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Graphical Review

The osmorespiratory compromise in the fish gill

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ABSTRACT

August Krogh made fundamental discoveries about both respiratory gas exchange and osmo/iono-regulation in fish gills. Dave Randall and co-workers identified a tradeoff between these two functions such that high functional surface area and low diffusion distance would favour O_2 uptake (e.g. exercise, hypoxia), whereas low functional surface area and high diffusion distance would favour osmo/iono-regulation (rest, normoxia). Today we call this concept the "osmorespiratory compromise" and realize that it is much more complex than originally envisaged. There are at least 6 mechanisms by which fish can change functional branchial area and diffusion distance. Three involve reorganizing blood flow pathways: (i) flow redistribution within the secondary (respiratory) lamellae; (ii) flow shunting between "respiratory" and "ionoregulatory" pathways in the filament; (iii) opening up more distal lamellae on the filament and closing non-respiratory pathways. Three more involve "reversible gill remodeling": (iv) proliferation of the interlamellar gill cell mass (ILCM); (v) proliferation of ionocytes up the sides of the lamellae; (vi) covering over the apical exposure of ionocytes by extension of pavement cells. In ways that remain incompletely understood, these mechanisms allow dynamic regulation of the osmorespiratory compromise, such that ion and water fluxes can be decoupled from O_2 uptake during continuous exercise. Furthermore, hypoxia-tolerant species can reduce branchial ion and water fluxes below normoxic levels despite hyperventilating during hypoxia. In marine fish, the osmorespiratory conflict is intensified by the greater ionic and osmotic gradients from seawater to blood, but underlying mechanisms remain poorly understood.

1. History

Among his many contributions to physiology, August Krogh was a pioneer in studying both respiration (Ege and Krogh, 1914) and osmoregulation (Krogh, 1939) by the gills of fish. Indeed, Krogh appreciated that rapid weight changes seen in eels transferred between freshwater and seawater represented osmotic water fluxes across the gills of stressed fish. Together with George de Hevesy and Hans Ussing, Krogh also introduced the use of isotopes into physiology, a powerful tool which others later used to measure unidirectional fluxes of ions and water across fish gills. Steen and Kruysse (1964), based on blood PO_2 measurements in eels and ground-breaking studies of the vascular fine structure of the gills in many species, concluded that "*gill respiration thus seems to include mechanisms for effecting a favourable compromise between respiratory and osmotic demands*".

Dave Randall et al. (1972) were the first to specifically articulate the potential conflicts between these two processes. This classic paper proposed "*Any change in the pattern of blood and water flow which reduces diffusion distance or increases the functional exchange area between blood and water will increase the rate of ion and water diffusion, unless there are compensatory reductions in the permeability of the gill epithelium*" and presented ^{22}Na efflux data from stressed trout, and trout treated with epinephrine, to support the idea. They concluded "*If those factors which increase the exchange of gases across the gills also cause concomitant increases in ion and water transfer, then conditions in the gills must be the result of continual compromise between the minimal rate of gas exchange required and the maximum rate of ion and water transfer that can be tolerated*". At about the same time, Stevens (1972) showed that fish gained weight with exercise in freshwater but lost it in seawater, demonstrating that the concept was true for water fluxes as well as Na^+ fluxes. Wood and Randall (1973a, 1973b) used ^{22}Na and urinary catheterization to separate branchial influx and efflux components from urinary excretion, showing that initial increases and later decreases in unidirectional branchial Na^+ efflux rate occurred with continuous exercise. Similarly,

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Abbreviations: AQP3, aquaporin3 protein; CVS, central venous sinus; ^3H_2O, tritiated (radiolabeled) water; IGR, ion-to-gas ratio (molar ratio of Na^+ loss to O_2 uptake); ILCM, interlamellar cell mass; ^{22}Na, radiolabeled sodium; PEG-4000, polyethylene glycol with molecular weight of 4000 Da; PO_2, partial pressure of oxygen.

