3 Gas Exchange

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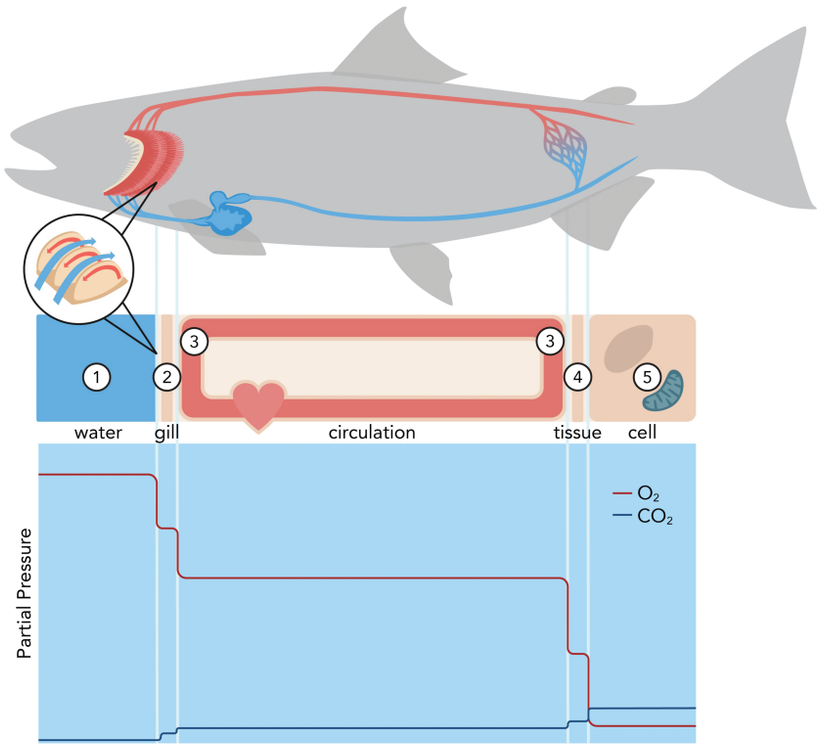
CONTENTS

3.1 INTRODUCTION

Oxygen is a prerequisite for life for all fish species with no known exceptions. Oxygen (O_2) uptake from the environment, transport across respiratory surfaces and through the circulatory system, and ultimately, delivery to metabolizing tissue, with the reverse for carbon dioxide (CO_2), which is produced in approximately equal amounts, have been topics of interest for fish physiologists for centuries. Yet, gas exchange is not just restricted to O_2 and CO_2. Ammonia (NH_3) excretion, also collectively part of gas exchange, is the key pathway for nitrogenous waste elimination for both marine and freshwater fishes. Therefore, gas exchange includes O_2, CO_2, and NH_3 and, at least in most adult fishes, primarily occurs at the gills. The skin and air-breathing organs can also be used for gas exchange, depending on life stage and species, and will be discussed briefly here but elaborated upon in other chapters. As gas exchange in fishes has been reviewed relatively recently in detail (Evans et al., 2005; Randall et al., 2014; Harter and Brauner, 2017), only the fundamentals are reviewed here (focusing on O_2 and CO_2) along with more recent advances in the field. In this chapter, we focus on the role of the gill in gas exchange, reviewing aspects related to ventilation, morphology, contact with the external environment, diffusion across membranes, blood flow and perfusion, and diffusion at the tissue level (Figure 3.1). Cellular metabolism, the next logical step in this cascade, is discussed at both the cellular and the whole-organism level in Chapter 10: Metabolism. Interactions between gases and effects on transport will be discussed here. Inter- and intra-specific differences and physiological and morphological adaptations to stress will also be examined throughout this chapter, especially with respect to contemporary issues such as pollution, climate change, and other anthropogenic disturbances, as these are significant areas for future research.

3.2 FROM ENVIRONMENT TO GILL – BRANCHIAL GAS TRANSFER

The external environment and lifestyle of the fish will dictate efficiencies or inefficiencies in gas exchange, which can be elaborated upon by discussing each step of the O_2 or respiratory gas transport cascade (Figure 3.1). Oxygen diffuses down its partial pressure gradient from the highest value in the environment to the tissues. Metabolically produced CO_2 is highest in the tissues and also diffuses down its partial pressure gradient to the environment for elimination. In both gases, a drop in partial pressure at each of the five steps of the gas transport cascade represents resistance due to convection and



① Convection: $\dot{V}_W = f \cdot V_S$

② Diffusion: $\dot{M}_{O_2} = \frac{K \cdot A \cdot \Delta P_{O_2}}{D}$

③ Convection: $\dot{M}_{O_2} = f \cdot V_S \cdot (C_aO_2 - C_vO_2)$

④ Diffusion: $\dot{M}_{O_2} = \frac{K \cdot A \cdot \Delta P_{O_2}}{D}$

FIGURE 3.1 The gas transport cascade for O_2 and CO_2 in a model fish, where the steps represent: 1, ventilation; 2, gill diffusion; 3, circulation; 4, tissue diffusion; and 5, cellular metabolism. Flows and gas fluxes can be calculated at each step by modifying the Fick equation as shown for O_2, where V_W = ventilator water flow, f = frequency, V_S= stroke volume, $\dot{M}O_2$ = oxygen uptake rate, K = Krogh's permeation coefficient, A = gill functional surface area, $\Delta PO_2 = O_2$ partial pressure gradient, D = diffusion distance, C_aO_2 and $C_vO_2 = O_2$ content of arterial and mixed-venous blood, respectively. See text for further information.

