J. exp. Biol. 143, 305–319 (1989)
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MAINTENANCE OF OXYGEN CONSUMPTION IN RESTING *SILURUS GLANIS* AT DIFFERENT LEVELS OF AMBIENT OXYGENATION

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Accepted 5 January 1989

Summary

The mechanisms of adaptation that allow the teleost *Silurus glanis* to maintain its resting oxygen consumption constant when the O_2 partial pressure (P_{O_2}) in the inspired water (PI_{O_2}) varied between 40 and 3 kPa were studied at 13 °C. Steady-state values of oxygen consumption, ventilatory and circulatory flow rates, P_{O_2} in the inspired and expired water, P_{O_2} and O_2 concentration in the arterial and venous blood, haematocrit and acid–base status in the arterial blood were determined after 1-day exposures at selected PI_{O_2} values. Whole-blood O_2-binding characteristics were also determined.

The key adaptation after 1 day of acclimation was maintenance of oxygen consumption by ventilatory adjustment with no change in blood flow rate or pH (no Bohr effect). At each PI_{O_2} value (i) the ventilatory adjustment was minimal as the O_2 extraction coefficient from water always remained around 80–90 % and (ii) Pa_{O_2} stayed constant at about 2 kPa. Data are compared with previous results in crayfish and other teleosts. It is concluded that the principle of a constant O_2 status in the *milieu intérieur* – independent of large changes in PI_{O_2} for a given state of activity – should be valid in many crustaceans and teleosts.

Introduction

In most water-breathers, oxygen consumption can be maintained constant in spite of large changes in ambient oxygenation. In the crayfish *Astacus leptodactylus* we reported the mechanisms which permit this maintenance at basal metabolism (Massabuau & Burtin, 1984). We showed that when this crustacean is exposed to different oxygenation levels, ventilation is adjusted so that the O_2 partial pressure (P_{O_2}) in the arterial blood (Pa_{O_2}) is maintained in a low and narrow range: it increased from $1 \pm 0{\cdot}2$ to $3{\cdot}5 \pm 0{\cdot}4$ kPa when the inspired P_{O_2} (PI_{O_2}) increased from 3 to 33 kPa. In absolute terms this Pa_{O_2} change appears rather small

Key words: fish, *Silurus*, respiration, regulation, oxygen consumption, acid–base balance, countercurrent.

compared to the $P_{I_{O_2}}$ increase. Concurrently, P_{O_2} in the expired water remained constant around 1 kPa. Other adaptations are a Bohr effect appearing below 10 kPa (Sakakibara *et al.* 1987) and an increase of blood flow rate below 5 kPa (Massabuau & Burtin, 1984). The relative constancy of P_{O_2} in the fluids leaving the gas exchanger is consistent with the existence of O_2 chemoreception in the branchial cavities (Massabuau & Burtin, 1984).

The aim of the present work was to learn whether this strategy for maintaining resting $\dot{M}_{O_2}$ constant – largely based on ventilatory control of Pa_{O_2} – is restricted to *A. leptodactylus* or part of a more general pattern in water-breathers. As noted by Shelton *et al.* (1986), there are data suggesting that in fishes with high O_2-affinity respiratory pigments, Pa_{O_2} 'may be little affected' by $P_{I_{O_2}}$ changes (see for example Eddy, 1974, fig. 2 and table 1; Itazawa & Takeda, 1978, table 1). There has been no comprehensive demonstration of this. We present data showing that this strategy exists in the wels (or sheat-fish), *Silurus glanis*. It is a nocturnal fish living in lakes and slow-flowing streams (Muus & Dahlstrom, 1978). During the daytime wels lie on the bottom in hollows or under stones and, like crayfish, rarely move, so that measurements can be made on animals that spontaneously remain at basal metabolism.

Materials and methods

Experiments were performed on 19 male and female wels, *Silurus glanis*, reared in captivity and acclimated in our laboratory for at least 2 months. Animals were fed with frozen fish and beef heart. During maintenance and experimental periods, the animals were supplied with water from the Strasbourg water table (see Table 1 for water ionic composition; $T = 13°C$; partial pressure of carbon dioxide, $P_{CO_2} \approx 0{\cdot}1$ kPa; pH ≈ 8·30–8·40; $P_{O_2} \approx 20$ kPa during the maintenance period, variable during experiments; O_2 capacity coefficient in the water, $\beta w_{O_2} = 15{\cdot}67\ \mu mol\ l^{-1}\ kPa^{-1}$). During experiments, acid–base balance in the water was controlled with a pH–CO_2-stat (Dejours *et al.* 1978). During experiments fishes were unfed. They were maintained under a natural rhythm of light conditions (dim light during the daytime) and could not see the experimenter. Five types of experiments were performed. All values are presented as mean

Table 1. *Ionic composition of the water used*

Ion	Concentration (mequiv l^{-1})	Ion	Concentration (mequiv l^{-1})
NH_4^+		$HCO_3^- + CO_3^{2-}$	4·44
Na^+	0·490	Cl^-	0·820
K^+	0·086	NO_2^-	0·002
Mg^{2+}	1·22	NO_3^-	0·058
Ca^{2+}	4·88	SO_4^{2-}	1·30
Sum of cations	6·676	Sum of anions	6·620

Strasbourg water table.

±1 standard error (S.E.). $P < 0{\cdot}05$ was taken as the fiducial limit of significance in paired *t*-tests.

O_2-binding curve of whole blood

These determinations were performed in winter on six animals weighing 685 ± 89 g. Blood was sampled by puncturing the caudal aorta or vein of anaesthetized fish (urethane 8 g l^{-1}). The heparinized blood was stored in a rotating system immersed in melting ice. Gas equilibration was performed at 13 °C in a bowl-shaped tonometer (Radiometer type) gently shaken for 30 min. Gas mixtures, $N_2/O_2/CO_2$, were obtained by using gas-mixing pumps (Wösthoff, Bochum). The O_2 concentration of equilibrated blood was measured with a modified Tucker chamber (Tucker, 1967) on 10 µl samples and pH was determined with a Radiometer 6299A capillary electrode at 13 °C.