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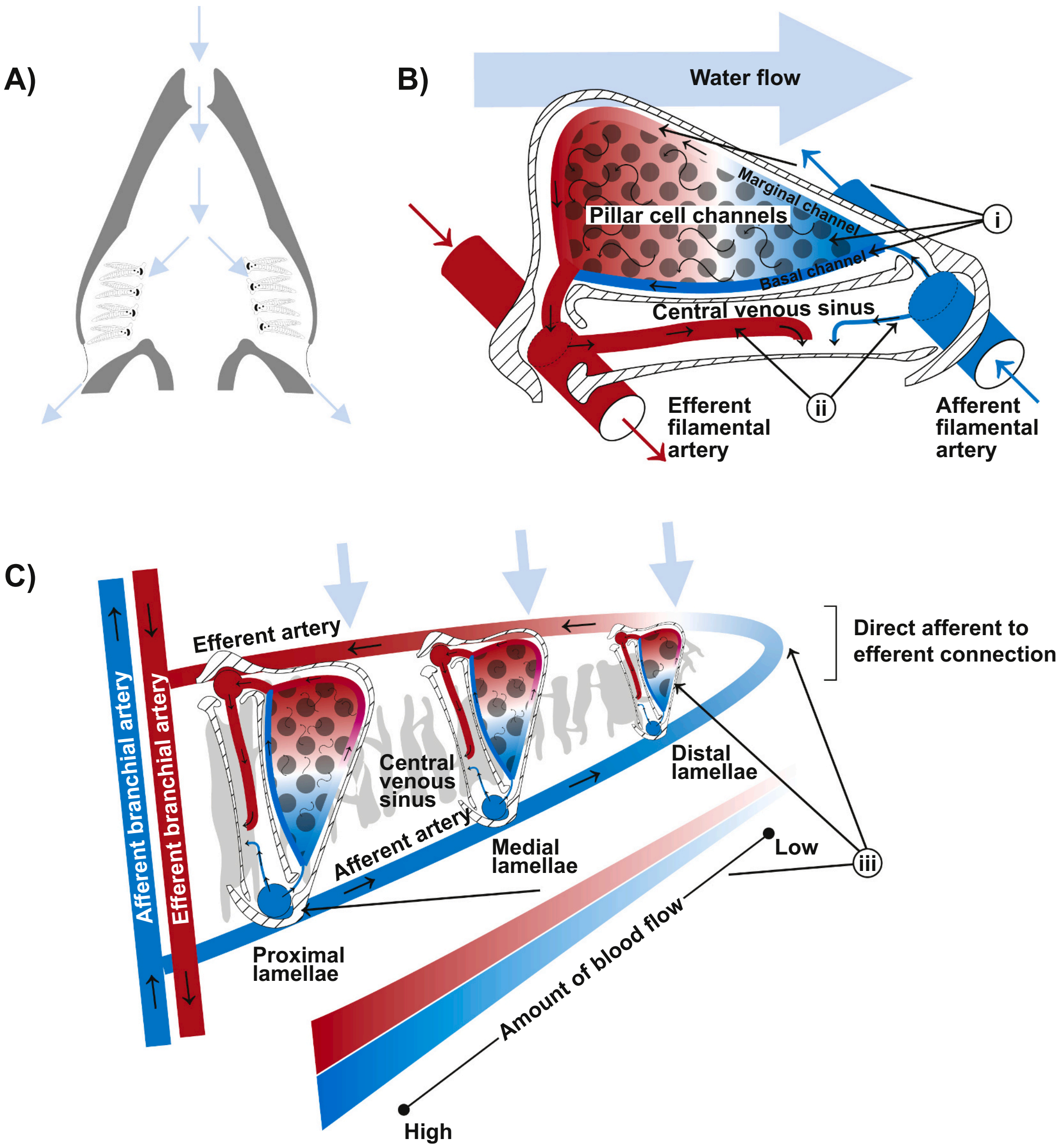

