The Journal of Experimental Biology 208, 1109-1116
Published by The Company of Biologists 2005
doi:10.1242/jeb.01505

Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*

Jørund Sollid[1,*], Roy E. Weber[2] and Göran E. Nilsson[1]

[1]*Department of Molecular Biosciences, University of Oslo, PO Box 1041, N-0316 Oslo, Norway* and
[2]*Department of Zoophysiology, Institute of Biological Sciences, University of Aarhus, C. F. Moellers Alle 131, DK-8000 Aarhus C, Denmark*

*Author for correspondence (e-mail: jorund.sollid@bio.uio.no)

Accepted 20 January 2005

Summary

We have previously found that the gills of crucian carp *Carassius carassius* living in normoxic (aerated) water lack protruding lamellae, the primary site of O_2 uptake in fish, and that exposing them to hypoxia increases the respiratory surface area of the gills ~7.5-fold. We here examine whether this morphological change is triggered by temperature. We acclimated crucian carp to 10, 15, 20 and 25°C for 1 month, and investigated gill morphology, oxygen consumption and the critical oxygen concentration at the different temperatures. As expected, oxygen consumption increased with temperature. Also at 25°C an increase in the respiratory surface area, similar to that seen in hypoxia, occurred. This coincided with a reduced critical oxygen concentration. We also found that the rate of this transformation increased with rising temperature. Goldfish *Carassius auratus*, a close relative to crucian carp, previously kept at 25°C, were exposed to 15°C and 7.5°C. At 7.5°C the respiratory surface area of its gills was reduced by development of an interlamellar cell mass as found in normoxic crucian carp kept at 10–20°C. Thus, both species alter the respiratory surface area in response to temperature. Rather than being a graded change, the results suggest that the alteration of gill morphology is triggered at a given temperature. Oxygen-binding data reveal very high oxygen affinities of crucian carp haemoglobins, particularly at high pH and low temperature, which may be prerequisites for the reduced gill respiratory surface area at low temperatures. As ambient oxygen and temperature can both induce the remodelling of the gills, the response appears primarily to be an adaptation to the oxygen demand of the fish.

Key words: teleost, crucian carp, goldfish, gill, morphology, temperature, Hb, haemoglobin, respiration, Q_{10}.

Introduction

Crucian carp and goldfish, two closely related species of the same genus *Carassius*, exhibit striking capacity of coping with low levels of oxygen and a wide range of ambient temperatures. Both species are anoxia tolerant and able to convert lactate to ethanol during severe hypoxia and anoxia, thus avoiding acidosis (Johnston and Bernard, 1983; Shoubridge and Hochachka, 1980; Shoubridge and Hochachka, 1983). Although this mechanism enables them to avoid lactate self-pollution during anoxia, the release of ethanol to the water is energetically very costly, due to the loss of this energy-rich hydrocarbon. Since their anoxic survival time is dependent on their glycogen stores (Nilsson, 1990), it must be advantageous being able to postpone the activation of anaerobic ethanol production, and rely on their aerobic metabolism, for as long as possible.

Being freshwater fish, crucian carp and goldfish are faced with a dilemma: they have to cope with a continuous ion loss and water influx over the respiratory surface area in the gills (Evans, 1979), but still maintain sufficient oxygen uptake. The water influx must be compensated by a large urine production resulting in an even greater loss of ions. These ion losses must be compensated by energetically demanding ion transport over the gills. Thus, being able to modulate the respiratory surface area in response to oxygen supply and demand should be of advantage.

We have previously shown that crucian carp kept in normoxia at 8°C lack protruding lamellae, but if exposed to hypoxia, a morphological alteration is triggered resulting in protruding lamellae and a 7.5-fold increase of respiratory surface area (Sollid et al., 2003). This caused a fall in the critical oxygen concentration ($[O_2]_{crit}$), i.e. the lowest ambient $[O_2]$ where the fish is able to sustain its resting oxygen consumption ($\dot{M}_{O_2}$). The gill remodelling is due to an induction of apoptosis and cell-cycle arrest in the mass of cells filling up the space between adjacent lamellae, causing this interlamellar cell mass (ILCM) to shrink. A reduction in respiratory surface area in normoxia should lead to lower water and ion fluxes and thus reduction of osmoregulatory costs. At the same time, the crucian carp's ability to maintain a sufficient rate of oxygen uptake without protruding lamellae indicates a very high

oxygen affinity of its haemoglobin (Hb), which has remained to be studied.

Fish are ectothermic organisms; hence increased temperature profoundly raises their metabolic rates. Increased temperature also decreases the amount of oxygen dissolved in the water. Temperature-related changes in metabolism are met with behavioural, respiratory, cardiovascular, hematological and biochemical adjustments (Aguiar et al., 2002; Burggren, 1982; Butler and Taylor, 1975; Caldwell, 1969; Fernandes and Rantin, 1989; Goldspink, 1995; Houston et al., 1996; Houston and Rupert, 1976; Maricondi-Massari et al., 1998). The responses to increased temperature may include air gulping, increased gill ventilation, increased lamellar perfusion, increased cardiac output, changes in Hb function and altered expression of metabolic enzymes. Studies related to gill morphology and temperature are scarce and only cover acute temperature changes (Hocutt and Tilney, 1985; Jacobs et al., 1981; Nolan et al., 2000; Tilney and Hocutt, 1987), which often reflect more pathophysiological responses that are not necessarily adaptive.

Changes in Hb function could result from changes in the levels of erythrocytic effectors such as organic phosphates (ATP, often supplemented by guanosine triphosphates in fish) or changes in Hb isomorphs (Weber, 2000). In addition to the 'standard' electrophoretically 'anodic' Hb components that display pronounced Bohr shifts, some fishes (salmonids, catfishes and eels) also have electrophoretically 'cathodic' Hbs, that have lower Bohr shifts and show divergent phosphate sensitivities (which are insignificant in salmonids and large in eels and catfishes). Hb composition of goldfish (that is closely related to crucian carp) changes with temperature: electrophoresis reveals two isoHbs in fish acclimated to 2°C, and three isoHbs in fish acclimated to 20°C and 35°C (Houston and Cyr, 1974). This modification also occurs in isolated cells and in hemolysates, suggesting that it is caused by altered aggregation of pre-existing subunits rather than *de novo* Hb synthesis (Houston and Rupert, 1976).

