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COMBINED EFFECTS OF ENVIRONMENTAL P_{O_2} AND TEMPERATURE ON VENTILATION AND BLOOD GASES IN THE CARP *CYPRINUS CARPIO* L.

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Summary

The effects of changes in environmental temperature and oxygen tension on gill ventilation, arterial P_{O_2}, P_{CO_2}, pH and $[HCO_3^-]$ were evaluated in carp (*Cyprinus carpio* L.). Gill ventilation was measured continuously in specimens acclimated to 10 or 20°C, combining the method of electromagnetic flow determination with the application of a rubber mask technique. After establishing control values in air-equilibrated water the environmental water P_{O_2} (Pw_{O_2}) was reduced from about 150 mmHg (20 kPa) during control conditions to 110 or 75 mmHg (14.7 or 10 kPa), respectively. Measurements of blood gases and acid–base parameters were performed repeatedly before, and 1 and 4 h after, initiation of hypoxia. Regardless of temperature, these moderately hypoxic conditions caused considerable and lasting increases in gill ventilation of about 70 % (Pw_{O_2}=110 mmHg/14.7 kPa) or 180 % (Pw_{O_2}=75 mmHg/10 kPa), relative to the respective normoxic control values of about 50 ml kg^{-1} min^{-1} at 10°C and 230 ml kg^{-1} min^{-1} at 20°C. These increases in ventilation reduced P_{CO_2} substantially, resulting in a rise in pHa by about 0.1 units at Pw_{O_2} of 110 mmHg (14.7 kPa) and by about 0.2 units at Pw_{O_2} of 75 mmHg (10 kPa). Arterial P_{O_2} was low under normoxic conditions at both temperatures (≈15 mmHg, ≈2 kPa). During hypoxia, Pa_{O_2} was marginally reduced, whereas the arterial O_2 content and saturation remained at normoxic levels, mainly because of the increase in the blood O_2-affinity induced by respiratory alkalosis. This lack of any clear relationship between arterial O_2 content and ventilatory response to moderate hypoxia contrasts with previously reported data for trout, and supports the hypothesis that a change in P_{O_2} is an adequate stimulus for the adjustment of ventilation in carp.

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The considerable ventilatory response, together with small and inconsistent reductions in arterial P_{O_2}, may also represent an expression of the action of water-facing oxygen receptors on the regulation of breathing. A striking feature of the regulation of ventilation in carp compared with that in air-breathing lower vertebrates is that the hypoxic response is maintained at low temperatures, possibly indicating a relatively small safety margin for complete tissue oxygen supply in fish.

Introduction

Fish may encounter large and rapid variations in environmental temperature and in the availability of oxygen. Several regulatory mechanisms, involving various steps in the O_2 transport chain, are utilized to cope with adverse conditions. Responses to acute environmental hypoxia include increased gill ventilation (Johansen, 1982) and improved branchial O_2 diffusing capacity, which is achieved by recruitment of secondary lamellae as well as by secondary lamellar distension owing to an increased perfusion pressure (Soivio and Tuurala, 1981). Oxygen transport by the blood is promoted by an increased O_2 capacity or by an elevation of the O_2 affinity, achieved by modulation of the internal environment of the red blood cells (Tetens and Lykkeboe, 1985).

Information concerning directly measured responses of gill ventilation in teleost fish exposed to hypoxia at different temperatures is scarce, and simultaneously determined blood gas data are not available (Dejours, 1981; Johansen, 1982). Lomholt and Johansen (1979) applied electromagnetic flowmeters to evaluate the effects of considerably decreased water P_{O_2} (Pw_{O_2}) on gas exchange and gill ventilation. Another study utilized the indirect estimation of ventilation by the Fick principle to describe the effects of changes in Pw_{O_2} on ventilation and blood gases in the carp (Itazawa and Takeda, 1978). The degree of hypoxia applied in these studies was considerable. Inspired water P_{O_2} was generally reduced to less than 50 mmHg (6.7 kPa, Itazawa and Takeda, 1978; Lomholt and Johansen, 1979) or, in some series, even to 25 mmHg (3.3 kPa, Itazawa and Takeda, 1978).

In contrast, the present study has focused on much less severe hypoxia, but has adopted temperature as an additional variable. This strategy was followed to determine whether the ventilatory response to hypoxia was temperature-dependent (Wood, 1982; Glass *et al.* 1983). Temperature-dependent regulation would be advantageous, given the background of an increasing oxygen uptake at higher temperatures and simultaneously falling blood oxygen-affinity. If conditions for oxygen delivery at the systemic capillary level are to be unchanged, these effects could be compensated for by an increased perfusion rate and, at least partially, also by improved branchial gas exchange conditions. A marked elevation of branchial gas exchange, comparable to the rise in oxygen consumption, could, however, only be brought about by a reduction of the inspired/average gill water P_{O_2} difference, induced by an increase in gill ventilation.

In amphibians and reptiles the ventilatory response to hypoxia is markedly decreased at lower body temperatures. This is consistent with a considerably

depressed ventilatory response in the air-breathing fish *Channa argus* during aerial hypoxia at low temperatures (Glass *et al.* 1986). Similarly, the water-breathing spangled perch *Leiopotherapon unicolor* essentially lacks any response in breathing frequency at reduced body temperatures (Gehrke and Fiedler, 1988). Breathing frequency alone, however, may be misleading. This is emphasized by studies of ventilation in rainbow trout (*Salmo gairdneri*), which demonstrated a response to hypoxia mainly by elevation of tidal volume, with essentially no change in breathing frequency (Smith and Jones, 1982). Likewise, no conclusion can be drawn from data obtained in bluegill sunfish *Lepomis macrochirus*, where breathing frequency increased during moderate hypoxia ($P_{W_{O_2}}$>60 mmHg/8 kPa) at 13 and 25°C, but did not change at 30°C (Spitzer *et al.* 1969). Uncalibrated estimates of tidal volume indicated that moderate hypoxia was associated with an increased ventilation amplitude at lower temperatures, but not at 30°C.

