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Review

Ontogeny and paleophysiology of the gill: New insights from larval and air-breathing fish☆

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ABSTRACT

There are large changes in gill function during development associated with ionoregulation and gas exchange in both larval and air-breathing fish. Physiological studies of larvae indicate that, contrary to accepted dogma but consistent with morphology, the initial function of the gill is primarily ionoregulatory and only secondarily respiratory. In air-breathing fish, as the gill becomes progressively less important in terms of O_2 uptake with expansion of the air-breathing organ, it retains its roles in CO_2 excretion, ion exchange and acid–base balance. The observation that gill morphology and function is strongly influenced by ionoregulatory needs in both larval and air-breathing fish may have evolutionary implications. In particular, it suggests that the inability of the skin to maintain ion and acid–base balance as protovertebrates increased in size and became more active may have been more important in driving gill development than O_2 insufficiency.

1. Introduction

The internal fish gill is one of the most complex organs found in vertebrates. In teleosts, the primary focus of this review, gill functions include oxygen (O_2) and carbon dioxide (CO_2) exchange, ionoregulation, acid–base balance, ammonia excretion, water balance, hormone production, activation/inactivation of circulating metabolites and immune defense (summarized in Rombough, 2007). This physiological complexity is matched by a corresponding anatomical complexity. The gills of teleosts consist of gill arches, filaments and lamellae. In adults, the lamellae are considered the definitive site of respiratory gas exchange but all three structural components may participate to some extent. Oxygen uptake occurs by simple diffusion as does the bulk of CO_2 excretion. Ion exchange and some ammonia excretion are mediated by specialized gill cells collectively called ionocytes or mitochondrion-rich cells (Dymowska et al., 2012; Wright and Wood, 2012). The major sites for ionocytes are on the gill filaments but in some species they are also located on the lamellae. Several subtypes of ionocytes with different functional properties have been identified (Evans, 2011; Hwang et al., 2011; see Dymowska et al., 2012). The number of subtypes and the specific ions exchanged by each subtype vary depending on the environment (e.g. in fresh or salt water; see Hiroi and McCormick, 2012; Kumai and Perry, 2012) and species. The circulation of the gill is also complicated. Three distinct blood pathways have been identified: a respiratory (arterio-arterial) pathway, an interlamellar pathway and a nutrient pathway (Olson, 2002). The respiratory pathway, as the name suggests, is involved in gas exchange. The interlamellar and nutrient pathways are part of the secondary circulation; their precise functions remain unclear. The gill is well-innervated with both afferent and efferent nerves. The afferent division includes O_2 and CO_2-sensing neuroepithelial cells (Jonz and Nurse, 2005; Qin et al., 2010; see Zachar and Jonz, 2012) while the efferent division appears to synapse with gill ionocytes suggesting that ion exchange is subject to direct nervous control (Jonz and Nurse, 2006). Gill cells also respond to hormones and other circulating factors. The anatomy of the gill musculature and associated ventilatory structures likewise is complex, as are the ventilatory control pathways.

Inherent in the physiological complexity of the gills is the potential for interactions and tradeoffs among the different functions. A currently topical area related to gill function is the interaction between ionoregulation and gas exchange at the gills, termed the osmorespiratory compromise (Nilsson, 1986). There is often some separation of function, for example ionocytes may be localized on the filament and gas exchange occurs primarily across the lamellae, but there is always some degree of interaction among the processes. Thus, gill morphology under a given condition will represent a compromise among these processes. Traditionally, gill morphology in adult fish was thought to be relatively non-plastic. However, recent

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work on several species of carp has shown that a reduction in O_2 availability or an increase in O_2 demand can result in large and rapid changes in gill morphology. In normoxia at low temperatures the interlamellar spaces of the carp gill are filled with cells resulting in a column-like appearance of the filaments. During exposure to hypoxia (Sollid et al., 2003; Matey et al., 2008), an increase in temperature (Sollid et al., 2005), hemolytic anemia, exposure to carbon monoxide (Tzaneva et al., 2011), or forced sustained exercise (Brauner et al., 2011; Fu et al., 2011) the interlamellar cell mass is lost through a combination of apoptosis and cell cycle arrest. The result is a dramatic increase in total gill surface area, in some cases exceeding control values by 7-fold (Sollid et al., 2003). There is also a reduction in blood–water diffusion distance (Matey et al., 2008), which in combination with the increase in SA greatly increases the diffusing capacity of the gill. The impact is particularly pronounced during exposure to hypoxia (Sollid et al., 2003; Fu et al., 2011). In some studies, this appears to be associated with negative effects on ionoregulation (Sollid et al., 2003; Matey et al., 2008; Mitrovic et al., 2009) indicating the nature of the trade-off between gas exchange and ionoregulation at the gills.

