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RESPIRATORY GAS EXCHANGE IN THE RESTING STARRY FLOUNDER, *PLATICHTHYS STELLATUS*: A COMPARISON WITH OTHER TELEOSTS

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SUMMARY

A wide range of respiratory, ventilatory, and cardiovascular parameters have been recorded under completely resting conditions in the starry flounder (*Platichthys stellatus*), a generally inactive benthic teleost. The results differ in a number of important respects from those of a previous study on the same species. The present data have also been compared with those reported for the active pelagic rainbow trout (*Salmo gairdneri*) and for other teleost species. Of particular note in the flounder, relative to the trout, are low arterial and venous P_{O_2}'s, a low arterial-venous O_2 content difference, a low transfer factor and high diffusion gradient for O_2 across the gills, a high *in vivo* blood O_2 affinity, a high cardiac output and stroke volume accompanied by a low peripheral vascular resistance, a low ventilation volume, a low ventilation–perfusion ratio, and a low capacity-rate ratio for O_2 exchange at the gills. Parameters of CO_2 transport and acid-base regulation appear conventional, though blood CO_2 contents and lactate concentrations are low. The respiratory strategies of inactive versus active, and benthic versus pelagic teleosts are discussed.

INTRODUCTION

As reference to any recent review will illustrate (e.g. Randall, 1970*a*, *b*; Johansen, 1971; Hughes & Morgan, 1973; Jones & Randall, 1978), the great advances over the past 15 years in our understanding of respiration, ventilation, and circulation in water-breathing teleosts are based to a very large extent on experiments with the rainbow trout, *Salmo gairdneri*, in freshwater. However, the results are unlikely to be representative of most other species since the trout is atypical in its extremely active habits and its usually well-oxygenated environment.

Recently, several studies employing modern analytical techniques have appeared on less active, more benthic fish: the starry flounder (*Platichthys stellatus*; Watters & Smith, 1973), the tench (*Tinca tinca*; Eddy, 1973, 1974) and the winter flounder (*Pseudopleuronectes americanus*; Cech *et al.* 1976, 1977). From the investigation of Watters & Smith (1973), it appears that *Platichthys stellatus* might make an ideal

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'standard' species representative of marine benthic forms for cardio-respiratory analysis. We have recorded a fairly comprehensive range of respiratory, ventilatory, and cardiovascular parameters from this species in an experimental situation designed to reproduce natural resting conditions as closely as possible, as a background for further studies (Wood, McMahon & Donald, 1979). Our results are in agreement with those of Watters & Smith (1973) in some areas but differ on a number of key points which we believe reflect methodological problems, especially non-resting conditions, in the earlier study.

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; 300–1200 g) were collected by otter-trawling a region of sandy substrate in East Sound of Orcas Island, Washington State. The fish were then held in large, sand-covered tanks for at least 10 days before use at Friday Harbor Laboratory, University of Washington. The acclimation conditions were those employed in subsequent experiments: running seawater, salinity = 26–28‰, temperature = 7·5–10·5 °C. Two series of experiments were performed. In the first ($N = 8$), which concentrated on ventilation and O_2 transport, the fish were fitted with arterial and venous blood catheters, inspired water catheters, ventilation collection masks, and opercular impedance leads. In the second ($N = 15$), which dealt primarily with CO_2 transport and acid-base regulation, only blood catheters (arterial and/or venous) were implanted.

1. *Preparation of animals*

Fish were anaesthetized on an operating table with 1:15000 MS-222. The caudal artery and the caudal vein were catheterized with Clay–Adams PE 50 as described by Watters & Smith (1973). These catheters were positioned to allow the withdrawal of arterial blood from a site just posterior to the union of the last efferent branchial arteries, and venous blood from the level of the kidney. The catheters were filled with Cortland saline (Wolf, 1963) adjusted to 160 m-equiv./l sodium concentration by addition of NaCl, and heparinized at 100 i.u./ml. The wound was dusted with the fish antibiotic 'Furanace' (Nifurpironol, Dainippon Pharmaceutical) and closed with silk sutures. A length of PE 160 was anchored in the buccal cavity for sampling of inspired water. Fine wires were implanted subcutaneously on either side of the upper opercular opening to detect ventilatory movements by means of an impedance converter (Biocom Inc.). In a few experiments, impedance leads were similarly implanted across the position of the heart to detect cardiac contraction. The wires were sewn to the skin at the point of exit and encased in PE 60 to avoid tangling.

The technique used for collection of the ventilatory water flow differed slightly from that described by Watters & Smith (1973). A mask designed to completely encompass the upper and lower opercular openings and pectoral fins was constructed to fit each fish from latex rubber dental dam cemented with cyanoacrylate glue. The mask was sewn securely to the skin with silk suture in a running rib stitch, a procedure which took 2–3 h. Particular care was taken to ensure that there was sufficient play in the mask to avoid any restriction of the ventilatory muscles. Both surfaces of the mask were attached to a common outflow, the barrel of a 5 ml plastic syringe, at the ventral apex of the fish. The barrel was fitted with a length of PE 160 for sampling of mixed expired water and could be connected to flow monitoring devices for measurement of ventilation volume (see below).