Measurements of oxygen consumption ($\dot{M}_{O_2}$) and estimation of water flow ($\dot{V}w$)

These experiments were performed in February and March on five *Silurus* weighing 127 ± 20 g. $\dot{M}_{O_2}$ was measured in an open-flow respirometer, volume 1300 ml, using the technique described by Massabuau *et al.* (1984). These measurements, together with the defined $P_{I_{O_2}}$ and the measured $P_{E_{O_2}}$ (see below), permitted calculation of ventilation, $\dot{V}w$, using the Fick principle (Saunders, 1962). Because the existence and importance of possible cutaneous oxygen uptake was not taken into account, the actual value of $\dot{V}w$ may have been somewhat overestimated. No allometric correction of $\dot{M}_{O_2}$ was made as in the studied range there was no significant difference with the 1 kg standard-mass correction. Each animal was placed in the respirometer at least 24 h before measurements began. It was then exposed for periods of 90 min to 24 h to five levels of $P_{I_{O_2}}$. The order of presentation was 20, 40, 10, 5 and 2 kPa. Because results were independent of the exposure period, all data at each $P_{I_{O_2}}$ were computed together.

Measurements of P_{O_2} in the expired water, $P_{E_{O_2}}$

Seven animals weighing 809 ± 43 g were used for this experiment performed in February and March. To sample the expired water, a catheter was fixed on the upper part of the operculum, above the pectoral fin, where the water flows out after having ventilated the apex of the gill arches (Fig. 1). A hole (≈1·5 mm in diameter) was drilled through the cleithrum, 1–2 mm anterior to the thin sheet of tissue that comes into contact with the body and prevents water reflux. A polyethylene catheter (i.d. 0·38 mm, o.d. 1·09 mm, length 50–55 cm), with the inner end shaped into a collar of 5 mm diameter, was slipped into the hole from the internal face of the operculum. A second catheter (length ≈ 5 mm), with the outer end shaped into a 5–7 mm diameter collar, was slipped over the first from outside the operculum. They were tied together with a thin stainless-steel wire. The inside of the assembly projected less than 0·5–1 mm into the branchial chamber. The surgery took about 5 min and was performed on anaesthetized animals. Fish were then acclimated for 2–3 days in the experimental tank (43 cm × 35 cm × 16 cm,

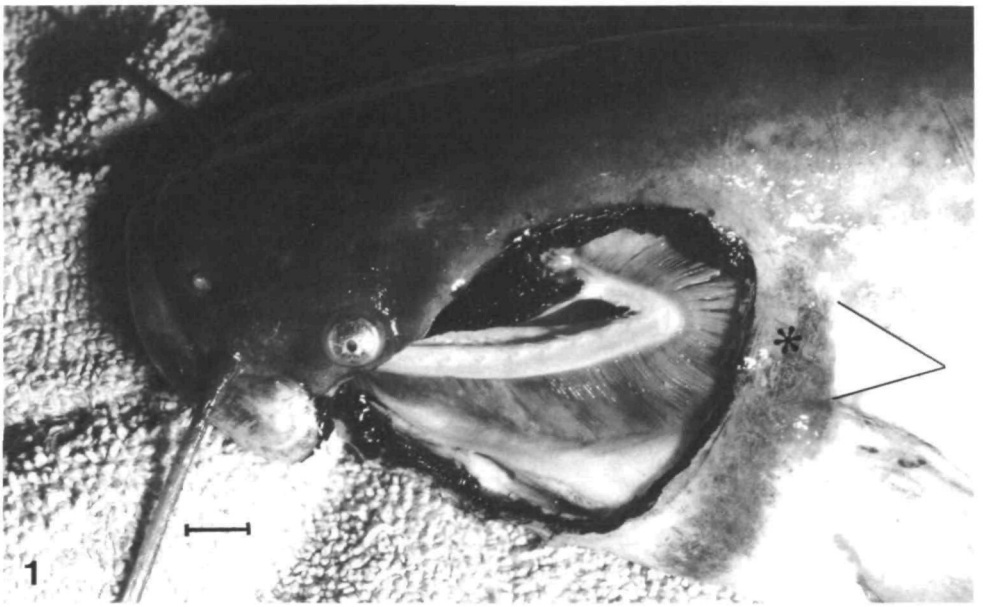

Fig. 1. Side view of the branchial cavity in a 1 kg *Silurus glanis* showing the shape of the gill arches (central part of the operculum removed). The white arrow shows the duct where the water is expired after having perfused the apex of the gill arches; the asterisk shows the site where expired water was sampled. Scale bar, 1 cm.

renewal rate $10\,l\,h^{-1}$) before measurements began. The catheter passed freely through the roof of the experimental box (in a 3 mm hole), and a small piece of Teflon prevented it falling into the tank. $P_{E_{O_2}}$ was continuously recorded during the daytime using a Radiometer polarographic electrode placed in series between the sampling catheter and a Gilson peristaltic pump (flow rate $0{\cdot}1\,ml\,min^{-1}$). At night, when the animal was active, the catheter was disconnected. The injection port of the P_{O_2} electrode was equipped with a T-tube operated by remote control so that it could be calibrated before and after every set of measurements without disturbing the fish. The order of $P_{I_{O_2}}$ presentation was as above and each plateau lasted about 24 h. Consequently an experiment with a single animal took about 1 week.

Measurements of acid–base balance and P_{O_2} in the arterial blood (Pa_{O_2})

This was performed in June and July on six *Silurus* weighing 775 ± 43 g. Animals were kept in the same apparatus as above and exposed to the same protocol of $P_{I_{O_2}}$ plateaus. Arterial blood was sampled following the technique described for crayfish by Massabuau & Burtin (1984). Its advantage is that it is a push–pull system which requires only a single catheter rather than the complete extracorporeal loop. A catheter was implanted in the caudal aorta 3–5 cm anterior to the caudal fin. It consisted of two parts: a silicone tube (i.d. 0·30 mm, o.d. 0·64 mm, length 2 cm) which was inserted in the aorta and a polyethylene catheter (i.d

