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Commentary

Gill remodeling in fish – a new fashion or an ancient secret?

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Summary

While a large respiratory surface area is good for gas exchange, it also poses several problems, including energetically unfavorable fluxes of water and ions. As a result, fishes appear to have a respiratory surface area that is matched to their oxygen demands. When faced with changes in their need for oxygen uptake, e.g. through altered physical activity or altered ambient oxygen levels, fishes have long been known to make two different adjustments: (1) to change the water flow over the gills or (2) to change the blood flow inside the gills. It has recently become clear that at least some teleosts have a third option: to reversibly remodel the gill morphology. Studies have shown that the lamellae of crucian carp *Carassius carassius* gills are embedded in a cell mass during normoxic conditions or at low temperature, while much of this cell mass dies off in hypoxia and at higher temperatures, thereby exposing a much larger respiratory surface area. Gill remodeling has subsequently been seen in two more cyprinids and in the mangrove killifish *Kryptolebias marmoratus*. In the latter case it appears to be an adaptation to periods of air exposure. Gill remodeling in response to changing respiratory requirements could be an ancient mechanism, occurring in many more teleosts than presently known. It is tempting to suggest that gill remodeling has been overlooked in many fishes, either because it is relatively subtle in some species, or because fishes are often kept at the warmer end of their temperature range where they need fully protruding lamellae.

Key words: crucian carp, hypoxia, respiration.

Pros and cons of having gills

The lamellae of the gills are the primary site for oxygen uptake in fish (Evans et al., 2005, for review). In his classical study, Gray (Gray, 1954) found that the total lamellar area (or gill area as he called it) in relation to body mass differed by more than an order of magnitude in 31 species of marine teleosts, with sluggish species having the smallest lamellar area and highly active fishes having the largest. Since then, numerous studies have confirmed the correlation between a large respiratory surface area and the requirement for high rates of oxygen uptake in various species of fish (e.g. Bernal et al., 2001). Similarly, hypoxia tolerance has been correlated with a large lamellar area in fishes (e.g. Fernandes et al., 1994; Chapman et al., 2000; Chapman and Hulen, 2001; Chapman et al., 2002). It is important to note that these relationships do not only show that fishes with high oxygen needs must have large lamellar surface areas. They simultaneously reveal that there must be selection pressures against a large respiratory surface area in less active or less hypoxia tolerant species, implying that there are significant drawbacks with having large gills.

Indeed, several problems are likely associated with a large lamellar surface area:

(1) ‘The osmorespiratory compromise’, which emphasizes that the gills are the main sites for water and ion fluxes, and that an increase in the respiratory surface area results in elevated fluxes that have to be counteracted by energetically expensive ion pumping (e.g. Nilsson, 1986; Gonzalez and McDonald, 1992). Indeed, estimated energetic costs for ion-regulation/osmoregulation in fish varies between 10 and 50% of the energy budget of the animal (Bæuf and Payan, 2001).

(2) The water may contain toxic substances and a large lamellar surface area may facilitate the entrance of many of these into the fish. Naturally occurring substances such as ammonia, algal toxins and metal ions may have provided a selection pressure for small gills for millions of years, and since the industrial revolution, manmade pollutants may provide additional selection pressures for small gills (Wood, 2001).

(3) Pathogens that enter the fish over the gills and parasites that attach to the gills should make it advantageous to have small gills.

(4) In fish, the whole cardiac output has to pass through the gills, making hemorrhage from gill injury immediately life threatening (Sundin and Nilsson, 1998a). Large gills are likely to be more fragile than small ones, so having large gills probably increases the risk of dying from gill injury.

(5) Since the gills take up space in the oral cavity, large gills may impede the capacity for feeding (Schaack and Chapman, 2003).

Consequently, for a particular species or population, it can be argued that natural selection should produce an optimal lamellar area that is matched to oxygen demands as closely as possible. In addition, the drawbacks of having large gills with a large lamellar surface area means that any trait that increases oxygen needs will need to have a fitness value that is high enough to compensate for these problems.

Altering the functional respiratory area the old fashioned way

An optimal gill size, produced by natural selection as a perfect match between the pros and cons of having gills, will unfortunately rarely be that optimal, since the oxygen requirements of a fish may vary considerably both in the short and long term. Most fishes live in variable environments, with significant daily and seasonal fluctuations in oxygen availability and temperature. Moreover, different stages in the life cycle may require different rates of oxygen uptake. For example, salmon at sea are likely to need considerably less oxygen than later in life when they have to fight against nearly overwhelming water currents on their route up river to reach the breeding grounds.

When fishes change their oxygen needs or are faced with variations in the water oxygen concentration, they have long been known to have two strategies to meet these challenges: (1) to alter gill ventilation (i.e. the water flow over the gills) by adjusting the volume and frequency of buccal pumping, or (2) to alter their functional respiratory surface area, i.e. the extent to which the lamellae are perfused with blood. In hypoxia, for example, fishes increase both the water flow over the gills (e.g. Hughes and Saunders, 1970) and the functional respiratory surface area (Booth, 1979; Soivio and Tuurala, 1981). The latter can be done by increasing the blood pressure to open up more of the lamellar vasculature (lamellar recruitment) through vasoconstriction on the efferent (outgoing) side of the gill vasculature and/or by dilating afferent (incoming) lamellar arterioles (Davis, 1972; Booth, 1978; Farrell et al., 1980; Taylor and Barrett, 1985). During periods of low oxygen need, much of the gill blood flow may pass through channels embedded in the body of the gill filaments relatively far from the water (Pärt et al., 1984). The functional respiratory surface area can probably also be regulated by changing the thickness of the vascular space inside the lamellae through contracting or relaxing the pillar cells within the lamellae (Sundin and Nilsson, 1998b; Stensløkken et al., 2006). Pillar cells are contractile column-like cells linking the two epithelial sheets that make up the outer surfaces of the lamellae. Numerous neural and humoral substances have been linked to the regulation of gill blood flow, including acetylcholine, adrenaline, adenosine, endothelin, serotonin, prostaglandins, and most recently, hydrogen sulfide (Sundin et al., 1995; Sundin and Nilsson, 1996; Sundin and Nilsson, 1997; Evans et al., 2005; Stensløkken et al., 2002; Stensløkken et al., 2006; Olson et al., 2006).