The fish were then transferred to individual chambers (60 × 35 cm and 15 cm deep in the first experimental series; 30 × 30 cm and 15 cm deep in the second) which were shielded from the investigators and filled to a depth of 6 cm with fine beach sand. Within 30 min of post-anaesthesia, the flounder buried in the sand until only the eyes and mouth were exposed. Except for occasional shifts in position, the animals remained buried in this fashion for the whole experimental period, a duration of up to 3 weeks. This behaviour paralleled that of unrestrained animals in the main holding tanks, so we believe that this closely duplicates the natural resting situation.

II. *Analytical techniques*

Ventilation volume ($\dot{V}_w$) was measured by connecting the outflow barrel of the mask to a constant level overflow constructed of wide-bore (I.D. = 22 mm) glass and rubber tubing. The hydraulic resistance of the system was negligible, for when artificially perfused at 250 ml/min (2–3 × the normal $\dot{V}_w$), the pressure drop across the tubing was less than 0·2 cm H_2O as measured by a Hewlett–Packard 267 BC differential pressure transducer. A manometer ensured that water levels in the fish and overflow chambers were identical. In one experiment, ventilation was also determined by attaching an electromagnetic blood-flow probe (I.D. = 7 mm; Biotronex Labs) to the outflow barrel of the mask. Values of $\dot{V}_w$ and O_2 uptake measured using the two techniques separately were virtually identical.

Inspired (P_{I,O_2}) and expired (P_{E,O_2}) water O_2 tensions and arterial (P_{a,O_2}) and venous (P_{v,O_2}) blood O_2 tensions were measured with a thermostatted Radiometer microelectrode, all samples being lead directly from the animal to the same electrode by a slight siphon. This blood was returned to the flounder after analysis. Arterial (P_{a,CO_2}) and venous (P_{v,CO_2}) CO_2 tensions were measured as described by Wood, McMahon & McDonald (1977). Because of possible deterioration during the long equilibration time in the CO_2 electrode, these blood samples were generally not returned to the animal. Arterial (C_{a,O_2}) and venous (C_{v,O_2}) blood O_2 contents were determined with a 'Lex-O_2-Con' analyser (Lexington Instruments) using a sample volume of 40 μl. We found that when calibrated with air as suggested by the manufacturer, our 'Lex-O_2-Con' significantly underestimated O_2 contents at meter readings less than about 5 vol %. Consequently, a calibration curve was constructed for this range using various volumes of distilled water of known O_2 content. Arterial (C_{a,CO_2}) and venous (C_{v,CO_2}) CO_2 contents, arterial (pH_a) and venous (pH_v) hydrogen ion levels, haematocrits (Ht); and venous lactate concentrations were all measured as described by Wood *et al.* (1977). All blood samples were handled anaerobically in ice-cold Hamilton syringes to minimize the effects of endogenous red blood cell metabolism.

Although the distinction is often ignored, oxygen capacity ($C^{\max}_{\mathrm{HbO_2}}$) strictly refers only to the amount of O_2 bound to haemoglobin when blood is equilibrated with air and does not include physically dissolved O_2 ($C^{\max}_{\mathrm{O_2\,diss}}$) (Bartels *et al.* 1973). At the rather low Ht's of flounder blood, the physically dissolved component can be a significant fraction of the total. Consequently, in each animal, the O_2 contents of both whole blood and plasma were measured when equilibrated with air. Blood or plasma samples (0·2–0·5 ml) were placed in 5 ml spinning tonometers gassed with room air ($P_{\mathrm{O_2}} \approx 155$ torr) which had been passed through 1 M NaOH for humidification and removal of all CO_2. An equilibration period of 30 min at the experimental temperature was employed; the samples were then analysed with the 'Lex-O_2-Con'. $C^{\max}_{\mathrm{HbO_2}}$ was calculated as the difference between the O_2 content of the whole blood and that of the plasma ($C^{\max}_{\mathrm{O_2\,diss}}$) and was expressed on a unit Ht basis (i.e. $C^{\max}_{\mathrm{HbO_2}}/\mathrm{Ht}$).

Arterial (BP_a) and venous (BP_v) blood pressures were recorded from the respective catheters with a Hewlett-Packard 267 BC pressure transducer whose output, along with that of the impedance converters and electromagnetic flowmeter, was displayed on Brush or Gilson oscillographs. Heart rate (f_H) was taken from the cardiac impedance recording or from the pulsatility of the BP_a trace, and ventilation rate (f_R) from the opercular impedance recording.

III. *Calculations*

O_2 uptake ($\dot{V}_{\mathrm{O_2}}$) and cardiac output ($\dot{V}_b$) were both calculated by the Fick principle:

$$\dot{V}_{\mathrm{O_2}} = (P_{I,\mathrm{O_2}} - P_{E,\mathrm{O_2}}) \cdot \beta_{w,\mathrm{O_2}} \cdot \dot{V}_w,$$

$$\dot{V}_b = \frac{\dot{V}_{\mathrm{O_2}}}{(C_{a,\mathrm{O_2}} - C_{v,\mathrm{O_2}})} \cdot 100,$$

where $\beta_{w,\mathrm{O_2}}$ is the solubility of O_2 in sea water.

