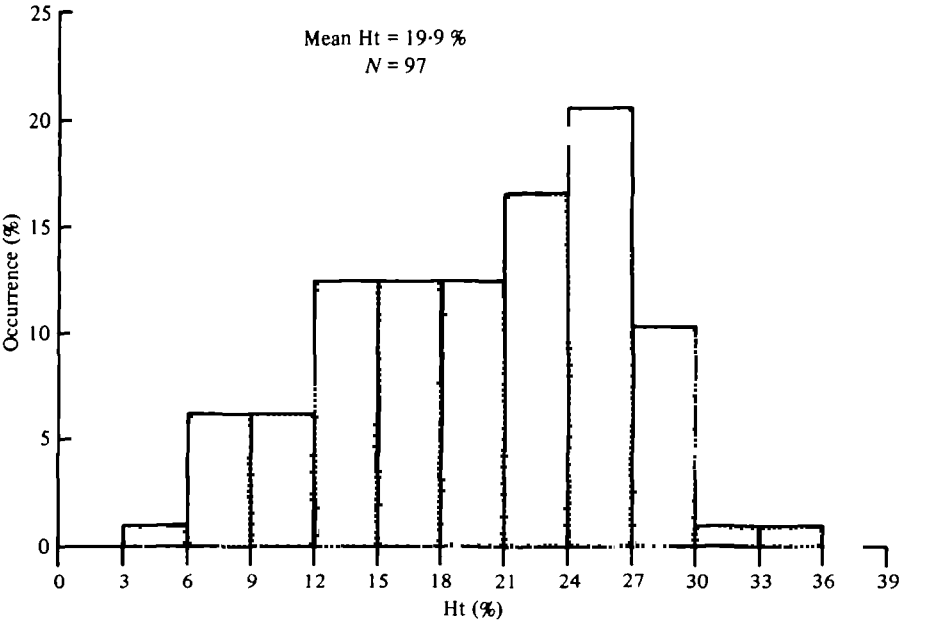

Fig. 11. The frequency distribution of haematocrits (Ht) naturally occurring in 97 starry flounder collected from the wild.

(Fig. 7A) in the face of the declining $C_{O_2}^{max}$, and there was no need for compensatory manipulation of $\dot{V}_b$ or other cardiorespiratory parameters. Below a Ht of about 5 %, $C_{a,O_2}-C_{v,O_2}$ declined, and a host of other changes were implemented in addition to the continuing decrease in P_{v,O_2}. These included increases in $\dot{V}_b$ (Fig. 7B) through elevation of $\dot{V}_{s,H}$ (Fig. 8 B), decreases in BP_a (Fig. 9 A) and increases in BP_v accompanied by a decline in R_s (Fig. 9B), increases in P_{a,O_2} (Fig. 5A), decreases in pH_a and pH_v (Fig. 6A, B), increases in $\dot{V}_w$ (Fig. 2A) through elevation of $V_{s,R}$ (Fig. 3B) and slight depression of f_R (Fig. 3A), and increases in P_{E,O_2} (Fig. 2B).

The frequency distribution of Ht in flounder recently captured from the wild (Fig. 11) indicates that anaemia is of significant natural occurrence. The physiological data on the responses to experimental anaemia would seem to explain this frequency distribution. The 'normal' Ht of healthy fish is probably in the region of the mode (i.e. above 24%), but large numbers of animals occur with Ht's as low as 6%, giving the distribution a strong skew to the left (Fig. 11). These moderately anaemic flounder have probably lost erythrocytes through branchial (or other) parasitism, or through injury. Red cell replacement is obviously a very slow process for we observed no recovery of Ht in experimentally anaemic flounder kept for up to 7 weeks after bleeding. Moderately anaemic flounder can survive in the wild because compensation involves minimal disturbance of the cardio-respiratory system (decreases in P_{v,O_2} only), at least in the resting state. However, only one fish (out of 97) was found in the wild with a Ht less than 6%. This cutoff point corresponds with the inflection point (5 %) seen in the physiological data. Below a Ht of about 5 %, the animals must invoke major compensations in order to maintain resting $\dot{V}_{O_2}$. Some of these, especially the increases in $\dot{V}_w$ [redacted] $\dot{V}_b$ may have considerable metabolic cost (Jones, 1971*b*), yet there is no accom-