Accordingly, ventilation was determined in this study by a method allowing simultaneous monitoring of tidal volume and breathing frequency. Gill ventilation together with arterial P_{O_2}, O_2 content and parameters of the acid–base status were measured in carp acclimated to normoxia ($P_{W_{O_2}}$≈150 mmHg/20 kPa) at two temperatures (10 and 20°C), and during subsequent exposure to acute hypoxia ($P_{W_{O_2}}$ of 110 or 75 mmHg/14.7 or 10 kPa).

Materials and methods

Specimens of carp (*Cyprinus carpio*, mass 1.3–3.3 kg; 2.3±0.1 kg, $\bar{x}$±S.E., N=32) were acclimated for 4–8 weeks to 10 or 20°C in large glass aquaria (about 100 l volume per fish) in air-equilibrated water, with a continuous flow-through of fresh Göttingen tap water at a rate of at least 100 ml min^{-1} $animal^{-1}$). The fish were fed daily on commercial carp food, and food was withheld only about 48 h before experimentation. Blood was sampled from all experimental animals (N=32); gill ventilation was measured in 24 fish.

Surgical preparations

Anaesthesia was initiated by immersion of the animals in buffered ($NaHCO_3$) MS 222 (1:10 000); after loss of reactivity the anaesthesia and the oxygen supply were maintained by irrigation of the gills with aerated, thermostatted water at a reduced MS 222 concentration (1:15 000). A catheter for blood sampling was inserted into the dorsal aorta by direct puncture, applying a modification of the Seldinger technique, and then secured with 3–5 atraumatic surgical polyamide sutures (for details see Claiborne and Heisler, 1984).

Gill ventilation was measured by application of a mask technique. To separate inspired from expired water flows, a funnel was constructed from parts of a surgical rubber glove (Fig. 1), the lumen of which was extended and supported by several rings. The smaller, open end of the funnel was sutured around the mouth of the carp by means of 20–30 small atraumatic polyamide sutures. The larger, open end of the funnel was fitted onto a device holding the electromagnetic flow

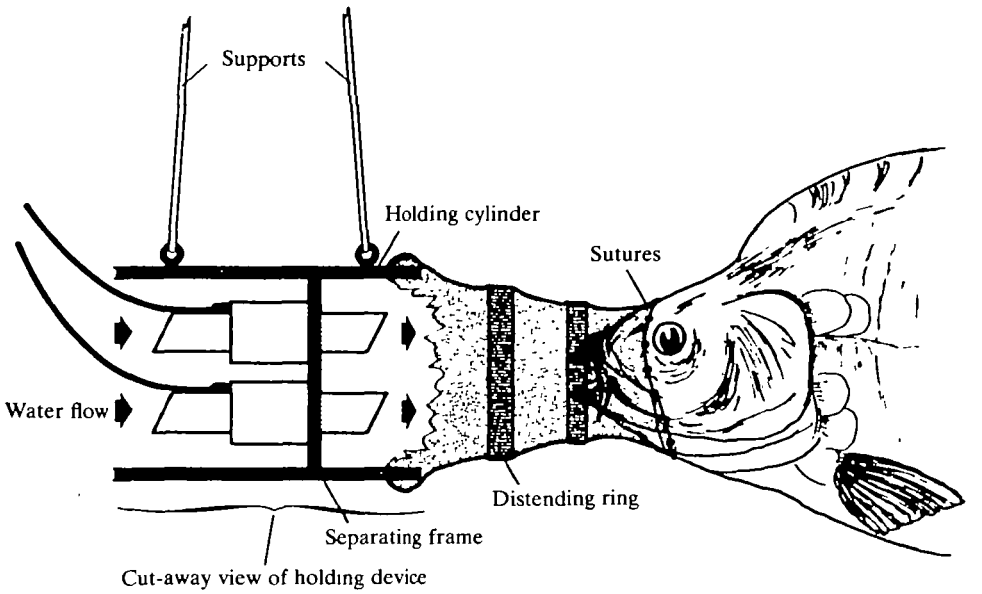

Fig. 1. Method for measuring inspired gill ventilation. The inspired water is directed through flowmeter probes and a funnel constructed from a rubber glove, providing a water-tight connection between a device holding electromagnetic flowmeter probes and the perimeter of the animal's forehead.

sensors, so that all inspired water was channelled through the probes. The holding device was constructed of light-weight polymers and suspended from a float by polymer supports. This arrangement allowed the carp a certain degree of freedom in the experimental chamber. After these preparations, the anaesthesia was terminated and when, after a few minutes, the animal resumed spontaneous breathing it was left undisturbed in the experimental apparatus for at least 48 h before the experiment began.

To exclude any disturbing effects of the mask on the establishment of a steady state, the blood gas values of carp fitted with masks were compared with those of specimens equipped with only the dorsal aortic catheter. No differences were found between the two groups.

Experimental apparatus and protocol

The experiments were conducted using a recirculation system consisting of a Plexiglas box (≈12 l), a gas equilibration column (≈6 l), a particle filter and a circulation pump, similar to the one described in detail by Claiborne and Heisler (1984). The gas equilibration column was supplied with normoxic or hypoxic gas mixtures at a rate of about 7 l min^{-1}. Equilibration gases were prepared by a microprocessor-controlled mixing system on the basis of electronic mass flow controllers. Water temperature was regulated to ±0.1 °C of the desired value (10 or 20 °C) by feedback regulation of thermostatting fluid flow through stainless-

steel heat exchangers in the system. The recirculation system was completely flushed frequently with fresh pre-thermostatted and aerated Göttingen tap water to avoid build-up of toxic waste substances.

Normoxic control values of gill ventilation and blood gas variables in animals exposed to well-aerated water were determined starting 48 h after surgery. When repeated sampling indicated that a steady state had been attained, the water P_{O_2} (Pw_{O_2}) was lowered to either 110 or 75 mmHg (14.7 or 10 kPa). Gill ventilation was monitored continuously and the blood variables were measured 1 and 4 h after initiation of water hypoxia.