Morphological remodeling of gills – the new fashion?

But what about changing the structure of the gills to morphologically alter the size of the respiratory surface area in response to environmental challenges? When faced with ionic or acid–base challenges, fishes have been found to adjust the cellular makeup of the gill surface (Perry, 1997; Perry, 1998; Goss et al., 1998). This may lead to altered oxygen diffusion distances since the number of relatively thick ion-pumping chloride cells (in relation to thin epithelial cells) on the lamellar surface may change. Moreover, fish that are exposed to hypoxia during development may end up with a significant, but relatively modest, increase in the respiratory surface area [an 18% increase in an African cichlid (Chapman et al., 2000)].

However, over the last few years, it has become clear that at least some fishes have much more drastic means for adjusting the gill structure. Thus, changes in environmental variables such as water oxygen content and temperature can cause fish to dramatically change the morphology of their gills in a rapid and reversible manner. The first fish found to have such abilities was the crucian carp *Carassius carassius* (Sollid et al., 2003), and subsequent studies have indicated that at least four fish species can make these striking adjustments.

When I took my first look at the gills of a crucian carp under a microscope, I got quite worried. I could not find any lamellae. After examining a larger sample of crucian carp from our aquarium facility, I was soon under the impression that I had spent much of my scientific career unknowingly studying severely malformed or diseased fish. All the crucian carp in our storage tanks appeared to show the same features: sausage like filaments without any protruding lamellae (Fig. 1A,B). This happened just a few weeks before I was to move from Uppsala (Sweden) to Oslo (Norway). Consequently, when I arrived in Oslo, one of the first things I did was to examine some crucian carp that my new Norwegian colleagues were keeping. These Norwegian fishes had gills that looked exactly the same as those from Uppsala, and I found some comfort in discovering that more scientists than me had unknowingly been studying malformed fish. However, there was also the possibility that this is what crucian carp gills actually look like. To cut a long story short, the odd looking gills of the crucian carp became a MSc project for Jørund Sollid, and in 2005 he defended his PhD on the same subject.

What we found was that crucian carp gills look like sausages when the fish are kept in cold ($\leqslant$20°C) aerated water, while hypoxia (water [O_2]=6–8% of air saturation) (Fig. 1C) or increased temperature ($\geqslant$25°C) (Fig. 1D) makes these gills transform into ‘normal’ gills with protruding lamellae. The process was found to be reversible, and a few days in relatively cold normoxic water will make them regain the sausage-like morphology (Sollid et al., 2003; Sollid et al., 2005a). Low oxygen levels and high temperatures have a common denominator: they increase the demand on the gills for oxygen uptake.

Mechanisms of gill remodeling in crucian carp

In a series of studies (Sollid et al., 2003; Sollid et al., 2005a; Sollid et al., 2005b; Sollid et al., 2006) (reviewed by Sollid and Nilsson, 2006), we found that the lamellae are actually present all the time, i.e. also during normoxia and low temperatures, but they are then embedded in a cell mass that we denoted an interlamellar cell mass (ILCM), which completely fills up the