0·38 mm, o.d. 1·09 mm, length 50–55 cm). If used every day it remained patent for 3–4 weeks before spontaneously falling out. After a 7- to 10-day recovery period Pa_{O_2} was measured once a day between 10.00 and 11.00 h. In brief, the system consisted of the arterial catheter, a thermostatted P_{O_2} electrode, a 2-m polyethylene tube acting as a blood reservoir and a Gilson peristaltic pump (blood flow rate 0·07 ml min^{-1}) placed in series. Pa_{O_2} was read exactly 6 min after the beginning of the sampling period. Before reinjection into the fish, 100 μl of blood was anaerobically sampled in capillary tubes for analysis of acid–base balance. This sample was immediately used to determine pHa (with a Radiometer 6299A capillary electrode thermostatted at 13 °C) and Ca_{CO_2}, the total CO_2 concentration (with a modified Cameron chamber; Cameron, 1971). From these values, arterial blood CO_2 partial pressure, Pa_{CO_2}, and bicarbonate concentration, $[HCO_3^-]_a$, were calculated using a CO_2 solubility of 0·396 mmol l^{-1} kPa^{-1}, $pK_1' = 6·21$ and $pK_2' = 9·68$ (J.-L. Rodeau & B. Burtin, unpublished data; throughout the text we use $[HCO_3^-]_a$ for $[HCO_3^-]_a + 2[CO_3^{2-}]_a$.

Measurement of O_2 concentration in mixed venous blood ($C\bar{v}_{O_2}$); estimation of blood flow rate ($\dot{V}b$) and venous P_{O_2} ($P\bar{v}_{O_2}$)

Five *Silurus* weighing 742 ± 49 g were examined in February and March. Surgery and experimental procedures were the same as those described above except that (i) mixed venous blood was sampled from the ventral aorta and (ii) C_{O_2} was measured instead of P_{O_2} because of the expected $P\bar{v}_{O_2}$ range and the shape of the O_2-binding curve (see Fig. 2). The animals were exposed to the same P_{O_2} plateaus as above, and 100 μl of venous blood was sampled once a day between 10.00 and 12.00 h. The O_2 concentration was immediately measured using a modification of Tucker's method (Tucker, 1967).

Blood flow rate was estimated by the Fick principle, using these $C\bar{v}_{O_2}$ values, Ca_{O_2} values obtained by graphical extrapolation on the O_2-binding curve recalculated for a haematocrit (Hct) of 14 % (see Results) and the $\dot{M}_{O_2}$ measurements. $P\bar{v}_{O_2}$ was estimated by graphical extrapolation of the same recalculated O_2-binding curve.

Results

Mean O_2-binding curves for whole blood in *Silurus* are presented in Fig. 2 at two P_{CO_2} and pH values. The curves are hyperbolic and P_{50} was 0·64 ± 0·03 kPa at pH = 7·96 ± 0·02 and P_{CO_2} = 0·2 kPa; it was 0·85 ± 0·04 kPa at pH = 7·69 ± 0·01 and P_{CO_2} = 0·7 kPa. There was a Bohr effect, $\Delta \log P_{50}/\Delta pH = -0·46 \pm 0·06$, but no visible Root effect at the studied pH. The haematocrit of the blood used for these determinations was 25·0 ± 1·6 %. In chronically cannulated fishes it was always lower, and decreased to 14·0 ± 0·8 % within 1 week. To take this into account – with the assumption that haemoglobin characteristics did not change – we recalculated O_2-binding curves for Hct = 14 % (dotted lines in Fig. 2). Following Roughton (1964), they are geometrically similar to the curves at

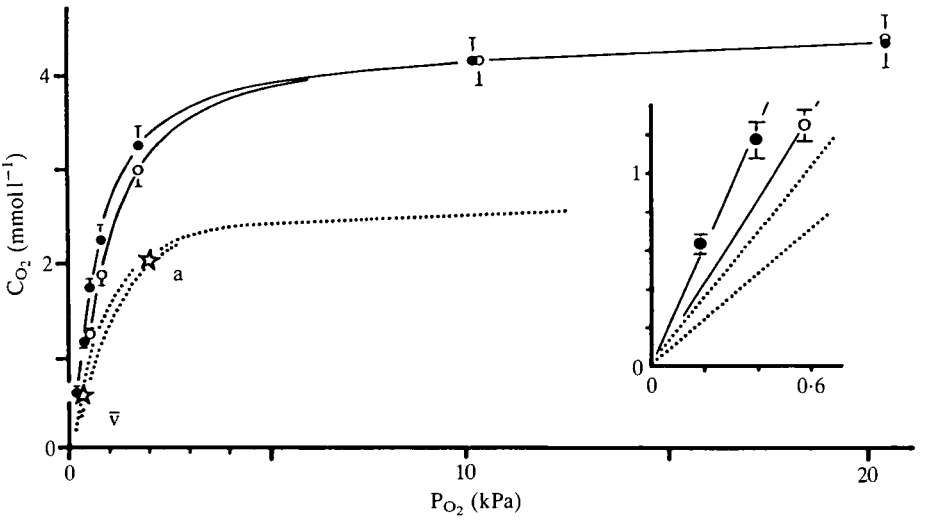

Fig. 2. Solid lines: *in vitro* O_2-binding curves at two experimental P_{CO_2} and pH values determined on blood sampled from anaesthetized fishes ($N = 6$; Hct = 25 %; T = 13 °C; (●), pH = 7·96 ± 0·02 and P_{CO_2} = 0·2 kPa; (○), pH = 7·69 ± 0·01 and P_{CO_2} = 0·7 kPa; means ± 1 S.E.). Dotted lines, recalculated curves for Hct = 14 %. Paired curves are geometrically similar; they have the same P_{50} (see text). O_2 solubility coefficient = 22 μmol l^{-1} kPa^{-1}. Inset: enlarged view of the origin area assuming the curves are hyperbolic. This graph was used to estimate $P\bar{v}_{O_2}$ from $C\bar{v}_{O_2}$ and Ca_{O_2} from Pa_{O_2}. a, arterial point and $\bar{v}$, venous point in Table 2.

Hct = 25 % (scale 14:25) and have the same P_{50}. Sixteen red cell counts in four animals at different times after manipulation showed that Hct was linearly related to cell count: number of red blood cell = 67 500Hct + 38 000.