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diffusion, and transport at each level of the gas transport cascade can be quantified by modifications of the Fick equation (Figure 3.1), as discussed later.

3.2.1 VENTILATION

Most species, as adults, possess four to seven gill arches on each side of the head that are collectively referred to as the branchial basket. The developmental timeline for gill formation and, in some species, the external to internal migration of the structures depend on myriad factors; as well as species and temperature, other abiotic factors may be influential. Water must pass over the gills via ventilatory flow (V_w), which is largely the same process ~~regardless of~~ species. For most teleost fishes, ventilation involves asynchronous buccal (mouth) and opercular (gill cover) pumping to generate pressure (Hughes, 1960). In contrast, ram-ventilators, such as some pelagic teleosts and many elasmobranch species (Emery and Szczepanski, 1986), generate dynamic pressure while swimming, which drives water over the gills. Some species may transition from buccal pumping to ram ventilating, with a change in swimming speed and/or environmental O_2 level likely due to the energetic savings (Steffensen, 1985) associated with gill water flow being powered by skeletal muscles rather than by the buccal pump muscles. ~~Ventilation~~ flow is determined by the product of the ventilator stroke volume (V_s) and breathing frequency (f) (Figure 3.1, step 1), which can vary by species, developmental stage, activity, and environmental conditions. For example, the stargazer (*Genyagnus monopterygius*) is a successful ambush

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predator because it can bury itself in the sand and remain largely undetected by exhibiting extremely shallow ventilatory movements (i.e., branchiostegal movements) even when water becomes hypoxic (Forster and Starling, 1982). Red drum (*Sciaenops ocellatus*) hyperventilate in response to relatively low levels of hypercapnia (elevated water CO_2; 1000 and 5000 μatm; Ern and Esbaugh, 2016), with many fishes responding to higher CO_2 levels (Gilmour, 2001). Increases in ventilatory ~~volume~~ during exposure to gill irritants, such as harmful algal blooms or toxicants, are often indicative of a reduction in O_2 uptake efficiency in conjunction with an increased metabolic rate (Bradbury et al., 1989; Svendsen et al., 2018). Moreover, some fishes can even appear to hold their breath, which may be an adaptation to fend off would-be predators. The deep-sea coffinfishes (Lophiiformes: Chaunacidae) use their unique buccal and gill anatomy to slowly ventilate and even cease ventilatory movements for as long as 245 s (Long and Farina, 2019). This strategy may save energy, as ventilation can consume as much as 15% of the oxygen budget of slow-moving fishes (Steffensen, 1985), and may also increase their body volume by 30%, thus deterring would-be predators. Once the water flows over the gills, with a ventilatory frequency averaging from 30 to 70 min^{-1} for most adult fish species (Roberts, 1975) and as low as 10 min^{-1} for newly hatched larvae with poorly developed gills (McDonald and McMahon, 1977), gases must equilibrate between the water and the blood. Thus, the structure and function of the gill filaments and lamellae greatly influence the efficiency of gas exchange.

3.2.2 Morphology

From a physical perspective, gill filaments and lamellae can act like a sieve, being numerous or even fused at the tips to maximize gas exchange with the water. Indeed, the morphology of the gill filaments and lamellae and how they contact the external environment have been well studied across species, lifestyles, habitat, and developmental stages, as histological and microscopy preparations have been long established. On each filament, lamellae are comprised of two epithelia separated by pillar cells for structural integrity and to enable blood flow through the lamellae (Figure 3.1). Lamellae are often very thin, sometimes only one or two cell layers thick. As a result, the potentially short diffusion distances between the environment and the blood and the large surface area ~~are thought to~~ facilitate effective O_2 uptake from the environment and gas exchange in general (Hughes and Morgan, 1973). For this reason, it was long accepted that fast-swimming marine fishes (e.g., mackerel, tuna) exhibit high lamellar densities of 26–31 per millimetre of filament and total lamellae approaching six million, whereas slow swimmers exhibit lower densities (e.g., European sea bass: 21–26 lamellae per millimetre of filament (Hughes, 1984; Gray, 1954). However, tropical coral reef fishes exhibit lamellar densities ranging from 28 to 34 lamellae per millimetre of filament and are not necessarily considered fast-swimming, active species (Bowden et al., 2014). It may be that species with high O_2 needs have the greatest surface areas, which then would include not only the top performers but also hypoxia-tolerant species (Chapman et al., 2000). While it was once thought that gill (lamellar) surface area scaled predictably with body mass (Hughes, 1984) and activity (Roberts, 1975), that may no longer be the case, as suggested here. Moreover, while it was once thought that the gill anatomy for adult fishes was relatively fixed, it is becoming apparent that a great degree of plasticity can exist in some species and environmental conditions (Nilsson et al., 2012; Gilmour and Perry, 2018); we elaborate on this later in discussions of the osmorespiratory compromise. Lamellar spacing along the filament (and thus, interlamellar distances) was also thought to scale with activity (Piiper and Scheid, 1982). However, we now know that interlamellar distances are uniform regardless of fish body mass, indicating optimal interlamellar distances for maximizing gas exchange (Park et al., 2014).