Measurements

Inspired gill ventilation was measured by means of two 'cannulating' electromagnetic flow probes (inner diameter 12 mm), controlled by two synchronized flowmeters (Gould/Statham SP2202). Two probes were used to reduce the breathing resistance. The signal from the flowmeters was recorded directly (Brush mark 260) and in integrated form for determination of ventilation minute volume. Calibration of the flowmeters and integrators was performed by pumping the experimental water at known rates (within the range of the inspired gill flow of carp) through the flow probes. The ventilation reported is based on the average gill ventilation determined during a period from 2 min before to 2 min after blood sampling.

Blood samples (1.5 ml), drawn from the dorsal aortic catheter, were analyzed for P_{O_2}, P_{CO_2} and pH by means of microelectrodes (Radiometer, Copenhagen) as described by Claiborne and Heisler (1984). Blood oxygen content was measured by a micro-method according to Tucker (1967). Blood saturation was calculated from O_2 and haemoglobin contents as described by Jensen *et al.* (1987). Haematocrit was determined in triplicate after centrifugation at 19 000 *g* in micro glass capillaries, and the supernatant plasma was analyzed for total CO_2 content with a Capni-Con III (Cameron Instruments Inc., Port Aransas, Texas, USA). CO_2 solubilities at 10°C and 20°C were calculated from the polynomials of Heisler (1984). (Note: the first sign of the last line of the α-formula is misprinted and should read '+'.) Blood lactate concentration was measured enzymatically (Boehringer GmbH, Mannheim, FRG), and haemoglobin concentration was determined by the cyanmethaemoglobin method (Jensen *et al.* 1987).

Results

Ventilation of carp in air-equilibrated water increased markedly when water temperature was raised from 10 to 20°C (Fig. 2). Correspondingly, the ventilatory responses to hypoxia at 20°C were 'scaled up' compared with those at the lower temperature. Referred to baseline values for normoxic conditions, however, the increase in gill ventilation during hypoxia was similar at both temperatures (Fig. 3). The responses to moderate hypoxia were significant at both temperatures

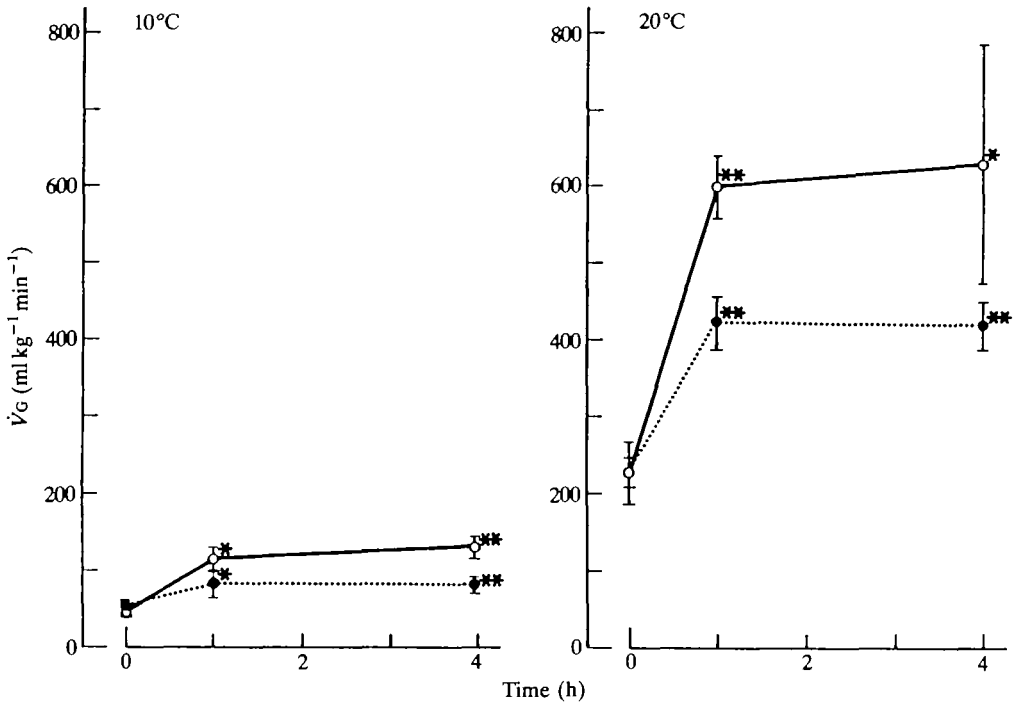

Fig. 2. Ventilatory response to moderate hypoxia at 10 and 20°C. Time=0 h represents control values for air-equilibrated water (Pw_{O_2}>150 mmHg, 20 kPa). Ventilation is plotted corresponding to the simultaneous blood sample after 1 and 4 h of moderate hypoxia (Pw_{O_2} 110 mmHg/14.7 kPa, closed points, dotted line or 75 mmHg/10 kPa, open points, solid line). $\bar{x}\pm$S.E., N=5 for 10°C; N=8 for 20°C (110 mmHg, 14.7 kPa), and N=6 for 20°C (75 mmHg, 10 kPa). A paired t-test was applied to evaluate the statistical significance of the difference between the average values during hypoxia and the respective normoxic control values (* P<0.10 and ** P<0.01).

and were maintained throughout the 4 h period of hypoxic exposure. Gill ventilation rapidly recovered to control values upon return to normoxia.

The breathing pattern of normoxic carp consisted of short breathing episodes of a few breaths alternating with breath-holds (Fig. 4). With rising temperature the number of breathing episodes per unit of time increased considerably. During normoxia the number of breathing episodes per minute was 2.4±0.5 at 10°C ($\bar{x}\pm$S.E., N=5), and it increased to 6.5±0.8 at 20°C ($\bar{x}\pm$S.E., N=6; P<0.01, grouped t-test). The number of breaths per episode remained constant (2.6±0.2 and 2.7±0.3 at 10°C and 20°C, respectively) (control values of 110 mmHg/14.7 kPa hypoxia series). The tidal volume of normoxic carp was smaller at 10°C than at 20°C, and the breathing frequency fell markedly, so that the changes in these respiratory parameters accounted for the temperature-related fall in normoxic ventilation (Table 1). Increased ventilation during hypoxia resulted partly from a larger tidal volume and partly from an elevated breathing