Table 2 shows all the respiratory variables measured and calculated in *Silurus* exposed to selected and fixed $P_{I_{O_2}}$ levels. Betwen 40 and 3 kPa, $\dot{M}_{O_2}$ was maintained constant, whereas $\dot{V}w$ was greater the lower the $P_{I_{O_2}}$. We believe that 2 kPa is about the lower limit of the regulation, as in one animal in which $\dot{M}_{O_2}$ was measured at a lower $P_{I_{O_2}}$ value it decreased linearly below this value. Fig. 3 is a typical example of the $P_{E_{O_2}}$ changes recorded during the daytime as fishes were kept at fixed $P_{I_{O_2}}$ values. $P_{E_{O_2}}$ was typically low but interspersed with transient peaks. The frequency of $P_{E_{O_2}}$ peaks – which, based on visual observations, corresponded to periodic 'sighs' – was independent of $P_{I_{O_2}}$, but their amplitude was higher when $P_{I_{O_2}}$ increased. At $P_{I_{O_2}}$ = 3–3·5 kPa, $P_{E_{O_2}}$ was constant and at 38 kPa it could remain steady at about 2 kPa for more than 1 h. Resting values were never as low as zero at any $P_{I_{O_2}}$. Values of $P_{E_{O_2}}$ were sampled every 6 min in all animals. Depending on technical problems the recording period covered between 6 and 8 h, i.e. 60–80 values per animal. The frequency distribution of $P_{E_{O_2}}$ values is shown in Fig. 4. The modal value of $P_{E_{O_2}}$ was also determined for each animal at every $P_{I_{O_2}}$ value. The mean of these is presented in Fig. 5A, together with the results of the Pa_{O_2} measurements and $P\bar{v}_{O_2}$ estimates. At values of $P_{I_{O_2}}$ betwee

Table 2. *Respiratory variables in* Silurus glanis *exposed to various oxygenation levels and constant acid–base balance status in the water (T = 13°C)*

	$P_{I_{O_2}}$ (kPa)				
	3·1 ± 0·2	5·2 ± 0·6	10·8 ± 0·3	19·2 ± 0·7	39·0 ± 0·8
$\dot{M}_{O_2}B^{-1}$ (μmol kg^{-1} min^{-1})	15·3 ± 1·3	15·3 ± 1·3	15·4 ± 1·3	15·4 ± 1·5	14·7 ± 2·0
$\dot{V}wB^{-1}$ (ml kg^{-1} min^{-1})*	416	212	112	56	26
$\dot{V}w\beta wB^{-1}$ (μmol kPa^{-1} kg^{-1} min^{-1})*	6·52	3·32	1·75	0·88	0·40
$\dot{V}bB^{-1}$ (ml kg^{-1} min^{-1})*	8·5	9·0	9·3	9·0	9·2
$\dot{V}b\beta a,\bar{v}B^{-1}$ (μmol kPa^{-1} kg^{-1} min^{-1})*	7·74	7·92	8·18	7·92	8·09
$\dot{V}w\dot{V}b^{-1}$*	49·0	23·5	12·0	6·2	2·8
$\dot{V}w\beta w\dot{V}b^{-1}\beta a,\bar{v}^{-1}$*	0·84	0·42	0·21	0·11	0·05
$\dot{V}w\dot{M}_{O_2}^{-1}$ (ml μmol^{-1})*	27·2	13·8	7·3	3·6	1·8
$\dot{V}b\dot{M}_{O_2}^{-1}$ (ml μmol^{-1})*	0·5	0·6	0·6	0·6	0·6
$P_{E_{O_2}}$ (kPa)	0·8 ± 0·2	0·7 ± 0·4	2·2 ± 0·5	2·1 ± 0·4	3·9 ± 1·4
$\Delta P_{I,E_{O_2}}$ (kPa)*	2·6 ± 1·2	5·0 ± 0·3	8·1 ± 1·5	17·7 ± 0·5	34·3 ± 1·7
Ew_{O_2}*	0·75 ± 0·06	0·88 ± 0·03	0·78 ± 0·05	0·89 ± 0·02	0·90 ± 0·04
Pa_{O_2} (kPa)	1·9 ± 0·5	1·6 ± 0·2	1·9 ± 0·2	2·3 ± 0·3	2·0 ± 0·3
Ca_{O_2} (mmol l^{-1})*	2·0 ± 0·1	1·9 ± 0·1	2·0 ± 0·1	2·1 ± 0·1	2·0 ± 0·1
$P\bar{v}_{O_2}$ (kPa)*	0·3	0·3	0·4	0·6	0·7
$C\bar{v}_{O_2}$ (mmol l^{-1})	0·4 ± 0·1	0·4 ± 0·1	0·4 ± 0·1	0·7 ± 0·1	0·9 ± 0·2
$\Delta Pa,\bar{v}_{O_2}$ (kPa)*	1·7	1·4	1·6	1·9	1·6
$\Delta Ca,\bar{v}_{O_2}$ (mmol l^{-1})*	2·7	2·8	2·9	2·7	2·5
Eb_{O_2}*	0·87	0·87	0·88	0·79	0·73
Sb*	0·43	0·73	0·85	0·91	0·97
pHa	7·93 ± 0·03	7·93 ± 0·03	7·96 ± 0·02	7·96 ± 0·02	7·92 ± 0·02
Ca_{CO_2} (mmol l^{-1})	2·79 ± 0·11	3·21 ± 0·17	4·04 ± 0·34	6·26 ± 0·47	8·81 ± 0·72
Pa_{CO_2} (kPa)*	0·13 ± 0·01	0·15 ± 0·02	0·18 ± 0·02	0·27 ± 0·02	0·44 ± 0·02
Hct (%)	13 ± 1·9	14 ± 2·6	13 ± 0·8	17 ± 1·4	13 ± 1·3

Unstarred values were directly measured, starred values were calculated.

$N = 5$–7, see text.