3.2.3 Diffusion across Membranes

Diffusion across the gills is directly proportional to the functional surface area of the gills (A) and inversely proportional to the diffusion distance between the blood and the environment (D; i.e., the diffusion distance; Figure 3.1, step 2). In addition, water flows across the lamellae, counter-current to blood flow (Figure 3.1; Randall and Daxboeck, 1984), maximizing the partial pressure gradient between the environment and the blood for gas exchange (i.e., ΔPO_2 for O_2; Figure 3.1, step 2). Indeed, counter-current flow is so efficient that it enables 80–90% O_2 extraction from the water compared with approximately 25% extraction in humans breathing air (Schmidt-Nielsen, 1997). It is thought that this combination helps to overcome the slower diffusion rates (up to 300,000 times slower) of O_2 in water and the lower O_2 content and higher viscosity (840 times) and density (60 times) of water compared with air (Schmidt-Nielsen, 1997). These three elements – functional surface area, diffusion distance, and the gas partial pressure gradient between the water and blood – as well as a fourth, the permeation coefficient (K), collectively dictate the

diffusion of gases (e.g., O_2 transfer; $\dot{M}O_2$) across these membranes via the Fick equation (Figure 3.1, step 2). The permeation coefficient (K) describes the mobility of the gas in question and takes into consideration the diffusion coefficient and the solubility of the gas. K varies with temperature; values are available in the literature. The K for O_2 moving through tissues, for example, is estimated to be approximately one-third the K (i.e., slower) of O_2 moving through water (Randall and Daxboeck, 1984).

Functional surface area (A) is referred to as such because it is unlikely that the entire surface area of the fish's gill is used for gas exchange at any given time. The area can be estimated using morphometric measurements to sum the areas of all lamellae. However, it must be considered that the area functioning for gas exchange may be only 70% of the total anatomical area (Piiper et al., 1986). Not all lamellae will be perfused, especially in resting fishes (Booth, 1978). Moreover, the bases of the lamellae have greater diffusion distances, and the tips of the filaments receive ~~different~~ water flow~~,~~ when compared with the rest of the ~~gill anatomy~~, making both regions (i.e., base and tips) minimally involved in gas exchange, if not largely non-respiratory (Tuurula et al., 1984). The partial pressure gradient of the gas between the environment and the blood (e.g., ΔPO_2) is the driving force for diffusion. For O_2, this can be estimated as the difference between the average of inspired and expired water PO_2 and the average of arterial and venous blood PO_2 (Wood and Perry, 1985), all of which can be measured using microelectrodes or fibre optic sensors. However, it should be noted that unstirred layers cannot be accounted for in this calculation; this caveat, in combination with differential lamellar recruitment and changes in blood flow, makes estimates of ΔPO_2 challenging. Finally, D describes the diffusion distance or thickness of the tissue separating the environment from the blood and can also be calculated from morphometric measurements. It is important to consider that increases in ventilation and blood pressure, differential dilation of arterioles, and lamellar recruitment or shunting will alter this relationship. All these variables may change with exposure to environmental stress and/or changes in activity.

3.2.4 The Osmorespiratory Compromise

As discussed, the functional surface area and the gas partial pressure gradient between the environment and the blood are the primary drivers for gas exchange, but alterations to increase the efficiency of gas exchange can come with trade-offs to other gill functions, such as ion- and osmo-regulation. Maximizing functional surface area while enhancing gas diffusion promotes ion loss and water influx for freshwater teleosts, and ion gain and water efflux for marine teleosts. For example, rainbow trout lose Na^+ to the environment with increases in $\dot{M}O_2$ (Gonzalez and McDonald, 1992); calculations suggest that Na^+ efflux can increase by 70% in freshwater rainbow trout during exercise (Wood and Randall, 1973). The opposite (i.e., Na^+ influx and water loss) occurs for marine teleosts (e.g., Coho salmon, *Oncorhynchus kisutch*; Brauner et al., 1992). It may be that the osmorespiratory compromise underpins improved recovery in fishes upon exercising in brackish or freshwater conditions (i.e., because of maintained or decreased plasma ion levels) compared with fishes recovering from exercising in seawater (e.g., Atlantic salmon, *Salmo salar*; Hvas et al., 2018), an area worthy of further investigation. Given that increased activity, especially under elevated temperatures, necessitates increased perfusion and potentially lamellar recruitment, but ion- and osmo-regulation processes may require between 5% and 50% of a fish's energy budget (Bæuf and Payan, 2001); balancing gas exchange with ion- and osmo-respiratory functions represents a potentially profound compromise (Randall et al., 1972). One of the most thorough examples to date where the trade-offs associated with gill remodelling and the osmorespiratory compromise have been examined has been in the cyprinids (reviewed in Nilsson et al., 2012 and Gilmour and Perry, 2018). During winter months and near-freezing temperatures, Crucian carp fill their interlamellar spaces, reducing functional gill surface area and ultimately, safeguarding ion- and osmo-regulation when energetic needs are low. When waters warm and/or during hypoxic conditions or exercise, the interlamellar cell mass (ILCM) disappears, revealing up to a sevenfold increase in functional surface area and a reduction in blood–water diffusion distance to support an elevated $\dot{M}O_2$ associated with changing conditions (reviewed in Nilsson et al., 2012). However, the loss of the ILCM is also associated with a large reduction in plasma ion levels (Matey et al., 2008). Indeed, the osmorespiratory compromise is a key consideration when investigating physiological constraints on fish performance and the role of environment, stress, activity, species, and life stage.

3.3 CIRCULATORY TRANSPORT OF RESPIRATORY GASES

While gill ventilation and diffusion represent important limitations in the respiratory gas transport cascade (Figure 3.1, steps 1 and 2), gill perfusion and blood convection represent the next important step in gas transport

and exchange (Figure 3.1, step 3). The convective transport of blood is determined by cardiac output, the product of heart rate (f) and stroke volume (V_S; Figure 3.1, step 3 and see Chapter 4: Cardiovascular System), and the ability of the blood to transport and exchange O_2 and CO_2 is largely associated with the characteristics of haemoglobin (Hb), which is encapsulated within red blood cells (RBCs), as described in the following section.