The aim of this study was to investigate if increased temperature, leading to an increased oxygen demand, can trigger the morphological response recently found in hypoxia-exposed crucian carp (Sollid et al., 2003). At our latitude the typical seasonal temperature range for the crucian carp habitat is 0°C to 25°C. We thus acclimated crucian carp to temperatures ranging from 10 to 25°C to examine the possible effects of changing oxygen demand on gill morphology. In addition goldfish were acclimated at 7.5, 15 and 25°C to see if the gill remodelling seen in crucian carp also is expressed in this closely related species when kept at low temperatures. Since goldfish normally are kept at room temperature, an ability to remodel the gills may not have been noticed. To identify adaptations in oxygen transport functions we also investigated Hb multiplicity in fish acclimated to the different temperatures, and measured the intrinsic oxygen-binding properties and effector sensitivities of crucian carp Hbs.

Materials and methods

Animals

Crucian carp *Carassius carassius* L. (weighing 12.5–31.5 g; all adults) were caught in June 2003 in the Tjernsrud pond, Oslo community. They were kept on a 12 h:12 h L:D regime in tanks (~100 fish per 500 l) continuously supplied with aerated and dechlorinated Oslo tapwater (10°C), and fed daily with commercial carp food (Tetra Pond, Tetra, Melle, Germany).

Goldfish *Carassius auratus* L. (weighing 8.0–16.5 g; all adults), bred and cultivated in Singapore, were bought from a commercial wholesaler. They where kept in a tank (~100 fish per 500 l) with aerated, ion strength adjusted to 500 μS cm^{-1} (dH-Salt, NOR ZOO, Bergen, Norway) and dechlorinated Oslo tap water (25°C) for 1 month before experiments. The light regime and feeding were the same as for crucian carp.

Temperature acclimation

Crucian carp were transferred to new holding tanks (~10 fish per 25 l) held at 10°C, 15°C, 20°C and 25°C, respectively, and acclimated 1 month before respirometry experiments (see below). The fish were fed until 24 h before respirometry. Each fish was placed in the respirometer with a continuous flow of aerated water until 12 h before commencing measurements. To examine if gill morphology was affected by the respirometry, four fish from each group were sampled before and after respirometry and the left first and second gill arches were dissected out. As a control for possible effects of the confinement in the respirometer, crucian carp kept at 15°C were placed in the respirometer for 24 h and continuously supplied with aerated and dechlorinated Oslo tapwater. After exposures, the fish were killed with a sharp blow to the head.

Goldfish were transferred to a new container (~10 fish per 25 l) with ion strength adjusted, aerated and dechlorinated Oslo tapwater (25°C) for 1 month, whereafter the gills of four fish, were sampled. Subsequently, the water temperature in the container was reduced to 15°C. After 5 days at this temperature four additional fish were sampled. The temperature was finally reduced to 7.5°C and the gills of four fish were sampled after 5 days and 1 month at this temperature. The fish were fed during temperature acclimation. The fish were killed for dissection of the left first and second gill arches and treated as the crucian carp.

Respirometry

$\dot{M}_{O_2}$ during falling water oxygen concentration was measured with closed respirometry, and the $[O_2]_{crit}$ was determined as described previously (Nilsson, 1992). The temperature in the 1 l respirometer was the same as the acclimation temperature. Oxygen levels in the respirometer were measured with an oxygen electrode (Oxi340i, WTW, Weilheim, Germany) and recorded on a laptop computer *via* an analog–digital converter (Powerlab 4/20, AD Instruments Ltd., Oxon, UK). The fish were removed from the respirometer for dissection of gills when the recorded oxygen content became 0 mg O_2 l^{-1}.

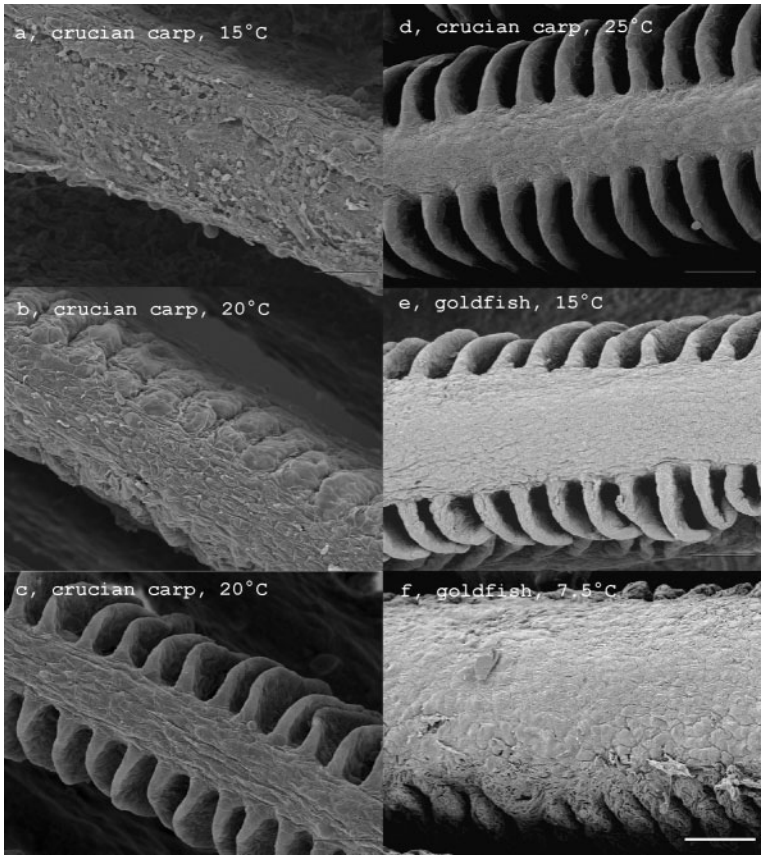

Fig. 1. (a–f) Scanning electron micrographs from the second gill arch of crucian carp and goldfish kept at different temperatures. At 15°C (a) and 20°C (b) the crucian carp gills do not have protruding lamellae. However, after respirometry at 20°C (c) crucian carp gill filament exhibited protruding lamellae, a response probably induced by the hypoxic period in the respirometer. At 25°C (d) the crucian carp developed protruding lamellae in normoxia. Goldfish gills at 15°C (e) showed protruding lamellae; however after 5 days at 7.5°C (f) the gill morphology of goldfish started to resemble that of normoxic crucian carp at 10–20°C. Scale bar, 50 μm.