Ventilatory ($V_{s,R}$) and cardiac ($V_{s,H}$) stroke volumes were calculated as

$$V_{s,R} = \frac{\dot{V}_w}{f_R}, \quad V_{s,H} = \frac{\dot{V}_b}{f_H}.$$

As with $C^{\max}_{\mathrm{HbO_2}}$, oxygen saturation strictly refers only to O_2 bound to haemoglobin (Bartels *et al.* 1973). Therefore arterial saturation ($S_{a,\mathrm{O_2}}$) was calculated in the following manner:

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

Venous saturation ($S_{v,\mathrm{O_2}}$) was estimated in an analogous fashion.

Mean arterial blood pressure (Burton, 1972) was calculated as:

$$\mathrm{BP}_a = \tfrac{1}{3}(1\ \text{systolic} + 2\ \text{diastolic}).$$

RESULTS

During the post-operative recovery period, most cardio-respiratory parameters tended to change with time. In particular, pH_a, pH_v, $C_{a,\mathrm{CO_2}}$, and $C_{v,\mathrm{CO_2}}$ increased, while $\dot{V}_w$, f_R, $V_{s,R}$, $P_{E,\mathrm{O_2}}$, $\dot{V}_{\mathrm{O_2}}$, $P_{a,\mathrm{O_2}}$, $P_{v,\mathrm{O_2}}$, f_H, BP_a, BP_v and Ht all declined. $C_{a,\mathrm{O_2}}$, $C_{v,\mathrm{O_2}}$, $C^{\max}_{\mathrm{HbO_2}}$, $C^{\max}_{\mathrm{O_2\,diss}}$, $P_{a,\mathrm{CO_2}}$, $P_{v,\mathrm{CO_2}}$, $S_{a,\mathrm{O_2}}$, $S_{v,\mathrm{O_2}}$ and $\dot{V}_b$ were not monitored during

Table 1. *Ventilatory, respiratory, and cardiovascular parameters in the resting flounder of the first experimental series (means* ± S.E., *N* = 8)*: a comparison with other studies*

	Platichthys stellatus (present study)	*Platichthys stellatus* (Watters & Smith, 1973)	*Pseudopleuronectes americanus* (Cech *et al.* (1976, 1977)	*Tinca tinca* (Eddy, 1973, 1974)	*Salmo gairdneri* (*a–e*)*
Weight (g)	683·5 ± 80·7	1340·5	635·0	150–400	100–725 *a–e*
Temp. (°C)	7·5–10·5	9·0–12·3	10·0	11·0–14·0	8·0–16·0 *a–e*
Ht (%)	14·5 ± 1·2	13·7	—	24·1	22·8 *b*
P_{I,O_2} (torr)	138·7 ± 1·7	126·3	(130·0)	145·0	160·3 *b*
P_{E,O_2} (torr)	43·5 ± 2·9	54·2	—	65·5	86·1 *b*
$\dot{V}_{O_2}$ (ml.kg^{-1}.min^{-1})	0·458 ± 0·028	0·391	0·390	0·500	0·645 *b*
$\dot{V}_w$(ml.kg^{-1}.min^{-1})	109·2 ± 5·9	141·5	—	132·0	171·4 *b*
f_R (no. min^{-1})	41·7 ± 1·6	—	—	—	73·8 *b*
$V_{s,R}$ (ml.kg^{-1}.stroke^{-1})	2·62 ± 0·11	—	—	—	2·34 *b*
P_{a,O_2} (torr)	34·9 ± 3·0	75·5	90·0	35·8	133·2 *b*
P_{v,O_2} (torr)	13·4 ± 1·5	42·9	31·0	7·0	31·9 *b*
C_{a,O_2} (vol. %)	4·60 ± 0·50	(4·74)	4·90	(7·50)	8·20 *c*
C_{v,O_2} (vol. %)	3·34 ± 0·51	(3·86)	3·10	(3·10)	(3·0–5·0) *a, c*
$C_{a,O_2} - C_{v,O_2}$ (vol. %)	1·26 ± 0·16	(0·88)	1·80	(4·40)	(3·0–4·0) *b, c*
S_{a,O_2} (%)	90·3 ± 3·9	88·6	—	85·0	95–100 *c, d*
S_{v,O_2} (%)	65·7 ± 5·6	72·1	—	40·0	50–75 *a, c, d*
$\dot{V}_b$ (ml.kg^{-1}.min^{-1})	39·2 ± 3·5	45·1	23·1	16·0	18·3 *b*
f_H (no. min^{-1})	35·1 ± 2·2	—	35·0	—	62·9 *b*
$V_{s,H}$ (ml.kg^{-1}.stroke^{-1})	1·15 ± 0·12	—	0·68	—	0·28 *b*
BP_a (cm H_2O)	25·0 ± 1·4	—	36·7	—	35·3 *e*
BP_v (cm H_2O)	2·94 ± 0·16	—	4·08	—	5·40 *e*
pH_a	7·872 ± 0·036	7·685	7·860	8·180	7·840 *c*
pH_v	7·869 ± 0·035	7·544	7·890	8·080	7·820 *c*
In vivo P_{50} (torr)	8·6 ± 0·7	—	—	≈ 7·0	(≈ 28·0) *a*
$C^{max}_{HbO_2}$/Ht (vol % . %$^{-1}$)	0·346 ± 0·006	—	—	(0·318)	0·311 *d*
$C^{max}_{O_2,diss}$ (vol %)	0·718 ± 0·029	—	—	—	0·700 *d*

* *a*, Cameron (1971); *b*, Cameron & Davis (1970); *c*, Eddy (1976); *d*, Holeton & Randall (1967); *e*, Wood (1974).

