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Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills

Jørund Sollid[1,*], Paula De Angelis[2], Kristian Gundersen[1] and Göran E. Nilsson[1]

[1]*Division of General Physiology, Department of Biology, University of Oslo, PO Box 1051, 0316 Oslo, Norway* and [2]*Institute of Pathology, Norwegian National Hospital, Oslo, Norway*

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

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Summary

We show that crucian carp (*Carassius carassius*) living in normoxic (aerated) water have gills that lack protruding lamellae, the primary site of O_2 uptake in fish. Such an unusual trait leads to a very small respiratory surface area. Histological examination showed that the lamellae (secondary lamellae) of these fish were embedded in a cell mass (denoted embedded lamellae). When the fish were kept in hypoxic water, a large reduction in this cell mass occurred, making the lamellae protrude and increasing the respiratory surface area by ~7.5-fold. This morphological change was found to be reversible and was caused by increased apoptosis combined with reduced cell proliferation. Carp with protruding lamellae had a higher capacity for oxygen uptake at low oxygen levels than fish with embedded lamellae, but water and ion fluxes appeared to be increased, which indicates increased osmoregulatory costs. This is, to our knowledge, the first demonstration of an adaptive and reversible gross morphological change in the respiratory organ of an adult vertebrate in response to changes in the availability of oxygen.

Key words: crucian carp, *Carassius carassius*, gill, secondary lamellae, morphology, hypoxia, apoptosis.

Introduction

In vertebrates, the need for gas exchange forces the animal to expose its interior milieu to the external world. For animals breathing with lungs, the large respiratory surface area can be problematic with respect to infection, heat and water loss. Fish respirating in seawater lose water over the gills to the hyperosmotic environment, while freshwater fish accumulate water and lose salts owing to the gradients between the animal and the environment (Maloiy, 1979). Large water uptake results in high rates of dilute urine excretion, whereas ion losses must be compensated for by energetically costly ion pumping. The structure of the respiratory organ is therefore likely to be a compromise between the need for gas exchange and a desire to minimise ion and water fluxes.

The crucian carp (*Carassius carassius*) is a North European freshwater fish that often inhabits small ponds that, due to ice coverage, become hypoxic and finally anoxic for several months every winter (Holopainen et al., 1986). Its exceptional hypoxia and anoxia tolerance make it the sole fish species in this habitat. In both Norwegian and Swedish populations of this carp, we have observed a lack of protruding lamellae, the respiratory units in fish (sometimes denoted secondary lamellae). This is a highly exceptional feature in fish since the lamellae are the primary site for gas exchange, making up most of the respiratory surface area of fish gills. However, a favourable aspect of a small gill area would be reduced water influx and ion losses.

The haemoglobin of *Carassius* has an extremely high affinity for O_2; a P_{50} for O_2 of 0.347 kPa has been measured in goldfish (*Carassius auratus*; Burggren, 1982). This may explain why, under normoxic conditions, crucian carp do not need a large respiratory surface area. Thus, the lack of protruding lamellae could be a morphological adaptation for reducing water and ion fluxes. However, such an unusual trait should limit the ability of the crucian carp to cope with falling environmental O_2 conditions, unless relatively rapid morphological changes can take place.

Here, we present evidence that such a change is actually taking place in the gills of crucian carp when exposed to severe hypoxia. The change is reversible, and we suggest that this plasticity is an adaptive mechanism that promotes aerobic metabolism under hypoxic conditions and reduces problems related to osmoregulation under normoxic conditions.

Materials and methods

Animals

Crucian carp (*Carassius carassius* L.; weighing 10–30 g; all adults) were caught in June 2000 and 2001 in the Tjernsrud pond, Oslo community. They were kept in tanks continuously supplied with aerated and dechlorinated Oslo tap water (8°C) and fed daily with commercial carp food (Tetra Pond, Tetra, Melle, Germany). The fish were not fed during experiments. The mean mass of the fish in the different exposure groups was

not significantly different (P>0.1). After exposures, the fish were killed with a sharp blow to the head.

Hypoxia exposure

During hypoxia exposure, the fish were kept in dark 25-litre plastic tanks continuously supplied with N_2-bubbled dechlorinated Oslo tap water. The oxygen level was monitored with an oxygen electrode (WTW, Weilheim, Germany), which also controlled a valve that varied the rate of N_2 bubbling according to the desired level of hypoxia. The water temperature was 8°C. The fish were exposed to hypoxia for up to 14 days. Fish were taken out for sampling at 0, 1, 3, 7 and 14 days of exposure. The O_2 level was kept at 0.75±0.15 mg l^{-1} (6–8% of air saturation). This is slightly below their critical O_2 concentration ($[O_2]_{crit}$), which is the level of O_2 where the fish can no longer meet their energy requirements through aerobic metabolism alone (Beamish, 1964). $[O_2]_{crit}$ of crucian carp is approximately 1.0 mg l^{-1} at 8°C (Nilsson, 1992). A group of fish were put back into normoxia for 7 days after 14 days of hypoxia exposure and were then sampled. Control fish (and reoxygenated fish) were kept in an identical tank, which was supplied with aerated water rather than N_2-bubbled water. $[O_2]$ in this tank was 10–11 mg l^{-1}. The hypoxia experiments where done from March to October 2000 and 2001.