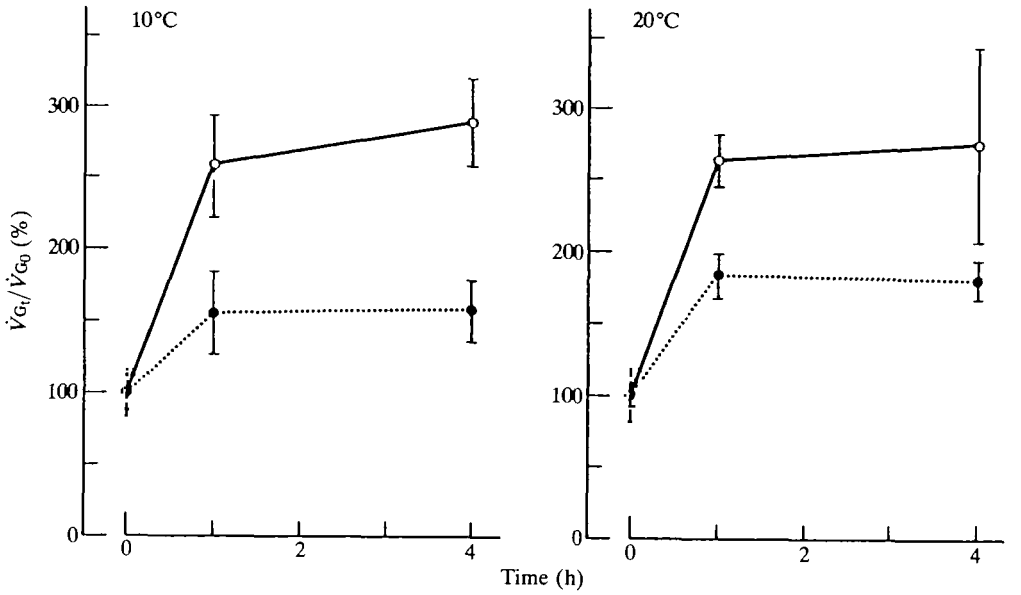

Fig. 3. Ventilatory responses to hypoxia expressed as percentage increases relative to baseline values, based on the data presented in Fig. 2. Symbols as for Fig. 2.

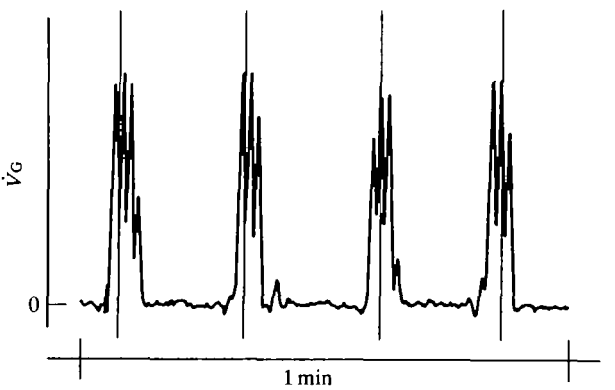

Fig. 4. Breathing pattern of a carp in air-equilibrated water at 20°C. Episodes of breathing activity are interlaced with periods of apnoea. Spike signals from an integrator are superimposed on the flow signal and are triggered for each 100 ml of water passing the flow probes.

frequency. Adjustment of tidal volume was more important at 10°C, whereas the frequency response dominated at 20°C (Table 1). This frequency response included a reduction in the duration of breath-holds, which often resulted in continuous, non-periodic ventilation at 20°C and inspired Pw_{O_2} of 75 mmHg (10 kPa).

Table 1. *Breathing pattern and tidal volume: effects of temperature (10 or 20°C) and hypoxia (inspired water oxygen partial pressure, Pw_{O_2}, 75 or 110 mmHg, 10 or 14.7 kPa)*

		10°C			20°C		
Time (h)	Pw_{O_2} (mmHg/kPa)	V_T (ml kg^{-1})	f (min^{-1})	$\dot{V}_G$ (ml min^{-1} kg^{-1})	V_T (ml kg^{-1})	f (min^{-1})	$\dot{V}_G$ (ml min^{-1} kg^{-1})
0†	≈150/20	9.3±0.5	5.4±0.9	50.2	15.0±1.4	16.1±2.8	241
1	75/10	12.0±2.5	10.9±2.6*	130.8	15.8±3.4	49.2±10.9*	777
4	75/10	13.2±2.0*	10.0±1.0*§	132.0	16.8±2.8	39.2±8.8**	659
0†	≈150/20	8.7±1.4	6.5±1.7	56.6	14.8±1.4	17.1±1.1	253
1	110/14.7	10.4±1.8	8.4±2.4	87.4	16.1±2.8	24.2±1.4*	369
4	110/14.7	11.8±1.6**	8.6±2.4	101.5	18.6±1.7	26.6±3.5*	494

Ventilation ($\dot{V}_G$) is calculated as the product of average values for tidal volume (V_T) and breathing frequency (f).
Values are means±S.E.
* Values significantly different (paired t-test, $P<0.1$).
** Values significantly different (paired t-test, $P<0.01$).
† Time=0 represents normoxic control values preceding hypoxia (Pw_{O_2}≈152 mmHg/20.3 kPa at 10°C and ≈154 mmHg/20.5 kPa at 20°C).
N=5 for both series at 10°C, N=6 for 75 mmHg/10 kPa at 20°C and N=8 for 110 mmHg/14.7 kPa at 20°C.