NOTE. Where data from other studies are quoted in parentheses, the value was calculated from other data reported in the study.

recovery. Most values had stabilized by 6–24 h, but $\dot{V}_w$, P_{E,O_2}, $\dot{V}_{O_2}$, P_{a,O_2} and P_{v,O_2} continued to fall for at least 48 h. Consistently stable levels for these parameters could only be recorded after 3 days, so a recovery period of at least 72 h was routinely employed for all resting state measurements. Furthermore, stable values could not be recorded from flounder denied access to sand. Fish held in bare tanks exhibited persistent abortive burying movements, with variable and generally high levels of $\dot{V}_w$, P_{E,O_2}, $\dot{V}_{O_2}$, P_{a,O_2} and P_{v,O_2}.

Resting state values for ventilatory, cardiovascular and O_2 transport parameters in the eight fish of the first experimental series are summarized in Table 1. In each animal, 3–17 separate sets of measurements were taken, and calculated parameters (e.g. $\dot{V}_{O_2}$, C_{a,O_2}–C_{v,O_2}) are based on these individual sets rather than on average values for each flounder. The data of Watters & Smith (1973) on the same species, and of other authors on the winter flounder, the tench, and the rainbow trout are included for comparison in Table 1.

There are a number of differences between the present results and those of Watters & Smith (1973) (Table 1). In particular, note the much lower values of P_{a,O_2} and P_{v,O_2} and higher values of pH_a and pH_v in the present study. These differences cannot be attributed to the mask used in the present study, for virtually identical values of pH_a and pH_v (see Table 2), P_{a,O_2} (range: 26–45 torr; $n = 2$) and P_{v,O_2} (8–23 torr; $n = 3$) were obtained in the second experimental series where the fish lacked masks. $\dot{V}_w$ was also somewhat lower, and the $P_{I,O_2}-P_{E,O_2}$ difference somewhat higher in the present work. In preliminary experiments on 3 fish, $P_{i,O_2}-P_{e,O_2}$ differences were also measured in completely unoperated animals via mobile cannulae positioned close to the mouth and the upper opercular vent. The values (P_{I,O_2} = 136–141 torr; P_{E,O_2} = 38–47 torr) were identical to those in fish with masks (Table 1), indicating that the mask did not constrain ventilation.

The general agreement in S_{a,O_2} and S_{v,O_2} between the two studies is fortuitous, for Watters and Smith based their calculations of S_{a,O_2} and S_{v,O_2} on their measured values of P_{a,O_2} and P_{v,O_2} and *in vitro* blood O_2 dissociation curves. The differences in P_{a,O_2} and P_{v,O_2} were offset by differences in the O_2 dissociation curves (see below). The agreement in C_{a,O_2}, C_{v,O_2}, and $C_{a,O_2}-C_{v,O_2}$ has the same basis; the values attributed to Watters and Smith in Table 1 were calculated by us on the basis of their mean reported $C^{max}_{HbO_2}$, S_{a,O_2}, and S_{v,O_2} figures.

The value of $C^{max}_{O_2,diss}$ measured in air-equilibrated plasma (Table 1) was identical to the intercept of an experimentally determined regression of total O_2 capacity on Ht (Wood *et al.* 1979), indicating that plasma physical solubility provides a good estimate of whole blood physical solubility for O_2. O_2 dissolved in the blood is not unimportant in the flounder. Physical solution accounts for about 12·5% of the O_2 content of air equilibrated blood (e.g. Fig. 1) and about 7·9% of O_2 delivery to the tissues, based on the mean values in Table 1.

In vivo blood O_2 dissociation curves were determined under resting conditions for each of the eight fish in the first experimental series via simultaneous determinations of blood P_{O_2}'s and C_{O_2}'s. A typical example is shown in Fig. 1. To obtain points at very low (i.e. < 10 torr) and very high (> 50 torr) P_{O_2}'s, the shielding was removed from the tank. This 'stress' usually caused an initial hypoventilation or apnoea (accompanied by low blood P_{O_2}'s) followed by a hyperventilation (with high blood P_{O_2}'s). The total blood pH variation accompanying these changes was small ($\leqslant$ 0·045). This 'stress' was applied only at the end of the day when all other resting state data had been recorded.

The mean curve of all points for all fish is plotted as S_{O_2} versus P_{O_2} in Fig. 2. The relationship was typically hyperbolic, with maximum variability occurring at the knee of the curve. *In vivo* P_{50}'s (haemoglobin-bound O_2 only) ranged from 5·0 to 11·5 torr in individual fish, and averaged 8·6 ± 0·7 torr (Table 1). Over the pH range recorded (7·765–8·021) there was no significant interaction between pH and P_{50}. However, the present relationships were very different from those determined *in vitro* by Watters & Smith (1973), who reported P_{50}'s of 15–20 torr at a comparable temperature. This difference may be attributed to the very low pH's (7·45–7·67) used by Watters and Smith in their equilibrations which were chosen to agree with their *in vivo* pH measurements (Table 1).