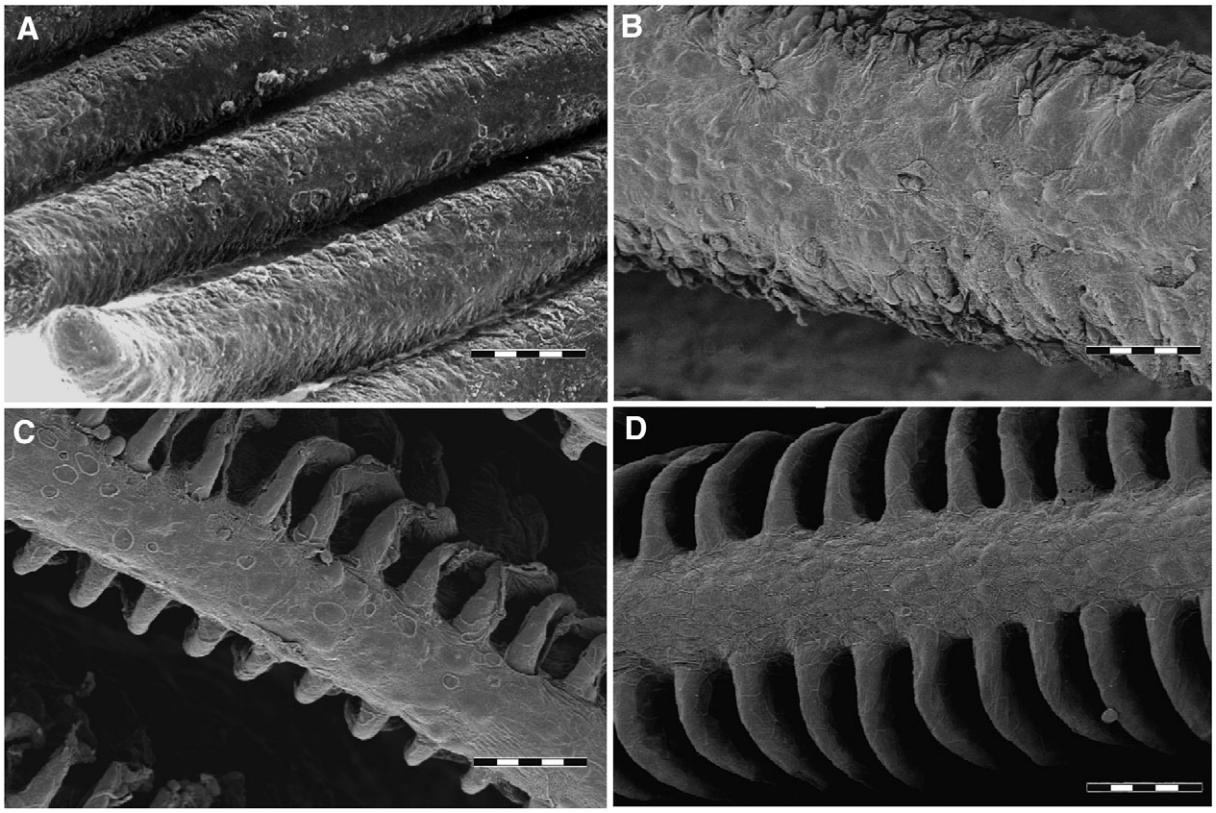

Fig. 1. (A–D) Scanning electron micrographs of gill filaments from crucian carp kept in normoxic water at 8°C (A,B), in hypoxic water at 8°C (C), or in normoxic water at 25°C (D). Scale bars, 150 μm (A); 50 μm in (B–D) (from Sollid et al., 2003; Sollid et al., 2005a).

space between the lamellae (Fig. 2A). An increased rate of apoptosis ('programmed cell death' or 'cell suicide') combined with suppressed mitosis was responsible for regression of the ILCM during hypoxia (Fig. 2B). At 8°C, this process took between 3 and 7 days (Sollid et al., 2003). Interestingly, in a later experiment, when we let crucian carp use up the oxygen in a closed respirometer at 20°C, resulting in a 6 h long hypoxia exposure, we found that most of the ILCM disappeared during this time. Since it is difficult to imagine that apoptosis alone could remove the ILCM in such a short time [it usually takes a day or so to remove cells though apoptosis in mammalian tissues (e.g. Rodriguez and Schaper, 2005)], we find it likely that the ILCM may detach from the gills during hypoxia at 20°C, possibly as an emergency response. In gills observed in scanning electron microscopy (SEM), we have occasionally noticed that segments of the ILCM appear to be ripped off during the histological preparation process, leaving the lamellae exposed. Thus, the ILCM may be attached relatively loosely to the gills.

With regard to the molecular mechanisms involved, the first possibility we examined was that the ILCM cells sense hypoxia and a rise in temperature through an increased level of the hypoxia inducible factor 1-alpha (HIF-1α). This transcription factor has been shown to be responsible for numerous cellular responses to hypoxia (for a review, see Nikinmaa and Rees, 2005). We therefore cloned HIF-1α from crucian carp and, in collaboration with Mikko Nikinmaa's group, measured the level of HIF-1α (mRNA and protein) in the gills of crucian carp exposed to hypoxia at various temperatures. The results were ambiguous. HIF-1α did indeed increase in concentration (both as protein and mRNA) in response to hypoxia (Sollid et al., 2006), but we subsequently found that HIF-1α also increases in concentration with falling temperature during normoxic conditions (Rissanen et al., 2006). This does not fit well with the finding that high temperature stimulates apoptosis in the ILCM. Thus, if HIF-1α is involved, it is unlikely to be the only signal for reducing the ILCM, and another mechanism has to be initiating the apoptosis induced by a rise in temperature. Moreover, the mRNA level of inducible nitric oxide synthase (iNOS), an enzyme induced by HIF-1α and known to trigger apoptosis (Dimmeler and Zeiher, 1997), was found to be unaffected by hypoxia in crucian carp gills (Sollid et al., 2006). This seems to rule out a role for iNOS in inducing apoptosis in the ILCM.

Thus, at present we have no good evidence for any particular molecular mechanisms responsible for inducing the gill remodeling in crucian carp. In fact, we do not even know if the signal is sensed by the ILCM cells themselves or if the cells receive a signal from elsewhere in the body. The latter possibility is a bit difficult to reconcile with their anatomical location. The ILCM cells are situated outside the lamellar epithelium and we have not observed any blood vessels in the ILCM in light microscopic and transmission electron microscopic examinations. We also find it unlikely that the ILCM is innervated, but this remains to be examined. Therefore, it is not clear if the fish has any humoral or neural means of