$P_{I_{O_2}}$, O_2 partial pressure, P_{O_2}, in the inspired water; $\dot{M}_{O_2}B^{-1}$, oxygen consumption per unit of body mass; $\dot{V}wB^{-1}$, ventilatory flow rate per unit of body mass; $\dot{V}w\beta wB^{-1}$, ventilatory conductance per unit of body mass; $\dot{V}bB^{-1}$, circulatory (or perfusive) flow rate per unit of body mass; $\dot{V}b\beta a,\bar{v}B^{-1}$, perfusive conductance per unit of body mass; $\dot{V}w\dot{V}b^{-1}$, ventilation/perfusion ratio; $\dot{V}w\beta w\dot{V}b^{-1}\beta a,\bar{v}^{-1}$, ventilatory/perfusive conductance ratio; $\dot{V}w\dot{M}_{O_2}^{-1}$, specific ventilation; $\dot{V}b\dot{M}_{O_2}^{-1}$, specific circulatory flow rate; $P_{E_{O_2}}$, mode of P_{O_2} in the expired water; $\Delta P_{I,E_{O_2}}$, P_{O_2} difference between inspired and expired water; Ew_{O_2}, extraction coefficient of water O_2; Pa_{O_2}, P_{O_2} in the arterial blood; Ca_{O_2}, O_2 concentration in the arterial blood; $P\bar{v}_{O_2}$, P_{O_2} in the venous blood; $C\bar{v}_{O_2}$, O_2 concentration in the venous blood; $\Delta Pa,\bar{v}_{O_2}$, P_{O_2} difference between arterial and venous blood; $\Delta Ca,\bar{v}_{O_2}$, C_{O_2} difference between arterial and venous blood; Eb_{O_2}, extraction coefficient of blood O_2; Sb, blood shunt fraction; pHa, pH in the arterial blood; Ca_{CO_2}, CO_2 concentration in the arterial blood; Pa_{CO_2}, CO_2 partial pressure in the arterial blood; Hct, haematocrit measured on the arterial blood. All symbols taken from Dejours (1981) and Piiper & Scheid (1984).

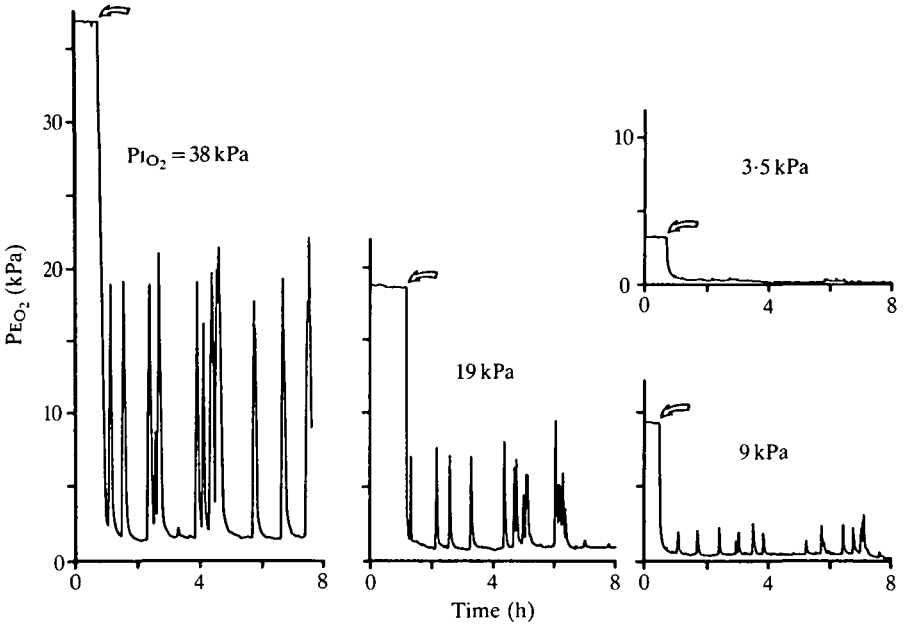

Fig. 3. Example of O_2 partial pressure changes in the expired water of *Silurus glanis* maintained at selected values of inspired P_{O_2} as a function of time. Switches of measurements from inspired to expired water are shown by arrows. The record is characterized by an alternation between a low steady $P_{E_{O_2}}$ value and peak transient values, except at $P_{I_{O_2}} = 3{\cdot}5$ kPa.

about 3 and 40 kPa, the modal value of $P_{E_{O_2}}$ increased from $0{\cdot}8 \pm 0{\cdot}2$ to $3{\cdot}9 \pm 1{\cdot}4$ kPa ($P < 0{\cdot}05$; paired *t*-test) whereas Pa_{O_2} and $P\bar{v}_{O_2}$ values did not change. The haematocrit remained constant and was independent of $P_{I_{O_2}}$. The corresponding changes in acid–base balance in the arterial blood are shown in Fig. 5B. Values of pHa were generally constant at varying levels of $[HCO_3^-]_a$ and Pa_{CO_2}. As a consequence of the maintenance of Pa_{O_2} and pHa (assuming no changes in haemoglobin characteristics), Ca_{O_2} remained constant at 2 mmol l^{-1}. On the O_2-binding curve this corresponds to 85 % saturation. As $C\bar{v}_{O_2}$ was also constant, the arteriovenous O_2 concentration difference ($\Delta Ca,\bar{v}_{O_2}$), $\dot{V}b$ and the O_2 capacity coefficient in the blood ($\beta a,\bar{v} = 880\ \mu$mol l^{-1} kPa^{-1}) did not vary.

Discussion

Comparison with previous data

The present study reports steady-state respiratory adaptations in the teleost *S. glanis* after 1-day acclimation periods at various levels of inspired P_{O_2}. Although many previous studies of water-breathers have been devoted to this subject, homeostatic mechanisms have received little attention (Dejours, 1988). Our aim was to learn whether the principles of breathing control we found in crayfish (see