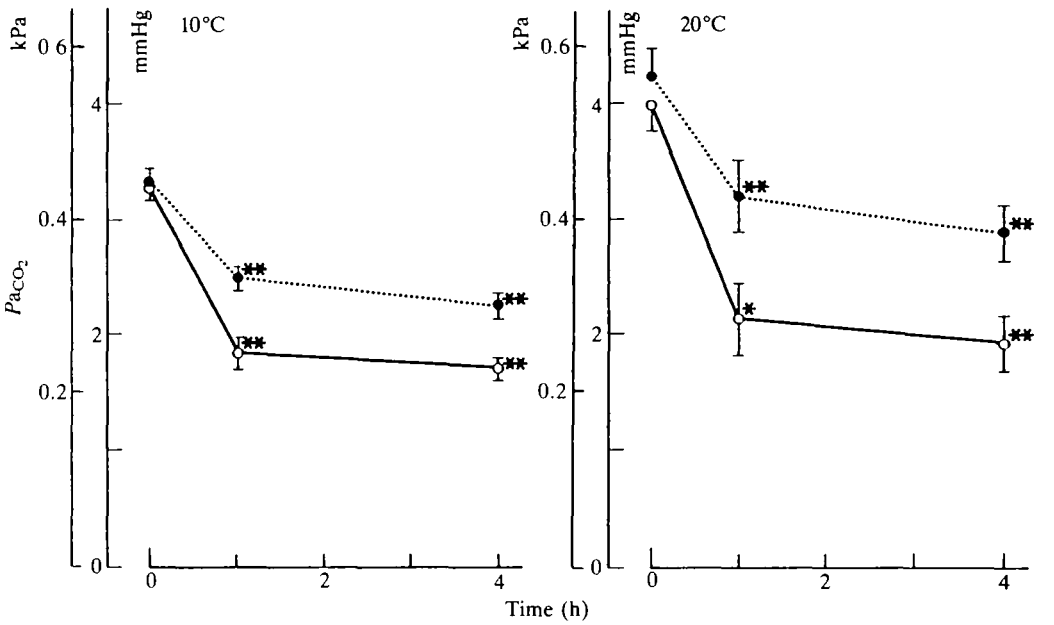

Fig. 5. Dorsal aortic P_{CO_2} as a function of time after exposure to hypoxia at 10 and 20°C ($\bar{x}\pm$S.E.; N=8). Symbols and statistical treatment as for Fig. 2.

The ventilatory responses greatly affected the acid–base status of the arterial blood. At an inspired water P_{O_2} of 75 mmHg (10 kPa), Pa_{CO_2} was reduced by almost half its normoxic values of about 3.3 mmHg (0.44 kPa, 10°C) and 4.0 mmHg (0.53 kPa, 20°C) (Fig. 5). This fall in P_{CO_2} resulted in an elevation of dorsal aortic pH by about 0.2 units compared with the normoxic values of 8.07 (10°C) and 8.01 (20°C) (Fig. 6). The rise in pH was accompanied by a compensatory fall in plasma [HCO_3^-] (Fig. 7). At both temperatures arterial P_{O_2} was low during normoxic conditions (approximately 15 mmHg, 2 kPa). The hypoxia-induced reduction was not consistently significant (Fig. 8). Also, no clear correlation existed between the extent of reduction of inspired P_{O_2}, and the fall in arterial P_{O_2}. The response of arterial P_{O_2} to inspired hypoxia varied considerably among individuals, so that in some cases the 4 h value exceeded even the normoxic control P_{O_2}. Hypoxia did not affect arterial O_2 content or O_2 saturation (Table 2). Likewise, arterial haematocrit and haemoglobin concentration were unaffected. Average pooled control values ($\bar{x}\pm$S.E., N=12) for haematocrit were 25.0±1.3% at 10°C and 25.7±0.9% at 20°C and average blood haemoglobin concentrations were 1.07±0.08 mmol l^{-1} (tetramer) at 10°C and 1.21±0.1 mmol l^{-1} at 20°C. Arterial lactate concentration rose under hypoxic conditions, in spite of the maintained blood oxygen saturation. The increase was most pronounced at 20°C with a rise from 1.36±0.35 mmol l^{-1} (normoxic controls) to 4.09±1.2 mmol l^{-1} during 4 h at an inspired P_{O_2} of 75 mmHg (10 kPa) ($\bar{x}\pm$S.E., N=7; P<0.02, paired t-test). At 10°C the same reduction of water P_{O_2} resulted in an increase in blood

[lactate] from 1.41±0.22 mmol l^{-1} to 2.10±0.32 mmol l^{-1} ($\bar{x}$±S.E., N=8; P<0.01, paired t-test).

Discussion

Carp maintain a markedly increased gill ventilation, with relatively small and

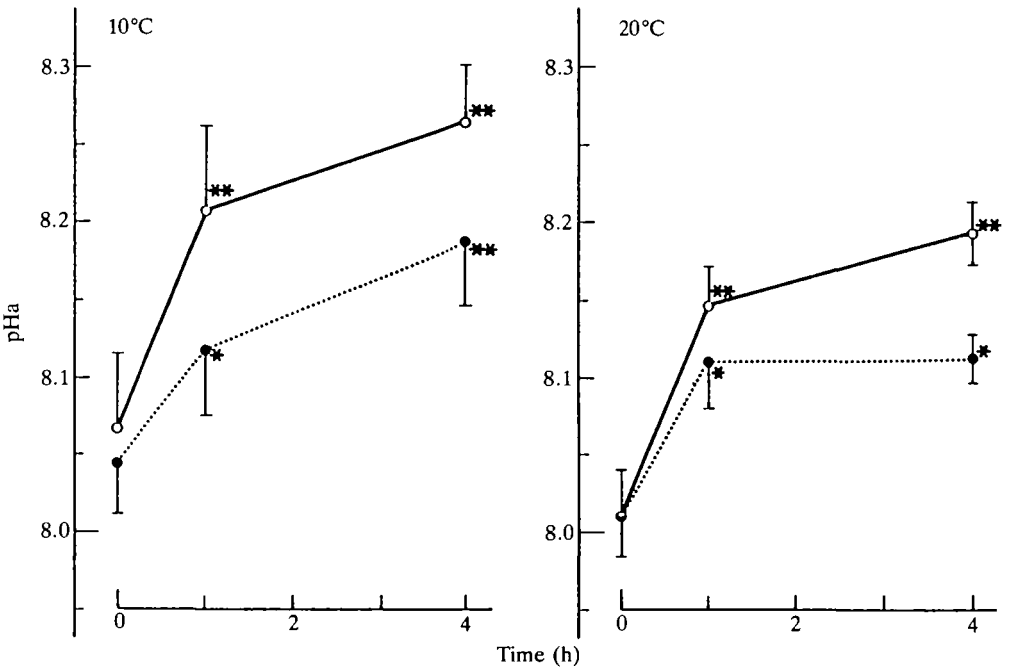

Fig. 6. Dorsal aortic pH as a function of time after exposure to hypoxia at 10 and 20°C ($\bar{x}$±S.E.; N=8). Symbols and statistical treatment as for Fig. 2.