In addition to the dramatic changes in gill morphology noted in carp, more subtle changes have been observed in adult fish of other species, including salmonids. During acclimation to ion poor waters (soft water), extensive proliferation of ionocytes in the branchial epithelium has been observed presumably to enhance ion uptake from the water (Greco et al., 1995, 1996; Perry, 1998). In salmonids in freshwater, ionocytes generally cover less than 10% of the total gill surface area and are often restricted to the interlamellar regions of the gill. Acclimation to soft water results in proliferation of ionocytes which may end up comprising as much as 30% of the total gill surface area (Perry, 1998) and resulting in a doubling of the blood–water diffusion distance (Greco et al., 1996). These changes while enhancing ion uptake appear to have a negative effect on gas exchange (see Perry, 1998). Dussault et al. (2008) noted that chronic exposure to soft water resulted in a 14% reduction in the critical swimming velocity (U_{crit}), of rainbow trout which they attributed to the reduction in gill diffusing capacity. Thus, ionocyte proliferation and the associated lamellar thickening are associated with a cost in terms of whole animal performance, again attesting to the trade-off between gas exchange and ionoregulation at the gills.

It is now clear that gill morphology and function in some species of fish is quite plastic and can be dramatically altered to meet changes in demands placed upon the gills by altered environmental conditions, such as low O_2 or ion levels. However, an area that has received less attention is the role of the gills during development, and how gill function changes at different developmental stages where selection pressures associated with gas exchange and ionoregulation may differ. This review will focus firstly upon larval fish, where a body of research now indicates that the dominant role of the gill may initially be ionoregulatory and secondarily respiratory. This review will then summarize changes in the gills of air-breathing fish during development as the primary function of the gill shifts away from gas exchange with the development of the air-breathing organ. Finally, the review will conclude with implications of these findings on the evolution of gills in early vertebrates.

2. Larval fish

2.1. The oxygen hypothesis

Although it has long been known that the gill is involved in processes other than gas exchange (e.g. Krogh, 1939), the role of the gill in O_2 uptake has been the primary focus of those interested in the ontogeny and evolution of the vertebrate gill. This emphasis seems to be a reflection of the fact that in most adult fish, O_2 transport is the immediate critical function of the gill. Adult fish placed in anoxic water typically die within a few minutes; the effects of selectively ablating the other functions of the gill take considerably longer to become apparent. The overarching importance of O_2 uptake in adult fish has been assumed to also apply during development when the gills are first forming. Developing fish, like all vertebrates, initially have no specialized respiratory structures and thus gas exchange occurs across the skin. This is possible because of the large surface area (SA) of the skin in embryos/larvae relative to their mass and O_2 consumption rate ($\dot{M}_{O_2}$). Body SA is roughly proportional to length squared, while $\dot{M}_{O_2}$ is proportional to the body volume, and thus length cubed. Thus, as animals grow, the SA: $\dot{M}_{O_2}$ ratio tends to decline in proportion to body mass$^{2/3}$ ($M^{2/3}$) (Rubner, 1883). Furthermore, with development, the skin becomes mineralized and thicker, increasing gas diffusion distance. At some point, the ability of the skin to supply the growing organism with O_2 becomes limiting which would lead to hypoxemia were it not for the development of the gills. Gills because of their anatomical complexity are not subject to the same geometric constraints as the skin (i.e. SA can expand at a rate $>M^{2/3}$). This scenario, first advanced by Krogh (1941), is termed the "oxygen hypothesis" and, until recently has been the accepted explanation for why fish develop gills.

Over the past few decades technological advancements in areas such as histochemical imaging and the design of gas- and ion-specific microelectrodes have facilitated research on the anatomy and physiology of developing fish. One emerging trend is that gas exchange may not be the first critical function to shift from the skin to gill. In studies where the ontogeny of gas and ion exchange have been investigated simultaneously, a consistent pattern has emerged indicating that ion exchange may be the first to transition to the developing gill (reviewed in Rombough, 2007). This scenario has been termed the ionoregulatory hypothesis.

2.2. The ionoregulatory hypothesis

The ionoregulatory hypothesis has been proposed as an alternative explanation for why fish initially develop gills. Li et al. (1995) appear to have been the first to formally propose that the gill of teleosts may play a dominant role in ionoregulation before gas exchange. This supposition was based on changes in gill morphology and histology in tilapia, *Oreochromis mossambicus*, larvae where ionocytes on the filament develop prior to gill lamellae. Since then, similar developmental sequences have been reported for a wide variety of marine and freshwater fish (reviewed in Rombough, 2007). Quantification of the total numbers of ionocytes on the skin and the gill in developing rainbow trout (Fig. 1) strongly indicate that the bulk of ionoregulatory activity shifts to the gill quite early in development and well before the gill begins to play a major role in gas exchange (Rombough, 1999). This observation has been supported using radioisotope fluxes to quantify unidirectional ion uptake further supporting the idea that the developing gill quickly becomes the dominant site of ion exchange (reviewed in Rombough, 2004). Functional ablation experiments using zebrafish larvae have shown that the gill becomes essential for ionoregulation about a week before it is needed for O_2 uptake (Rombough, 2002). Most recently, Fu et al. (2010) were able to directly demonstrate that the bulk of Na^+ uptake shifts to the gill well before O_2 uptake in developing rainbow trout. These studies and others are elaborated upon below.