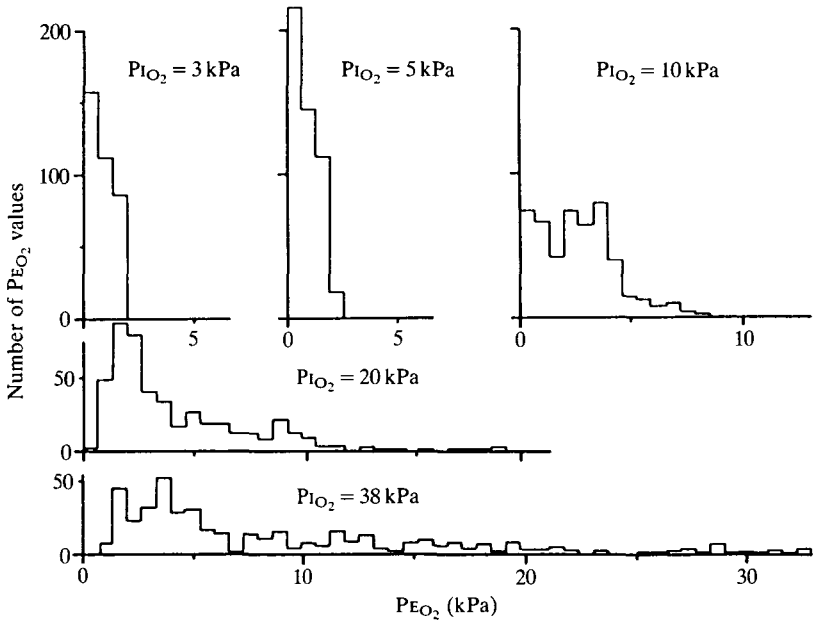

Fig. 4. Distribution of O_2 partial pressures values in the expired water, P_{EO_2}, of seven *Silurus glanis* exposed to selected values of inspired P_{O_2}, P_{IO_2}. The number of P_{EO_2} values counted in each P_{EO_2} class (on the abscissa) of 0·67 kPa are shown.

Introduction) could be extended to teleosts. We did not intend to study the acute phases of adaptation but rather the results of the adaptation. In humans and birds (Bouverot, 1985), as in crayfish (Massabuau & Burtin, 1984), it is generally agreed that the early respiratory changes result from the O_2 stimulation of peripheral chemoreceptors. In teleosts there are strong arguments in favour of the existence of such peripheral O_2 chemoreceptors located in, or close to, the branchial cavity (Eclancher, 1972, 1975; Eclancher & Dejours, 1975; Bamford, 1974; Milsom & Brill, 1986).

Our measurements of blood characteristics are comparable to those of Albers *et al.* (1981) in *Silurus glanis* and those of Haws & Goodnight (1962) in the related freshwater species *Ictalurus nebulosus* and *Ictalurus punctatus*. We found similar hyperbolic O_2-binding curve and P_{50} values. The oxygen capacity we report is comparable to those of *I. punctatus* and *I. nebulosus*, but $\Delta\log P_{50}/\Delta pH$ is lower than that given by Albers *et al.* (1981). Our haematocrit values (25·0 ± 1·6 %) and red blood cell count measured on the sample taken in the anaesthesized animals are comparable to the values reported by Albers *et al.* (1981) in the same experimental conditions. In the resting state they differ little from the 16 ± 2 % reported in chronically cannulated dogfish by Baumgarten-Schumann & Piiper (1968). It is likely that these differences were related to the stress of surgery and anaesthesia, as we observed negligible blood loss. Following severe exercise induced by chasing, fish can exhibit a Hct increase of 40 %, due mainly to

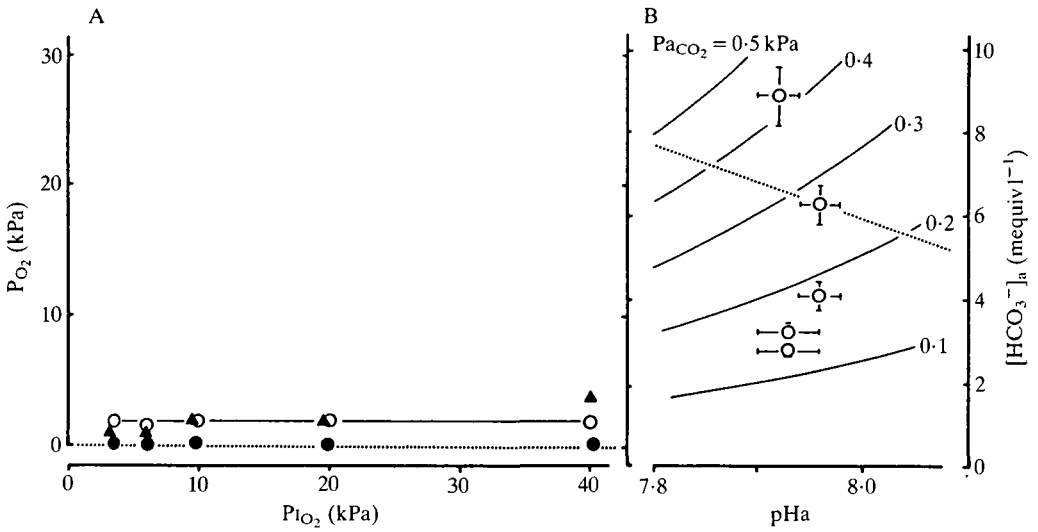

Fig. 5. (A) Steady-state values of O_2 partial pressure, P_{O_2}, in arterial (○) and mixed venous (●) blood and in expired water (▲) after, or during, 24 h at selected O_2 partial pressures in the inspired water, PI_{O_2}. All values remained in a very low and narrow range independent of PI_{O_2} ($N = 5$–7; see Table 2 for S.E.). (B) Steady-state values of arterial acid–base status in six *Silurus* after 24 h at various PI_{O_2} values (from top to bottom PI_{O_2} = 40, 20, 10, 6 and 3·5 kPa). The dotted line gives the slope of the buffer line determined *in vitro*. pH values are not statistically different (means ± 1 S.E.). See Table 2 for exact values.