Fig. 1. Mechanisms (i, ii, iii – see text) dependent on blood flow redistribution for changing functional surface area and/or blood-to-water diffusion distance in the gills. (A) General organization of the four gill arches, each with two hemibranchs, in the waterflow pathway. (B) Within the secondary lamellae ("respiratory pathway), blood may be diverted preferentially (mechanism i) - to the partially buried basal channel, to the wider, longer, fast-flowing marginal channel, or through the network of narrower, slower-flowing pillar cell channels. Blood flow may also be diverted into the central venous sinus (CVS: "ionoregulatory pathway") of the filament that underlies most of the ionocytes (mechanism ii). A small amount of blood flow may be diverted by pre-lamellar arterio-venous anastomoses completely away from the "respiratory pathway" into the CVS. A larger portion may be diverted to the ionoregulatory pathway after it has passed through the respiratory pathway of the lamellae by post-lamellar arterio-venous anastomoses. (C) Blood flow may also be redistributed among secondary lamellae (mechanism iii). Under control resting conditions, proximal lamellae close to the base of the filament preferentially receive most of the blood flow, and blood flow may also be diverted away from the respiratory pathway through direct connections between afferent and efferent filamental arteries at the filament tip. When O_2 demand, cardiac output, and water flow all increase, more distal lamellae become well-perfused and the afferent-to-efferent connection is closed.

there were initial increases in net water entry across the gills and later net water effluxes through the kidney (Wood and Randall, 1973c).

Today, we know this concept as the "osmorespiratory compromise". Both Steen and Kruysse (1964) and Randall et al. (1972) had used the term 'compromise", but the origin of the new word "osmorespiratory" remains unclear. It is often incorrectly attributed to Nilsson (1986), but he actually used "respiratory-osmoregulatory compromise". The term appears in the seminal study of Gonzalez and McDonald (1992) which introduced the concept of the ion-to-gas ratio (IGR = molar ratio of Na^+ loss to O_2 uptake) as a parameter that the fish may dynamically regulate. Thorough reviews of various aspects of the osmorespiratory compromise have been presented by Perry (1998), Gonzalez (2011), Nilsson et al. (2012), and Gilmour and Perry (2018). It has been studied most extensively with respect to exercise and generalized stress, and to a lesser extent with respect to hypoxia and temperature, all of which alter O_2 demand and/or O_2 availability. Most focus has been on the trans-epithelial movements of O_2 and ions, with less attention to the fluxes of water and other respiratory gases (CO_2, ammonia).

2. Changes in gill functional surface area and diffusion distance

A key part of the concept is that fish can alter the functional surface area and/or blood-to-water diffusion distance. We know of at least 6 different mechanisms; there may be more.

The first three (Fig. 1) involve blood flow redistribution and are controlled by blood pressure, autonomic nervous innervation and