Measurements relating to acid-base regulation and CO_2 transport in resting fish

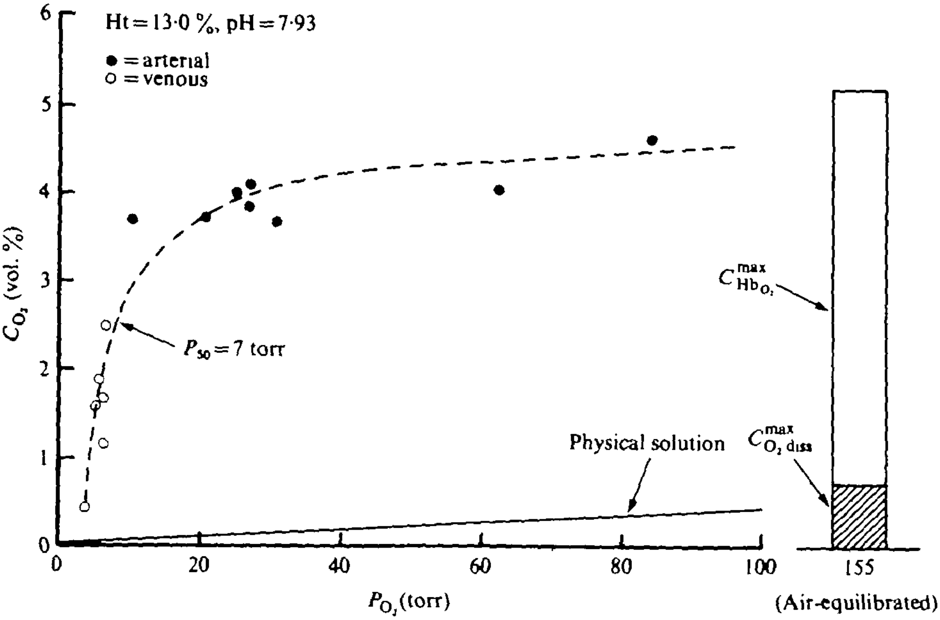

Fig. 1. A typical *in vivo* blood O_2 dissociation curve in a resting starry flounder (weight = 441 g) at 10 °C. The bar represents the O_2 content of air-equilibrated blood from the same animal, divided into haemoglobin-bound ($C^{max}_{HbO_2}$) and physically dissolved ($C^{max}_{O_2\,diss}$) components. The dissociation curve of physical solution is also shown.

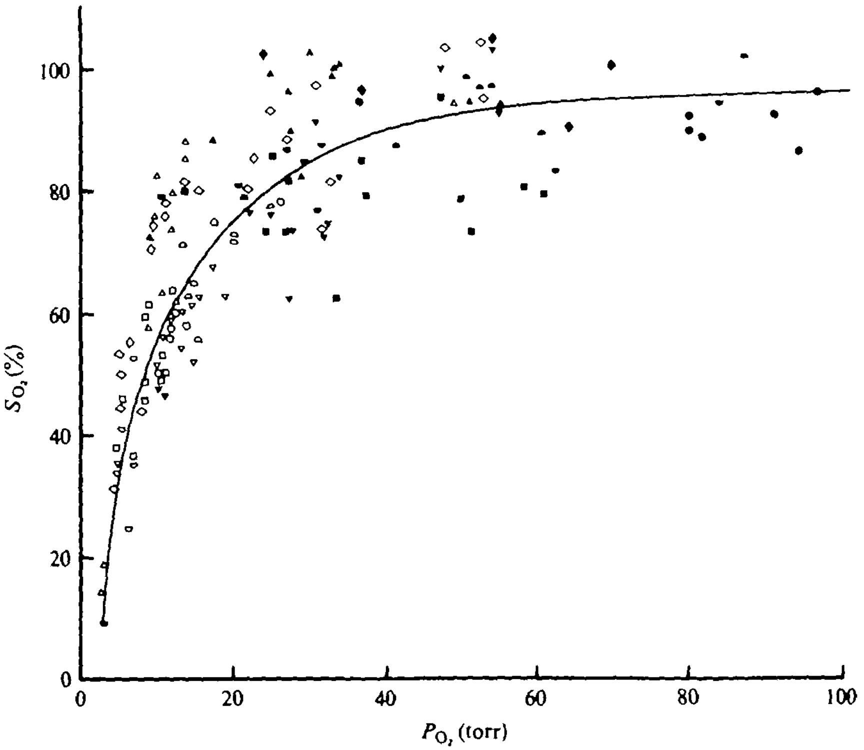

Fig. 2. The mean *in vivo* blood O_2 dissociation curve in the resting starry flounder constructed from all simultaneous determinations of blood C_{O_2} and P_{O_2} in the 8 fish of the first experimental series. C_{O_2} has been converted to S_{O_2} in order to express all results on a common basis. A different symbol is used for each fish. Closed symbols = arterial, open symbols = venous. The line has been fitted by eye.