Scanning and transmission electron microscopy (SEM and TEM)

Gills were fixed in 3% glutaraldehyde in 0.1 mol l^{-1} Na-cacodylate buffer. For SEM, the gills were dehydrated and dried with Blazers Critical Point Drier (Hypervision, Fremont, USA) before being AuPd coated with an SEM coating unit (E5000; Polaron Equipment Limited, Watford, UK). For TEM, the gills were post-fixated in 2% osmium in 0.1 mol l^{-1} Na-cacodylate buffer with 1.5% potassium-ferri-cyanide before *en bloc* incubation in 1.5% uranylacetate. The tissue samples were then dehydrated before being embedded in Epoxy embedding medium (Fluka, Buchs, Switzerland). The microscopes used were a JSM 6400 from JEOL (Peabody, USA) for SEM and a CM100 from Phillips (Potomac, USA) for TEM.

Morphometry

The area of the portion of the lamellae in contact with water was calculated. To do this, three measurements were taken on randomly selected gill filaments from four normoxic and four hypoxic fish (see Fig. 1; lowercase letters denote measurements in normoxia, and uppercase letters denote measurements in hypoxia): (1) the basal length of the part of the lamellae in contact with water was measured using SEM (l and L, respectively); (2) the mean height of the lamellae in contact with water was measured using light microscopy on the sections obtained in the BrdU and TUNEL experiments (see below); the latter was done on cross-sections where one-third of the measurements were done when the central venous sinus was visible and two-thirds were done when it was not; thus, one-third were from the central portion of the lamellae

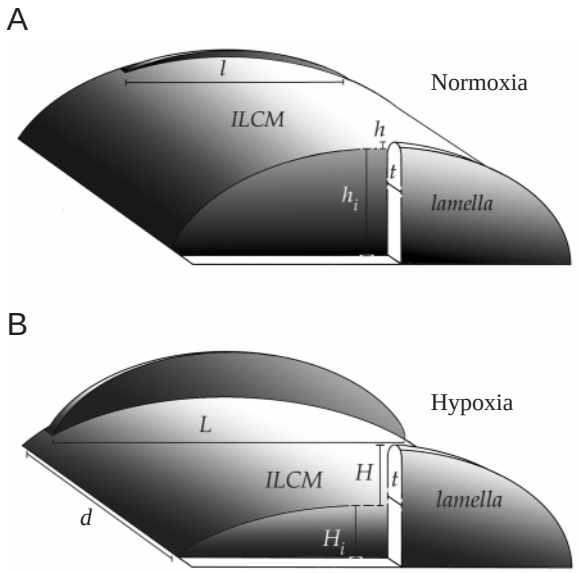

Fig. 1. Graphic illustration of the sites where lamellar diameter (d), height of protruding lamellae (h and H) and basal length of protruding lamellae (l and L) were measured in (A) normoxic and (B) hypoxic fish. Lowercase letters denote measurements in normoxia, and uppercase letters denote measurements in hypoxia. ILCM, interlamellar cell mass (see text for explanation).

(h_c and H_c, respectively) and two-thirds were from the edges (h_e and H_e, respectively); (3) the mean lamellae thickness (t) was also measured using light microscopy on BrdU and TUNEL sections.

The area of the normoxic lamella (a) was approximated by calculating the lamellar tip area, $a=pl$, where l is the protruding basal length and p is the ellipse perimeter formula divided by two:

$$p = \frac{2\pi\sqrt{\frac{1}{2}(r^2 + h^2)}}{2}, \quad (1)$$

where $r=t/2$ and $h=(2h_e+h_c)/3$.

The total lamellar area in hypoxia (A) was approximated by multiplying the mean protruding height minus the mean height of the lamella tip (obtained in the measurements of normoxic lamellae) by the mean basal length and finally adding the area of the lamellar tip:

$$A = pL + 2L(H - h), \quad (2)$$

where $H=(2H_e+H_c)/3$. The approximate volume (V) of the interlamellar cell mass (ILCM; see below for explanation) was obtained by measuring the distance between two adjacent lamellae (d) and multiplying by the measured height of the ILCM (h_i or H_i) and the lamellar basal length in hypoxia (L):

$$V = dh_iL, \quad (3)$$

where $h_i=(2h_{i,e}+h_{i,c})/3$.