Scanning electron microscopy (SEM)

The gill morphology of all groups was investigated as previously described (Sollid et al., 2003). In brief, gills were fixed in 3% glutaraldehyde in 0.1 mol l^{-1} sodium cacodylate buffer before dried, AuPd coated, and examined using a JSM 6400 electron microscope (JEOL, Peabody, USA).

Hb oxygen binding

IsoHb composition was probed using PhastSystem (Amersham Biosciences, Piscataway, NJ, USA) by isoelectrofocusing on polyacrylamide gels in the 5–8 pH range. The crucian carp had been acclimated for 1 month at 16°C or 26°C prior to blood samples.

Crucian carp Hb for oxygen-binding studies was prepared from washed red cells as previously described (Weber et al., 1987). The Hb was 'stripped' of ionic effectors by column chromatography on Sephadex G25 Fine gel (Berman et al., 1971). Major isoHbs were separated using preparative isoelectric focusing using Pharmacia ampholytes (0.22% pH 5–7, 0.22% pH 6–8 and 0.11% pH 6.7–7.7). Retrieved pools were concentrated using Amicon Ultra-15 (molecular weight cut-off 10.000) filters. All Hb samples were subsequently dialyzed for at least 24 h against three changes of 10 mmol l^{-1} Hepes buffer containing 0.5 mmol l^{-1} EDTA. All preparation procedures were carried out at 0–5°C. Samples were frozen at –80°C and freshly thawed for subsequent analyses. O_2 equilibrium measurements at different pH values and in the presence of 0.1 mol l^{-1} KCl were carried out using a modified gas diffusion chamber as previously detailed (Weber, 1981; Wells and Weber, 1989).

Statistics

All values are given as means ± S.E.M. and statistically significant differences were detected with a one-way ANOVA test with Tukey's test as post test using GraphPad InStat (GraphPad, San Diego, CA, USA).

Results

Morphology

In crucian carp that were originally kept at 10°C, and then exposed to higher temperatures (15, 20 and 25°C), the gill morphology only changed in the 25°C group prior to the respirometry (Fig. 1a–c). Thus, the threefold increase of $\dot{M}_{O_2}$ from 10 to 20°C, did not trigger a change of gill morphology (Table 1).

Throughout closed respirometry, the oxygen tension in the respirometer drops, eventually to a level below the $[O_2]_{crit}$. Hence the fish will experience a hypoxic environment and finally anoxia (0 mg O_2 l^{-1}). At 8°C an increase of respiratory surface in hypoxia takes 3 days before it is pronounced (Sollid et al., 2003). The present results show, that this time period is dramatically reduced at higher temperatures. In the respirometer at 15 and 20°C the fish experienced hypoxia and anoxia on average 6 h before sampled. Crucian carp at 15°C

Table 1. *Respirometry data from the crucian carp*

Groups	Temp. (°C)	$\dot{M}_{O_2}$ (mg kg^{-1} h^{-1})	$[O_2]_{crit}$ (mg l^{-1})	$\dot{M}_{O_2}$:$[O_2]_{crit}$
A	10	$38.9\pm4.5^{b,c,d}$	$1.43\pm0.13^{b,c,d}$	$27.4\pm2.1^{b,c,d}$
B	15	$88.2\pm7.9^{a,d}$	$2.45\pm0.18^{a,c,d}$	$36.0\pm2.0^{a,d}$
C	20	$122.7\pm12.2^{a,d}$	$3.55\pm0.29^{a,b}$	$34.5\pm1.1^{a,d}$
D	25	$209.5\pm15.1^{a,b,c}$	$4.02\pm0.27^{a,b}$	$52.1\pm1.6^{a,b,c}$

Significant temperature-dependent changes in the mean values for rate of oxygen consumption ($\dot{M}_{O_2}$), critical oxygen tension ($[O_2]_{crit}$) and the ratio between the two at the different temperatures, are indicated by an ANOVA ($P<0.0001$). The superscripted letters (a,b,c,d) denote significant differences ($P<0.05$) between groups (A,B,C,D) within a variable. An increase of $\dot{M}_{O_2}$:$[O_2]_{crit}$ ratio indicates an improvement of the capacity for oxygen uptake.