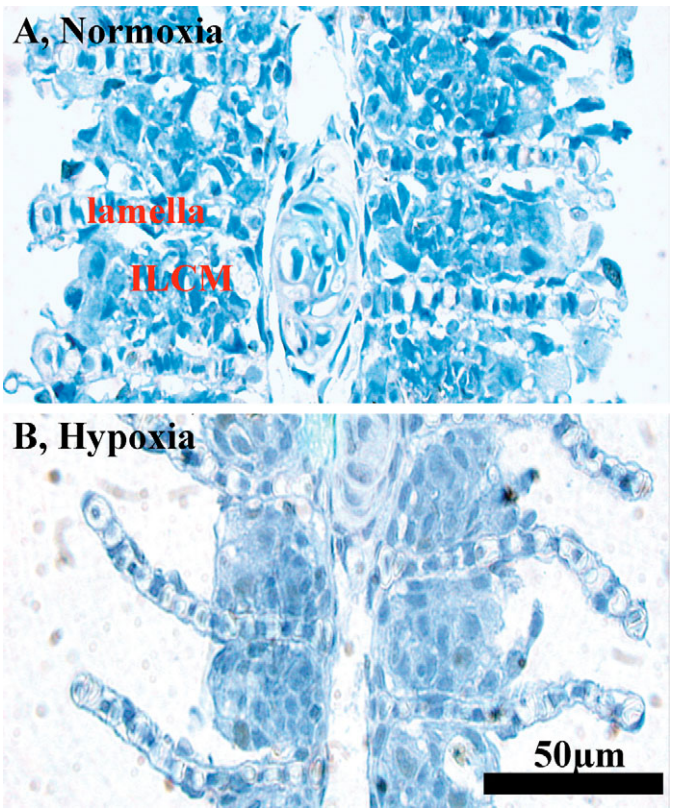

Fig. 2. (A,B) Light micrographs of gills from crucian carp kept in normoxic (A) or hypoxic (B) water at 8°C. Note that the lamellae are present in both conditions but that a regression of the interlamellar cell mass (ILCM) during hypoxia makes the lamellae protrude, thereby greatly increasing the respiratory surface area. A filament arteriole (with blood clots) is seen running vertically in the center of each micrograph. Scale bar, 50 µm (from Sollid et al., 2003).

signaling to the ILCM cells that they should start dividing or undergo apoptosis. Still, it is tempting to suggest that the signal comes from the fish. As mentioned previously, a low oxygen level in the water and a high temperature have a common denominator: they increase the demand on the gills for oxygen uptake. An insufficient oxygen uptake could, for example, be sensed through a fall in blood oxygen levels.

Moreover, the apparent lack of vasculature in the ILCM also presents another unanswered question: how do these cells receive nutrients for their energy metabolism? At present, we have to admit that most questions regarding the nature and regulation of the ILCM remain to be answered. A first aim will be to characterize the cells that make up the ILCM.

Pros and cons of gill remodeling

Important clues to understanding the advantages of gill remodeling in crucian carp should lie in its life history and physiology. There is one physiological trait that distinguishes the crucian carp from most other fishes: it is extremely hypoxia tolerant. In fact, it even tolerates long periods of anoxia (for a review, see Nilsson and Lutz, 2004). In the winter, many crucian carp living in Northern Europe endure a life under the ice of small ponds that become hypoxic and finally anoxic for several months every winter (Holopainen and Hyvärinen, 1985; Vornanen and Paajanen, 2006). During the anoxic period, the crucian carp uses glycolysis to produce ATP, with ethanol as the major end product. The ethanol is released to the water over the gills (Shoubridge and Hochachka, 1980; Nilsson, 1988). Thus, while producing ethanol has the advantage of being pH neutral (thereby avoiding lactate acidosis), it is an energetically wasteful mechanism since ethanol is a highly energetic hydrocarbon that is forever lost to the water. Moreover, to avoid ethanol intoxication, they probably have to maintain a high rate of gill blood flow, which is the likely explanation for why the crucian carp has the same cardiac output in anoxia as in normoxia (Stecyk et al., 2004).

Thus, having to rely on anaerobic glycolysis is energetically very costly for this fish and puts it in danger of running out of fuel, even if it has enormous glycogen stores (Hyvärinen et al., 1985; Vornanen and Paajanen, 2006). Therefore, it must be beneficial for the crucian carp to make the period where it utilizes anaerobic glycolysis as short as possible. This could be the reason why crucian carp remain physically active at low oxygen levels, and even in anoxia (Nilsson et al., 1993; Nilsson, 2001), since this will allow them to seek out oxygen throughout the habitat. Limiting the anaerobic period could also be a major selection pressure for remodeling the gills. The anoxic period in the winter must be preceded by hypoxia. Here, gill remodeling in the direction of increased lamellar surface area will allow the crucian carp to maintain aerobic ATP production for a longer period. Sollid et al. (Sollid et al., 2003; Sollid et al., 2005a) showed that crucian carp with protruding lamellae were able to run aerobic metabolism at significantly lower oxygen levels than when the lamellae were imbedded in an ILCM.

In line with its extreme hypoxia tolerance, the crucian carp blood contains haemoglobin with an extremely high O_2 affinity. Sollid et al. (Sollid et al., 2005a) found that crucian carp haemoglobin is 50% saturated with O_2 already at a P_{O_2} of 0.8 or 1.6 mmHg (=P_{50} at 10°C and 20°C, respectively), which appears to be a record low P_{50} for a vertebrate haemoglobin. In addition to being useful for oxygen uptake at very low water P_{O_2}, this high O_2 affinity may be a prerequisite for the extensive gill remodeling displayed by the crucian carp. In normoxic water, the high oxygen affinity probably allows the haemoglobin to accumulate enough O_2 even over relatively long diffusion distances between water and blood, thereby allowing this fish to fully cover its lamellae with a cell mass.