Note that most histological procedures are likely to distort the tissue, and comparisons between studies should be made with caution.

Respirometry

Fish kept in normoxia (with embedded lamellae) were compared with fish exposed to hypoxia (with protruding lamellae). The latter group had been exposed to hypoxia for 7 days and then kept in normoxic water for 24 h to minimise the possibility that the fish suffered from hypoxia-induced energy deficiency, even if this is unlikely for crucian carp. The rate of O_2 consumption ($\dot{V}_{O_2}$) during falling water [O_2] was measured with closed respirometry, and the $[O_2]_{crit}$ was determined as described previously (Nilsson, 1992).

High salinity exposure

Fish with embedded and protruding lamellae were obtained as described above for the respiration experiment. Fish were then subjected to normoxic saline waters (containing 50% seawater and 50% Oslo tap water, giving a salinity of 16 p.p.m.) for 0, 1.5 and 6 h. Blood samples were taken from the caudal vessels by heparinised 1 ml syringes. The blood plasma chloride content was analysed using a CMT10 Chloride Titrator (Radiometer, Lyon, France).

Measurement of mitotically active cells by 5′-bromodeoxyuridine (BrdU)

BrdU has previously been successfully used to stain mitotically active cells (cells in the S phase) in fish gills (Tsai and Hwang, 1998; Uchida and Kaneko, 1996). BrdU was injected intraperitoneally at a dose of 100 μg g^{-1}. After 24 h, the fish were killed with a sharp blow to the head, and the gills were dissected out, fixated in 4% formaldehyde in 0.1 mol l^{-1} phosphate-buffered saline (PBS) and embedded in liquid paraffin before being serially cross-sectioned (thickness, 2 μm). One slide from each fish was rehydrated before being placed in 2 mol l^{-1} HCl for 30 min. They were quenched in 3% H_2O_2 and subsequently incubated for 30 min in 5% blocking solution (denoted BS; 5% bovine serum albumin and 0.3% Triton X-100 in PBS, pH 7.2). 65 μl of anti-BrdU mouse IgG, diluted 1:5 in 0.5% BS (BS diluted 10× with PBS, pH 7.2) were applied and incubated for 1 h in a humidified chamber. 70 μl of biotinylated goat anti-mouse IgG, diluted 1:20 in 0.5% BS, were applied and incubated for 30 min in a humidified chamber. 70 μl of peroxidase conjugate, diluted 1:20 in 0.5% BS, were applied and incubated for 30 min in a humidified chamber. The slides were washed three times with PBS (pH 7.2). 65 μl of diaminobenzidine (DAB) solution (6 mg DAB, 10 ml PBS and 30 μl 30% H_2O_2) were then applied for 3–10 min before the slides were washed, put briefly (3–5 s) in haematoxylin and mounted. Between all the steps, from placing in 2 mol l^{-1} HCl to incubating with peroxidase conjugate, a double wash with PBS/saponin (1 g saponin:l ml PBS) was used. All solutes were from Sigma-Aldrich (St Louis, USA), except anti-BrdU (Becton Dickinson, Franklin Lakes, USA). Sections from the gastrointestinal tract of the crucian carp were used as positive controls. The staining pattern agreed well with what is known about gastrointestinal cell proliferation in the tract (not shown). As a negative control, BrdU-containing sections from normoxic fish were used. The sections were incubated without anti-BrdU. The pre- and post-treatments were conducted as above. This test did not show any non-specific binding of the secondary or tertiary antibodies.

Detection of apoptotic cells using TdT-dUTP-nick end labelling (TUNEL)

An ApopTag®Plus Peroxidase *In Situ* apoptosis detection kit from Intergen (Temecula, USA) was used. No modifications of the protocol included in the kit were necessary. The gills were dissected, fixed, embedded and sectioned as described in the BrdU method. The sections were counterstained with haematoxylin before being mounted. In the rodent mammary gland, extensive apoptosis occurs 3–5 days after weaning of rat pups. Sections of this tissue were included in the kit and were used as positive controls. 1–2% of the total number of cells on the slides was apoptotic, as predicted in the kit instructions. A section from the mammary gland and a section from the gills were used as negative (substitution) controls. This was done by excluding the TdT but including the proteinase K digestion to control for non-specific incorporation of nucleotides or for non-specific binding of enzyme conjugate. Equilibration buffer was substituted for the volume of TdT enzyme reagent. No stained cells were seen in the negative controls. The TUNEL method distinguishes apoptosis from necrosis by specifically detecting DNA cleavage and chromatin condensation associated with apoptosis. However, there were some instances where cells exhibiting necrotic morphology were diffusely stained. Therefore, stained cells were only counted if they showed morphological criteria consistent with apoptosis (nuclear condensation, cell shrinkage and apoptotic bodies). This was assessed using light microscopy. Initially, TEM was used to confirm apoptosis morphologically (Fig. 2) in a few sections. The number of stained cells without apoptotic morphology was below 1% of the total number of stained gill cells in hypoxia-exposed fish, clearly indicating that apoptosis, and not necrosis, is involved in reducing the ILCM.