2.3. Evidence for the ionoregulatory hypothesis

The geometric constraints that provide the theoretical basis for the O_2 hypothesis can also be applied to the ionoregulatory

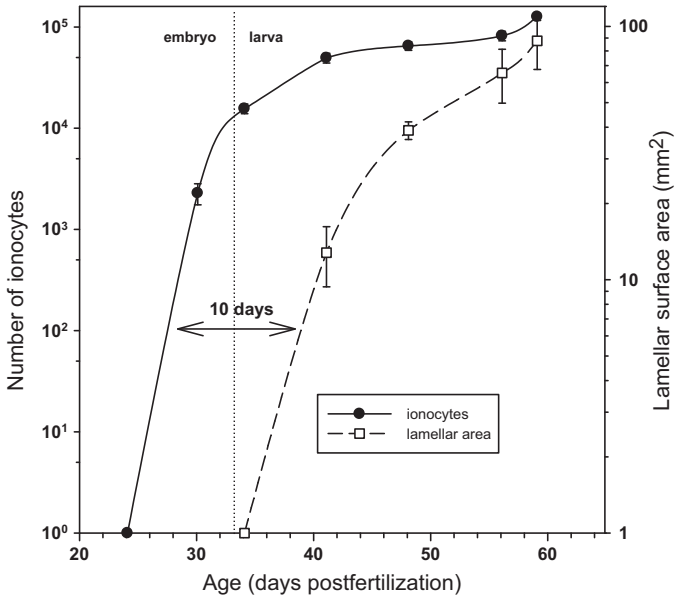

Fig. 1. Changes in total number of ionocytes (left axis) and the total surface area of lamellae (right axis) on the gills of rainbow trout during the course of embryonic/larval development. The vertical dashed line indicates median hatch time. Expansion of branchial ionocytes precedes the expansion of lamellar surface area by about 10 days.

Data from Rombough (1999).

hypothesis. Ion exchange, like O_2 uptake, is a surface phenomenon and, as such becomes progressively constrained as surface area:volume ratios decline and the skin thickens. Indeed, there are reasons to believe that ion exchange is even more affected than O_2 uptake in this regard. The rate of O_2 uptake decreases as the skin thickens but does so progressively and relatively independently of capillary density (Malvin, 1988). Cutaneous ionoregulation requires that ionocytes have their apical membrane exposed to the external environment and their basal membrane in close contact with a capillary or blood sinus (Alderdice, 1988). This becomes less likely with development as the skin thickens and capillary density declines. The net result is a sharp decline in the density of skin ionocytes during larval development (Fig. 2). For example, cutaneous ionocyte density drops from about 1800 mg^{-1} at hatch to ~300 mg^{-1} by the start of exogenous feeding in rainbow trout *Oncorhynchus mykiss* (Rombough, 1999). In sea bass, *Dicentrachus labrax*, 29% of skin surface area is occupied by ionocytes at hatch; by the time larvae are 26 mm long, ionocytes occupy only about 2% of skin surface area (Varsamos et al., 2002). Despite the sharp decline in the relative number of ionocytes on the skin, there is no corresponding decline in whole body ionoregulatory capacity. This is because the rapid proliferation of branchial ionocytes more than compensates for the loss of cutaneous ionocytes.

Ionocytes are evident on the developing gill soon after the gill begins to form in most species. For example in rainbow trout, ionocytes were first observed on the gill at about the same time as the gills form distinct arches (Gonzalez et al., 1996). In sea bass, ionocytes appear on the gill arches shortly after the mouth opens and well before the filaments develop (Varsamos et al., 2002). Indeed in every species that has been looked at to date, ionocytes appear on the gill well in advance of the lamellae (reviewed in Rombough, 2007). Some gas exchange undoubtedly takes place across the filaments and arches before the lamellae form but net flux is probably small because of the relatively large diffusion distances. In newly hatched Atlantic salmon, *Salmo salar*, for example, the harmonic mean diffusion distance is about 3.8-times greater for filaments than for lamellae (14 μm vs. 3.7 μm, respectively; Wells and Pinder, 1996a). Branchial ionocytes, in contrast, initially appear to be more active than they are later in life. Li et al. (1995) reported that in tilapia gill mass-specific Na^+, K^+-ATPase (NKA) activity was 390-times higher in 10 day old larvae than in adults. Consistent with this, the apparent mass-specific Na^+ uptake capacity in zebrafish is about 6-fold greater in larvae than in adults (Rombough and Brauner, unpublished data). Taken together, the low O_2 diffusing capacity and high NKA activity of the early gill indicates it may initially play a much greater role in ion exchange than gas exchange.

Direct measurements of blood PO_2 levels in developing rainbow trout support the view that the gill initially plays a minor role in whole body O_2 uptake. Rombough (1992) used microelectrodes to measure PO_2 values in blood vessels leading toward the gill (anterior vitelline vein) and away from the gill (efferent branchial artery) in spontaneously ventilating rainbow trout larvae. At the two stages tested, immediately after hatch and just before mid-yolk absorption, blood PO_2 values were actually lower in the efferent branchial artery implying that at this stage, the gill is more of a sink for O_2 than as a source for the rest of the body. Partitioning studies to quantify gill and skin O_2 uptake revealed that the gills were responsible for 20–30% of total O_2 uptake during this period (Rombough, 1998). This O_2 must have been used locally, likely to power gill ionoregulatory activity due to the high NKA activity of the larval gill, given that it does not show up in the systemic circulation. At present there are no definitive data regarding the relative cost of ionoregulation in developing fish. Estimates for adult fish generally range from 2 to 20% of routine metabolism (Febry and Lutz, 1987) but values as high as 40% have been reported (Wood et al., 2007).