During the summer and autumn, a lack of protruding lamellae should allow the fish to shift parts of its energy budget from osmoregulatory tasks to building up glycogen stores (assuming the water temperature is not so high that it would make the lamellae protrude). Large glycogen stores would reduce the risk of the fish running out of fuel during the anoxic period in the winter. Similarly, in the spring, when water oxygen levels are restored and it is time to spawn, reducing the lamellar surface area will mean that the crucian carp can devote more energy into producing gametes, which will directly promote its fitness.

In addition, having a small lamellar surface area for most of the year is likely to make the crucian carp less vulnerable to

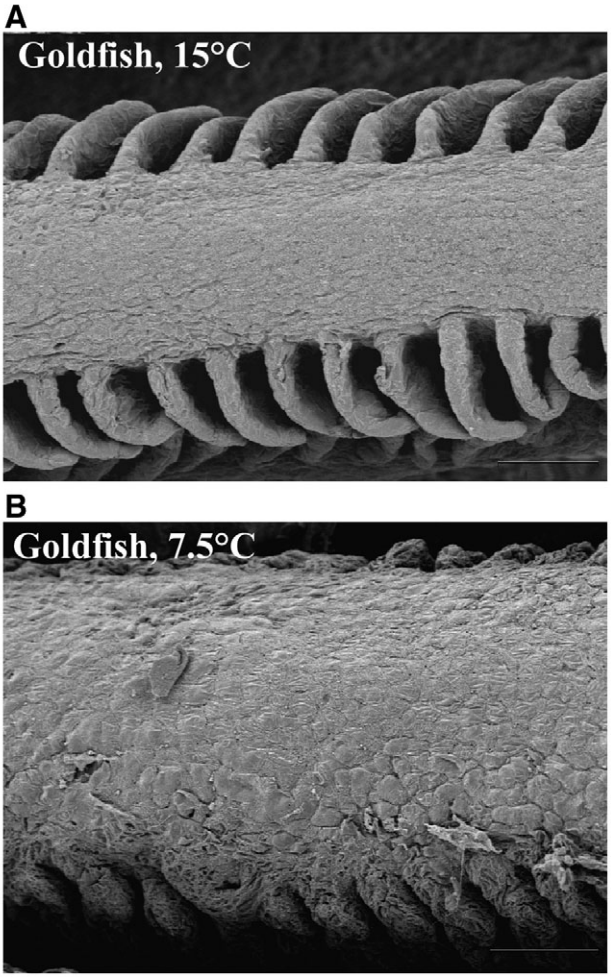

Fig. 3. (A,B) Scanning electron micrographs of gill filaments from goldfish kept in normoxic water at 15°C (A) and 7.5°C (B). Scale bars, 50 μm (from Sollid et al., 2005a).

pathogens and toxic substances that may enter the body over the gills.

More fishes do it

The crucian carp has a famous close relative, the goldfish *Carassius auratus*. Like the crucian carp, the goldfish is extremely hypoxia tolerant and has a haemoglobin with a very high oxygen affinity (Burggren, 1982). In the literature, there is no shortage of studies involving microscopic examinations of goldfish gills, and if these can be remodeled, it initially struck us as odd that no one had noticed it. Indeed, in all the published pictures we saw, goldfish gills looked very much like 'normal' fish gills with protruding lamellae. However, virtually all goldfish that have had their gills examined are the domesticated variants that have been in the hands of aquarists for hundreds of years, and for which the main selection pressures have been for odd shapes and colors, and for prolific breeding in captivity (the crucian carp is notoriously difficult to breed in captivity). Thus, we could not exclude that the goldfish in the aquarium trade had accidentally lost their capacity for gill remodeling during centuries of domestication.

Another possibility that struck us was that gill remodeling could have been overseen in goldfish because they are traditionally kept and studied at room temperature (20–25°C). At such a high temperature, crucian carp also have 'normal' gills with protruding lamellae. We tested this possibility in some pretty goldfish imported from a dealer in Singapore and acclimated to 25°C, by chilling down their water to 15°C for 5 days and subsequently to 7.5°C for 5 days. While goldfish at 25 and 15°C showed 'normal' gills with protruding lamellae (Fig. 3A), those that had been at 7.5°C for 5 days had acquired filaments virtually without protruding lamellae (Fig. 3B), being strikingly similar to those of crucian carp kept below 20°C. Clearly, like crucian carp, goldfish are able to remodel their gills, and the reason that this had been overlooked is probably that no one has previously examined the gills of goldfish held at a low temperature.

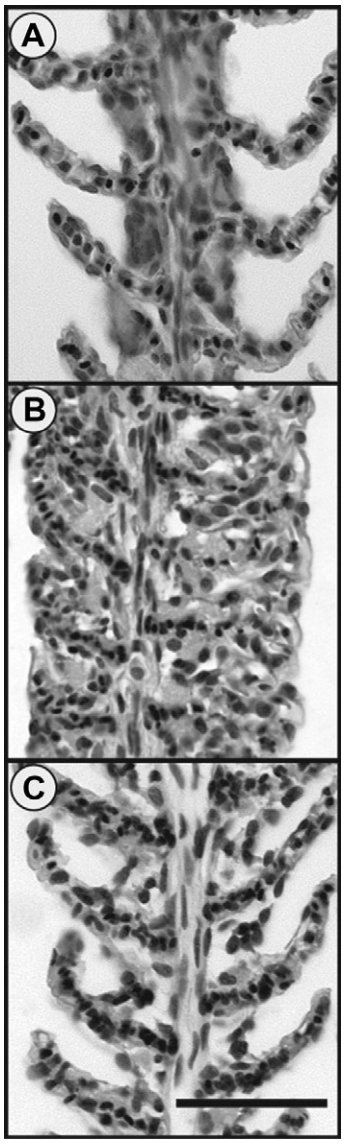

Fig. 4. (A–C) Light micrographs of gills from the mangrove killifish kept in water (A), in air for 1 week (B), and in water for 1 week after air exposure (all at 25°C). Note that the lamellae are present both in water and in air, but that an interlamellar cell mass fills up the space between the lamellae during air exposure. Scale bar, 40 μm (from Ong et al., 2007).