Table 2. *Arterial blood O_2 content (C_{O_2}) and saturation (S_{O_2})*

		10°C		20°C	
Time (h)	Pw_{O_2} (mmHg/kPa)	C_{O_2} (vol%)	S_{O_2} (%)	C_{O_2} (vol%)	S_{O_2} (%)
0†	≈150/20	7.16±0.42	63.0±2.5	6.77±1.0	50.6±3.9
1	75/10	6.72±0.33	65.2±4.0	5.90±0.57	50.2±3.6
4	75/10	6.55±0.29	64.1±2.6	6.36±0.77	64.1±3.4
0†	≈150/20	6.90±0.53	72.3±3.1	8.18±0.44	66.3±2.8
1	110/14.7	6.62±0.35	65.0±3.3	7.48±0.48	64.2±3.3
4	110/14.7	6.95±0.22	68.2±2.7	7.60±0.84	62.3±2.4

Values are means±S.E.
† Normoxic control values. See Table 1 for exact values.
N=8 for all series.
Pw_{O_2}, inspired water oxygen partial pressure.

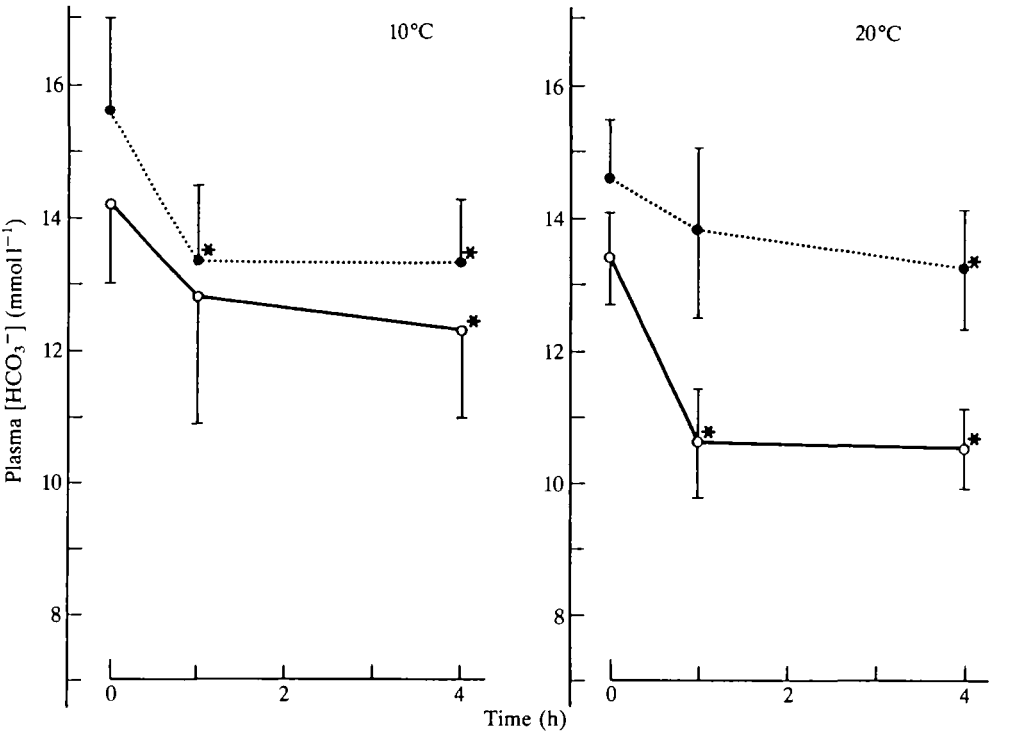

Fig. 7. Dorsal aortic plasma bicarbonate concentration as a function of time after exposure to hypoxia at 10 and 20°C ($\bar{x}\pm$S.E.; N=8). Symbols and statistical treatment as for Fig. 2.

inconsistent decreases in Pa_{O_2} and unchanged arterial O_2 saturation and O_2 content, in the face of hypoxia. These data resemble a pattern described by Hughes *et al.* (1983), in which Pa_{O_2} of carp changed little during moderate hypoxia and even increased when the P_{O_2} of the inspired water was reduced from 130 to 90 mmHg (17.3 to 12 kPa). Another finding of the present study is that the ventilatory response to hypoxia was graded according to the degree of decrease in inspired water P_{O_2}, although the effects on Pa_{O_2} did not differ for the two hypoxic conditions. These findings are difficult to interpret owing to a large variation between individuals and correspondingly large standard deviation. Non-arterial chemoreceptors, however, may be involved, a notion supported by direct and circumstantial evidence for the existence of extra-arterial chemoreceptor sites (including venous and external locations) (Randall and Daxboeck, 1984; Milsom and Brill, 1986).

The exact nature of the breathing stimulus in fish exposed to hypoxia is not yet certain. Some data favour the hypothesis that blood O_2 content rather than P_{O_2} is the decisive factor (Randall, 1982). Studying ventilatory responses to hypoxia and hypercapnia in the rainbow trout (*Salmo gairdneri*), Smith and Jones (1982) demonstrated an inverse relationship between arterial O_2 content and gill