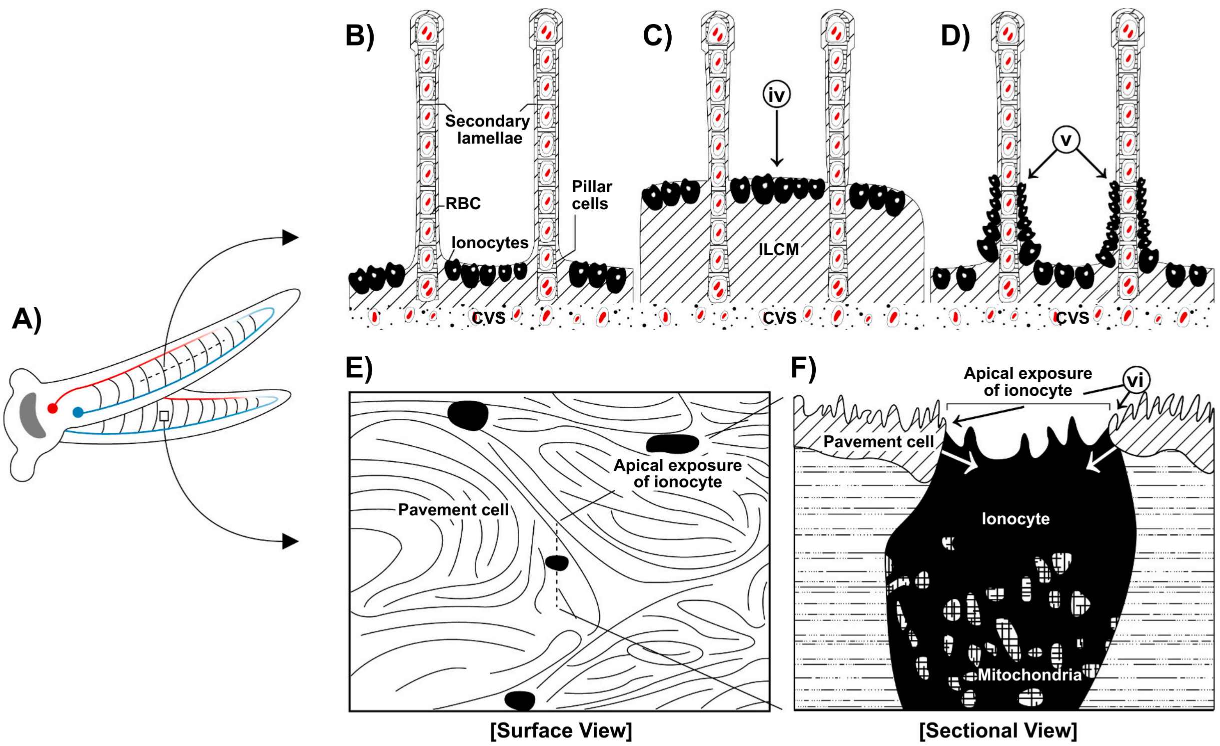

Fig. 2. Mechanisms (iv, v, vi – see text) dependent on "reversible gill remodeling" that involve physical blockage for changing functional surface area and/or blood-to-water diffusion distance in the gills. (A) Gill filaments of two hemibranchs, each bearing respiratory lamellae. (B) Longitudinal section through a filament under normal conditions, showing two lamellae, with red blood cells in pillar cell channels, and with ionocytes located mainly in the interlamellar region, overlying the central venous sinus (CVS). (C) At times of low O_2 demand, undifferentiated cells proliferate, forming the interlamellar cell mass (ILCM) (mechanism iv). This buries the lower pillar cell channels, reduces total surface area, and increases diffusion distance to the CVS and pillar cell channels. Note that the ionocytes retain contact with the water as they ride up on the surface of the ILCM. (D) During exposure to ion-poor softwater or chronic stress, ionocytes proliferate from the interlamellar region, and move up the sides of the lamellae overlying the pillar cell channels, and increasing the blood-to water diffusion distance (mechanism v). (E) Surface view of the interlamellar region, showing the apical exposures of ionocytes in between pavement cells that are ornamented with surface ridges. (F) Sectional view showing pavement cells covering over an ionocyte, pushing it downwards and reducing its area of apical exposure (mechanism vi). Under other circumstances, the pavement cells may retract, increasing the apical exposure of the ionocyte.

humoral factors including catecholamines (Steen and Kruysse, 1964; Wood, 1974; Booth, 1979; Nilsson, 1986; Olson, 1991):

Mechanism (i). The pathway of blood flow through individual secondary lamellae ("respiratory pathway") can be modified by the contractile pillar cells, which form the inner structure of the lamellae, and by pressure-driven stretching, thereby widening passages and thinning the overlying pavement cells (Steen and Kruysse, 1964; Wood, 1974). Blood flow may be preferentially directed to more basal channels, where the lamella is thicker and the channels are partially or completely buried (see below), so diffusion distances are greater. Alternately, blood can flow evenly through the whole lamellar sheet (probably most efficient for exchange), or through the much wider, longer marginal channel at the outer lamellar border (Wood, 1974), where both blood flow and water flow are probably faster, yet the time available for diffusive equilibration may be shorter and mean diffusion distance greater (Fig. 1B).

Mechanism (ii). Blood flow may be diverted ("shunted") into the central venous sinus of the filament ("ionoregulatory pathway") (Steen and Kruysse, 1964; Nilsson, 1986). Pre-lamellar anastomoses (which divert blood completely away from the respiratory pathway) seem to be relatively rare, whereas post-lamellar anastomoses (which divert blood after it has passed through the respiratory pathway) are much more common (Olson, 1991). The real significance of these anastomoses is that they direct more or less blood flow to the central venous sinus (CVS) of the filament which directly underlies the ionocytes (also called "mitochondria-rich cells" or "chloride cells"). After leaving the CVS, this "ionoregulatory" blood flow drains into the venous system ("arterio-venous pathway") via filamental and branchial veins whereas post-lamellar blood leaving via the efferent filamental arteries ("arterio-arterial pathway") continues via the dorsal aorta to the systemic circulation (Fig. 1B, C).