Table 3. *Some derived expressions of O_2 exchange and transport in the resting starry flounder before and after severe anaemia (means ± 1 S.E.; N): a comparison with the rainbow trout*

		Platichthys stellatus (present study)			*Salmo gairdneri* (Cameron & Davis, 1970)	
		Control	Anaemia	*P*	Control	Anaemia
% utilization – water	$U_{w,O_2} = \frac{P_{I,O_2}-P_{E,O_2}}{P_{I,O_2}} \times 100\%$	67·6 ± 1·5 (6)	42·3 ± 6·8 (6)	0·01	46·9 ± 1·5 (18)	39·1 ± 2·4 (16)
% utilization – blood	$U_{b,O_2} = \frac{C_{a,O_2}-C_{v,O_2}}{C_{a,O_2}} \times 100\%$	27·9 ± 2·9 (6)	82·2 ± 2·4 (6)	0·001	•	•
% effectiveness – water	$E_{w,O_2} = \frac{P_{I,O_2}-P_{E,O_2}}{P_{I,O_2}-P_{v,O_2}} \times 100\%$	75·9 ± 3·8 (6)	44·5 ± 7·1 (6)	0·01	(57·8)†	(45·7)†
% effectiveness – blood	$E_{b,O_2} = \frac{C_{a,O_2}-C_{v,O_2}}{C_{I,eq,O_2}-C_{v,O_2}} \times 100\%$	54·9 ± 8·3 (6)	65·3 ± 7·8 (6)	n.s.	•	•
Ventilation-perfusion ratio	$\frac{\dot{V}_w}{\dot{V}_b}$	2·78 ± 0·15 (6)	2·06 ± 0·21 (6)	0·05	10·45 ± 0·88 (18)	3·62 ± 0·44 (16)
Capacity-rate ratio	$\frac{P_{a,O_2}-P_{v,O_2}}{P_{I,O_2}-P_{E,O_2}}$	0·245 ± 0·025 (6)	1·576 ± 0·371 (6)	0·02	1·190 ± 0·089 (18)	1·770 ± 0·018 (16)
P_{O_2} gradient (torr)	$\Delta P_{O_2} = \frac{[P_{I,O_2}-P_{a,O_2}]-[P_{E,O_2}-P_{v,O_2}]}{\ln[(P_{I,O_2}-P_{a,O_2})/(P_{E,O_2}-P_{v,O_2})]}$	57·7 ± 4·1 (6)	63·3 ± 4·1 (6)	n.s.	(54·8)†	(53·4)†
Transfer factor (ml $O_2 \cdot kg^{-1} \cdot min^{-1} \cdot torr^{-1}$)	$T_{O_2} = \frac{\dot{V}_{O_2}}{\Delta P_{O_2}}$	0·0083 ± 0·0009 (6)	0·0067 ± 0·0012 (6)	n.s.	(0·0118)†	(0·0106)†
Convection requirement for water (l.mmol O_2^{-1})	$\frac{\dot{V}_w}{\dot{M}_{O_2}}$	5·37 ± 0·19 (6)	9·95 ± 2·00 (6)	0·05	(5·95)†	(7·12)†
Convection requirement for blood (l.mmol O_2^{-1})	$\frac{\dot{V}_b}{\dot{M}_{O_2}}$	1·97 ± 0·15 (6)	4·78 ± 0·72 (6)	0·02	(0·64)†	(1·94)†

Note: *P* determined by Student's paired two tailed *t*-test, using each animal as its own control.

• Values not reported by Cameron & Davis (1970), but blood P_{O_2} data indicate little or no change in the parameters during anaemia.

† Values calculated from other data presented by Cameron & Davis (1970).

anying rise in $\dot{V}_{O_2}$ (Fig. 1). Thus a greater fraction of resting $\dot{V}_{O_2}$ must be devoted to fuelling the branchial and cardiac muscles, and less will be available for other maintenance functions. This condition can be tolerated by the fish in the laboratory where it simply lies buried in the sand the whole time. However in the wild, far greater demands are placed on the animal, and the condition presumably becomes unviable.