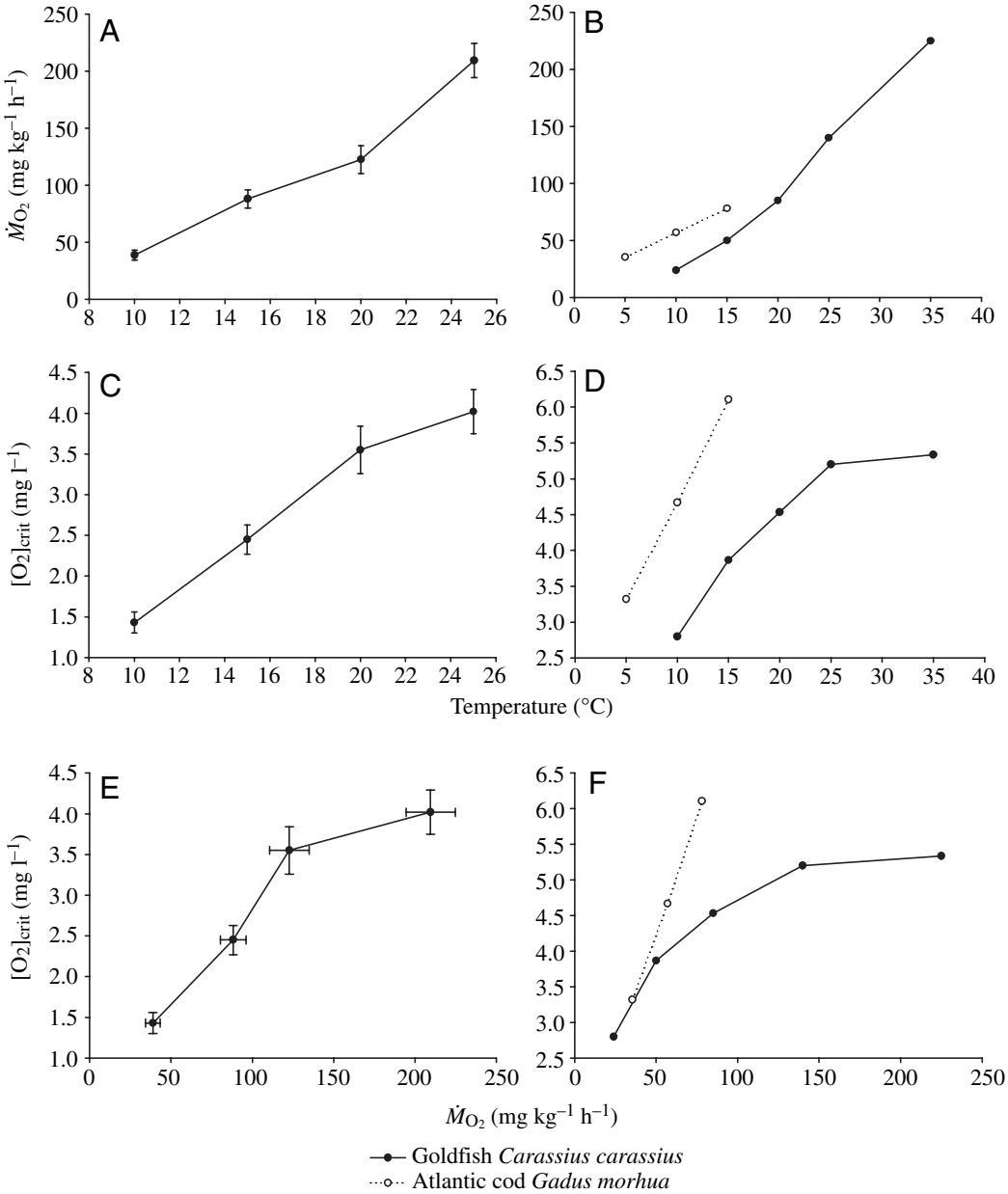

Fig. 2. (A–F) Respirometry data from the present study of crucian carp (left), and previous studies (right) on goldfish (Fry and Hart, 1948) and Atlantic cod (Schurmann and Steffensen, 1997) showing the effect of temperature on $\dot{M}_{O_2}$ (A) and (B), the alteration of critical oxygen concentration ($[O_2]_{crit}$), in response to different temperatures (C) and (D), and how the different species alter their oxygen uptake capabilities at different $\dot{M}_{O_2}$ (E) and (F).

(not shown) and 20°C (Fig. 1d) underwent the characteristic remodelling of their gills to increase the respiratory surface area during these few hours in the respirometer. This change was not due to confinement (not shown).

In our previous study, morphometric measurements indicated a ~7.5-fold increase in the lamellar area exposed to water in crucian carp kept in hypoxia (Sollid et al., 2003). The gill morphological changes of crucian carp kept at 25°C, and exposed to 15°C and 20°C in the respirometer in this study appeared to be identical in extent to those seen after hypoxia in our previous study. However, since the gills were only examined by SEM in the present study, no quantitative morphometrical measurements were attempted.

Goldfish at 20°C had protruding lamellae (not shown), which were indistinguishable from those seen in the 15°C group (Fig. 1f). However, in goldfish exposed to 7.5°C, a clear change in the gill filament morphology occurred. This was clearly visible after 5 days (Fig. 1e) and no further changes were apparent after 1 month (not shown). The space between adjacent lamellae was partially filled with a cell mass, as seen in crucian carp, although slightly less pronounced, as the edges of the lamellae were still visible.

Table 2. *Q_{10} values for crucian carp, goldfish and Atlantic cod*

Q_{10} regimes	Crucian carp	Atlantic cod	Goldfish
5–10°C		2.6	
10–15°C	5.1	1.9	4.3
15–20°C	1.9		2.9
20–25°C	2.9		2.7

Q_{10} values (i.e. the increase of $\dot{M}_{O_2}$ observed at 10°C higher temperatures, here given for 5°C intervals) for crucian carp (present study), goldfish (Fry and Hart, 1948) and Atlantic cod (Schurmann and Steffensen, 1997).

Respiration

The respirometry data for the crucian carp showed, as expected, that $\dot{M}_{O_2}$ increased with temperature (P<0.0001, Fig. 2A). A temperature rise from 10°C to 25°C increased the $\dot{M}_{O_2}$ more than fivefold, from 38.9±4.5 mg kg^{-1} h^{-1} to 209.5±15.1 mg kg^{-1} h^{-1} (P<0.001, Table 1). The increase of $\dot{M}_{O_2}$, from 10°C to 25°C, lead to an increase of $[O_2]_{crit}$ from 1.43±0.13 kPa to 4.02±0.27 kPa (P<0.001, Table 1). However, there was a strikingly small increase in $[O_2]_{crit}$ between 20°C and 25°C (Fig. 2B). This corresponds well with the transformation in gill morphology that occurred between these two temperatures (Fig. 1b,d).