Mechanism (iii). At rest, the larger proximal lamellae close to the filament base appear to receive most of the blood flow, and the more distal, smaller lamellae closer to the filament tip are poorly perfused (Booth, 1979). Additionally, some blood may flow through direct connections from the afferent-to-efferent filamental arteries at the filament tip, thereby bypassing the respiratory lamellae (Steen and Kruysse, 1964). As O_2 demand increases, these distal lamellae start to receive more blood perfusion and the connections at the filament tip may be shut down (Fig. 1C).

The other three mechanisms (Fig. 2) involve physical blockage of diffusion pathways, and can all be considered as types of "reversible gill remodeling":

Mechanism (iv). The interlamellar regions on the filament surface where most of the ionocytes normally reside (Fig. 2B) may become infilled with undifferentiated cells, the interlamellar cell mass (ILCM; Nilsson et al., 2012) (Fig. 2C). This increases not only the water-to-blood diffusion distance for the CVS, but also for the secondary lamellae, so functional surface area is greatly reduced. At its extreme, the ILCM may completely bury the lamellae, converting the filaments into sausage-like tubes. Remarkably, the ionocytes are not buried but rather stay on the ILCM surface as it advances and regresses, and most remain innervated. They maintain access to the water but lose direct contact with the blood, but are presumably still connected through the interstitial fluid. Originally identified in extremely hypoxia-tolerant cyprinids under conditions of high O_2 availability and low O_2 demand (e.g. rest, cold

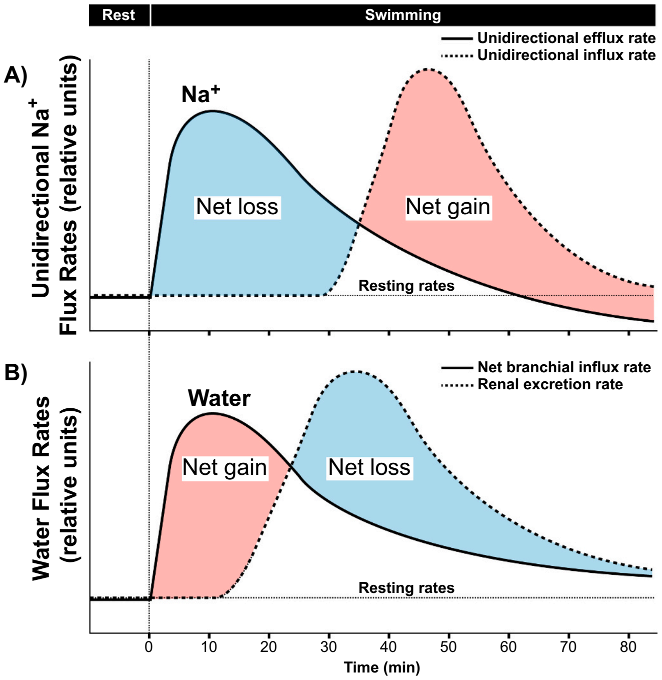

Fig. 3. Models of dynamic regulation of (A) gill Na^{+} permeability (based on data from Wood and Randall, 1973b and Postlethwaite and McDonald, 1995) and (B) gill water permeability (based on data from Wood and Randall, 1973c and Hofmann and Butler, 1979) during continuous aerobic exercise in rainbow trout, and the compensating mechanisms that are used to achieve a new internal condition as swimming continues. In (A), as O_2 uptake increases at the start of exercise, the rate of unidirectional Na^{+} efflux by diffusion at the gills also increases greatly, resulting in net Na^{+} loss. However, a later increase in the rate of active unidirectional Na^{+} influx at the gills more than compensates. While diffusive Na^{+} efflux eventually falls below resting levels, active Na^{+} influx remains elevated above resting levels so a net Na^{+} gain occurs as exercise continues. In (B) the rate of osmotic water uptake at the gills also rapidly increases at the start of exercise, resulting in net water gain. This is subsequently compensated by a large increase in urine flow rate (renal excretion rate) that results in net water efflux. Both rates gradually decline in a coupled fashion as exercise continues but remain above the resting rate.

temperature, normoxia), the ILCM is now known to occur in many species in a variety of circumstances (Gilmour and Perry, 2018). In addition to reducing osmoregulatory costs, it may serve protective functions against pollutants, reactive O_2 species, and parasites. It may also provide structural support and minimize evaporation during emersion in amphibious fish, and play a role in normal development in air-breathing species (e.g. pirarucu) that shut down the respiratory function of the gills as they get older. The ILCM normally grows and regresses slowly (days-weeks) by the balance between mitosis and apoptosis, but may be sloughed within minutes-to-hours if O_2 demand is suddenly increased by high temperature, hypoxia, or enforced exercise. The ILCM itself is not innervated and its growth seems to be directly keyed to O_2 availability versus O_2 demand, though how this balance is sensed remains unknown (Nilsson et al., 2012; Gilmour and Perry, 2018).