Despite the great differences in blood O_2 transport capacity between normal and severely anaemic flounder, the responses of the two groups to enforced activity were remarkably similar (Fig. 10). This finding supports our previous contention that O_2 transport is not the primary factor limiting exercise (Wood *et al.* 1977). Rather, exertion may be limited by the acute acidosis which quickly lowers blood pH to an unacceptable level. This rapid acidosis is almost entirely respiratory in origin (i.e. due to P_{CO_2} increase; Wood *et al.* 1977). The pH_v changes during this period (0–4 h after exercise) were very similar in the two groups (Fig. 10). As the non-bicarbonate buffer capacity (β) was reduced about 30% by severe anaemia (Wood *et al.* 1977), a larger pH_v decline for the same P_{v,CO_2} rise might have been expected in the anaemic fish. That this did not occur may mean that the critical pH limiting further exercise was reached at a lower P_{CO_2} in the anaemic animals. Only late in recovery (6–24 h) were there significant differences between the two groups. The slower return of pH_v to pre-exercise values in the anaemic fish was associated with higher blood lactate levels at this time. The slow time course of lactic acid release into the bloodstream ensures that this metabolic acid makes a significant contribution to the pH_v depression only late in the recovery period (Wood *et al.* 1977). The eventually higher blood lactates in the anaemic group do indicate a greater reliance on anaerobic metabolism, but the differences were not great (Fig. 10). In any event, exercise metabolism in this sluggish species may normally be largely anaerobic. The accumulation of other anaerobic end-products besides lactate is also possible (Hughes & Johnston, 1978).

Comparison of the responses of starry flounder to severe anaemia with those of the rainbow trout (Cameron & Davis, 1970) reveals both similarities and differences in the mechanisms of adaptation (Table 1). A number of derived parameters based on gas exchange equations have also been summarized in Table 3 to aid in this comparison. A detailed analysis of the gas exchange strategies of the two species in the resting state has been presented earlier (Wood *et al.* 1979).

Like the flounder, the trout maintains normal resting $\dot{V}_{O_2}$ during severe anaemia (Table 1), but the compensatory mechanisms appear much less complex. The anaemic trout simply elevates $\dot{V}_b$ by increasing $V_{s,H}$ and decreasing R_s, thereby maintaining $\dot{V}_{O_2}$ constant despite a greatly reduced $C_{a,O_2}-C_{v,O_2}$. No adjustments are made in ventilation, P_{E,O_2}, or blood O_2 tensions (Table 1). There are therefore no changes in the percentage utilizations of O_2 from the water by the blood (U_{w,O_2}) or from the blood by the tissues (U_{b,O_2}), in the percentage effectiveness of O_2 removal from the water (E_{w,O_2}) or uptake by the blood (E_{b,O_2}), in the mean P_{O_2} gradient (ΔP_{O_2}) or transfer factor for O_2 (T_{O_2}) across the branchial epithelium, or in the convection requirement for water ($\dot{V}_w/\dot{M}_{O_2}$) (Table 3). However this strategy does mean that the convection requirement for blood ($\dot{V}_b/\dot{M}_{O_2}$) must increase while the ventilation/perfusion ratio ($\dot{V}_w/\dot{V}_b$) falls sharply in such a fashion as to hold the capacity-rate ratio of the two convective media almost constant at a value close to unity (Table 3). This effectively keeps the exchange system at maximal efficiency in terms of O_2 transfer per unit total flow of blood and water (Shelton, 1970).