Table 2. *Parameters of CO_2 transport and acid-base regulation in the resting flounder of the second experimental series (means* ± S.E.; *N): a comparison with other studies*

	Platichthys stellatus (present study)	*Tinca tinca* (Eddy, 1973, 1974)	*Salmo gairdneri* (Eddy, 1976)
Weight (g)	445·5 ± 35·4 (15)	150–400	300–600
Temp. (°C)	8·0–10·0	11·0–14·0	15·0
Ht (%)	15·6 ± 1·1 (15)	24·1	—
pH_a	7·896 ± 0·020 (6)	8·180	7·840
pH_v	7·899 ± 0·011 (14)	8·080	7·820
P_{a,CO_2} (torr)	2·49 ± 0·16 (6)	3·30	3·20
P_{v,CO_2} (torr)	3·02 ± 0·13 (10)	5·00	4·20
C_{a,CO_2} (mM.l^{-1})	6·78 ± 0·44 (5)	—	9·39
C_{v,CO_2} (mM.l^{-1})	7·15 ± 0·27 (10)	—	10·62
$C_{a,CO_2} - C_{v,CO_2}$ (vol. %)	1·40 ± 0·39 (5)	—	—
Blood lactate (mM.l^{-1})	0·26 ± 0·03* (12)	1·56†	1·14†, ‡

Note the difference in units between C_{a,CO_2} and C_{a,CO_2}–C_{v,CO_2}.

* Venous. † Arterial. ‡ Wood (unpublished results).

of the second experimental series are summarized in Table 2. No comparable data for flounder exist in the literature, but values for the tench and the trout have been included for comparison. P_{I,CO_2} was less than 1 torr and could not be measured. Blood pH's and P_{CO_2}'s in flounder appeared conventional, while lactate concentrations were extremely low. C_{CO_2} levels (largely reflective of HCO_3^- concentrations) were also fairly low so bicarbonate as well as non-bicarbonate buffering capacity (Wood *et al.* 1977) is small.

Only a few simultaneous C_{a,CO_2}–C_{v,CO_2} measurements were obtained (Table 2), but the mean value was comparable to that for C_{a,O_2}–C_{v,O_2} (Table 1), indicating an R value close to 1·0 at the gills.

DISCUSSION

The assumption has been made that the blood sampling sites used in the present study provide 'mixed' arterial and venous samples representative of blood entering and leaving the gills. There is little doubt that this is correct for arterial samples, but the equivalence between caudal vein blood (taken at the level of the kidney) and true pre-branchial venous blood is unsure. The assumption is safe in trout (Wood, unpublished results) and other fish (see Watters & Smith, 1973), but it has not been validated in flounders. We tried a variety of procedure to chronically sample ventral aortic blood, but were unsuccessful due to the fragile nature and inaccessibility of this vessel in the flounder. Limited success was achieved with needle-tipped cannulae inserted through the body wall directly into the ventricle. However, both P_{a,O_2} and P_{v,O_2} values from these fish were far higher than from fish cannulated in the conventional manner, while both pH_a and pH_v values were far lower, indicating considerable trauma associated with the procedure. We therefore elected to use caudal vein samples. Any systematic difference between caudal vein and true 'mixed' venous blood will therefore be incorporated into our calculations and comparisons. However, *a priori*, we can see no reason to suspect such differences.

Differences between the present results and those of Watters & Smith (1973), wh used identical cannulation techniques, can probably be attributed to two factors: (i) Watters and Smith employed a recovery period of 24 h, while we used a 72 h period; (ii) in the present study, the flounder were allowed to bury in sand, while Watters and Smith employed a bare tank. After only 24 h recovery in animals in sand, we observed values of P_{a,O_2} (63–104 torr; $n = 8$), P_{v,O_2} (28–51 torr; $n = 4$), $\dot{V}_w$ (108–207 ml.kg^{-1}.min^{-1}; $n = 6$) and P_{E,O_2} (42–93 torr; $n = 6$) very similar to those of Watters and Smith. We also observed similar values after 72 h recovery in animals denied access to sand. Blood pH's similar to those of Watters and Smith were observed 1–2 h after exhausting activity (Wood *et al.* 1977). Therefore we feel that their data can be taken as representative of flounder in a moderate state of activity, rather than of resting fish.

There is fair agreement between the present results and those of Cech *et al.* (1976, 1977) on the winter flounder (Table 1), but again P_{a,O_2} and P_{v,O_2} were much higher in the latter study. Cech *et al.* used recovery and tank conditions similar to those of Watters and Smith, so the difference could be due to the difference in method. The blood P_{O_2} data of Eddy (1973, 1974) on the benthic freshwater tench are very similar to the present results, but differences reflective of the much higher Ht (and therefore $C^{\max}_{\mathrm{HbO}_2}$) of tench blood are apparent (i.e. greater C_{a,O_2}, C_{v,O_2} and C_{a,O_2}–C_{v,O_2}, and lower $\dot{V}_b$ – Table 1).

A source of error in the present study, and perhaps in all studies involving anaesthesia and cannulation, was a reduction in Ht below normal levels. The mean Ht of uncannulated starry flounder was 19·9% (Wood *et al.* 1979) compared to 14·5–15·6% (Tables 1, 2) in the experimental animals. This Ht loss has also been documented by Watters & Smith (1973) in the same species, by Wood & Randall (1971) in the southern flounder, *Paralichthys lethostigma*, and by Houston, DeWilde & Madden (1969) in the brook trout, *Salvelinus fontinalis*. However, our studies on experimentally induced anaemia in *Platichthys stellatus* indicate that the only parameters likely to have been affected by this slight anaemia were C_{a,O_2} and C_{v,O_2} (both slightly depressed); C_{a,C_2}–C_{v,O_2} was probably unchanged (Wood *et al.* 1979).