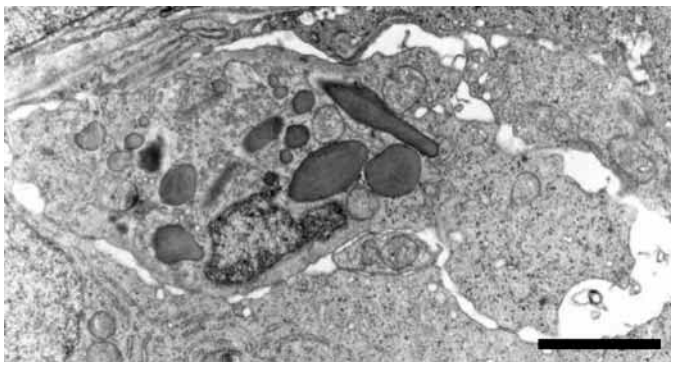

Fig. 2. Transmission electron micrograph of a typical apoptotic cell in the interlamellar cell mass, displaying cell shrinkage and nuclear fragmentation. Scale bar, 2 μm.

Counting of cells

Stained (BrdU and TUNEL) and non-stained cells in the ILCM (all cells between two adjacent lamellae) were counted in randomly selected areas on the sections by two persons to a total number of 500–600 cells per fish in all immunohistochemical analyses, giving between 15 and 25 ILCMs per fish.

Statistics

All data were analysed with a non-parametric Kruskal–Wallis test with Dunn's test as a post-test, except for morphometry, which was analysed with an unpaired Student's *t*-test. The data were analysed by the program GraphPad InStat (GraphPad, San Diego, USA).

Results and discussion

Morphology

The exposure to hypoxia triggered a striking change in gill morphology (Fig. 3): the gills displayed protruding lamellae. The change was visible after 1 day of hypoxia and reached its greatest extent after 7 days. No further alteration was apparent after a further 7 days of hypoxia (Fig. 3A–E). The change was completely reversible, since, after an additional week in normoxia, the gills were indistinguishable from their initial state, showing no protruding lamellae (Fig. 3F). The changes occurred on all four gill arches and over the whole length of the gill filament. Moreover, the response was seen during the whole experimental period (March–October).

Morphometry

Light microscopic examination of serially cross-sectioned gill arches showed that the lamellae were also present in normoxia but that the space between the lamellae was then completely filled by an interlamellar cell mass (ILCM). After 7 days in hypoxia, the mean area of the portion of the lamellae that was in contact with water increased by ~7.5-fold, from 1195 μm^2 per lamella in normoxia to 8898 μm^2 per lamella in hypoxia (Table 1). This resulted from the reduction of the ILCM, which was reduced by ~52% from 2.01×10^5 μm^3 per interlamellar space in normoxia to 9.79×10^4 μm^3 in hypoxia.

The reduction of the ILCM and the resultant exposure of a much larger lamellar area to water will greatly increase the part of oxygen transfer aided by convective water movements. However, some oxygen can be expected to diffuse through the ILCM to reach the blood inside the lamellae. We do not know how important this route is but it is likely to contribute to some of the oxygen uptake, especially in carp with embedded lamellae. However, in those fish, it is possible that most of the O_2 is taken up by erythrocytes passing through the outer marginal channel, which runs immediately inside the edge of the lamellae of fish gills (Laurent and Dunel, 1980). In the carp with embedded lamellae, about half of the lamellae had their outer edges not covered by ILCM cells. Fishes appear to have the ability to redirect blood flow from the lamellar sheet to the outer marginal channel (Stensløkken et al., 1999) and such a mechanism could be in operation in carp with embedded lamellae. Still,