Gill ablation experiments indicate that in developing zebrafish, gills are required for ionoregulation well before they become absolutely necessary for O_2 uptake. Rombough (2002) used a variety of chemical and physical methods to block gill ventilation in zebrafish larvae that were placed in physiological saline to mitigate the impact of impaired ionoregulatory activity, in hyperoxia to compensate for impaired O_2 uptake, or in a combination of the two. Survival times under these conditions were compared with survival times in normal freshwater (Fig. 3). Immediately after hatch (3 days postfertilization, dpf), none of the treatments improved survival. Indeed, blocking gill ventilation in newly hatched larvae appeared to have no noticeable effects. At 7 dpf, physiological saline but not hyperoxia significantly improved survival. At 14 dpf, survival times were significantly longer in both treatments but by 21 dpf, only hyperoxia improved survival. These results were interpreted to indicate that cutaneous exchange on its own was sufficient to meet the ionoregulatory requirements of larvae until about 7 dpf and O_2 requirements until about 14 dpf. This difference of 7 dpf is considerable when one considers that larvae begin to feed exogenously by 5–6 dpf. The time during development when the gills first become necessary to supplement cutaneous ion and gas exchange agrees well with histological observations. Ionocytes appear on the zebrafish gill at about 5 dpf; gill lamellae do not begin to form until 9–12 dpf (Rombough, 2002; Jonz and Nurse, 2006). Branchial ionocytes appear to be innervated by the efferent division of the peripheral nervous system as early as 5 dpf (Jonz and Nurse, 2006). In contrast the afferent O_2-chemosensory pathways do not become established until about 7 dpf.

Partitioning studies, where ventilatory flow over the gills is physically separated from water flow over the rest of the body, are considered "the gold standard" when it comes to estimating the relative importance of cutaneous and branchial exchange processes. It is extremely difficult to separate flows with most larvae because of their small size. The larvae of salmonids appear to be just big enough. To date, the method has been used successfully to look at O_2 uptake in three species: chinook salmon, *Oncorhynchus tshawytscha* (Rombough and Ure, 1991), Atlantic salmon (Wells and Pinder, 1996b) and rainbow trout (Rombough, 1998; Fu et al., 2010).

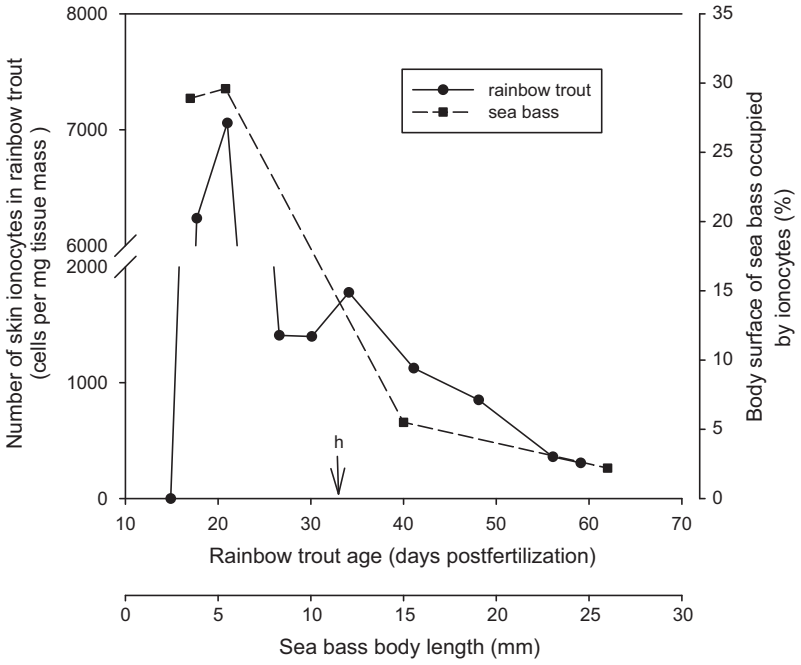

Fig. 2. Age related declines in the relative density of ionocytes on the skin of rainbow trout and sea bass. Arrow labeled "h" indicates median hatching time for rainbow trout. Data for rainbow trout from Rombough (1999); data for sea bass from Varsamos et al. (2002).

In all three, the gills account for about 20% of O_2 uptake at hatch. The relative gill O_2 uptake increases gradually in a hyperbolic fashion during larval development, accounting for about 50% of total uptake by mid-yolk absorption and 70–80% by the end of yolk absorption. Only one study has looked at how ion uptake changes during larval development but this study is particularly interesting because it simultaneously determined O_2 uptake allowing the direct comparison of the trajectories for the two processes (Fu et al., 2010). The underlying pattern for Na^+ uptake was similar to that for O_2; low branchial uptake at hatch followed by a gradual increase toward asymptotic levels with development (Fig. 4). The key difference was that the shift from predominantly cutaneous to predominantly branchial uptake occurred significantly earlier for Na^+ than for O_2. By 15.5 days posthatch (dph), 50% of total Na^+ uptake had shifted to the gill; it took until 27 dph before the gill was responsible for 50% of O_2 uptake. This difference (11.5 days) is highly significant when one considers it represents about 1/3 the total length of the larval developmental period.