The advantages of *in vivo* blood O_2 dissociation curves (as recorded in the present study) over *in vitro* relationships have been pointed out by Eddy (1974). In particular, blood buffering capacity is greater *in vitro* than *in vivo*; thus under similar P_{O_2}'s and P_{CO_2}'s, blood *in vivo* will be less saturated. Eddy (1974) recorded an *in vitro* $P_{50} = 3$ torr and an *in vivo* $P_{50} = 7$ torr under similar P_{CO_2}'s. The latter is very similar to the *in vivo* P_{50} of the starry flounder (8·6 torr; Table 1). These high blood O_2 affinities in benthic teleosts may be compared with an *in vitro* $P_{50} = 18{\cdot}5$ torr and an *in vivo* $P_{50} \approx 28$ torr (estimated) under comparable conditions ($P_{\mathrm{CO}_2} =$ 3–6 torr, 10 °C) in the rainbow trout (Cameron, 1971).

To compare the gas exchange strategies of the starry flounder with those of other benthic teleosts and the rainbow trout, a number of expressions commonly employed in respiratory analysis have been calculated (Table 3). For reference, values have also been included from Watters & Smith (1973); note the major differences for capacity–rate ratio, ΔP_{O_2}, T_{O_2}, and convection requirement for water. The comparisons below for the starry flounder refer only to the results of the present study. Whenever possible, the data of Cameron & Davis (1970) for *Salmo gairdneri* have been quoted, for their work appears to be the most careful investigation to date in resting trout.

Table 3. *Some derived expressions of* O_2 *exchange and transport in the resting starry flounder of the first experimental series (means* ± 1 S.E., $N = 8$). *A comparison with other studies*

		Platichthys stellatus (present study)	*Platichthys stellatus* (Watters & Smith, 1973)	*Pseudo-pleuronectes americanus* (Cech *et al* 1976, 1977)	*Tinca tinca* (Eddy, 1973, 1974)	*Salmo gairdneri* (*a–d*)*
% Utilization – water	$U_{w,O_2} = \frac{P_{I,O_2} - P_{E,O_2}}{P_{I,O_2}} . 100\%$	67·8 ± 2·7	(57·1)	—	54·9	46·0 *a*
% Utilization – blood	$U_{b,O_2} = \frac{C_{a,O_2} - C_{v,O_2}}{C_{a,O_2}} . 100\%$	30·3 ± 4·7	(18·6)	(36·7)	(58·7)	(30–40) *b*, *c*
% Effectiveness – water	$E_{w,O_2} = \frac{P_{I,O_2} - P_{E,O_2}}{P_{I,O_2} - P_{v,O_2}} . 100\%$	75·8 ± 2·8	78·4	—	(57·6)	(30–58) *a*, *d*
% Effectiveness – blood	$E_{b,O_2} = \frac{C_{a,O_2} - C_{v,O_2}}{C_{I,eq,O_2} - C_{v,O_2}} . 100\%$	56·8 ± 6·2	60·6	—	(81·5)	(90–100) *b*, *d*
Ventilation-perfusion ratio	$\dot{V}_w / \dot{V}_b$	2·94 ± 0·30	4·07	—	(8·25)	10·45 *a*
Capacity-rate ratio	$\frac{P_{a,O_2} - P_{v,O_2}}{P_{I,O_2} - P_{E,O_2}}$	0·229 ± 0·022	(0·452)	—	(0·362)	1·190 *a*
Mean P_{O_2} gradient (torr)	$\Delta P_{O_2} = \frac{P_{I,O_2} + P_{E,O_2}}{2} - \frac{P_{a,O_2} + P_{v,O_2}}{2}$	67·0 ± 4·2 *e*	31·0	—	80·0	(40·7) *a*
Transfer factor (ml $O_2 . kg^{-1} . min^{-1} . torr^{-1}$)	$T_{O_2} = \frac{V_{O_2}}{\Delta P_{O_2}}$	0·0069 ± 0·005 *e*	0·0288	—	0·0061	(0·0159) *a*
Convection requirement for water (l. mmole O_2^{-1})	$\frac{\dot{V}_w}{\dot{M}_{O_2}}$	5·34 ± 0·16	(8·11)	—	(5·91)	(5·95) *a*
Convection requirement for blood (l. mmole O_2^{-1})	$\frac{\dot{V}_b}{\dot{M}_{O_2}}$	1·96 ± 0·19	(2·59)	(1·33)	(0·72)	(0·64) *a*

NOTE. Where data from other studies are quoted in parentheses, the value was caluclated from other data reported in the study.

* *a* = Cameron & Davis (1970), *b* = Eddy (1976), *c* = Holeton & Randall (1967), *d* = Randall *et al.* (1967), *e* = ΔP_{O_2} is 59·1 ± 3·1 torr and T_{O_2} is 0·0079 ± 0·0007 ml $O_2 . kg^{-1} . min^{-1} . torr^{-1}$ by the logarithmic formulae of Hughes (1972) and Scheid & Piiper (1976).

Kiceniuk & Jones (1977) have recently reported generally similar data for the rainbow trout, but their results were obtained under very different conditions.