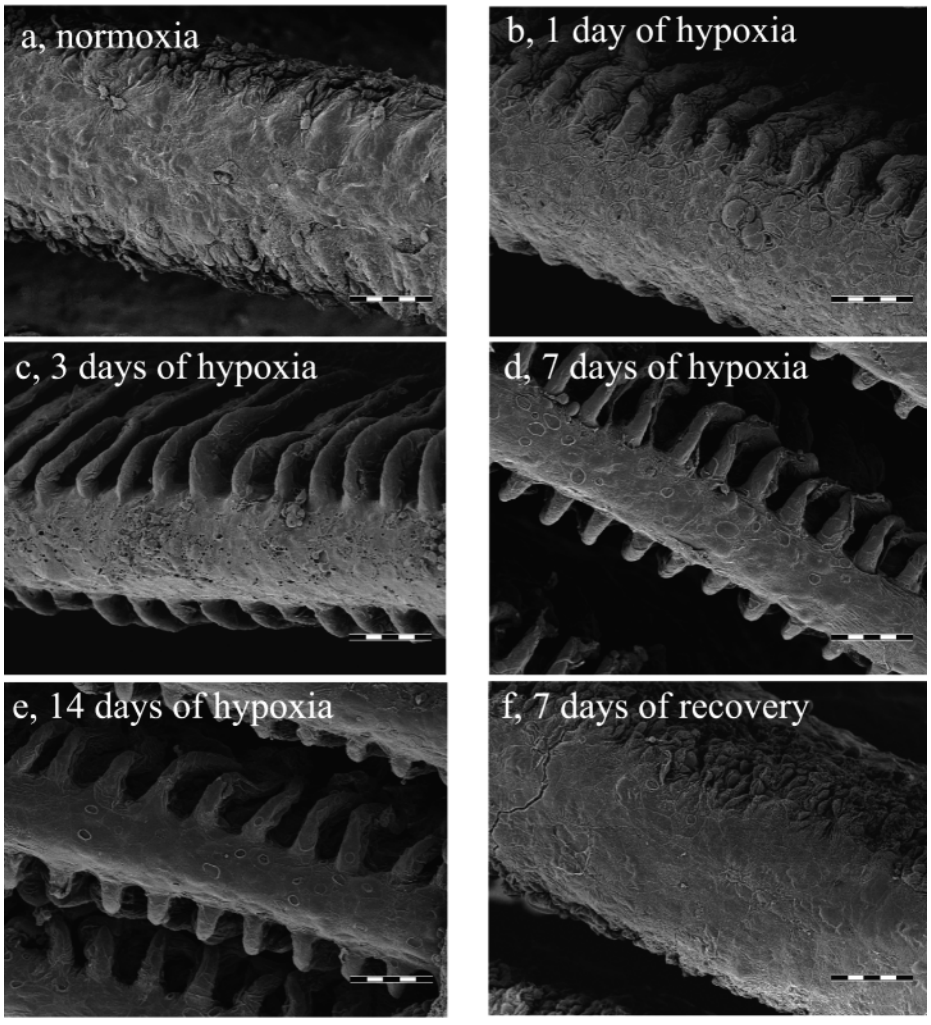

Fig. 3. Scanning electron micrographs from the 2nd gill arch of crucian carp kept in normoxic or hypoxic water. (a) In normoxia, the gill filaments have no protruding lamellae. (b) The morphology has already changed after 1 day of hypoxia exposure (0.75 ± 0.15 mg O_2 l^{-1}). (c,d) The change progresses for up to 7 days in hypoxia, but (e) there were no further changes with subsequent exposure. (f) When the fish were moved to normoxic water, the morphological changes were reversed within 7 days. Scale bar, 50 μm.

Table 1. *Height, length and surface are of the protruding lamellae, and ILCM height and volume, in fish in normoxia and 7 days of hypoxia*

	Morphometry	
	Normoxia	Hypoxia
Protruding lamellar height	4.7±0.1 (*N*=45) (*h*)	33.4±1.1 (*N*=54) (*H*)
Protruding lamellar basal length	91.8±1.4 (*N*=30) (*l*)	118.2±0.6 (*N*=21) (*L*)
ILCM height	55.8±2.2 (*N*=45) (h_i)	27.1±1.2 (*N*=54) (H_i)
Protruding lamellae area	1195 μm^2	8898 μm^2
ILCM volume	$2.01*10^5$ μm^3	$9.79*10^4$ μm^3

Values are means ± S.E.M. The lamellar thickness (*t*) was 7.1±0.2 μm (*N*=30), and the distance between two adjacent lamellae (*d*) was 29.1±0.3 μm (*N*=31). All differences between normoxia and hypoxia were significant ($P<0.0001$).

the increased lamellar area must significantly increase the capacity for oxygen uptake, and it is also likely to increase water and ion fluxes between water and blood. Indeed, such effects were clearly indicated by the subsequent experiments described below.