3.3.1 Blood

3.3.1.1 Oxygen

Most O_2 carried by the blood is bound to the respiratory pigment Hb, which is encapsulated within RBCs. As a tetrameric protein, Hb consists of two α (141 amino acid) and two β (146 amino acid) subunits (globins), each containing a porphyrin ring with an iron (Fe^{2+}) haem centre, the site of O_2 binding (Perutz et al., 1960; Nikinmaa, 1990). Exceptions to this molecular structure of Hb are found in the lampreys and hagfishes, which both possess monomeric Hbs. In fishes in general, less than 5% of O_2 is transported physically dissolved in the blood; the remainder is bound to Hb (Nikinmaa, 1990). The only exception is the Antarctic icefishes, which lack both Hb and RBCs (Suborder Notothenioidei, Family Channichthyidae; Sidell and O'Brien, 2006). However, these Hb-less species are thought to compensate via large blood volumes and increased nitric oxide, thereby influencing vasodilation/constriction, angiogenesis, and mitochondrial biogenesis (Sidell and O'Brien, 2006). Compensation also occurs via increased cardiac stroke volume and power output (Pellegrino et al., 2003) and low metabolic rates, all of which may only be possible due to the stable, cold environment they inhabit. But for all other fishes, under specific conditions, Hb binds O_2 at the respiratory surface, releases it to tissues in exchange for CO_2, and transports CO_2 back to the respiratory surface for removal.

Due to its key role in both O_2 and CO_2 transport, Hb has become one of the most well-studied proteins to date. Hoppe-Seyler (1866) was the first to determine that Hb could reversibly bind O_2. Then, during the early 1900s, the reversible binding relationship between Hb and CO_2 was investigated by Bohr, Hasselbalch, and Krogh (1904), thus linking this molecule to the respiratory gas cascade. Bohr et al. (1904) determined that ~~CO_2 binding by Hb was not affected by O_2, but~~ the extent of Hb-O_2 binding was reduced in the presence of CO_2. This is because CO_2 decreases blood pH due to H^+ formation from CO_2 hydration, and Hb was discovered to be sensitive to pH, which is now generally known as the Bohr effect. The key to Bohr and colleagues' early studies was that the effects of CO_2 on Hb–O_2 binding were investigated at low *and* high O_2 tensions, whereas earlier studies had only investigated atmospheric O_2 tensions. Several decades later, Perutz et al. (1960) examined the binding properties of these two respiratory gases and Hb and determined that binding was largely determined by the structural and conformational changes incurred by the protein upon exposure to O_2, CO_2, and other ligands. When ligands break salt bridges, shifting the structural conformation of Hb from a tense (T) to a relaxed (R) state, binding sites for oxygenation are revealed. Breaking salt bridges is energetically costly, especially for the number of salt bridges that must be broken to reveal the first O_2 binding site. However, after the first O_2 molecule binds, subsequent O_2 binding at other haem groups is easier, because fewer salt bridges have to be broken to reveal binding sites. This is termed *cooperativity* and ultimately results in an oxygenated, high-affinity Hb (Bonaventura et al., 2004). While there were seminal studies that linked O_2 and CO_2 transport to the Hb protein, Perutz (1960) linked the physiochemical structure of the Hb protein with respiratory function.

An O_2 equilibrium curve (OEC) represents O_2 binding to Hb (expressed as % Hb-O_2 saturation), which depends on the PO_2 to which the system is equilibrated. The shape and position of the OEC (Figure 3.2) have been topics of intense investigation (Kobayashi et al., 1994; Rummer and Brauner, 2015). The OEC shape is dictated by the way in which individual Hb subunits interact upon binding. Cooperative binding is described by the Hill coefficient (n_H) and calculated from the slope of the line when $[\log(\text{Hb-O}_2)]/[1 - (\text{Hb-O}_2)]$ is plotted against $\log PO_2$ (Hill, 1910). A sigmoidal OEC has a higher n_H and therefore a higher degree of cooperative Hb subunit binding than a hyperbolic OEC. With high cooperativity, substantial decreases in Hb-O_2 can occur during capillary blood transit with only very small decreases in PO_2, maintaining a relatively constant driving force for O_2 unloading/delivery (Lapennas and Reeves, 1983; Nikinmaa, 1990). The affinity of Hb for O_2 is generally quantified by the PO_2 at which 50% of Hb is oxygenated (P_{50}). The P_{50} differs markedly between and within organisms and therefore has important implications for gas exchange (Figure 3.2).

The Bohr effect can be illustrated by the change in the position of the OEC due to an increase in H^+ concentration and/or CO_2 (Figure 3.2). In the presence of acidosis (e.g., metabolic CO_2 production at the tissues), a low-affinity Hb conformation is favoured, and the OEC shifts to the right (Figure 3.2), thus enhancing O_2 delivery to tissues. An increase in blood pH (e.g., at the gill due to CO_2 removal) shifts the OEC back to the left,