Since goldfish and crucian carp are close relatives, it was in the end not very surprising to find that both species can remodel their gills in response to increased demands for oxygen uptake, either brought about by hypoxia or by a high environmental temperature. But what about gill remodeling in other fishes? At about the same time as we found that goldfish can remodel their gills, we heard from Colin Brauner (University of British Columbia) that he had found the Qinghai carp *Gymnocypris przewalskii* to be able to remodel its gills in a similar fashion as the *Carassius* species, although maybe not as extensively (C. J. Brauner, unpublished results). The Qinghai carp is a cyprinid, so it is tempting to conclude that gill remodeling could be a trait shared by many cyprinids.

At an APS Conference in Virginia Beach in October 2006, Patricia A. Wright (University of Guelph) announced that her research group had found gill remodeling in the mangrove killifish *Kryptolebias marmoratus* in response to air exposure. This small cyprinidont fish was found to have an ILCM that reacted strongly to air exposure by increasing in volume to fill up most of the interlamellar space within a week. The process was found to be reversible and seemed to increase the ability of the killifish to take up oxygen from air, possibly by stabilizing the lamellae so that they would not stick together (Ong et al., 2007). This killifish is an excellent air breather that frequently ventures into the terrestrial environment. Histologically, the change in gill structure displayed by the killifish, involving a dynamic ILCM and a maintained lamellar structure (Fig. 4), appears to be very similar to that displayed by the cyprinids. Thus, reversible and adaptive gill remodeling is not exclusive to cyprinids.

An additional example of gill remodeling is provided by *Arapaima gigas*, a giant Amazonian fish, which starts off its life as a water breather with 'normal' gills with protruding lameallae. Later in life, it becomes an obligate air breather, using its swimbladder for oxygen uptake, while simultaneously developing gills without protruding lamellae (Brauner et al., 2004). However, in this fish, gill remodeling is a developmental change that is unlikely to be reversible. Moreover, histological examination of *A. gigas* gills at different life stages indicate a more profound reorganization of gill structure where the lamellae are no longer present. Thus, its cellular mechanisms for changing the gill morphology are possibly different from those operating in the cyprinids and cyprinodonts.

Finally, it should be mentioned that the exposure to aluminum in acid water can cause gross morphological changes in gills, which are generally considered to be pathological, but it cannot be excluded that they may reflect protective changes. In largemouth bass *Micropterus salmoides*, an enormous hypertrophy and vacuolization of the epithelial cells on the lamellae occurs (Leino and McCormick, 1993), while in brook trout *Salvelinus fontinalis*, undifferentiated cells and mucous producing cells have been seen filling up the interlamellar space after 7–10 days of aluminum exposure at pH 5.2, while these cells disappeared as the gill morphology showed a surprising recovery after a further week of aluminum exposure (Mueller et al., 1991). In the latter case, the cell mass filling up the interlamellar space may have provided a protective function as the fish appeared to acclimate to the aluminum, and the histology was not totally unlike that of crucian carp lamellae embedded in a ILCM.

New fashion or ancient secret?

As mentioned, the mode of gill remodeling in the killifish (Cyprinodontidae), involving a dynamic ILCM, appears to be virtually identical to that of the crucian carp, goldfish and Qinghai carp (Cyprinidae). Both families belong to the Euteleostei, but in spite of the similarities in their Latin family names, cyprinodontids and cyprinids are only distantly related. It was at least some 150 million years ago, possibly longer, that the cyprinids, which belong to the suborder Ostariophysi, and the cyprinodontids, which belong to the suborder Acanthopterygii, had a common ancestor (Briggs, 2005). This was, for example, long before any primates walked the earth. Thus, it is possible that gill remodeling is a very ancient trait in euteleosts. If it turns out that the gill remodeling of *Arapaima gigas* is based on a similar mechanism, then we would have to add a few more hundred million years to the age of this mechanism since *Arapaima* belongs to the Osteoglossiformes, one of the most primitive groups of teleosts. However, we will obviously need to characterize the mechanisms involved in the gill remodeling much better, and preferentially find more examples of fishes with this capacity, before we can draw any firm conclusions about how old (and widespread) this mechanism is. Until then, it remains an open question if gill remodeling is a relatively new fashion that has independently evolved at least twice in euteleosts, or if it is a many-million-years-old secret that is about to be revealed.

Arguably, many fish species would benefit from remodeling their gills to match oxygen uptake capacity with oxygen needs in the face of changing environmental conditions. However, to cover the lamellae almost completely with a cell mass in cold, well-oxygenated water, as the crucian carp and goldfish do, is unlikely to be an option for most fishes, as it possibly demands an extremely high blood oxygen affinity. Moreover, gill remodeling is probably quite costly, considering the apoptotic and mitotic processes involved. Thus, doing this many times a week in response to short-term changes in the habitat could be energetically unfavorable. This relatively slow and drastic mechanism is probably best suited for responding to relatively long-lasting changes in the environment, like annual wintertime hypoxia (as in crucian carp), or week-long expeditions into the terrestrial environment (as in the killifish). Adjusting gill ventilation and blood flow patterns in the gills are probably the mechanisms of choice for matching the functional respiratory surface area to short-term variations in ambient oxygen levels, temperature and oxygen needs.