The severely anaemic flounder similarly elevates $\dot{V}_b$ by increasing $V_{s,H}$ and decreasing R_s; the latter presumably helps to minimize the increase in cardiac work. However the flounder also manipulates ventilation, P_{E,O_2} and blood O_2 tensions (Table 1). Although the experimental anaemia was more pronounced in the flounder (Ht = 0·8 %) than in the trout (Ht = 3·8 %), the relative increase in $\dot{V}_b$ was lower (two- versus three-fold). Indeed Cameron & Davis (1970) present data indicating that at a comparably low Ht, the anaemic trout increases $\dot{V}_b$ seven- to ten-fold. This reflects a much lower resting $\dot{V}_b$ in the control trout and therefore a much greater scope for raising $\dot{V}_b$. In fact, the anaemic trout becomes remarkably similar to the control flounder in terms of $\dot{V}_b$, $V_{s,H}$, C_{a,O_2}–C_{v,O_2} (Table 1), and the $\dot{V}_w/\dot{V}_b$ and $\dot{V}_b/\dot{M}_{O_2}$ ratios (Table 3). Control flounder apparently operate at high $\dot{V}_b$ and low C_{a,O_2}–C_{v,O_2} in the resting state, thereby gaining cardiac efficiency but sacrificing high O_2 transport capacity during exercise or severe anaemia (Wood *et al.* 1979). In this light, it is perhaps remarkable that the anaemic flounder is able to increase $\dot{V}_b$ and $\dot{V}_b/\dot{M}_{O_2}$ as much as it does (Tables 1, 3).

Because of the flounder's limited scope for raising $\dot{V}_b$, the anaemic animal has to lower P_{v,O_2} and raise P_{a,O_2} to maintain O_2 transport, thereby increasing both the unloading of O_2 from the haemoglobin at the tissues and the delivery of O_2 by physical solution (Table 1). The success of these manoeuvres is reflected in an approximate tripling of U_{b,O_2} and maintenance of E_{b,O_2} during severe anaemia (Table 3). If necessary, the trout also could conceivably lower P_{v,O_2} and thereby further augment its scope for O_2 delivery; there is evidence that this occurs during exercise (Kiceniuk & Jones, 1977). However, the option of raising P_{a,O_2} is probably only available to benthic fish such as the flounder and tench (Eddy, 1974) which saturate their haemoglobin at low P_{a,O_2} under control resting conditions. P_{a,O_2} is already so high in the control trout that further increases seem unlikely.

The most surprising feature of the flounder's response is the increase in $\dot{V}_w$, since the metabolic cost of ventilation is considerable in flatfish (Edwards, 1971). The elevations of $\dot{V}_w$ and $\dot{V}_b$ are about equal, so the $\dot{V}_w/\dot{V}_b$ ratio falls only slightly in contrast to the trout (Table 3). As the increase in $\dot{V}_w$ is accompanied by a rise in P_{E,O_2} in the classical manner (Fig. 2; Table 1), it seems to make little sense, at first glance, for the animal to pump more water across its gills yet derive the same total amount of O_2 from that flow – a virtual doubling of the $\dot{V}_w/\dot{M}_{O_2}$ ratio (Table 3). E_{w,O_2} falls, and O_2 expenditure is presumably increased without raising O_2 uptake. An explanation for this anomaly may lie in the great importance of physical solution in blood O_2 transport during anaemia (Table 1), for which the P_{a,O_2} rise (Fig. 5A) is of critical importance. A scatter diagram of all simultaneously determined values of P_{E,O_2} and P_{a,O_2} shows a strong positive correlation ($r = 0{\cdot}65$; $P < 0{\cdot}001$) between the two parameters (Fig. 12). The regression relationship is:

$$P_{a,O_2} = 0{\cdot}81\ P_{E,O_2} + 11{\cdot}9$$

which is only slightly different from the line of equality. In other words P_{a,O_2} normally comes close to equilibrium with P_{E,O_2}. By raising $\dot{V}_w$ and therefore P_{E,O_2} through the decreases in water transit time, the anaemic flounder can raise P_{a,O_2} and thereby maintain blood O_2 transport. This strategy involves no change in the mean ΔP_{O_2} driving O_2 diffusion across the branchial epithelium and there