The relationship between $\dot{M}_{O_2}$ and $[O_2]_{crit}$ in crucian carp (Fig. 2C) was similar to literature data for goldfish (Fig. 2F). Both species show relatively low $[O_2]_{crit}$ at high temperatures, which indicates an improvement of their oxygen uptake capabilities that is likely to coincide with the remodelling of the gills. By contrast, the Atlantic cod (Schurmann and Steffensen, 1997) shows a steady increase in $[O_2]_{crit}$ with rising $\dot{M}_{O_2}$ (Fig. 2F), indicating that this species is incapable of any major morphological or physiological adjustments to improve its O_2 uptake capacity at high temperatures. Also, crucian carp showed lower $[O_2]_{crit}$ values than the goldfish. For example at a $\dot{M}_{O_2}$ of approximately 85 mg kg^{-1} h^{-1}, the $[O_2]_{crit}$ values were 2.4 kPa and 3.9 kPa for crucian carp and goldfish, respectively. This indicates an ability of crucian carp to extract more oxygen from the surrounding water than goldfish. The Q_{10} was also similar between the two species (Table 2). However, in contrast to goldfish, crucian carp exhibited a higher Q_{10} between 20–25°C than between 15–20°C (Table 2).

Hb and oxygen binding

The thin-layer isoelectrofocusing of Hbs from fish acclimated to 14 or 26°C (Fig. 3) showed at least three major bands. Importantly no consistent differences were seen in the number or relative intensities of the bands between fish acclimated to the two temperatures.

As shown (Fig. 4A,B), stripped crucian carp Hbs show an extremely high oxygen affinity (P_{50}=0.8 and 1.8 at pH 7.6 at 10 and 20°C, respectively). The Bohr effect that approximates –0.7 at pH 7.0 decreases markedly with increasing pH and is virtually absent at pH above 7.7 at 20°C. Interestingly, cooperativity increased with decreasing pH over the entire range investigated (8.4–6.4, Fig. 4A), whereas n_{50} values at low pH fall to unity and lower (reflecting anticooperativity) in fish Hbs that express Root effects (Brittain, 1987). The oxygen affinities decrease with increasing temperature (in agreement with the exothermic nature of haem oxygenation). As expressed by the heats of oxygenation (ΔH=58 and 49 kJ mol^{-1} at pH 7.6 and 7.0, respectively) the temperature sensitivity of P_{50} decreases with pH. This correlates with the parallel increase in the Bohr effect and, thus, in the endothermic dissociation of the Bohr protons. By contrast, the ATP sensitivity of the Hb decreases with increasing pH (Fig. 4A) in accordance with the associated decrease in positive charge of the phosphate binding sites.

Crucian carp red cells contain at least three major isoHbs (II, III and IV) and two minor ones (I and V). The elution profile (Fig. 4C) indicates relative abundance of 6% HbI, 27% HbII, 62% HbIII+IV and 5% HbV, and that Hbs I, II, III and IV are isoelectric at pH values of 6.7, 6.4, 6.9 and 5.8, respectively. All components exhibit similar, high oxygen affinities and similar Bohr effects (P_{50} of 1.5–1.8 mmHg at pH 7.6 and 20°C, and $\nu\cong$–0.30). These properties correspond with those of stripped hemolysates, indicating the absence of functionally significant interaction between the isolated components.

Discussion

The results show that both crucian carp and goldfish have the capacity to remodel their gills in response to temperature, hence altering the respiratory surface area. That hypoxia and high temperature induce apparently identical changes, i.e.

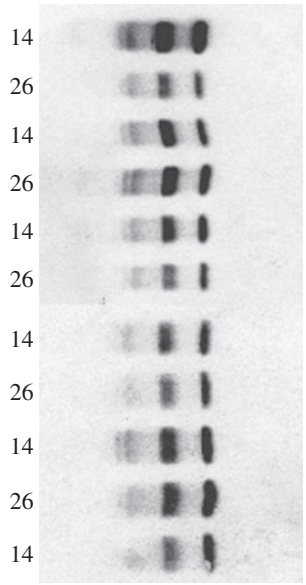

Fig. 3. Thin-layer isoelectrofocussing gels of Hbs from individual crucian carp specimens acclimated to either 14 or 26°C (as indicated) for 1 month, showing correspondence in isoHb compositions of the two groups.