contraction of the spleen and a shift of water out of the plasma (Yamamoto *et al.* 1980). The low values of Pa_{O_2} we report are in the range for other quiescent water-breathers, such as resting eels (Steen & Kruysse, 1964) and crayfish (Massabuau & Burtin, 1984), whereas much higher values have been observed in excited animals (Steen & Kruysse, 1964; Baumgarten-Schumann & Piiper, 1968). Notice that (i) Pa_{O_2} is adjusted to a value (≈2 kPa) very close to the minimum required to ensure intracellular O_2 supply in single-cell suspensions of rat hepatocytes (Jones & Kennedy, 1982) and (ii) $P\bar{v}_{O_2} \approx 0{\cdot}2$ kPa give a mean '*in vivo*' estimate of the intracellular P_{O_2} in *S. glanis* at basal metabolism. In *Silurus*, $P_{50} = 0{\cdot}6$ kPa, the values of Pa_{O_2} and $P\bar{v}_{O_2}$ we observed give the same Ca_{O_2} and $C\bar{v}_{O_2}$ values as those in 'normoxic' dogfish which have higher Pa_{O_2} and $P\bar{v}_{O_2}$ values but a P_{50} of 2·13 kPa ($T = 17$°C and $P_{CO_2} = 0{\cdot}2$ kPa; Baumgarten-Schumann & Piiper, 1968). The value of $\dot{M}_{O_2}$ reported here is similar to the value we reported in *A. leptodactylus* (13·8 ± 0·8 μmol kg^{-1} min^{-1}; Massabuau & Burtin, 1984) kept in identical water conditions. It is also similar to values obtained in eel (16·80 ± 0·79 μmol kg^{-1} min^{-1} at 11·5°C by Kirsch & Nonnotte, 1977), tench (20·2 ± 1·16 μmol kg^{-1} min^{-1} at 13°C by Nonnotte, 1981) and dogfish (20·7 ± 1·8 μmol kg^{-1} min^{-1} at 15°C) by Butler & Taylor, 1975; 28·4 ± 7·6 μmol kg^{-1} min^{-1} at 15–17°C by Baumgarten-Schumann & Piiper, 1968). All experiments were performed in winter, except for the arterial acid–base balance and P_{O_2} measurements which were performed i

summer. This raised the problem of comparing respiratory parameters measured at different times of the year and at potentially different metabolic levels. In resting carp there is no significant variation in Pa_{O_2} between 24·5 °C (3·3 ± 1 kPa in Itazawa & Takeda, 1978) and 10 °C (3·8 ± 2·1 kPa or 1 ± 0·6 kPa in Garey, 1967). Consequently it is unlikely that a potential increase of resting metabolism in summer interfered with our Pa_{O_2} measurements.

Mechanism of $\dot{M}_{O_2}$ *maintenance in resting* Silurus

The key point in the respiratory adaptation of *Silurus* is that when PI_{O_2} varies between 40 and 3 kPa, steady-state $\dot{M}_{O_2}$ after 1 day of acclimation appears to be maintained exclusively by ventilatory adjustment with no change of blood flow rate or pH (no Bohr effect). This corresponds to an adaptation based on a principle of economy, because $\dot{V}w$ – even though it increases 16-fold between $PI_{O_2} = 40$ and $PI_{O_2} = 3$ kPa – remains close to its minimum possible value at each PI_{O_2} value. Indeed, the O_2 extraction coefficient is always around 80–90 %. As a result of this $\dot{V}w$ adaptation, the value of Pa_{O_2} remains constant at about 2 kPa. It is likely that Pa_{O_2} must be the controlled variable, by analogy with what is known from higher vertebrates (Bouverot, 1985). The capacity to function at such low Pa_{O_2} values must be related to the very high haemoglobin O_2-affinity in *Silurus* (Fig. 2). The effect of the high O_2-affinity on extraction and ventilation in fishes has recently been discussed by Malte & Weber (1987). During inactive periods, *Silurus* rests in an environment that can be hypoxic, and the problem of O_2 uptake from the medium is obviously a priority. The details of this mechanism should be different in fishes with lower blood O_2-affinity, which presumably facilitates O_2 release at the cellular level (Krogh & Leitch, 1919), both in more active fishes (like trout) and in nonactive fishes (like dogfish) living in nonhypoxic environments where there is no problem of O_2 uptake. However, the principle of an oxygenation status that is independent of PI_{O_2} over a wide range must remain valid in steady states, either at rest or at a given level of activity. This latter point is illustrated by data from Garey & Rahn (1970), who measured P_{O_2} in gas pockets of *Salmo gairdneri* swimming freely in a fishery (Fig. 6A). The fishery was supplied by a river with a high photosynthetic rate. In these conditions, although PI_{O_2} varied between 30 and 6 kPa and temperature between 8 and 17 °C, P_{O_2} in the gas pockets (which is a closed estimate of P_{O_2} in the surrounding tissues and the venous blood draining them, Rahn, 1957; Piiper, 1965) was independent of PI_{O_2}. Trout can live perfectly well in poorly oxygenated waters. In eastern France we found a population of *Salmo trutta fario* living in the spring of a river in which the year-round PI_{O_2} is about 6–7 kPa at 10·0 ± 0·2 °C (Massabuau & Fritz, 1984). Ott *et al.* (1980) reported that *Salmo gairdneri* can maintain its resting $\dot{M}_{O_2}$ constant down to 2–3 kPa, independently of the temperature between 10 and 20 °C. Fig. 6B shows data redrawn from Lomholt & Johansen (1979) which corroborate our results on PE_{O_2}. These authors measured oxygen extraction coefficients in carp exposed to hypoxia. We recalculated the original PE_{O_2} values from their results. It is clear that they remain in a narrow range, although the mean tends to increase slightly with

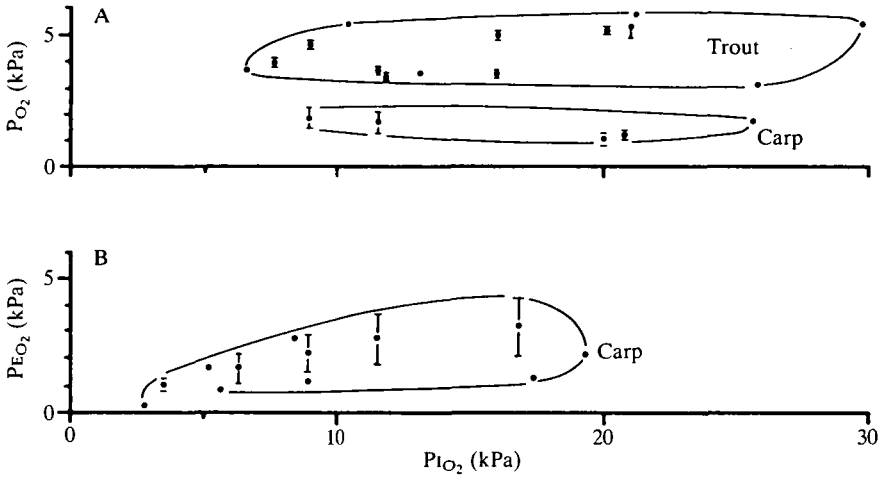

Fig. 6. (A) Changes of O_2 partial pressure, P_{O_2}, in gas pockets of trout and carp freely swimming in a fishery where the inspired P_{O_2}, $P_{I_{O_2}}$, varied spontaneously from about 30 to 6 kPa during daytime (redrawn from Garey & Rahn, 1970). The gas pocket P_{O_2} was independent of $P_{I_{O_2}}$. (B) Changes of O_2 partial pressure in the expired water, $P_{E_{O_2}}$, of carp exposed to various $P_{I_{O_2}}$ values (redrawn from Lomholt & Johansen, 1979; see text). Means ± 1 S.E.