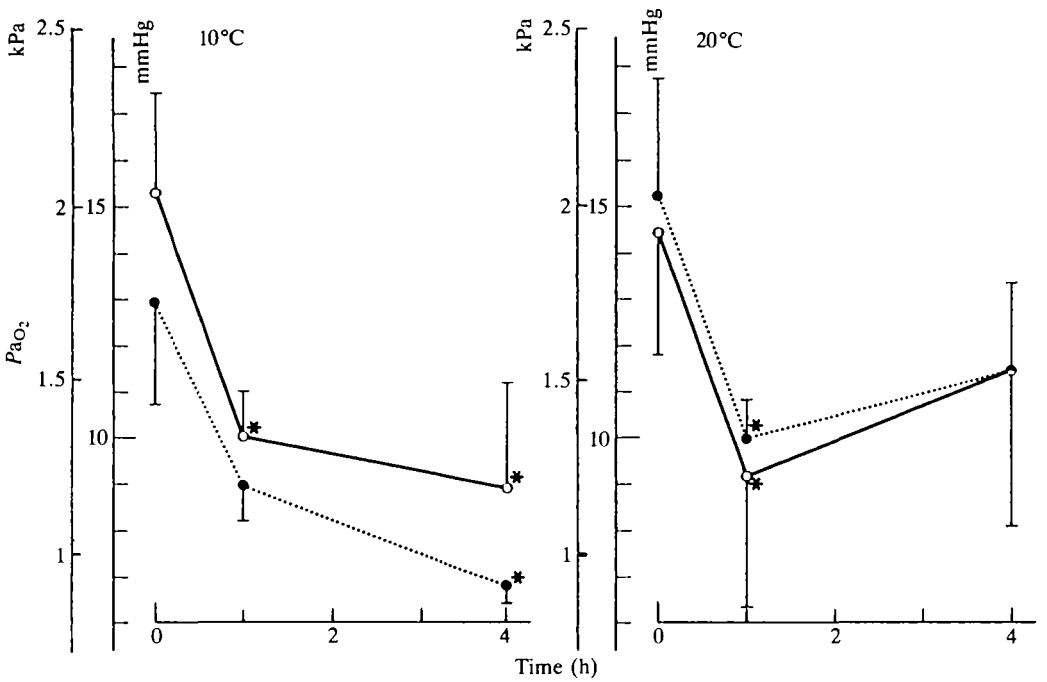

Fig. 8. Dorsal aortic P_{O_2} as a function of time after exposure to hypoxia at 10 and 20°C ($\bar{x}\pm$S.E.; N=8). Symbols and statistical treatment as for Fig. 2.

ventilation. Hypercapnia in the trout resulted in a reduction of O_2 content due to the Root effect, while Pa_{O_2} remained at normocapnic levels. Concomitantly, gill ventilation increased, suggesting that the O_2-mediated stimulus to breathing depended on content rather than partial pressure. In contrast, the present data for carp do not favour arterial O_2 content as a key factor, since substantial increases in ventilation occurred, and were maintained, while O_2 content remained at normoxic levels.

Regardless of the precise nature of the stimulus and the location of the receptor system, the regulatory system is capable of preventing a significant fall in Pa_{O_2} and oxygen saturation, relative to the characteristically low values during normoxia (Table 3). Although water P_{O_2} exceeded 150 mmHg (20 kPa) in air-equilibrated water, Pa_{O_2} of the carp was only about 15 mmHg (2 kPa), i.e. the difference between water and arterial oxygen partial pressure ($Pw_{O_2}-Pa_{O_2}$) was greater than 135 mmHg (18 kPa). The attained Pa_{O_2} permitted an oxygen saturation of barely 50 % at 20°C, and somewhat higher at 10°C because of the temperature-related increase in blood O_2-affinity. Below about 50 %, oxygen saturation may approach a critical threshold for complete tissue oxygen supply (Glass *et al.* 1983). Accordingly, we expected a ventilatory response to decreases of inspired P_{O_2}, although the extent of hypoxia applied in this study was much less severe than those of previous investigations (Table 3). A response was expected particularly

Table 3. *Ventilation and blood gases of carp*

Mass (kg)	Temperature (°C)	Pw_{O_2} (mmHg/kPa)	$\dot{V}_G$ (ml kg^{-1} min^{-1})	Pa_{O_2} (mmHg/kPa)	Pa_{CO_2} (mmHg/kPa)	pH	$[HCO_3^-]$ (mmol l^{-1})	Method for measurement of ventilation	Reference
2.34	10	≈150/20	50–57	15.3/2.04	3.3/0.44	8.07	14.2	Direct, by electromagnetic probes	Present study**
2.34	10	75/10	132†	8.9/1.18	1.7/0.23	8.27	12.3		
2.34	20	≈150/20	241–253	14.4/1.92	4.0/0.53	8.01	13.4		
2.34	20	75/10	659†	11.4/1.52	1.9/0.25	8.19	10.5		
1	20	>100/13.3	195*	...	...	...	...	Direct, by electromagnetic probes	Lomholt and Johansen (1979)
1	20	<40/5.3	1122	...	...	...	...		
0.56	25	141/18.8	244	24.8/3.3	3.9/0.52	7.89	...	Indirect estimation by Fick principle	Itazawa and Takeda (1978)
0.56	25	52/6.9	917	18.7/2.5	1.7/0.23	...	...		
0.56	25	25/3.3	2908	5.4/0.72	<1.0/0.13	...	...		
0.17	20	high	327	...	...	...	...	Indirect estimation	Saunders (1962)
0.17	20	low	3065	...	...	...	...		
1.78	15	≈150/20	...	44.4/5.92	4.8/0.64	7.87	13.8		Claiborne and Heisler (1984)
1.73	15	≈150/20	...	24.0/3.2	3.0/0.4	8.01	12.2		Jensen *et al.* (1987)
3.2	10	≈130/17.3	...	8.0/1.07	3.5/0.47	7.91	12.7		Garey (1967)
...	20	≈150/20	...	43.1/5.74	5.0/0.67	7.78	...		Fuchs and Albers (1988)

*Carp pre-acclimated to normoxia; † 4 h-values.
** Data for $\dot{V}_G$ from Table 1.

because the O_2 extraction of carp is quite high even in normoxia, precluding any substantial increase in this variable to alleviate hypoxic conditions (Lomholt and Johansen, 1979). Consequently, during hypoxia the oxygen demand has to be satisfied by an increase in gill ventilation. In spite of its reputation as being highly tolerant to lack of O_2 (Blazka, 1958), the carp regulates oxygen supply to the tissues to maintain an aerobic metabolic energy production. Curiously, the blood lactate levels, found to be in the same range as in earlier studies on carp and other fish species (Albers *et al.* 1983; Fuchs and Albers, 1988; Heisler, 1984), increased during hypoxia, although Pa_{O_2} was little affected and arterial O_2 content remained constant. This pattern is consistent with data for the channel catfish *Ictalurus punctatus* (Burggren and Cameron, 1980), and may be indicative of a small safety margin for complete oxygen supply in fish.