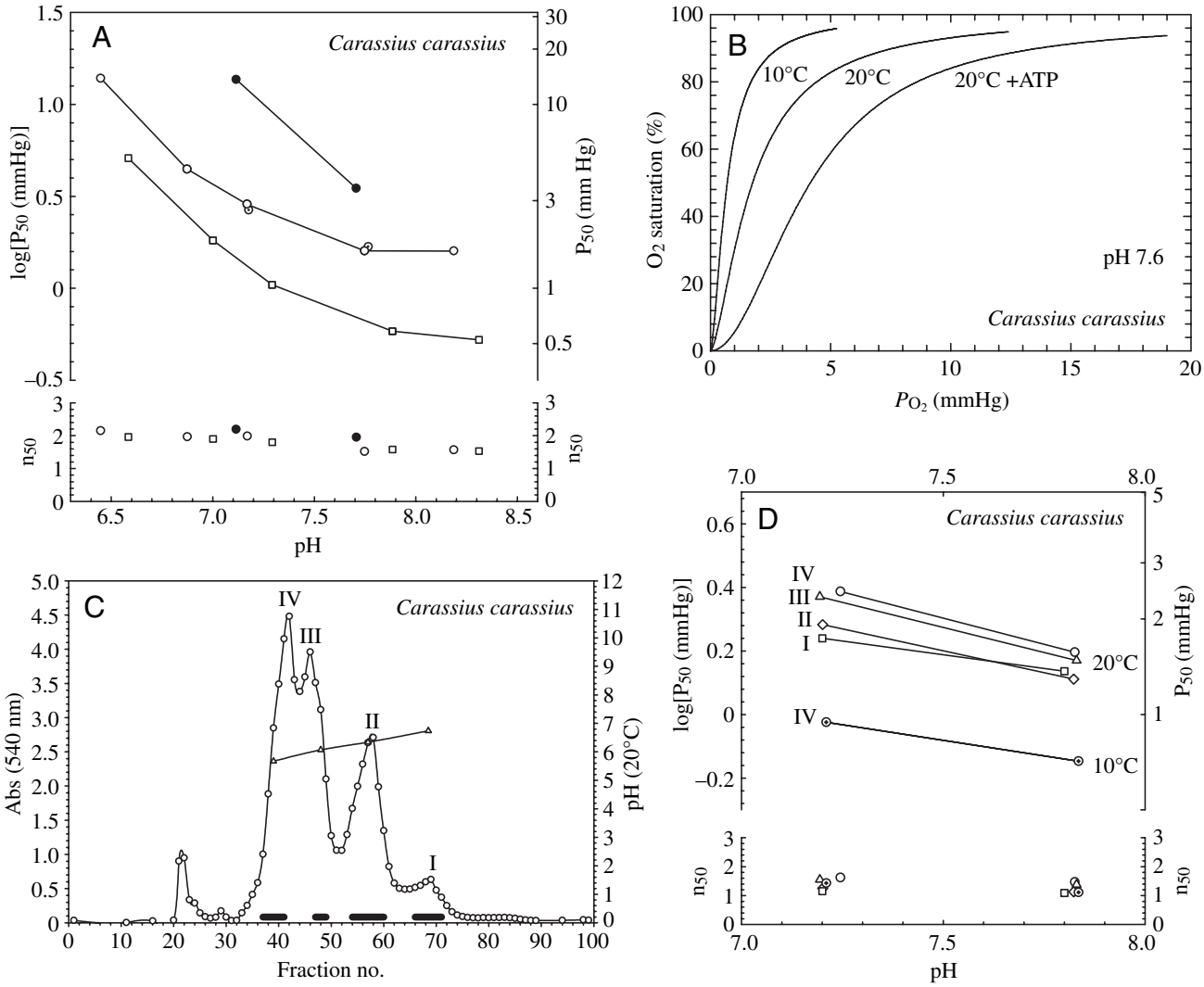

Fig. 4. Oxygen-binding characteristics and isoHb differentiation of crucian carp Hb, measured in the presence of 0.1 mol l^{-1} KCl and 0.1 mol l^{-1} Hepes buffers. (A) Oxygen tensions and Hill's cooperativity coefficients at 50% saturation (P_{50} and n_{50} of stripped hemolysates and their pH dependence (Bohr plots) at 10°C (□) and 20°C (○) and of the lysate in the presence of saturating concentration of ATP (ATP/tetrameric Hb ratio, 9.6), (●), [haem], 0.50 mmol l^{-1}. (B) Oxygen equilibrium curves at 10°C, 20°C and 20°C in the presence of saturating ATP (interpolated from data in A). (C) Isoelectric focusing profile, showing absorptions at 540 nm (○) and pH values at 25°C (△) of eluted fractions, and the presence of three major (II, III and IV) and two minor (I and V) isoHbs. (D) Bohr plots of isoHbs I–IV, at 10 and 20°C.

causing the gill lamellae to protrude, suggests that the actual trigger is the oxygen demand of the fish. Another possibility is that high temperature and hypoxia independently trigger the transformation of the gills.

The increase of respiratory surface area of crucian carp kept at 25°C coincided with a relatively low $[O_2]_{crit}$ at this temperature, indicating an increase in the capacity for oxygen uptake (Fig. 2B,C). This was clearly reflected in the high $\dot{M}_{O_2}$:$[O_2]_{crit}$ ratio of crucian carp kept at 25°C (Table 1). The relationship between temperature, $\dot{M}_{O_2}$ and $[O_2]_{crit}$ of crucian carp observed in the present study resemble that found in a study on goldfish (Fig. 2A–C) in more than half a century ago (Fry and Hart, 1948), which also showed an unexpectedly low $[O_2]_{crit}$ at higher temperatures. These results can now be explained by the present finding that goldfish have protruding lamellae at high, but not low, temperatures. The reason why this transformation of gill morphology has not been observed in goldfish earlier is most likely that goldfish are traditionally kept at rather high temperatures, usually at room temperature.

By contrast, Atlantic cod, a species that presumably does not have the ability to adjust the respiratory surface area to its oxygen needs, shows a linear relationship between $[O_2]_{crit}$ and $\dot{M}_{O_2}$ (Fig. 2C; see also Schurmann and Steffensen, 1997).

The results suggest that the oxygen demand of crucian carp does not trigger a remodelling of the gills unless the water temperature reaches 25°C, which is near the highest temperature that crucian carp normally experiences in its habitat for short periods during the summer months (J. S.,

G. E. N., unpublished observations from the Oslo area). This indicates that gills with non-protruding lamellae are able to supply the crucian carp with sufficient oxygen to sustain aerobic metabolism at 20°C where its $\dot{M}_{O_2}$ is around 120 mg kg^{-1} h^{-1} (Table 1). The capacity to sustain a high $\dot{M}_{O_2}$ with a small respiratory surface area could rely on a high O_2 affinity of the Hb. We measured oxygen affinity in the presence of 0.1 mol l^{-1} KCl, which decreases the oxygen affinity, mimicking the intracellular condition. Our data show that the high oxygen affinity (P_{50}=1.8 mmHg at pH 7.7 and 20°C) increases markedly with falling temperature (P_{50}=0.7 mmHg at 10°C) due to the pronounced temperature sensitivity at high *in vivo* pH (7.7), where the phosphate sensitivity is low (Fig. 4A,B). These properties that appear to characterise all major isoHbs (Fig. 4D) witness a high blood oxygen affinity as previously recorded in goldfish (P_{50}=2.6 mmHg at pH 7.56 and 26°C; Burggren, 1982).

The remodelling of the gills appears to be rapid, since we did not observe any intermediate stages in crucian carp kept at 15°C or 20°C. Thus, it appears to be an 'on/off' response that is triggered either by hypoxia or high temperature, or maybe by their common denominator: an increased demand for oxygen uptake. When we reduced the acclimation temperature for goldfish to 7.5°C, they remodelled their gills to a state with almost no protruding lamellae. Since no intermediate stages were seen in goldfish gills during the 25°C to 15°C transfer, it seems, like in crucian carp, that this is an 'on/off' response that is triggered by either temperature or $\dot{M}_{O_2}$.