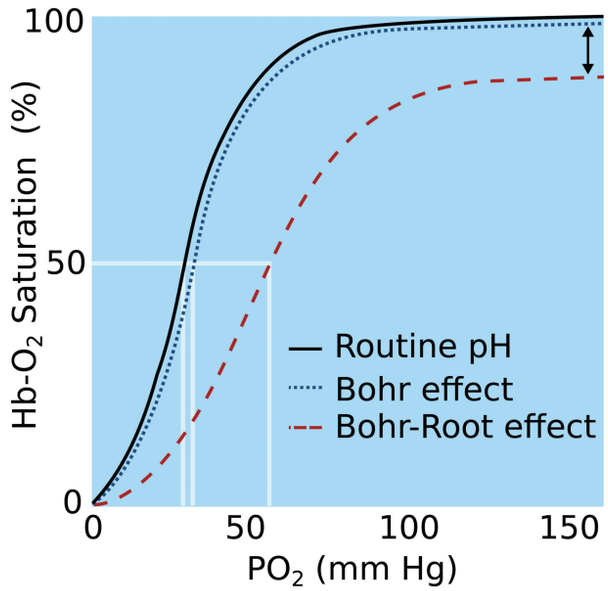

FIGURE 3.2 Theoretical oxygen equilibrium curve (OEC) depicting the relationship between haemoglobin oxygen saturation (%Hb-O_2) and blood partial pressure for O_2 (PO_2) at a routine pH value (black line) and at a reduced pH, resulting in either a Bohr effect (blue dashed line) right-shift or a combined Bohr–Root effect (red dashed line) rightward and downward shift. Thin white drop lines depict P_{50} values, the PO_2 at which 50% of Hb is saturated with O_2, for each of the three OECs.

where a lower PO_2 is required to saturate Hb with O_2 (Figure 3.2), thus enhancing O_2 uptake. The Bohr effect is quantified by the Bohr coefficient (Φ), which describes the degree to which the OEC shifts for a given decrease in pH (Figure 3.2; Bohr et al., 1904; Nikinmaa, 1990). This is usually calculated for a single point (typically P_{50}) on the OEC using the following equation:

$$\Phi = \Delta \log P_{50} / \Delta \mathrm{pH}$$

If the Φ is known, the equation can be rearranged such that the magnitude of the right-shift in the OEC (i.e., ΔPO_2, in mmHg) at a constant Hb-O_2 saturation (e.g., P_{50}) and a proposed ΔpH (e.g., the arterial and venous blood pH change) can be determined. Intuitively, for a given ΔpH, a large Φ results in a greater ΔPO_2. However, the ΔPO_2 can only be calculated in this manner if the Φ is linear over a wide range of Hb-O_2 saturations (20–80%) across the OEC, which is really only the case for air-breathing vertebrates. Teleost fishes exhibit a non-linear Bohr effect over the OEC (Jensen, 1986; Brauner and Randall, 1998), which makes modelling O_2 release from the Hb (i.e., to the tissues) in a teleost at different blood oxygenation levels easiest by directly interpolating ΔPO_2 from OECs, as has been done in model species such as the rainbow trout, *Oncorhynchus mykiss* (Rummer and Brauner, 2015).

Of all vertebrates, teleosts possess Hbs that are typically the most pH-sensitive. A decrease in pH reduces not only Hb–O_2 affinity but also the maximum carrying capacity of Hb for O_2 (Figure 3.2; Root, 1931). Thus, even at atmospheric O_2 tensions, Hb will not be fully saturated with O_2 at low pH. Although it is possible that the Root effect is an extension of a unique, non-mammalian Bohr effect (Berenbrink et al., 2005), it cannot be regarded simply as an exaggerated Bohr effect. Structure–function analyses have demonstrated specific locations where amino acid substitutions exist, supporting distinct molecular differences between Bohr and Root effects in fishes and the Bohr effect in other vertebrates, including human Hb-A (Berenbrink et al., 2005; Bonaventura et al., 2004; Mylvaganam et al., 1996). Conserved in Root effect Hbs are three elements: the βN-terminus, an arginine for lysine at 21β, and histidine at 3β. Removal of any of these can result in a decrease in the magnitude of the Root effect by 50%. It is also thought that a serine to cysteine substitution at the β93 position allows salt bridges to form with the C-terminal histidine residue to stabilize the T-state. However, additional substitutions have since been identified that may be involved in stabilizing the T-state at low pH (Berenbrink et al., 2005; Brittain, 2005).

Until the early 21st century, Root effect Hbs were only really discussed in terms of enhancing O_2 delivery to the eye and swimbladder of fishes. At both of these locations, dense capillary networks – *retia* – localize and magnify an acidosis that greatly elevates arterial PO_2 (Scholander and Van Dam, 1954; Wittenberg and Wittenberg, 1974). In the eye, the high arterial PO_2 serves to overcome great diffusion distances to oxygenate the metabolically active, yet poorly vascularized, retinal tissue (Wittenberg and Wittenberg, 1974). At the swimbladder, the gas gland acidifies incoming blood, and the high arterial PO_2 inflates the swimbladder against large pressure gradients (>50 atm) associated with depth, thus providing fish with precise buoyancy regulation (Scholander and Van Dam, 1954). Across the evolutionary trajectory of Percomorpha, one of the most advanced lineages among teleost fishes, as the magnitude of the Root effect increased, Hb buffer values decreased to a low plateau in the last common ancestor of *Amia* (Figure 3.3). This was also the time when the choroid rete appeared (Figure 3.3) and later, the swimbladder *rete mirabile* (Berenbrink et al., 2005). The liability of the Root effect, however, is that during a generalized acidosis, as occurs following exhaustive exercise or exposure to environmental hypoxia or hypercapnia,