Respiration

We hypothesised that this large increase in the respiratory surface area was beneficial for the ability to take up O_2 in hypoxia. Indeed, our closed respirometry experiment showed that the group with protruding lamellae had a 50% lower $[O_2]_{crit}$ (0.5±0.1 mg l^{-1}) compared with that of the group with embedded lamellae (1.0±0.1 mg l^{-1}; $P<0.01$; Table 2). The normoxic rate of O_2 consumption ($\dot{V}_{O_2}$) was 96±8 mg kg^{-1} h^{-1} in fish with embedded lamellae and 84±7 mg kg^{-1} h^{-1} in fish with protruding lamellae. These rates were not significantly different ($P>0.05$), which indicates that the difference in $[O_2]_{crit}$ was not caused by differences in $\dot{V}_{O_2}$. Thus, we attribute the lower $[O_2]_{crit}$ in fish with protruding lamellae to their larger respiratory surface area. However, the presumed increase in costs for osmoregulation in crucian carp with protruding lamellae was apparently not large enough to be detected in this experiment. This may relate to the relatively large variability in $\dot{V}_{O_2}$. Osmoregulatory costs in fish are thought to account for approximately 10% of the energy budget (Boeuf and Payan, 2001), and a reduction or increase in this figure may therefore not be readily detected with respirometry. Another possibility is that the fish recently exposed to hypoxia are metabolically depressed, and this could mask an increase in $\dot{V}_{O_2}$ related to increased osmoregulation costs.

Our data indicate that the crucian carp can take advantage of the very high ability of its haemoglobin to extract oxygen in such a way that it can reduce its gill surface under normoxic conditions without depressing $\dot{V}_{O_2}$ and, in this way, reduce osmoregulatory costs.

Since the crucian carp is an exceptionally anoxia-tolerant fish, one may argue that it would not need to increase its capacity for oxygen uptake when faced with hypoxia. However, in anoxia, crucian carp produce ethanol as the main anaerobic end product (Nilsson, 2001). The ethanol is lost to the water, making this an energetically wasteful strategy. Thus, it should be advantageous for it to avoid utilising anaerobic ATP production for as long as possible, particularly since its survival during anoxia is ultimately limited by the total depletion of its glycogen stores (Nilsson, 1990).

There is a report indicating that juvenile largemouth bass overwintering in 4°C have gills with thickened primary epithelia

Table 2. *Oxygen consumption ($\dot{V}_{O_2}$) and critical oxygen tension ($[O_2]_{crit}$) in crucian carp with embedded lamellae and protruding lamellae*

	Respiratory measurements	
Groups	$\dot{V}_{O_2}$ (mg O_2 kg^{-1} h^{-1})	$[O_2]_{crit}$ (mg O_2 l^{-1})
Fish with embedded lamellae	96±8	1.0±0.1*
Fish with protruding lamellae	84±7	0.5±0.1*

Values are means ± S.E.M. of eight fish. Asterisks denote significant difference between the groups ($P<0.01$).

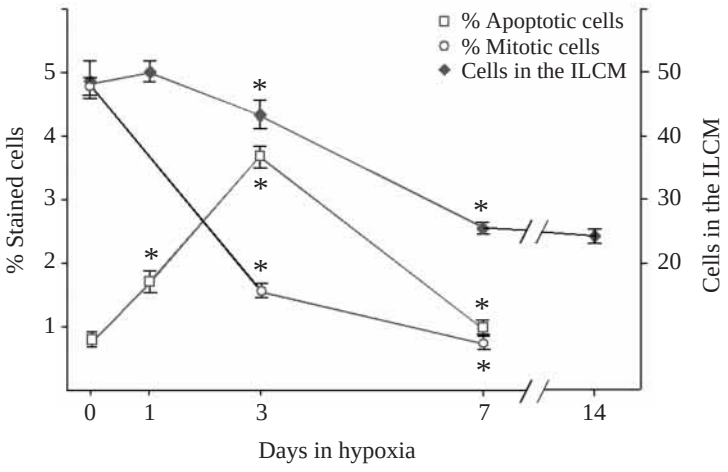

Fig. 4. The percentage of apoptotic and S-phase cells (left *y*-axis) and the total cell number in a central cross-section of the interlamellar cell mass (ILCM; right *y*-axis) during hypoxia exposure. Values are means ± S.E.M. from six fish. Values significantly different from previous values are marked with an asterisk ($P<0.01$).

Table 3. *Effect of high salinity (16 p.p.m.) exposure for different time periods on blood plasma chloride concentration (mmol l^{-1})*

	Salinity exposure		
Groups	0 h	1.5 h	6 h
Fish with embedded lamellae	99.0±1.3[a]	95.8±1.1[a]	160.6±4.8[c]
Fish with protruding lamellae	89.2±0.9[b]	97.1±0.8[a]	189.5±5.0[d]

Values are means ± S.E.M. of 10 fish. Groups with different letters (a–d) are significantly different (P<0.01).