Intriguingly, Isaia (1972) showed that the water flux across the goldfish gills increased more than five times from 5 to 25°C, which is much greater than would be expected from a diffusion process. It is tempting to suggest that at least part of this increased water flux was caused by an increase in the respiratory surface area. Indeed, Isaia (1972) suggested that the 'results must indicate either an important change in the branchial permeability during adaptation or the functioning of a greater respiratory surface at an increased temperature'. Moreover, it has been found that the common carp *Cyprinus carpio*, exposed to chronic hypoxia, is able to extract a higher percentage of the available oxygen than normoxic carp (Lomholt and Johansen, 1979). This could imply that the common carp has the ability to alter its respiratory surface area, possibly in a manner similar to that found in its cyprinid cousins: crucian carp and goldfish. Moreover, a capacity for gill remodelling to increase or decrease oxygen uptake and water fluxes may not be limited to cyprinids. A gill morphology characterised by thickened lamellae with epithelial cells being cuboidal or columnar instead of squamous has been seen in juvenile largemouth bass kept at over-wintering temperatures close to 4°C (Leino and McCormick, 1993).

The present data showed that the change from non-protruding to protruding lamellae occurs between 20 and 25°C in crucian carp, and between 7.5 and 15°C in goldfish. This may reflect species or population differences. Each year, the crucian carp we studied face a severely hypoxic and anoxic environment during the long winter period. Hence, they are more dependent on their glycogen stores than goldfish for survival. Thus, saving energy is likely to be a more critical feature for crucian carp. A small respiratory surface area over a large temperature interval will reduce osmoregulatory costs and, thereby, save energy that can be stored for surviving the long winter. There was also an apparent difference in the ability of these two species to handle soft water. The crucian carp population is well adapted to soft water, and do well in Oslo tapwater (20–50 μS cm^{-1}), whereas goldfish did not do well (did not feed and were lethargic) in Oslo tapwater. Upon recommendation from the importer, we increased the conductivity in the goldfish water to 500 μS cm^{-1}, which had a striking positive effect of the welfare of the goldfish. It is possible that these differences in water conductivity could be related to the difference seen in the temperature where gill remodelling takes place between the two species. However, at present we can only speculate.

It has been found previously that crucian carp acclimated to hypoxia has higher $\dot{M}_{O_2}$ than normoxic crucian carp (Johnston and Bernard, 1984). This increase of $\dot{M}_{O_2}$ could be due to increased ventilation rates and/or elevated osmoregulatory costs for having a larger respiratory surface area. Similarly, in the present study, the crucian carp displayed a larger difference in $\dot{M}_{O_2}$ between 20°C and 25°C (Q_{10}=2.9) than between 15 and 20°C (Q_{10}=1.9) (Table 2), which could be explained by the presence of protruding lamellae in the 25°C group, causing elevated osmoregulatory costs. By contrast, ectothermic animals generally show Q_{10} values that fall with increasing temperature (Prosser, 1986; Withers, 1992). Interestingly, between 10–15°C and 15–20°C, the Q_{10} values in goldfish (Fry and Hart, 1948) (Table 2) decrease less than in the crucian carp, which may be explained by the goldfish remodelling its gills at a lower temperature than the crucian carp.

To conclude, the present study shows that both crucian carp and goldfish have the ability to remodel their gills by changing the size of the ILCM between the lamellae. Moreover, the response, which has previously shown to be triggered by hypoxia, can also be triggered by temperature. Thus, at high temperatures both goldfish and crucian carp display gills with clearly protruding lamellae. The remodelling of the gills to gain protruding lamellae is caused by increased apoptosis and cell-cycle arrest in the ILCM (Sollid et al., 2003). In the light of the present results, it is possible that the signals that trigger this change could include both hypoxia and high temperature, or their common denominator: the need for extracting more oxygen from the water. The ability to match the respiratory surface area to oxygen needs may provide a means of reducing water and ion fluxes and, thereby, the osmoregulatory costs. However, our observations suggest that this is a sharp 'on/off' response rather than a graded change, since no intermediate stages are seen except during the short transition from one state to the other. While this transition took several days in hypoxia at 8°C (Sollid et al., 2003), the present study showed that, at 20°C, it could be completed during the few hours that the fish were exposed to hypoxia in the respirometer.

We are grateful to Anny Bang for assistance with the Hb–oxygen-binding measurements. We thank the Research Council of Norway and the Danish Natural Science Research Council for financial support.

References

Aguiar, L. H., Kalinin, A. L. and Rantin, F. T. (2002). The effects of temperature on the cardio-respiratory function of the neotropical fish Piaractus mesopotamicus. *J. Therm. Biol.* **27**, 299-308.

Berman, M., Benesch, R. and Benesch, R. E. (1971). The removal of organic phosphates from hemoglobin. *Arch. Biochem. Biophys.* **145**, 236-239.

Brittain, T. (1987). The Root effect. *Comp. Biochem. Physiol.* **86B**, 473-481.

Burggren, W. W. (1982). 'Air gulping' improves blood oxygen transport during aquatic hypoxia in the goldfish *Carassius auratus*. *Physiol. Zool.* **55**, 327-334.

Butler, P. J. and Taylor, E. W. (1975). Effect of progressive hypoxia on respiration in dogfish (*Scyliorhinus Canicula*) at different seasonal temperatures. *J. Exp. Biol.* **63**, 117-130.

Caldwell, R. S. (1969). Thermal compensation of respiratory enzymes in tissues of goldfish. *Comp. Biochem. Physiol.* **31**, 79-93.

Evans, D. H. (1979). Fish. In *Comparative Physiology of Osmoregulation in Animals*, vol. 1 (ed. G. M. O. Maloiy), pp. 305-349. London: Academic Press.

Fernandes, M. N. and Rantin, F. T. (1989). Respiratory responses of *Oreochromis niloticus* (*Pisces, Cichlidae*) to environmental hypoxia under different thermal conditions. *J. Fish Biol.* **35**, 509-519.