Still, considering the potential benefits that gill remodeling may have for both survival and fitness, one may speculate that a capacity for some degree of gill remodeling is widespread among fishes. If it is less extensive than in crucian carp, it could have easily been missed by fish physiologists. Moreover, if gill remodeling occurs under conditions where we rarely keep the fish, then even extensive gill remodeling, like that shown by goldfish at 7.5°C, may be overlooked.

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References

Bæuf, G. and Payan, P. (2001). How should salinity influence fish growth? *Comp. Biochem. Physiol.* **130C**, 411-423.

Bernal, D., Dickson, K. A., Shadwick, R. E. and Graham, J. B. (2001). Analysis of the evolutionary convergence for high performance swimming in lamnid sharks and tunas. *Comp. Biochem. Physiol.* **129A**, 695-726.

Booth, J. H. (1978). The distribution of blood flow in the gills of fish: application of a new technique to rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **73**, 119-130.

Booth, J. H. (1979). The effect of oxygen supply, epinephrine and acetylcholine on the distribution of blood flow in trout gills. *J. Exp. Biol.* **83**, 31-39.

Brauner, C. J., Matey, V., Wilson, J. M., Bernier, N. J. and Val, A. L. (2004). Transition in organ function during the evolution of air-breathing; insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *J. Exp. Biol.* **207**, 1433-1438.

Briggs, J. C. (2005). The biogeography of otophysan fishes (Ostariophysi: Otophysi): a new appraisal. *J. Biogeogr.* **32**, 287-294.

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

Chapman, L. J. and Hulen, K. G. (2001). Implications of hypoxia for the brain size and gill morphometry of mormyrid fishes. *J. Zool.* **254**, 461-472.

Chapman, L. J., Galis, F. and Shinn, J. (2000). Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecol. Lett.* **3**, 387-393.

Chapman, L. J., Chapman, C. A., Nordlie, F. G. and Rosenberger, A. E. (2002). Physiological refugia: swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region. *Comp. Biochem. Physiol.* **133A**, 421-437.

Davis, J. C. (1972). An infrared photographic technique useful for studying vascularization of fish gills. *J. Fish. Res. Board Can.* **29**, 109-111.

Dimmeler, S. and Zeiher, A. M. (1997). Nitric oxide and apoptosis: another paradigm for the double-edged role of nitric oxide. *Nitric Oxide* **1**, 275-281.

Evans, D. H., Piermarini, P. M. and Choe, K. P. (2005). The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiol. Rev.* **85**, 97-177.

Farrell, A. P., Sobin, S. S., Randall, D. J. and Crosby, S. (1980). Intralamellar blood flow patterns in fish gills. *Am. J. Physiol.* **239**, R428-R436.

Fernandes, M. N., Rantin, F. T., Kalinin, A. L. and Moron, S. E. (1994). Comparative study of gill dimensions of 3 Erythrinid species in relation to their respiratory function. *Can. J. Zool.* **72**, 160-165.

Gonzalez, R. J. and McDonald, D. G. (1992). The relationship between oxygen consumption and ion loss in a freshwater fish. *J. Exp. Biol.* **163**, 317-332.

Goss, G. G., Perry, S. F., Fryer, J. N. and Laurent, P. (1998). Gill morphology and acid base regulation in freshwater fishes. *Comp. Biochem. Physiol.* **119A**, 107-115.

Gray, I. E. (1954). Comparative study of the gill area of marine fishes. *Biol. Bull.* **107**, 219-255.

Holopainen, I. J. and Hyvärinen, H. (1985). Ecology and physiology of crucian carp (*Carassius carassius* (L.)) in small Finnish ponds with anoxic conditions in winter. *Verh. Internat. Verein Limnol.* **22**, 2566-2570.

Hughes, G. M. and Saunders, R. L. (1970). Responses of the respiratory pumps to hypoxia in the rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **53**, 529-545.

Hyvärinen, H., Holopainen, I. J. and Piironen, J. (1985). Anaerobic wintering of crucian carp (*Carassius carassius* L.). I. Annual dynamics of glycogen reserves in nature. *Comp. Biochem. Physiol.* **82A**, 797-803.

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. *Can. J. Zool.* **71**, 531-543.

Mueller, M. E., Sanchez, D. A., Bergman, H. L., McDonald, D. G., Rhem, R. G. and Wood, C. M. (1991). Nature and time course of acclimation to aluminium in juvelile brook trout (*Salvelinus fontinalis*). II. Gill histology. *Can. J. Fish. Aquat. Sci.* **48**, 2016-2027.

Nikinmaa, M. and Rees, B. B. (2005). Oxygen-dependent gene expression in fishes. *Am. J. Physiol.* **288**, R1079-R1090.

Nilsson, G. E. (1988). A comparative study of aldehyde dehydrogenase and alcohol dehydrogenase activity in crucian carp and three other vertebrates: apparent adaptations to ethanol production. *J. Comp. Physiol. B* **158**, 479-485.

Nilsson, G. E. (2001). Surviving anoxia with the brain turned on. *News Physiol. Sci.* **16**, 218-221.

Nilsson, G. E. and Lutz, P. L. (2004). Anoxia tolerant brains. *J. Cereb. Blood Flow. Metab.* **24**, 475-486.

Nilsson, G. E., Rosén, P. and Johansson, D. (1993). Anoxic depression of spontaneous locomotor activity in crucian carp quantified by a computerized imaging technique. *J. Exp. Biol.* **180**, 153-163.