Mechanism (v). The ionocytes themselves may also proliferate from their normal interlamellar location up onto the lateral surfaces of the secondary lamellae, resulting in increased water-to-blood diffusion distance for the pillar cell channels (Perry, 1998) (Fig. 2D). This is commonly seen when freshwater fish are exposed to very “soft”, ion-poor water and/or are chronically stressed. In both cases, cortisol mobilization may be an important cue, and the proliferation serves to increase active ion uptake (Perry and Wood, 1985). O_2 uptake is simultaneously impeded as evidenced by reduced hypoxia tolerance and exercise performance (Perry, 1998).

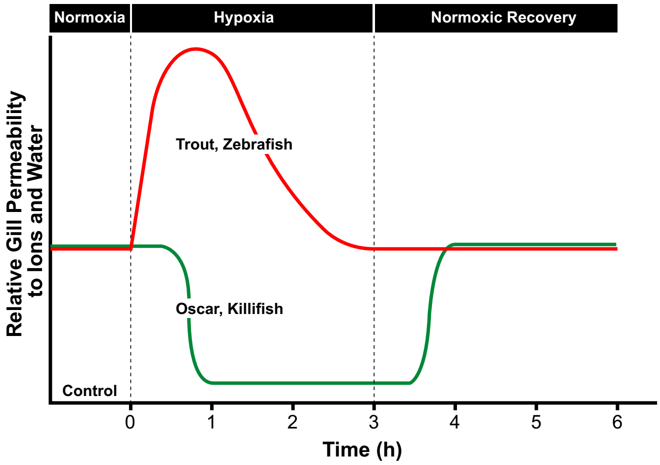

Fig. 4. Models of two strategies of dynamic regulation of the permeability of the gills to ions and water during acute exposure to hypoxia. In less hypoxia-tolerant species such as trout and zebrafish (Iftikar et al., 2010; Onukwufor and Wood, 2018, 2020), permeability initially increases greatly, but is regulated back towards control levels as hypoxia exposure continues. In very hypoxia-tolerant species such as oscars and killifish (Wood et al., 2007, 2009; Wood et al., 2019; Giacomin et al., 2020), permeabilty drops within about 1 h and stays low as hypoxia continues, but recovers back to control levels within about 1 h of restoration of normoxia. In both strategies, hyperventilation continues throughout the period of hypoxia exposure.

Mechanism (vi). The morphological relationships between ionocytes and the adjacent, partially overlying pavement cells (Figs. 2E,F), may change very quickly (minutes-to-hours) (Matey et al., 2011). In the hypoxia-tolerant Amazonian oscar, a fish that actually reduces ion and water fluxes during acute hypoxia, the pavement cells extend laterally, partially or completely covering the ionocytes and pushing them downwards so that their exposure area to the external water is greatly reduced (Wood et al., 2009). Conversely the hypoxia-intolerant rainbow trout increases ion and water fluxes during acute hypoxia, and this is correlated with an outward bulging of the ionocytes and increased exposure area, facilitated by pavement cell retraction (Iftikar et al., 2010). These pavement cell extensions and retractions are reflected in their surface “fingerprint” micro-ridges which become respectively wider apart and closer together. As water-to-blood diffusion distance is not affected, this mechanism provides a way of reducing osmoregulatory costs without affecting respiratory gas exchange. The proximate signals remain unknown.