Fry, F. E. J. and Hart, J. S. (1948). The relation of temperature to oxygen consumption in the goldfish. *Biol. Bull.* **94**, 66-77.

Goldspink, G. (1995). Adaptation of fish to different environmental-temperature by qualitative and quantitative changes in gene expression. *J. Therm. Biol.* **20**, 167-174.

Hocutt, C. H. and Tilney, R. L. (1985). Changes in gill morphology of *Oreochromis mossambicus* subjected to heat stress. *Environ. Biol. Fish.* **14**, 107-114.

Houston, A. H. and Cyr, D. (1974). Thermoacclimatory variation in the haemoglobin systems of goldfish (*Carassius auratus*) and rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **61**, 455-461.

Houston, A. H., Dobric, N. and Kahurananga, R. (1996). The nature of hematological response in fish – studies on rainbow trout *Oncorhynchus mykiss* exposed to stimulated winter, spring and summer conditions. *Fish Physiol. Biochem.* **15**, 339-347.

Houston, A. H. and Rupert, R. (1976). Immediate response of the hemoglobin system of the goldfish, *Carassius auratus*, to temperature change. *Canadian J. Zoology-Revue Canadienne De Zoologie* **54**, 1737-1741.

Isaia, J. (1972). Comparative effects of temperature on sodium and water permeabilities of gills of a stenohaline freshwater fish (*Carrassius auratus*) and a stenohaline marine fish (*Serranus scriba, Serranus cabrilla*). *J. Exp. Biol.* **57**, 359-366.

Jacobs, D., Esmond, E. F., Melisky, E. L. and Hocutt, C. H. (1981). Morphological changes in gill epithelia of heat stressed rainbow trout, *Salmo gairdneri* – Evidence in support of a temperature induced surface area change hypothesis. *Can. J. Fish. Aquatic Sci.* **38**, 16-22.

Johnston, I. A. and Bernard, L. M. (1983). Utilization of the ethanol pathway in carp following exposure to anoxia. *J. Exp. Biol.* **104**, 73-78.

Johnston, I. A. and Bernard, L. M. (1984). Quantitative study of capillary supply to the skeletal muscles of Crucian carp *Carassius carassius* L – effects of hypoxic acclimation. *Physiol. Zool.* **57**, 9-18.

Leino, R. L. and McCormick, J. H. (1993). Responses of juvenile largemouth bass to different pH and aluminum levels at overwintering temperatures – effects on gill morphology, electrolyte balance, scale calcium, liver glycogen, and depot fat. *Canadian J. Zoology-Revue Canadienne De Zoologie* **71**, 531-543.

Lomholt, J. P. and Johansen, K. (1979). Hypoxia acclimation in carp – how it affects O-2 uptake, ventilation, and O-2 extraction from water. *Physiol. Zool.* **52**, 38-49.

Maricondi-Massari, M., Kalinin, A. L., Glass, M. L. and Rantin, F. T. (1998). The effects of temperature on oxygen uptake, gill ventilation and ECG waveforms in the nile tilapia, *Oreochromis niloticus*. *J. Therm. Biol.* **23**, 283-290.

Nilsson, G. E. (1990). Long term anoxia in crucian carp – Changes in the levels of amino acid and monoamine neurotransmitters in the brain, catecholamines in chromaffin tissue, and liver glycogen. *J. Exp. Biol.* **150**, 295-320.

Nilsson, G. E. (1992). Evidence for a role of GABA in metabolic depression during anoxia in crucian carp (*Carassius carassius*). *J. Exp. Biol.* **164**, 243-259.

Nolan, D. T., Hadderingh, R. H., Spanings, F. A. T., Jenner, H. A. and Bonga, S. E. W. (2000). Acute temperature elevation in tap and Rhine water affects skin and gill epithelia, hydromineral balance, and gill Na^+/K^+-ATPase activity of brown trout (*Salmo trutta*) smolts. *Can. J. Fish. Aquatic Sci.* **57**, 708-718.

Prosser, C. L. (1986). Temperature. In *Adaptational Biology* (ed. C. L. Prosser), pp. 260-321. New York: John Wiley and Sons.

Schurmann, H. and Steffensen, J. F. (1997). Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *J. Fish Biol.* **50**, 1166-1180.

Shoubridge, E. A. and Hochachka, P. W. (1980). Ethanol – novel end product of vertebrate anaerobic metabolism. *Science* **209**, 308-309.

Shoubridge, E. A. and Hochachka, P. W. (1983). The integration and control of metabolism in the anoxic goldfish. *Mol. Physiol.* **4**, 165-195.

Sollid, J., De Angelis, P., Gundersen, K. and Nilsson, G. E. (2003). Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *J. Exp. Biol.* **206**, 3667-3673.

Tilney, R. L. and Hocutt, C. H. (1987). Changes in gill epithelia of *Oreochromis mossambicus* subjected to cold shock. *Environ. Biol. Fish.* **19**, 35-44.

Weber, R. E. (1981). Cationic control of O_2 affinity in lugworm erythrocruorin. *Nature* **292**, 386-387.

Weber, R. E. (2000). Adaptations for oxygen transport: lessons from fish hemoglobins. In *Hemoglobin Function in Vertebrates, Molecular Adapation in Extreme and Temperate Environments* (ed. G. Di Prisco, B. Giardina and R. E. Weber), pp. 22-37. Milano, Italy: Springer-Verlag.

Weber, R. E., Jensen, F. B. and Cox, R. P. (1987). Analysis of teleost hemoglobin by Adair and Monod-Wyman-Changeux models. Effects of nucleoside triphosphates and pH on oxygenation of tench hemoglobin. *J. Comp. Physiol. B* **157**, 145-152.

Wells, R. M. G. and Weber, R. E. (1989). The measurement of oxygen affinity in blood and haemaglobin solutions. In *Techniques in Comparative Physiology* (ed. C. R. Bridges and P. J. Butler), pp. 279-303. Cambridge: Cambridge Univerisity Press.

Withers, P. C. (1992). Temperature. In *Comparative Animal Physiology* (ed. P. C. Withers), pp. 122-191. New York: Saunders College Publishing.