Nilsson, S. (1986). Control of gill blood flow. In *Fish Physiology*: *Recent Advances* (ed. S. Nilsson and S. Holmgren), pp. 87-101. London: Croom Helm.

Olson, K. R., Dombkowski, R. A., Russell, M. J., Doellman, M. M., Head, S. K., Whitfield, N. L. and Madden, J. A. (2006). Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. *J. Exp. Biol.* **209**, 4011-4023.

Ong, K. J., Stevens, E. D. and Wright, P. A. (2007). Gill morphology of the mangrove killifish (*Kryptolebias marmoratus*) is plastic and changes in response to terrestrial air exposure. *J. Exp. Biol.* **210**, 1109-1115.

Pärt, P., Tuurala, H., Nikinmaa, M. and Kiessling, A. (1984). Evidence for a non-respiratory intralamellar shunt in perfused rainbow trout gills. *Comp. Biochem. Physiol.* **79A**, 29-34.

Perry, S. F. (1997). The chloride cell: structure and function in the gills of freshwater fishes. *Annu. Rev. Physiol.* **59**, 325-347.

Perry, S. F. (1998). Relationships between branchial chloride cells and gas transfer in freshwater fish. *Comp. Biochem. Physiol.* **119A**, 9-16.

Rissanen, E., Numminen, H. K., Sollid, J., Nilsson, G. E. and Nikinmaa, M. (2006). Temperature regulates hypoxia-inducible factor-1 (HIF-1) in a poikilothermic vertebrate, crucian carp (*Carassius carassius*). *J. Exp. Biol.* **209**, 994-1003.

Rodriguez, M. and Schaper, J. (2005). Apoptosis: measurement and technical issues. *J. Mol. Cell. Cardiol.* **38**, 15-20.

Schaack, S. and Chapman, L. J. (2003). Interdemic variation in the African cyprinid *Barbus neumayeri*: correlations among hypoxia, morphology, and feeding performance. *Can. J. Zool.* **81**, 430-440.

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

Soivio, A. and Tuurala, H. (1981). Structural and circulatory responses to hypoxia in the secondary lamellae of *Salmo gairdneri* gills at two temperatures. *J. Comp. Physiol. B* **145**, 37-43.

Sollid, J. and Nilsson, G. E. (2006). Plasticity of respiratory structures – adaptive remodeling of fish gills induced by ambient oxygen and temperature. *Respir. Physiol. Neurobiol.* **154**, 241-251.

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.

Sollid, J., Weber, R. E. and Nilsson, G. E. (2005a). Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. *J. Exp. Biol.* **208**, 1109-1116.

Sollid, J., Kjernsli, A., De Angelis, P. M., Røhr, Å. K. and Nilsson, G. E. (2005b). Cell proliferation and gill morphology in anoxic crucian carp. *Am. J. Physiol.* **289**, R1196-R1201.

Sollid, J., Rissanen, E., Numminen, H., Thorstensen, T., Vuori, K. A. M., Nikinmaa, M. and Nilsson, G. E. (2006). HIF-1a and iNOS in crucian carp gills during hypoxia induced transformation. *J. Comp. Physiol. B* **176**, 359-369.

Stecyk, J. A. W., Stensløkken, K.-O., Farrell, A. P. and Nilsson, G. E. (2004). Maintained cardiac pumping in anoxic crucian carp. *Science* **306**, 77.

Stensløkken, K. O., Sundin, L. and Nilsson, G. E. (2002). Cardiovascular effects of prostaglandin F2α and prostaglandin E2 in Atlantic cod (*Gadus morhua*). *J. Comp. Physiol. B* **172**, 363-369.

Stensløkken, K.-O., Sundin, L. and Nilsson, G. E. (2006). Endothelin receptors in teleost fish: cardiovascular effects and branchial distribution. *Am. J. Physiol.* **290**, R852-R860.

Sundin, L. and Nilsson, G. E. (1996). Branchial and systemic roles of adenosine in rainbow trout, an *in vivo* microscopy study. *Am. J. Physiol.* **271**, R661-R669.

Sundin, L. and Nilsson, G. E. (1997). Neurochemical mechanisms behind gill microcirculatory responses to hypoxia in trout: *in vivo* microscopy study. *Am. J. Physiol.* **272**, R576-R585.

Sundin, L. and Nilsson, G. E. (1998a). Acute defence mechanisms against haemorrhage from mechanical gill injury in rainbow trout. *Am. J. Physiol.* **275**, R460-R465.

Sundin, L. and Nilsson, G. E. (1998b). Endothelin redistributes blood flow through the lamellae of rainbow trout gills: evidence for pillar cell contraction. *J. Comp. Physiol. B* **168**, 619-623.

Sundin, L., Nilsson, G. E., Block, M. and Löfman, C. O. (1995). Control of gill filament blood flow by serotonin in the rainbow trout *Oncorhynchus mykiss*. *Am. J. Physiol.* **268**, R1224-R1229.

Taylor, E. W. and Barrett, D. J. (1985). Evidence of a respiratory role for the hypoxic bradycardia in the dogfish *Scyliorhinus canicula* L. *Comp. Biochem. Physiol.* **80A**, 99-102.

Vornanen, M. and Paajanen, V. (2006). Seasonal changes in glycogen content and Na+-K+-ATPase activity in the brain of crucian carp. *Am. J. Physiol.* **291**, R1482-R1489.

Wood, C. M. (2001). Toxic responses of the gill. In *Target Organ Toxicity in*