The extent to which rainbow trout are representative of fish generally remains to be established. The larvae of most species are much smaller than those of rainbow trout and thus the rubber dam method used to study partitioning in salmonids is unlikely to work for the majority of fish species. One way to circumvent this problem is to find morphological indicators that accurately reflect exchange capacities. Wells and Pinder (1996b) found that the anatomical diffusing factor (mass-specific surface area divided by mean diffusion distance) provided a fairly good estimate of how O_2 uptake was partitioned in Atlantic salmon. Determining anatomical diffusing factors is time-consuming but feasible for small larvae. One possible indicator for ion exchange capacity is the relative number of ionocytes on the skin and gill. In rainbow trout, the percent of total ionocytes located on the gill gradually increased during larval development and reached 50% at 15.7 dph (Rombough, 1999). This is remarkably close to the age (15.5 dph) where direct measurements determined that ≥50% of larval Na^+ uptake occurred across the gills (Fu et al., 2010) suggesting that tissue ionocyte distributions may be a good indicator of ionoregulatory capacity. Another possible alternative is to use chemical or physical means to block gill exchange. This seemed to work with zebrafish larvae (Rombough, 2002) although there is the possibility that blocking gill ventilation might lead to compensatory increases across the skin that would not occur if the gill was still being ventilated.

Clearly more research is required to understand the role of the developing larval fish gill, not only in terms of ionoregulation and gas exchange, but also in terms of the other processes taking place at the gill. Acid–base balance and ammonia excretion are particularly interesting in this regard in that they are highly

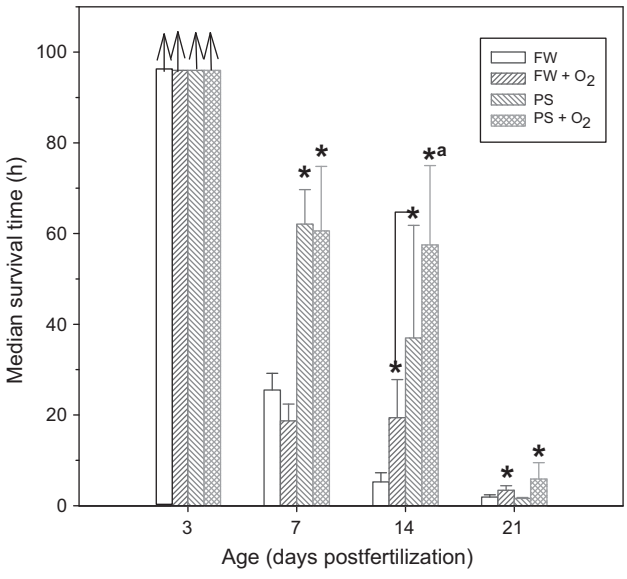

Fig. 3. Survival of non-ventilating zebrafish larvae in normoxic fresh water (FW), hyperoxic fresh water (FW+ O_2), normoxic 50% physiological saline (PS) and hyperoxic 50% physiological saline (PS + O_2). *Significant increase in median survival time compared with FW. "↑" indicates greater than 100 h survival. Joined asterisks indicate treatment effects were not significantly different. "a" indicates a significant additive effect.

Data from Rombough (2002).

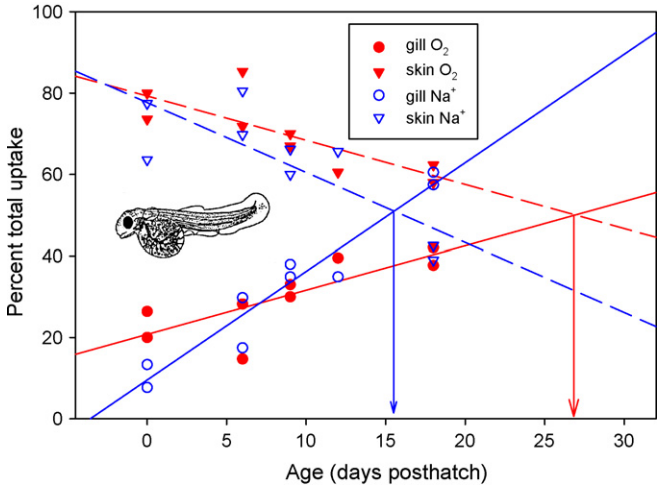

Fig. 4. Relative contributions of the gill and skin to total O_2 and Na^+ uptake in rainbow trout larvae. Vertical arrows indicate the age at which 50% of total Na^+ (15.5 days) or O_2 (27 days) uptake has shifted to the gills (after Fu et al., 2010 where data for soft and hard water were combined).

interconnected with ionoregulation and with each other. To date, no one has attempted to tease these processes apart in the developing gill. What has been referred to euphemistically as "ionoregulation" in terms of early gill function could turn out to be more directly related to acid–base balance or ammonia excretion than the exchange of the mineral ions (e.g. Na^+, Cl^-) which is generally implied by the term ionoregulation. Most of the studies described above, including the partitioning studies, were conducted in resting fish, often lightly anaesthetized, and reared under fairly standard conditions. Further studies are required to understand how gill developmental trajectories are affected by a change in environmental conditions that alters demands on gas exchange (e.g. hypoxia, hyperoxia) or ionoregulation (e.g. pH, ion composition of the water), as well as conditions that alter overall developmental rate (e.g. temperature) or gill demand (e.g. activity levels). This information will be important in determining the degree of plasticity that exists in the gills during development in water-breathing larval fish.