The lack of any effect of hypoxia on arterial oxygen saturation in carp can partly be explained on the basis of hyperventilation-induced increases in pHa combined with the large Bohr factor ($\Delta \log P_{O_2}/\Delta pH \approx -1.0$) (Ultsch *et al.* 1981; Albers *et al.* 1983). In addition, the O_2 affinity of carp blood may become considerably elevated owing to the readjustment of the intracellular pH of erythrocytes induced by decreased nucleoside triphosphate (NTP) concentrations (Lykkeboe and Weber, 1978). However, this is unlikely to have been a major factor in the present study. The absence of any increase in haematocrit or haemoglobin concentration during acute moderate hypoxia is consistent with data for rainbow trout, in which more severe hypoxia ($Pw_{O_2} \approx 30$ mmHg, 4 kPa) is required to increase haematocrit, caused both by swelling of erythrocytes and by an elevation in red cell haemoglobin content (Tetens and Lykkeboe, 1985).

Comparatively low Pa_{O_2} values are characteristic of carp, although data in the literature are inconsistent (Table 3). This scatter is probably unrelated to the methodological approach, because differing values have been obtained using identical techniques (Claiborne and Heisler, 1984; present study). The variability may rather be due to unidentified biological influences, as discussed earlier in relation to the effects of hypoxia on O_2 uptake (Hughes *et al.* 1983). A number of conditions may account for the low Pa_{O_2} of carp. The typically high O_2 extraction from the water in the gills of carp (Saunders, 1962; Lomholt and Johansen, 1979) sets an upper limit for the range of P_{O_2}. Full blood oxygen saturation is not achieved, which could result partly from a mismatch between ventilation and perfusion, or from blood bypassing the functional gas-exchange surfaces (Piiper and Scheid, 1984). Regardless of the mechanisms involved, incomplete blood saturation implies low Pa_{O_2} values because of the high oxygen affinity (low P_{50}) of carp blood (Wood, 1982; Albers *et al.* 1983).

In contrast to the data on arterial oxygen partial pressure, consistent values for gill ventilation have been reported for carp (Table 3). Indirect estimates (Itazawa and Takeda, 1978) based on the Fick principle (Rahn, 1966) agreed well with direct measurements of ventilation (Lomholt and Johansen, 1979; present study), whereas an indirect approach based on the differences in P_{O_2} between inspired and expired water sampled from buccal and opercular catheters, respectively, resulted

in a somewhat higher value (Saunders, 1962). This relative consistency between techniques is somewhat fortuitous considering the problems resulting from periodic ventilation, from an uneven P_{O_2} distribution in the expired water stream (Garey, 1967) and from the potential cutaneous uptake of oxygen from the water (Feder and Burggren, 1985).

The periodic breathing pattern of carp (Peyraud and Serfaty, 1964; Lomholt and Johansen, 1979; Hughes *et al.* 1983; present study), with its regular interruptions of breathing activity by periods of apnoea, is characteristic of ectothermic air-breathers. This periodicity also occurs in other teleost fish (Gehrke and Fiedler, 1988; Roberts and Rowell, 1988), and in elasmobranchs during hyperoxia (Heisler *et al.* 1988), although the rate of ventilation is much greater than in air-breathing lower vertebrates, matching the lower O_2 content of water relative to air (Rahn, 1966). The periodic breathing pattern leaves a considerable margin for periods of elevated ventilation brought about by changes in ventilatory frequency, without the necessity for increasing the flow velocity of the viscous breathing water during individual breaths. Although the ventilatory adjustments described in this study are also mediated by increases in tidal volume, at least at 10°C, tidal volume was rather constant at 20°C. This may be related to the fact that any further elevation of the flow velocity of the comparatively viscous water is energetically impossible on the basis of the much higher normoxic ventilation rate at 20°C (see Table 1). At any rate, these data clearly indicate that measurement of breathing frequency alone is insufficient for an assessment of control of breathing in fish. This is also evident from data in the literature on breathing frequency and O_2 uptake measured during small changes in water temperature occurring over a few minutes (Moffitt and Crawshaw, 1983). The resulting temperature-induced changes in O_2 uptake were accompanied by rapid adjustments of breathing frequency (Q_{10}=2.6, consistent with this study) as well as by a reduction in tidal volume which, however, could not be quantified by the techniques used.

Several recent studies have investigated the effects of temperature on the ventilatory response to hypoxia in ectothermic air-breathers. Comparable data are scarce for fish (Gehrke and Fiedler, 1988), although this aspect of respiratory control is apparently highly relevant, considering the variability of temperature and O_2 availability in the aquatic environment. The data provided by this study do not allow a generalized conclusion to be drawn about the differences in the regulation of ventilation between air-breathing and water-breathing ectothermic vertebrates, but some obvious characteristics may be listed tentatively. In carp, consistent with data for some other teleost fish (Burggren and Cameron, 1980; Rantin and Johansen, 1984), a considerable ventilatory response is provoked by relatively moderate falls in inspired Pw_{O_2}, whereas several-fold larger decreases in inspired P_{O_2} are required for a comparable response in ectothermic air-breathers (Glass *et al.* 1983). Also, carp retain a relatively large ventilatory response at 10°C, whereas hypoxic responses at similarly low temperatures are virtually absent in reptiles and amphibians (Glass *et al.* 1983, 1986). In contrast to air-breathers, the more than twofold increase of ventilation in carp exposed to an inspired P_{O_2} of

75 mmHg (10 kPa) is barely correlated with small and rather inconsistent reductions in Pa_{O_2}. These differences are presumably related to the lower oxygen content of the breathing medium at the same level of inspired P_{O_2}, which can barely be completely compensated by generally enhanced levels of ventilation, higher blood oxygen-affinity and larger ratios of perfusion over oxygen consumption of systemic tissues. Accordingly, the safety margin for maintaining complete oxygen supply in fish is probably much smaller than in air-breathers, which must favour development of a predominantly O_2-transport-oriented control of breathing to match the frequently changing and generally lower environmental O_2 availability.

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