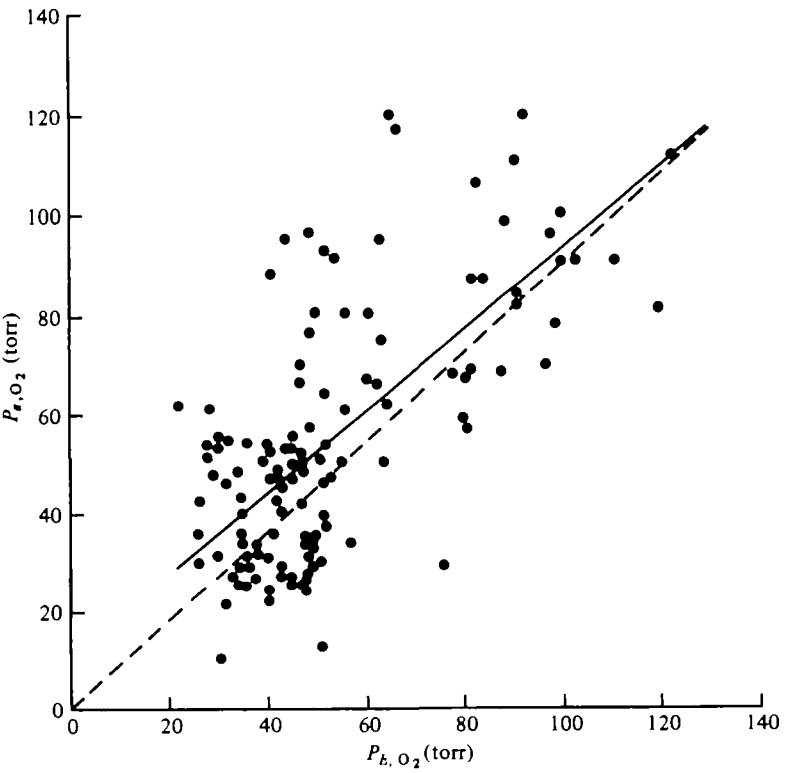

Fig. 12. The relationship between all simultaneously measured values of arterial O_2 tension (P_{a,O_2}) and expired O_2 tension (P_{E,O_2}) in starry flounder. The equation of the fitted regression line (solid line) is: $P_{a,O_2} = 0{\cdot}81\ P_{E,O_2} + 11{\cdot}9$ ($r = 0{\cdot}65$, $P < 0{\cdot}001$). The dotted line represents the line of equality.

necessitates no (possibly unfavourable) alteration of the rather low T_{O_2} (Table 3; Wood *et al.* 1979). An additional benefit of the $\dot{V}_w$ elevation may lie in the capacity-rate ratio approaching 1·0 (Table 3).

Both pH_a and pH_v declined quite markedly during anaemia in the flounder (Fig. 6; Table 1), a compensation which may have aided blood O_2 transport by the Bohr effect (see Results). Cameron & Davis (1970) did not report on blood pH in the anaemic trout, but Cameron (personal communication) has indicated that pH_a did not change with Ht in their study. More recently Haswell & Randall (1978) have confirmed that pH_a and P_{a,CO_2} remain constant during severe anaemia in *Salmo gairdneri* (Table 1). The acidosis observed in the flounder could be associated with the accumulation of acidic end products of anaerobic metabolism in the blood, with the reduced non-bicarbonate buffer capacity of low Ht blood (Wood *et al.* 1977), with a rise in blood P_{CO_2} levels, or with any combination of these and other factors. The phenomenon is currently under investigation, and preliminary results point to an elevation of blood P_{CO_2} levels during anaemia. Janssen & Randall (1975) have shown that a rise in P_{a,CO_2} acts as a direct ventilatory stimulus in fish. A rise in P_{a,CO_2} in the flounder but not in the trout (Haswell & Randall, 1978) would therefore explain the difference in the ventilatory responses of the two species to anaemia.

We wish to thank the Director, Dr A. O. D. Willows, and staff of Friday Harbor Laboratories, University of Washington, for their assistance and hospitality. Mr G. Hewlett, of the Vancouver Public Aquarium, kindly supplied 'Furanace'. Financial support was provided by grants from the National Research Council of Canada.

REFERENCES

ANTHONY, E. H. (1961). Survival of goldfish in presence of carbon monoxide. *J. exp. Biol.* **38**, 109–125.

CAMERON, J. N. & DAVIS, J. C. (1970). Gas exchange in rainbow trout (*Salmo gairdneri*) with varying blood oxygen capacity. *J. Fish. Res. Bd. Canada* **27**, 1069–1085.