Another interesting model system for understanding the ontogeny of gill function is that of air-breathing fish where selection pressures on the gill change during development as reliance upon aerial respiration progressively increases. This is the focus of the remainder of this review.

3. Air-breathing fish

3.1. Gill function during development of air-breathing

Air-breathing in fish is thought to have evolved independently almost 70 times and consequently there is both unity and diversity in the anatomy and physiology of the structures and adaptations associated with air-breathing (see Graham, 1997 for a review). In fish that possess air-breathing organs (ABO) it is well established that the ABO is primarily specialized for O_2 uptake and in obligate air-breathers (those that air-breath even in O_2 saturated waters), it is the dominant site for O_2 uptake. Air-breathing fish, especially obligate air-breathers, have a reduced mass-specific total gill surface area relative to non-air-breathers which is a result of having both fewer and smaller gill filaments and lamellae (see Graham, 1997 for a review). In many air-breathing fish, the blood–water diffusion distance is also higher than in water-breathing fish. This reduction in gill diffusing capacity is thought to be important in reducing diffusive loss of O_2 across the gills from blood that is oxygenated in the ABO prior to gill perfusion (Graham, 1997). However, despite the great reduction in gill diffusion capacity, the gills in air-breathing fish seem to remain the dominant structure for CO_2 elimination, indicating that they still play an important role in metabolic gas exchange. The gills also appear to remain the dominant structure for ionoregulation, acid–base balance and nitrogenous waste excretion although these functions have rarely been investigated directly an area clearly worthy of study.

Most of our current understanding of the influence of air-breathing on fish gill structure and function is based upon inter-specific comparisons between water-breathing fish and closely related air-breathing fish that differ in their degree of dependence on aerial respiration (e.g. facultative vs. obligate air-breathers; Fernandes et al., 1994). However, a powerful and underutilized approach is to investigate intra-specific changes in gill structure and function associated with growth and development in air-breathing fish. All air-breathing fish initially appear to be water-breathers, if only briefly. Graham (1997) concluded that 10 mm represents the minimum body size at which air-breathing commences. This is larger than the size at which the gills become the dominant site for gas exchange in most purely water-breathing fish and implies that even in air-breathing fish there is a period where the gills may be the major site of O_2 uptake.

The greatest post-larval ontogenetic changes in gill structure and function associated with air-breathing would be expected in fish that are obligate air-breathers as adults. One such fish that has been looked at in this regard is *Arapaima gigas*. Early in development, *A. gigas* is a water-breather, but at approximately 9 dph when it reaches a length of about 18 mm it starts to breathe air (Lüling, 1964). As an adult, *A. gigas* is impressive in many regards. It can reach a mass of 250 kg and length of 4.5 m (Salvo-Souza and Val, 1990; Graham, 1997) making it one of the largest freshwater fish in the world. Furthermore, it secures up to 80–95% of its O_2 uptake from its ABO (Brauner and Val, 1996; Stevens and Holeton, 1978; Sawaya, 1946) and reportedly drowns within 10 min without access to air (Val and Almeida-Val, 1995) making it one of the most, if not the most, aerially dependent air-breathing fish known. During development, *A. gigas* exhibits large and dramatic changes in gill morphology. As will be discussed, the functional significance of these changes is still unclear.

3.2. Morphological changes in the gills during development in A. gigas

With the progressive onset of air-breathing during development the functional requirement for O_2 uptake across the gills is reduced and in some extreme obligate air breathers, eventually almost completely lost. Effectively removing O_2 uptake as a selection pressure driving gill development may influence gill morphology. It has long been known that adult air-breathing fish have a reduced total gill surface area compared to water breathing fish (Graham, 1997). However, given that all air-breathing fish are water-breathers initially (during larval or juveniles stages) as discussed above, large changes in gill morphology may be expected throughout development. This was explored by Brauner et al. (2004) using *A. gigas* as a model. At 10 g, the smallest size examined, *A. gigas* are already obligate air-breathers. At this stage the gills possessed well developed, albeit stubby lamellae, typical of water breathing fish (Fig. 5A and B). One to two months later, when the fish weighed 70–100 g, the lamellae had become much less discernible (Fig. 5C and D) with much of the interlamellar space partially filled with cells including ionocytes. By the time the fish were between 0.7 and 1 kg, the lamellae were no longer visible, the filaments possessed a smooth column like appearance and the interlamellar space was completely filled (Fig. 5E and F). The filamental ionocyte density was two-fold higher in a 1 kg fish than in a 10 g fish (Brauner et al., 2004);