3. Dynamic regulation of gill permeability during exercise

The IGR (Na^{+} loss per unit O_2 consumption) increased greatly during exercise, and fell below resting levels during recovery in many freshwater species (Gonzalez and McDonald, 1992, 1994). In trout, unidirectional Na^{+} efflux rate increased markedly at the start of exercise, but fell below control levels as exercise continued (Wood and Randall, 1973b), whereas active unidirectional influx exhibited a delayed elevation (Postlethwaite and McDonald, 1995) (Fig. 3A). Net water entry across the gills similarly increased greatly at the start of exercise and thereafter slowly declined although it remained above control levels as urine flow compensated during continued swimming (Wood and Randall, 1973c; Hofmann and Butler, 1979) (Fig. 3B). These are perhaps the best of many examples showing that exercising fish are not just victims of the osmorespiratory compromise, but can actively adjust it over time. It is surprising that we do not know which of the many possible mechanisms (Section 1) are used. Many authors have suggested that this is accomplished by regulation of the paracellular pathway - i.e.

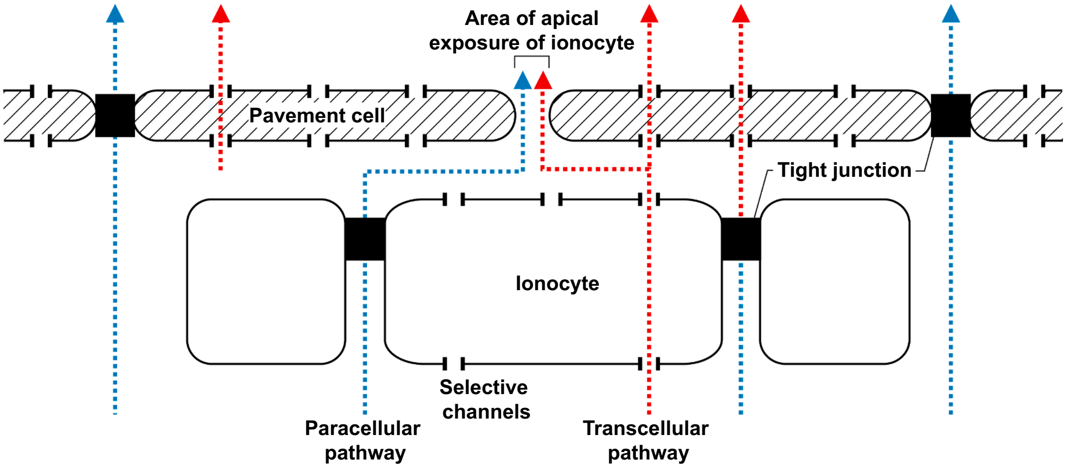

Fig. 5. A conceptual model illustrating paracellular and transcellular pathways by which substances may move across the gills, and how changes in the morphological relationships between pavement cells and the underlying ionocytes may blur the distinction between the two pathways. Thus, closure of the apical exposure of the ionocytes could cause a "morphological channel arrest", restricting both pathways. Modulation of the activity of individual substance- selective channels on apical and basolateral surfaces on both cell types may also modulate the transcellular permeability to specific molecules.

tight junctions between cells. However, we are not aware of any evidence that the paracellular pathway, rather than transcellular channels, is actually the major route of ion and water fluxes in fish gills. Indeed, while permeability to the paracellular marker polyethylene glycol MW 4000 (PEG-4000) did increase during exercise in trout (Robertson and Wood, 2014), it was much lower and did not change in oscars subjected to similar exercise, yet unidirectional Na^+ efflux rate still increased (Robertson et al., 2015a).

4. Dynamic regulation of gill permeability during hypoxia

While there are fewer hypoxia studies, here the evidence is stronger for dynamic regulation of branchial permeability, and for involvement of transcellular pathways. There appear to be two strategies. In the first, hypoxia-tolerant freshwater species such as the oscar (Wood et al., 2007, 2009), common killifish (Wood et al., 2019; Giacomin et al., 2020) and tambaqui (Robertson et al., 2015b) reduce unidirectional fluxes of Na^+ and net fluxes of other substances within about 1 h during severe hypoxia, even though they are clearly hyperventilating (Fig. 4). The changes are reversed within 1 h of restoration of normoxia. These flux reductions occur not only for Na^+ and Cl^-, but also for substances known to pass preferentially through transcellular pathways such as ^3H_2O (via aquaporins), ammonia (Rh glycoproteins), urea (UT proteins) and potassium (K^+ channels). The involvement of the paracellular pathway is less clear, as PEG-4000 permeability was reduced in only one of the two studies on killifish (Wood et al., 2019; Giacomin et al., 2020), and not in the oscar (Wood et al., 2009). In the second strategy, less hypoxia-tolerant species such as trout (Iftikar et al., 2010; Onukwufor and Wood, 2018) and zebrafish (Onukwufor and Wood, 2020) exhibit elevated Na^+ and ^3H_2O fluxes at the start of acute hypoxia, but these are then differentially attenuated during continued exposure and normoxic recovery (Fig. 4).