CAMERON, J. N. & WOHLSCHLAG, D. E. (1969). Respiratory response to experimentally induced anaemia in the pinfish (*Lagodon rhomboides*). *J. exp. Biol.* **50**, 307–317.

DEJOURS, P. (1975). *Principles of Comparative Respiratory Physiology*. Amsterdam, North Holland.

EDDY, F. B. (1974). Blood gases of the tench (*Tinca tinca*) in well aerated and oxygen-deficient waters. *J. exp. Biol.* **60**, 71–83.

EDWARDS, R. R. C. (1971). An assessment of the energy cost of gill ventilation in the plaice (*Pleuronectes platessa* L.). *Comp. Biochem. Physiol.* **40A**, 391–398.

FLETCHER, G. L. (1975). The effect of capture, 'stress', and storage of whole blood on the red blood cells, plasma proteins, glucose, and electrolytes of the winter flounder (*Pseudopleuronectes americanus*) *Can. J. Zool.* **53**, 197–206.

HASWELL, M. S. & RANDALL, D. J. (1978). The pattern of carbon dioxide excretion in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **72**, 17–24.

HOLETON, G. F. (1971*a*). Oxygen uptake and transport by the rainbow trout during exposure to carbon monoxide. *J. exp. Biol.* **54**, 239–254.

HOLETON, G. F. (1971*b*). Respiratory and circulatory responses of rainbow trout larvae to carbon monoxide and to hypoxia. *J. exp. Biol.* **55**, 683–694.

HOLETON, G. F. (1977). Constancy of arterial blood pH during CO-induced hypoxia in the rainbow trout. *Can. J. Zool.* **55**, 1010–1013.

HUGHES, G. M. & JOHNSTON, I. A. (1978). Some responses of the electric ray (*Torpedo marmorata*) to low ambient oxygen tensions. *J. exp. Biol.* **73**, 107–117.

JANSSEN, R. G. & RANDALL, D. J. (1975). The effects of changes in pH and P_{CO_2} in blood and water on breathing in rainbow trout, *Salmo gairdneri*. *Resp. Physiol.* **25**, 235–245.

JONES, D. R. (1971*a*). The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **55**, 541–551.

JONES, D. R. (1971*b*). Theoretical analysis of factors which may limit the maximum oxygen uptake of fish: the oxygen cost of the cardiac and branchial pumps. *J. theor. Biol.* **32**, 341–349.

KICENIUK, J. W. & JONES, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247–260.

NICLOUX, M. (1923). Action de l'oxyde de carbone sur les poissons et capacité respiratoire du sang de ces animaux. *C.r. Seanc. Soc. Biol.* **89**, 1328–1331.

SCHLICHER, J. (1926). Vergleichend Physiologische Untersuchungen der Blutkorperchenzahlen bei Knockenfischen. *Zool. Jb.* **43**, 121.

SHELTON, G. (1970). The regulation of breathing. In *Fish Physiology*, vol. IV (ed. W. S. Hoar and D. J. Randall). New York: Academic Press, Inc.

WATTERS, K. W. Jr. & SMITH, L. S. (1973). Respiratory dynamics of the starry flounder *Platichthys stellatus* in response to low oxygen and high temperatures. *Marine Biology* **19**, 133–148.

WOLF, K. (1963). Physiological salines for freshwater teleosts. *Progr. Fish. Cult.* **25**, 135–140.

WOOD, C. M. (1974). A critical examination of the physical and adrenergic factors affecting blood flow through the gills of the rainbow trout. *J. exp. Biol.* **60**, 241–265.

WOOD, C. M., MCMAHON, B. R. & MCDONALD, D. G. (1977). An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus*. *J. exp. Biol.* **69**, 173–185.

WOOD, C. M., MCMAHON, B. R. & MCDONALD, D. G. (1979). Respiratory gas exchange in the resting starry flounder, *Platichthys stellatus*: a comparison with other teleosts. *J. exp. Biol.* **78**, 167–179.

WOOD, C. M. & RANDALL, D. J. (1971). The effect of anaemia on ion exchange in the southern flounder (*Paralichthys lethostigma*). *Comp. Biochem. Physiol.* **39A**, 391–402.