The percentage utilization of O_2 from the ventilatory water flow (U_{w,O_2}) is somewhat higher in the starry flounder and tench than in the trout (Table 3). However, because of the higher P_{I,O_2} in the trout study, the actual differences in $P_{I,O_2}-P_{E,O_2}$ are much smaller than those in U_{w,O_2} (Tables 1, 3). Resting $\dot{V}_{O_2}$ is higher in the trout, but this is largely due to the higher $\dot{V}_w$ (Table 1). Consequently, the convection requirements for water ($\dot{V}_w/\dot{M}_{O_2}$; Dejours, 1972) are very similar in the three species (Table 3). What differs amongst the species is not the actual removal of O_2 from the water, but the way in which it is removed.

Relative to the trout, the flounder pumps a lot more blood through the gills (i.e. $\dot{V}_b$) but takes up a much smaller amount of O_2 per unit blood flow (i.e. $C_{a,O_2}-C_{v,O_2}$) (Table 1). This difference is clearly illustrated by the much larger convection requirement for blood ($\dot{V}_b/\dot{M}_{O_2}$; Dejours, 1972) (Table 3). The phenomenon is due both to the lower $C^{max}_{HbO_2}$ and to slightly lower values of S_{a,O_2} and slightly higher values of S_{v,O_2} (Table 1). In other words, in the face of a low $C^{max}_{HbO_2}$, the flounder elects to maintain a high S_{v,O_2} at the cost of a high resting $\dot{V}_b$. $C_{a,O_2}-C_{v,O_2}$ is lower by the same approximate proportion as C_{a,O_2}, so the percentage utilization of O_2 from the blood by the tissues (U_{b,O_2}) is similar in the two species (Table 3). The high $\dot{V}_b$ of the flounder is accompanied by a much lower f_H, so $V_{s,H}$ is about 4-fold that in the trout (Table 1). Blood pressures are lower in the flounder (Table 1) so an extremely low peripheral vascular resistance must facilitate this high $\dot{V}_b$ by minimizing heart work. This strategy means that the ventilation–perfusion ratio ($\dot{V}_w/\dot{V}_b$) of the flounder is less than one-third that of the trout (Table 3). The much higher percentage effectiveness of O_2 removal from the water (E_{w,O_2}) and much lower percentage effectiveness of O_2 uptake by the blood (E_{b,O_2}) (Randall, Holeton & Stevens, 1967) in the flounder than in the trout results from this difference in $\dot{V}_w/\dot{V}_b$ ratio (Table 3). In terms of the above points, the tench would appear more similar to the trout than to the flounder (Tables 1, 3).

These differences can probably be related to the normal activity levels of these animals. The starry flounder is generally inactive and can be exhausted by brief enforced swimming (Wood *et al.* 1977). The trout, however, is capable of considerable sustained activity (Bainbridge, 1962), as should be the tench if it behaves like the related carp (Johnston & Goldspink, 1973) and goldfish (Smit *et al.* 1971). Cameron & Davis (1970) argue that the efficiency of the teleost heart increases with $\dot{V}_b$. It appears that the sluggish flounder has elected to 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 transport capacity during infrequent exercise. The trout and tench, on the other hand, operate less efficiently at rest with a low $\dot{V}_b$ and high $C_{a,O_2}-C_{v,O_2}$, but can call upon a considerable scope for increase in O_2 transport during frequent exercise by raising $\dot{V}_b$ to more efficient levels (e.g. Stevens & Randall, 1967). The capacity for increasing $\dot{V}_b$ in the starry flounder is small (Wood *et al.* 1979); short periods of exercise are probably supported by anaerobic metabolism (Wood *et al.* 1977) and by depletion of the considerable venous O_2 reserve.

By virtue of a much higher blood O_2 affinity, the flounder at low P_{a,O_2} maintains

S_{a,O_2} at a level only slightly lower than that in the trout at a much higher P_{a,O_2} (Table 1). This means that the flounder can maintain a much higher mean P_{O_2} gradient across the branchial epithelium (ΔP_{O_2}) and a much lower transfer factor (T_{O_2}) (Table 3). In these respects the tench appears more similar to the flounder than to the trout (Tables 1, 3). These differences could reflect a thickening of the branchial epithelium and/or a reduction in gill area in benthic forms as a protection against the abrasive substrate which is continually ventilated. Unfortunately the branchial morphometric data of Hughes (1966, 1972) on flatfish and tench do not support this idea. An alternative explanation may be that the high blood O_2 affinity is simply adaptive to frequent environmental hypoxia. Under normoxic conditions, the low P_{50} allows the animal to maintain a low T_{O_2} yet still saturate the arterial blood, thereby keeping a low branchial permeability and reducing unfavourable ion and water fluxes (Wood & Randall, 1973). This consideration may be especially important in view of the euryhalinity of the starry flounder.

Finally, the capacity–rate ratio (Shelton, 1970) is extremely low in both the flounder and tench relative to the trout (Table 3). The similarity between the flounder and the tench here is deceptive, because in the former the ratio is low largely because of the high $\dot{V}_b$, while in the latter it is low largely because of the high solubility coefficient for O_2 in the blood over the normal P_{a,O_2}–P_{v,O_2} range (see Shelton, 1970). A capacity–rate ratio close to unity, as in the trout, is indicative of a gas exchanger which is maximally efficient in terms of O_2 exchange per unit total flow of blood and water. The marked deviation from unity in both the flounder and the tench for different reasons indicates that other influences may be of greater importance in setting the capacity–rate ratio.

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