Rapid changes in the morphological relationships between pavement cells and ionocytes (mechanism vi of Section 2) may explain these adjustments (Wood et al., 2009; Iftikar et al., 2010; Matey et al., 2011). Fig. 5 shows a conceptual model. Coverage of the ionocytes by the pavement cells (Fig. 2E, F) may cause a "morphological channel arrest" for both transcellular and paracellular pathways (in hypoxia-tolerant species), whereas pavement cell uncovering would have exactly the opposite effect in hypoxia-intolerant species. These effects may be modulated over time. Additionally, individual membrane channels may be regulated, as recently reported for aquaporin AQP3 during hypoxia and normoxic recovery in the killifish (Ruhr et al., 2020). By these cell-level mechanisms, permeabilities to multiple substances can be altered, and the distinction between paracellular and transcellular pathways may be blurred (Fig. 5). All this may occur with minimal effect on the effective O_2 permeability as water-to-blood diffusion distance is negligibly changed.

There may also be complex, as yet poorly understood, interactions between the ILCM (mechanism iv) and mechanism vi. In goldfish, the presence of the ILCM decreases permeability to ammonia (Smith et al., 2012) and Na^+ (Bradshaw et al., 2012), but curiously not to PEG-4000 or Cl^- (Mitrovic et al., 2009). Although hypoxia causes regression of the ILCM, which might be expected to increase permeability, it actually reduces the fluxes of all four substances, suggesting recruitment of mechanism vi.

5. The Osmorespiratory compromise in seawater fish

Most of the relevant studies to date have been on freshwater fish, yet we might expect the osmoregulatory side of the compromise to be under greater threat in seawater fish. For teleosts, absolute water-to-blood ionic and osmotic gradients are 2- to 3- fold greater in seawater, yet the respiratory gas gradients are about the same. Thus, in euryhaline coho salmon acclimated to a range of salinities for more than a year, disturbances in plasma osmolality caused by acute hypoxia were greatest in 100% seawater (Damsgaard et al., 2020). Seawater-acclimated killifish exhibited lower ^3H_2O permeability than freshwater-acclimated conspecifics under normoxia, but unlike the latter, were not able to decrease it under acute hypoxia (Wood et al., 2019; Giacomin et al., 2020), a difference which correlated with AQP3 protein abundance in the gills (Ruhr et al., 2020). Nevertheless, the absolute osmotic fluxes of water incurred during hypoxia were the same in the two salinities (net gain in freshwater, net loss in seawater), despite the much greater gradient in the latter, showing an enhanced ability to cope in seawater. Even marine fish that have "solved" the osmoregulatory problem by being approximately isosmotic to the environment exhibit penalties associated with the compromise. Elasmobranch dogfish sharks lost large amounts of urea (their primary, metabolically expensive osmolyte) across the gills during acute hypoxia (Zimmer and Wood, 2014) and high temperature exposures (Giacomin et al., 2017), suggesting failure of the retention mechanism at the gills. Agnathan hagfish, which have exceptionally high ^3H_2O permeability under normoxia, actually reduced it during hypoxia (like the oscar), but exhibited large increases when challenged with high temperature (Giacomin et al., 2019a; Giacomin et al., 2019b). In 2011, Gonzalez concluded that "*it is difficult to reach any conclusion of the osmorespiratory compromise in* *seawater fish*". Since then, new findings have demonstrated that it is real and very important, but patterns are variable and mechanistic understanding remains thin. This is a ripe area for future investigation, one which August Krogh would have undoubtedly tackled if he were alive today, in light of his fascination with the osmoregulatory challenges facing seawater fishes (Krogh, 1939).

Conflicts of interest

The authors declare no competing or financial interests.

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