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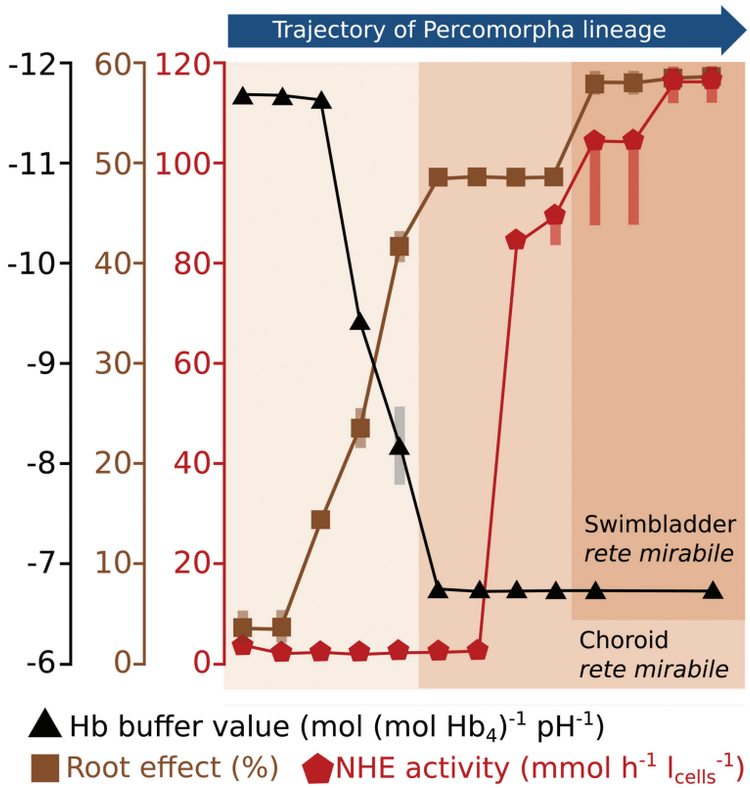

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FIGURE 3.3 Evolutionary changes in specific Hb buffer value, Root effect, and βNHE across the trajectory of the Percomorpha lineage. Beginning from the left of the figure with Chondrichthyes, Sarcopterygii, and Polypteriformes, Hb buffer values were highest (ancestral state), and both the magnitude of the Root effect (maximal % decrease in O_2 saturation of Hb) and βNHE activity were minimal. While Hb buffer values decreased, Root effect magnitude increased in Polypteriformes to plateau in *Amia*, Osteoglossomorpha, Elopomorpha, and Otocephala, only to increase again and plateau in *Oncorhynchus*, *Esox*, *Gadus*, and Smegmamorpha. Activity of βNHE first increased between Osteoglossomorpha and Elopomorpha. Red and blue fields indicate the presence of a choroid or swimbladder rete, respectively (the latter has been secondarily lost in the *Oncorhynchus* lineage). (Adapted from Berenbrink, M., et al., *Science*, 307, 1752–1757, 2005.)

O_2 uptake at the gills could be greatly impaired through a pH-induced reduction in Hb-O_2 carrying capacity. Fishes that possess a large Root effect generally possess RBC β-adrenergic Na^+–H^+ exchangers (βNHE; Nikinmaa, 1990) (Figure 3.4). The βNHE is activated by catecholamines released during stress. The stimulation of RBC βNHE leads to extrusion of H^+ that exceeds the rate of the associated HCO_3^-/Cl^- exchange and results in a large disequilibrium state across the RBC membrane, tightly regulating intracellular RBC pH (pH_i) despite the generalized reduction in blood pH (Figure 3.4). Thus, O_2 uptake at the gills is secured. This system depends upon the lack of plasma-accessible carbonic anhydrase (CA), which would otherwise short-circuit the response by permitting the rapidly catalysed conversion of plasma H^+ and HCO_3^- to CO_2, which would back-diffuse into the RBC, thus reducing pH_i (Figure 3.4). Teleosts in general are thought to lack plasma-accessible CA at the gills and possess circulating plasma CA inhibitors (e.g., with the strongest degree of inhibition in salmonids; Henry et al., 1997) to inhibit CA upon RBC lysis (Randall et al., 2014; Harter and Brauner, 2017). Yet, according to an evolutionary reconstruction, the Root effect evolved long before the *retia* of the swimbladder or eye (Berenbrink et al., 2005). Given this evolutionary trajectory, it was proposed that Root effect Hbs may have evolved to enhance general O_2 delivery in teleosts (Rummer et al., 2013; Randall et al., 2014).

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3.3.1.2 Carbon Dioxide

CO_2 transport in fishes has recently been reviewed (Brauner et al., 2019; Morrison et al., 2015) and thus, is only briefly summarized here. Metabolically produced CO_2 diffuses from the tissues down its partial pressure gradient through the gas transport cascade for ultimate elimination at the gills (Figure 3.1). Each step of the cascade is facilitated by the enzyme CA, which rapidly catalyses the conversion of CO_2 to