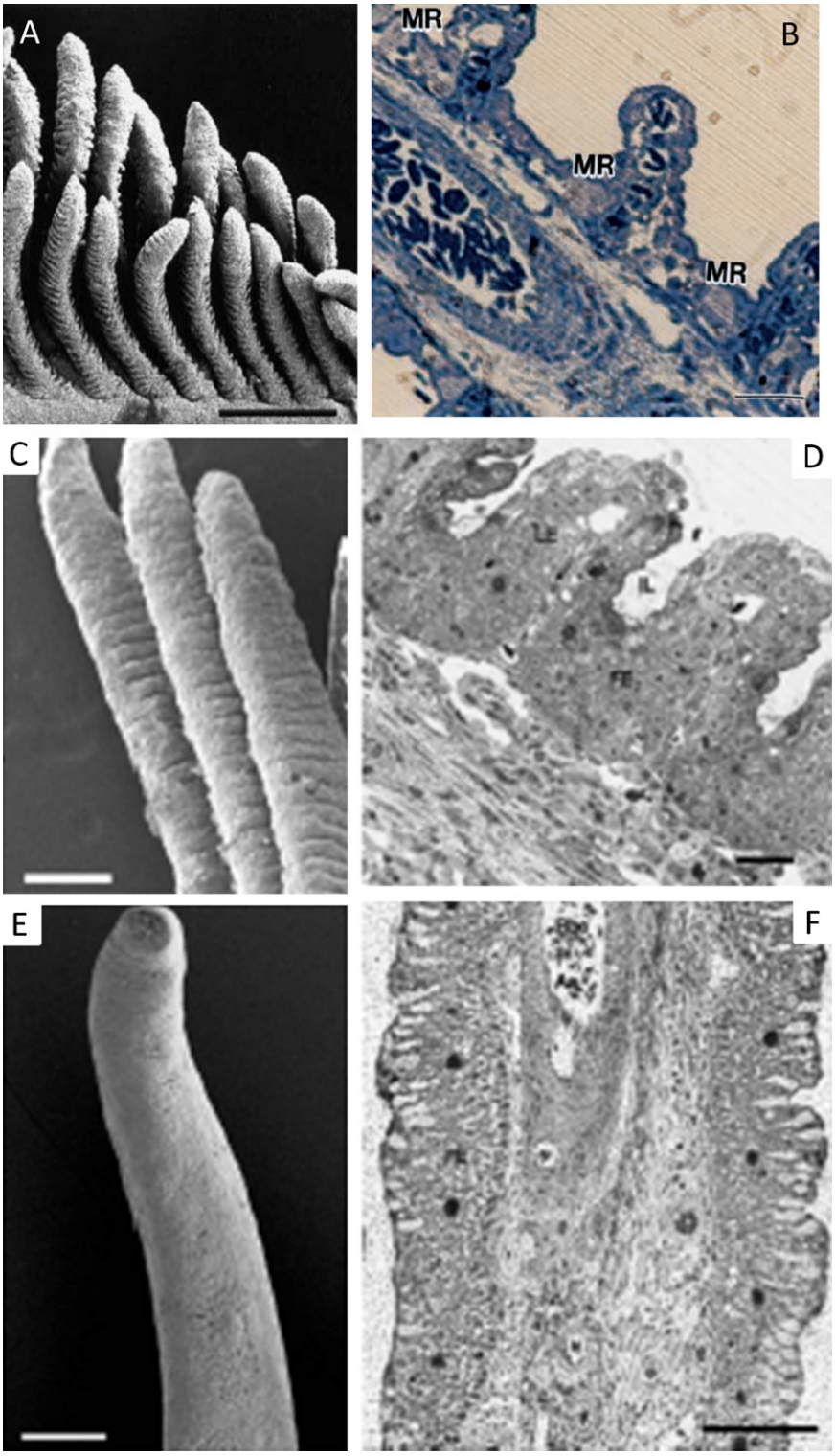

Fig. 5. Scanning electron and light microscopy views of the gills of small (A and B; 10 g), medium (C and D; 70 g) and larger (E and F; 700 g) *Arapaima gigas*. (A) Lateral view of gill filaments, with distinctive lamellae separated by interlamellar spaces. (B) Longitudinal section through a gill filament. In (C) and (D) note the thick filament and lamellar epithelia, short protruding lamellae, and narrow interlamellar spaces. (E and F) Note columnar appearance of filament with no lamellae evident. Scale bars: (A, C and E) 500 μm, (B and D) 20 μm, and in (F) 100 μm.

(A) and (B) are from Brauner et al. (2004), (C)–(F) are from Gonzalez et al. (2010).

the blood–water diffusion distance was four-fold greater (Gonzalez et al., 2010). Thus, with increased dependence upon aerial respiration and less dependence on water-breathing, there are dramatic changes in gill morphology. Morphologically, it appeared that the gill shifted from being primarily a gas exchange structure to an ion or acid-base regulatory organ (Brauner et al., 2004). It would be interesting to determine whether the timing and extent of the changes in gill morphology are influenced by environmental rearing conditions, such as environmental hypoxia or hyperoxia and whether these changes are reversible, as in carp. Furthermore, the

degree to which similar changes are seen during development in other air-breathing fish is worthy of further investigation.