$P_{I_{O_2}}$. This is because of the use of an arithmetic mean for a probably non-normal distribution (see Fig. 4 in present paper and fig. 6 in Massabuau & Burtin, 1984). These results are consistent with the constancy of P_{O_2} in carp gas pockets (Fig. 6A; Garey & Rahn, 1970) and data from Garey (1967), Eddy (1974) and Itazawa & Takeda (1978), who showed that changes of $P_{I_{O_2}}$ between 3–3·5 and 20 kPa did not alter the lowest measured Pa_{O_2} values in carp and tench.

In the teleost gill, the countercurrent model is generally accepted to describe the functioning of the gas exchanger (Hughes, 1984). In a system of this type, complete equilibration between inspired water and arterial blood and between expired water and venous blood is theoretically possible. In *Silurus* at rest, at least at the highest $P_{I_{O_2}}$, our present results show that equilibration between $P_{I_{O_2}}$ and Pa_{O_2} is far from complete, whereas $P_{E_{O_2}}$ is close to $P\bar{v}_{O_2}$ (Fig. 5A). Although the latter suggests that diffusion limitation must be very low, the former shows that gas exchange is ventilation-limited (Piiper & Scheid, 1984). The functional basis of this limitation can be attributed to a mismatch between ventilatory and perfusive conductance at all studied $P_{I_{O_2}}$ values (Table 2). This is in agreement with the general strategy of *Silurus* in hypoxia, which is based exclusively on the reduction of this ventilatory limitation. Some lamellae are likely not to be ventilated at rest but only perfused. This would lead to the equivalent of a 'mismatch blood shunt' (Piiper & Scheid, 1984). True shunt bypassing of the gills has not been described in teleosts (Dunel & Laurent, 1980). The magnitude of the shunt, Sb, can be estimated from the ratio $(P_I - Pa)/(P_I - P\bar{v})$, which is the amplitude of the non-equilibration divided by the P_{O_2} difference between inspired water and venou

blood. In normoxia Sb was 0·9 (Table 2). The decrease of Sb with hypoxia may correspond to an increase in the number of ventilated lamellae. These changes in ventilation–perfusion inhomogeneities affect gas exchange so that $P_{E_{O_2}}$ can be either lower or higher than $P_{a_{O_2}}$ or equal to it (see Piiper & Scheid, 1984, for a theoretical analysis). However, this type of observation, based on small $P_{E_{O_2}}$ changes, must be considered with caution as there are several uncertainties in our $P_{E_{O_2}}$ measurements (this is also valid for all the calculated variables in Table 2 where $P_{E_{O_2}}$ has been used). First, although we can be confident in our blood sampling from carefully chosen vessels, this is not true of our sampling of expired water. A perfectly defined channel exists only in a few species. Some problems of expired water mixing or contamination by backward gas diffusion may exist in *Silurus*, despite the anatomical arrangement of the branchial cavity (Fig. 1). Second, given the variability of $P_{E_{O_2}}$ (Figs 3 and 4), we chose the modal value as representing the actual value. Although the modal value is satisfactory in a study of a controlled system, it introduces a bias in the analysis of the overall gas exchanges. Indeed, all the water passing over the gills participates in the gas exchanges. The modal value clearly underestimates (at least at the highest $P_{I_{O_2}}$) the ideal measurement that would be performed on all the collected and mixed expired water.

Changes in acid–base balance

When $P_{I_{O_2}}$ varied, $P_{a_{CO_2}}$ changed as a consequence of the ventilatory adaptation. In *Silurus* this led either to a hypocapnic alkalosis or a hypercapnic acidosis, which were fully compensated within 1 day. This was achieved by metabolic means but also possibly – in the hyperoxic direction – by transient ventilatory adjustments that are likely to occur in dogfish exposed to hyperoxia (Heisler *et al.* 1988). Burtin *et al.* (1986) demonstrated that $\dot{V}w$ can participate in regulation of acid–base balance in water-breathers.

In conclusion

In the teleost *S. glanis*, as in the crayfish *A. leptodactylus*, $\dot{V}w$ plays a key role in maintaining resting $\dot{M}_{O_2}$ constant while $P_{I_{O_2}}$ varies. The main result of the $\dot{V}w$ adaptation is that $P_{a_{O_2}}$ remains constant in *Silurus* and in a narrow range in *Astacus*. Consequently, the homeostasis of the *milieu intérieur*, in terms of O_2, is fulfilled. But our data further show that the countercurrent arrangement of the fish gill is more efficient in achieving this result than is the crosscurrent design (Massabuau, 1983) of the crayfish gill. This has already been proposed on theoretical grounds by Piiper & Scheid (1984). Indeed, when $P_{I_{O_2}}$ decreased from 40 to 3 kPa, $P_{a_{O_2}}$ decreased slightly in crayfish (see Introduction), as to be expected in a crosscurrent system maintaining constant $P_{E_{O_2}}$. Also, a Bohr effect appeared at $P_{I_{O_2}}$ values below 10 kPa and $\dot{V}b$ was increased at 3·3 kPa (Massabuau & Burtin, 1984; Sakakibara *et al.* 1987). In *S. glanis* in the same $P_{I_{O_2}}$ range, $P_{a_{O_2}}$ stays constant. There is no Bohr effect and no $\dot{V}b$ increase. O_2 supply is maintained simply by ventilatory adjustments.

The authors wish to thank Dr D. C. Jackson for help in preparing the English manuscript and E. Pionnier for the kind supply of *Silurus*. The experiments were partially financed by funds of the programme PIREN-Eau/Alsace (CNRS) and Dr B. Burtin was supported by the Ministère de la Recherche et de la Technologie.

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