(Leino, 1993). This may be linked to reduced metabolic needs of the fish, which is torpid at this temperature. Thus, it is possible that several fish species utilise morphological alterations of the respiratory surface area as a strategy to minimise ion loss and water influx at times of low oxygen needs. It could also be mentioned that hypoxia may influence gill morphology in developing fish larvae (McDonald and McMahon, 1977).

Osmoregulation

We hypothesised that embedded lamellae might be beneficial to the osmoregulatory ability of the fish. Plasma [Cl^-] of saline-exposed fish with embedded lamellae was therefore compared with that in fish with protruding lamellae (Table 3). The groups already differed in the control blood samples (before exposure to hypersaline water), since [Cl^-] of the blood plasma was significantly lower in the group with protruding lamellae compared with the group with embedded lamellae (Table 3; P<0.01). This indicates that when the crucian carp develop protruding lamellae they do not fully compensate for the presumably increased water influx and/or ion loss over the enlarged gill surface. After 1.5 h of hypersaline exposure, there was a significant increase in the blood plasma Cl^- content of the group with protruding lamellae (P<0.01), while the Cl^- content of the embedded lamellae group was unaltered (Table 3; P>0.05). Exposure to saline water for 6 h increased the Cl^- blood plasma content significantly in both groups, but the increase was significantly higher in the group with protruding lamellae (Table 3; P<0.01). All these data suggest that an increased respiratory surface area has a significant effect on water and ion fluxes and probably increases the cost of the ion pumping needed to maintain homeostasis.