3.3. Functional changes in the gills during development in A. gigas

The changes in gill morphology with development in *A. gigas* appear to indicate an increase in the ionoregulatory capacity of the gills in larger fish. Gonzalez et al. (2010) conducted a suite of experiments to investigate the physiological implications of these morphological changes on aspects of gas exchange, ionoregulation and ammonia excretion by comparing small (70 g) and large (700 g) fish. Only the results for ionoregulation will be discussed here. In resting fish, the mass-specific unidirectional Na^+ uptake rate of the 700 g fish was approximately 3-fold greater than that of the 70 g fish in low Na^+ water. The higher uptake rate in larger fish was balanced by a 3-fold higher rate of Na^+ efflux. Interestingly, the mass-specific gill NKA activity of the larger fish was 40% lower than that of smaller fish even though their Na^+ uptake rate was higher. Na^+ uptake kinetic analysis revealed a very low-affinity and low-capacity transport system in *A. gigas* with Na^+ uptake rates at the low [Na^+] typical of Amazonian waters that were only about 5–10% of those measured in water-breathing Amazonian fish. Taken together, these data do not support the hypothesis that the gills of the larger fish have a higher Na^+ uptake capacity, despite the higher gill ionocyte density in larger fish. Indeed, it appears that active Na^+ uptake across the gill was not sufficient to balance Na^+ efflux (i.e. there was a net loss of ions across the gills). Gonzalez et al. (2010) concluded that *A. gigas* depend upon a "terrestrial" mode of ionoregulation which emphasizes dietary uptake of ions and renal conservation to maintain ion homeostasis.

The one striking finding regarding the ionoregulatory capacity of *A. gigas* was its ability to resist losing ions across the gill at low pH. Na^+ efflux was only modestly elevated in *A. gigas* at a water pH of 3.5, a level of acidity that dramatically increases Na^+ efflux in most fish. Even when compared with cardinal and neon tetras that can survive for weeks at a pH of 3.5, Na^+ efflux in *A. gigas* was far less affected by high H^+ levels. This makes them among the most acid resistant fish investigated to date (Gonzalez et al., 2002). It is possible that the driving pressure behind the reduced gill area and increased blood–water diffusion distance in large *A. gigas* with development has more to do with resisting ion loss in the low pH waters of the Amazon than in enhancing ion uptake. This raises the possibility that the morphological changes seen in the larger fish could somehow be related to acid–base balance; whether this is the case remains to be determined.

4. Implications

Clearly, there are large changes in gill function during development associated with ionoregulation and gas exchange in both larval and air-breathing fish. In larval fish, the initial function of the gill appears to be primarily ionoregulatory and secondarily respiratory. In air-breathing fish, as the need for branchial O_2 uptake diminishes with development of the air-breathing organ, there is a remodeling of the gill consistent with that of larval fish: fewer lamellae and a predominance of ionocytes. In both conditions, gill morphology and function is strongly influenced by ionoregulatory needs and this observation may have evolutionary implications.

A variant of the oxygen hypothesis has been used to explain why gills initially originated in vertebrates. It is generally accepted that the internal gill of vertebrates is homologous with the branchial basket of filter-feeding protochordates (e.g. Romer and Parsons, 1986; Gans, 1989). The branchial basket of *Amphioxius*, an extant cephalochordate anatomically similar to early Cambrian protochordates such as *Haikouella* and *Pikaia*, is primarily a feeding structure. Morphological studies indicate that it likely plays a very minor role in O_2 uptake (Schmitz et al., 2000). The transition from an ancestral filter-feeding organ to a respiratory organ is generally assumed to have been driven by increased demand for O_2 as protovertbrates became larger and more active combined with a trend toward heavy mineralization of the skin (e.g. Gans, 1989; Mallatt, 1996; Mallatt and Chen, 2003). According to this view, O_2 uptake was the initial factor driving gill development; all other functions of the gill are assumed to have arisen secondarily once the basic structure of the gill was in place. However as has been discussed in this review, when extant fish are faced with a decline in $SA:MO_2$ and thickening of the skin during development it seems to be ionoregulatory activity not gas exchange that first shifts from the skin to the gill. This raises the intriguing possibility that processes other than gas exchange may have influenced the evolution of the gill during early vertebrate evolution. In particular, it may be that the inability of the skin to maintain ion and acid–base balance as protovertebrates increased in size and became more active was the critical driving force behind gill development.

In the most basal craniate, the hagfish, the gills appear to account for only 20% of resting O_2 uptake (based upon calculations from Steffensen et al., 1984). Although hagfish are osmoconformers and maintain plasma ions at essentially the same concentrations as in sea water (Sardella et al., 2009), they possess gill ionocytes (Mallatt et al., 1987) and have a tremendous capacity for acid–base regulation (Brauner and Baker, 2009), which as discussed previously is a form of ionoregulation. Based on observations from hagfish, it has been proposed that the ionoregulatory functions of the gill initially may have evolved for acid–base regulation rather than for ion exchange per se (Mallatt et al., 1987; Evans, 1984). The acid–base relevant Na^+ and/or Cl^- exchange capability of the gill is thought to be an expectation that eventually permitted vertebrates to invade fresh water where the ability to take up ions from the medium independent of acid–base status is essential (Evans, 1984; Wright, 2007). This scenario is based on observations of adult fish. As discussed in this review, the trade-off between gas and ion exchange that occurs in the gills in adults is not necessarily consistent with early life stages or evolutionary history. Unfortunately, we know very little about the ontogeny of acid-base regulation and ion uptake in agnathans. The technical challenges of obtaining such information are great, but so will be the rewards.

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