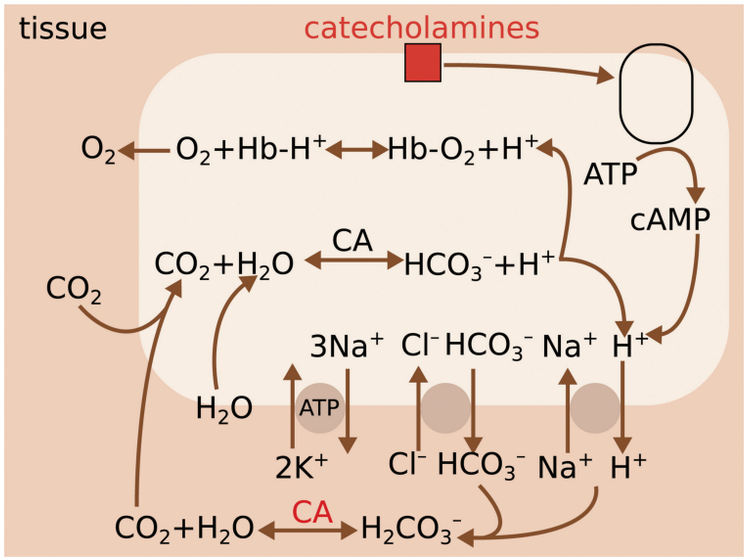

FIGURE 3.4 During stress, catecholamine release in a model fish activates the sodium and proton (Na^+/H^+) exchanger (e.g., the βNHE) on the RBC membrane through a G-protein-activated cascade including adenylate cyclase and 3′,5′-cyclic monophosphate (cAMP). The βNHE removes H^+ (produced via carbonic anhydrase [CA]-catalysed CO_2 hydration) from the cell in exchange for Na^+. With H^+, bicarbonate (HCO_3^-) is rapidly produced inside the cell via the CA-catalysed reaction and removed via anion exchange for Cl^- but at a slower rate than H^+. The H^+ removed from the RBC acidifies the plasma, resulting in a decrease in extracellular pH (pH_e) without plasma-accessible CA. Subsequent elevation of intracellular Na^+ and Cl^- results in RBC swelling through the movement of osmotically obliged water and activation of Na^+,K^+,ATPase. If CA is plasma-accessible, the βNHE is short-circuited by the catalysed conversion of plasma H^+ with HCO_3^- to form CO_2, which back-diffuses into the RBC. The RBC is then re-acidified when CO_2 is hydrated with CA inside the RBC to form H^+, which displaces O_2 from the Hb, thus enhancing O_2 release to the tissue. (Adapted from Rummer, J.L. and Brauner, C.J., *Journal of Experimental Biology*, 214, 2319–2328, 2011.)

HCO_3^- (CO_2 hydration) and vice versa (HCO_3^- dehydration) through the simplified equation

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

In the absence of CA, this reaction proceeds very slowly relative to blood transit times, and thus, CA is crucial for CO_2 transport and excretion. When CO_2 enters the blood, it diffuses into the RBC, where it is rapidly hydrated in the presence of RBC CA, and HCO_3^- is transported out of the RBC in exchange for Cl^- via an anion exchanger (AE). Some of the resulting H^+ is then either buffered by Hb or bound to Hb as O_2 is delivered to the tissues (Haldane effect). Any remaining H^+ then reduces pH and induces the Bohr effect, further facilitating O_2 unloading, and thus, there is an interaction between O_2 and CO_2 through the Bohr–Haldane effects (Figure 3.5; Brauner and Randall, 1998). At physiological pH, 90–95% of the total CO_2 is transported as HCO_3^- – the majority in the plasma – with the remainder transported as physically dissolved CO_2 (Perry, 1986; Brauner and Randall, 1998).

At the gills, the reverse occurs; counter-current exchange of blood and water facilitates CO_2 removal into the ventilated water. The reduction in PCO_2 promotes intracellular HCO_3^- dehydration in the presence of RBC CA. Protons released upon Hb oxygenation further facilitate CO_2 formation and excretion. Depletion of RBC HCO_3^- promotes plasma HCO_3^- entry into the RBC in exchange for Cl^-, and this is thought to be the rate-limiting step in CO_2 excretion (Figure 3.5). In teleosts, the lack of plasma-accessible CA at the gills restricts all HCO_3^- dehydration to the RBC and due to the large Bohr–Haldane effect in teleosts, results in a tight interaction between O_2 uptake and CO_2 removal (Brauner and Randall, 1998). One exception to this pattern in teleosts is in icefish (*Champsocephalus gunnari*), which, as mentioned earlier, lack Hb and RBCs. To compensate, they possess plasma-accessible CA in the gill, a trait thought to be absent in most teleosts, which represents yet another interesting adaptation that permits all CO_2 to be excreted directly from the plasma compartment (Harter et al., 2018b). In elasmobranchs and hagfishes that also possess plasma-accessible CA, some CO_2 excretion can occur directly from the plasma compartment (Morrison et al., 2015; Nikinmaa et al., 2019).