Cell proliferation and apoptosis

We next proceeded to investigate the cellular mechanisms underlying the

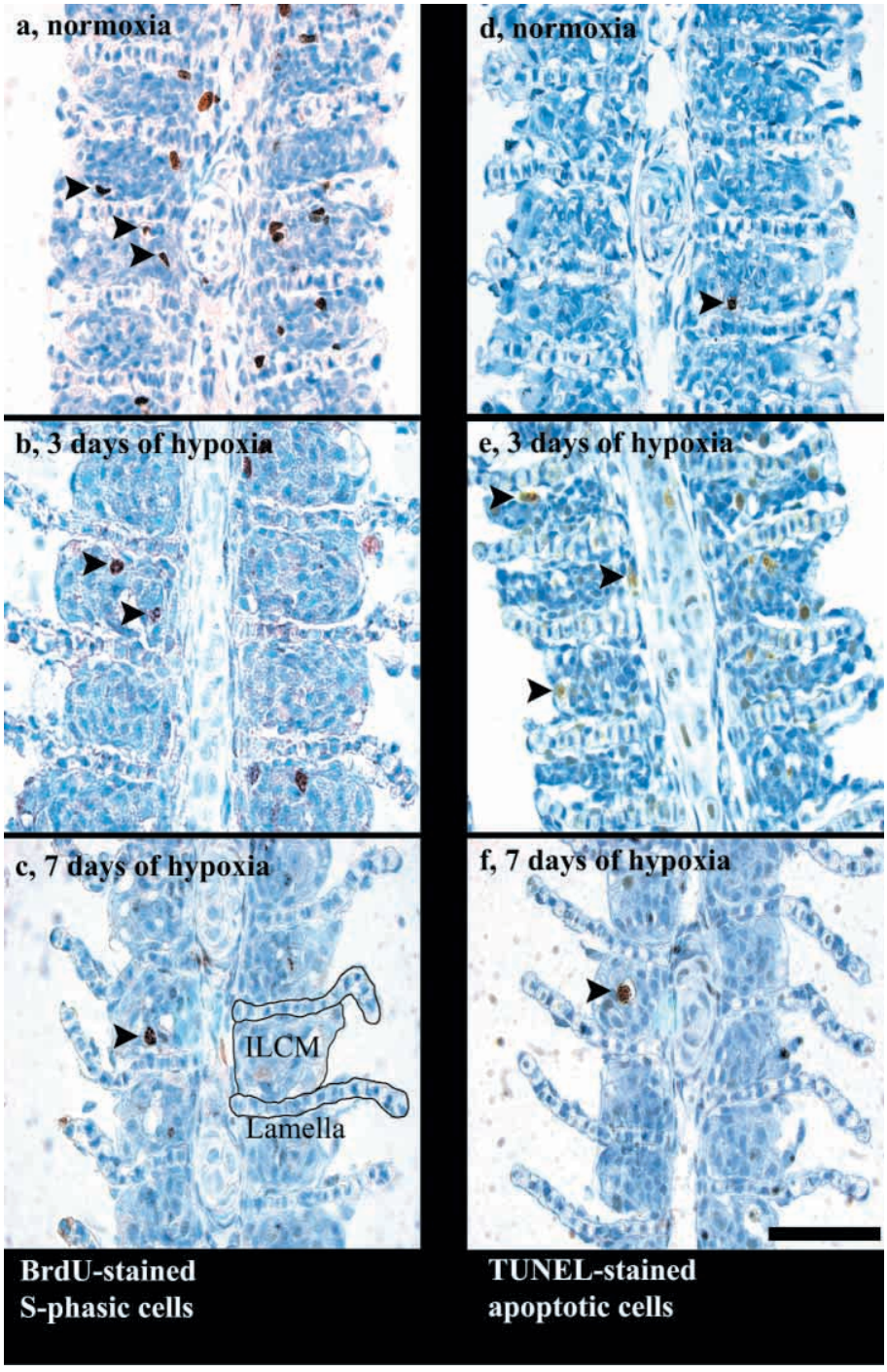

Fig. 5. Light micrographs of gills stained for (a–c) S-phase cells (BrdU) and (d–f) apoptotic cells (TUNEL). Picture series starts with normoxia (a,d), 3 days of hypoxia (b,e) and 7 days of hypoxia (c,f). Arrows point out some of the stained cells seen on the micrographs. ILCM, interlamellar cell mass. Scale bar, 50 μm.

morphological changes. The total number of ILCM cells in each interlamellar space, counted in the mid one-third of the filaments from the 2nd gill arch, fell from 50±2 to 26±1 after 7 days of hypoxia (Fig. 4; $P<0.001$). From 7 to 14 days of hypoxia, the number of cells did not decrease significantly ($P>0.05$), confirming the chronicle of change seen in the SEM micrographs (Fig. 3D,E).

To further examine the dynamics of the ILCM, we investigated the mitotic activity by injecting the fish with BrdU, a synthetic analogue of thymidine, and subsequently detecting cells in the S phase with an antibody against BrdU (Alfei et al., 1993; Tsai and Hwang, 1998; Uchida and Kaneko, 1996). Under normoxic conditions or in fish exposed to hypoxia for 3 or 7 days, BrdU-labelled cells were rarely found in the lamellar epithelia, suggesting that hypoxia has little effect on cell proliferation in the lamellae *per se*. By contrast, ILCM cells showed mitotic activity under all conditions (Fig. 4A–C). In fish kept in normoxic water, S-phase cells made up 4.82±0.15% of the total cell number in the ILCM (Fig. 5). This fraction decreased significantly after 3 days (1.42±0.12%; $P<0.01$) and 7 days (0.58±0.07%; $P<0.01$) of hypoxia exposure, suggesting that reduced cell proliferation in the ILCM is involved in reducing the size of this tissue and thereby causing the lamellae to protrude after exposure to hypoxia.

Next, we examined whether apoptosis could be involved in reducing the ILCM during hypoxia. TUNEL was used to visualize apoptotic cells (Gavrieli et al., 1992). Apoptosis was rarely found in the lamellar epithelia, again suggesting that this tissue was relatively stable. By contrast, the ILCM displayed a significant apoptotic activity (Fig. 4D–F). Thus, under normoxic conditions, 0.78±0.09% of the cells in the ILCM were TUNEL labelled (Fig. 5). After 1 day of exposure to hypoxia, the number of labelled ILCM cells was already significantly increased (1.69±0.17%; $P<0.01$). The apoptotic activity peaked after 3 days in hypoxia (3.79±0.13%; $P<0.01$). Subsequently, the incidence of apoptosis fell and, after 7 days of hypoxia, the degree of apoptosis was down to pre-hypoxic levels (0.98±0.09%; $P>0.05$ compared with normoxia). Thus, apoptosis was most frequent during the time when the ILCM was undergoing a reduction in size, suggesting that apoptosis in the ILCM is involved in reducing the size of this tissue, thereby causing the lamellae to protrude after exposure to hypoxia.

Conclusions

We conclude that the morphological change occurring after exposure to hypoxic conditions is caused by a combination of reduced cell proliferation and an induction of apoptosis. To our knowledge, these results are the first evidence of hypoxia-induced apoptosis in fish. Interestingly, S-phase and apoptotic cells were spread throughout the ILCM (Fig.4A–F), indicating that factors such as type of cell or position in cell cycle, rather than localisation, decide the fate of the ILCM cell. At present, we do not know if this morphological hypoxia response occurs in all crucian carp populations. So far, we have seen crucian carp with embedded lamellae in two populations some 500 km apart: Uppsala in Sweden and Oslo in Norway.

A likely evolutionary reason for having the capacity to alter the respiratory surface area is that it will make the fish able to minimise water and ion fluxes and maybe also reduce the risk of infections and uptake of toxic substances.

The ability of the ILCM cells to rapidly alter their apoptotic and mitotic activity could make them interesting models for studying cellular mechanisms involved in responses to different oxygen levels, particularly if they, like other gill cells (Pärt et al., 1993), can be cultured.

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