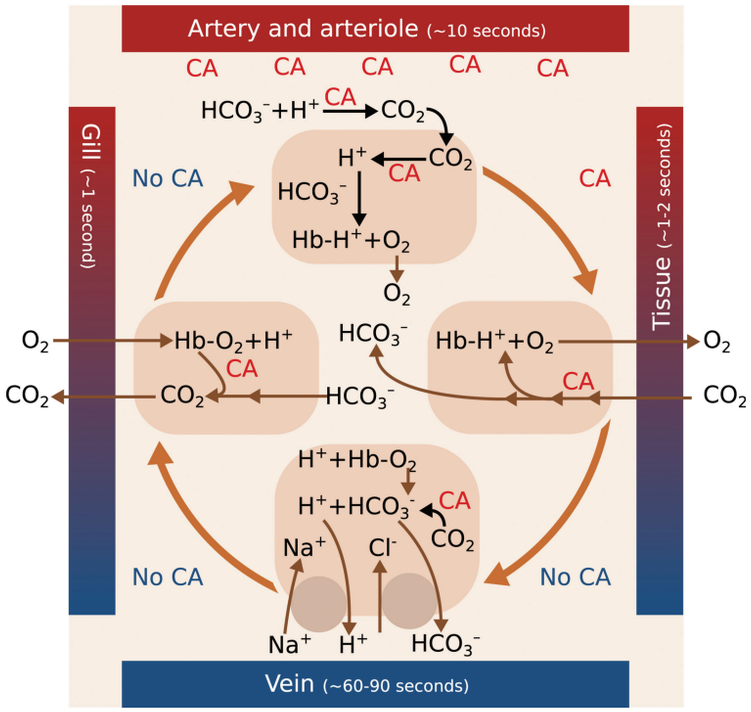

FIGURE 3.5 The transfer of oxygen (O_2), carbon dioxide (CO_2), protons (H^+), and bicarbonate (HCO_3^-) between the plasma and RBCs as blood flows through the gills, arteries, tissues, and venous circulation. The approximate transit time (seconds) for blood through these portions of the circulation is in brackets. There is no CA available to plasma in the gills and veins, but CA is available in the arteries and arterioles. (Adapted from Randall, D.J., et al., *Journal of Experimental Biology* , 217, 1205–1214, 2014.)

3.3.2 Blood Flow and Perfusion

After the blood leaves the gills and is circulated to the rest of the body (see Chapter 4: Cardiovascular System), factors such as the proportion of RBCs in circulation (i.e., haematocrit [Hct]) and the mean cell (corpuscular) Hb concentration (MCHC) are important considerations in matching gas exchange to the metabolic requirements of the fish. Hct varies from 15% to 40% across species and is thought to be highest in the most active species (e.g., tuna and mackerel) and lowest in sedentary species (e.g., hagfish), but even this has been debated (Brill and Bushnell, 1991). Some studies have demonstrated that Hct is often higher in male compared with female fishes, increases incrementally with fish size up to a certain size, and is higher in pre-spawning compared with spawning fishes (e.g., Indian shad, *Tenualosa ilisha*; Jawad et al., 2004). Theoretically, a higher Hct should confer a greater capacity for O_2 transport. However, with increasing Hct comes increasing blood viscosity, which at some point, is thought to limit performance due to the subsequent increase in cardiac output needed to pump viscous blood. Therefore, there must be an Hct where O_2 carrying capacity is maximized but without compromising cardiac output (i.e., the optimal Hct hypothesis; Richardson and Guyton, 1961). However, studies, including those where Hct is experimentally manipulated, suggest that other factors, such as a species' capacity for vasodilation and regulating cardiac output, plasma viscosity, and levels of activity, will also influence this relationship (Gallaugher et al., 1995). Moreover, stress can change Hct via splenic contraction (i.e., releasing more RBCs into circulation; Perry and Kinkead, 1989), RBC swelling (e.g., via adrenergic stimulation of RBC βNHE; Nikinmaa, 1990), and plasma skimming and/or use of secondary circulation (Steffensen and Lomholt, 1992; Rummer et al., 2014). Neither splenic release of RBCs nor plasma skimming will, in theory, change MCHC, but RBC swelling will. It is important to consider both changes in Hct and Hb concentration, both of which can be used to calculate MCHC, when determining responses at the level of the blood relative to O_2 transport.

3.4 DIFFUSION AT THE TISSUE LEVEL

Gas exchange at the tissues is determined by perfusion of the tissues with blood and diffusion across capillary membranes to and from the metabolizing tissues. As at the gills, tissue diffusion is enhanced by an increase in total capillary surface area, by a reduction in diffusion

distance, and by maximizing the partial pressure gradient between the blood and the cytosol (Figure 3.1, step 4). It has been known since the beginning of the 20th century that in vertebrates, metabolically produced CO_2 diffuses from the tissues to the blood, inducing the Bohr effect, elevating venous PO_2, and enhancing O_2 unloading (Bohr et al., 1904). The magnitude of this response is the product of the pH_{a-v} difference and the Bohr coefficient. However, recently, it has been shown that the latter may be especially important in some teleosts, such as salmonids, and perhaps more generally among teleosts relative to other vertebrates. As discussed earlier, during stress, catecholamines are released to protect RBC pH_i during a generalized acidosis to secure O_2 binding at the gills. While it is generally thought that plasma-accessible CA is absent at the gills, there is evidence for its presence in some tissues, such as the white (Wang et al., 1998) and red muscle (Rummer et al., 2013) of rainbow trout, the atrium of coho salmon (Alderman et al., 2016, reviewed in Harter and Brauner, 2017), and probably other species (Figure 3.5). When adrenergically stimulated RBCs come into contact with plasma-accessible CA, this rapidly short-circuits the βNHE and acidifies the RBC. This, combined with the high pH sensitivity of Root effect Hbs, results in a large increase in P_aO_2, which has been proposed to double O_2 unloading with no change in tissue perfusion (Rummer et al., 2013). When the RBC leaves the tissues and enters the venous system, there is sufficient time (60–90 s) for RBC pH_i to recover through RBC βNHE (Randall et al., 2014; Harter et al., 2018a; Figure 3.5) prior to gill perfusion. In Atlantic salmon forced to sustain different swimming speeds, injection of a membrane-impermeable CA inhibitor (C18) either impaired exercise or was associated with a 30% increase in cardiac output to maintain a given exercise intensity and metabolic rate (Harter et al., 2019). This mechanism of enhanced O_2 unloading in fish depends on three criteria: 1) a Hb with a high pH sensitivity, such as a Root effect; 2) RBC pH_i regulation, such as RBC βNHE or possibly other transporters; and 3) a heterogeneous distribution of plasma-accessible CA (i.e., presence in the tissues and absence in the gills). All three criteria may apply to salmonids specifically and may be central to their tremendous athletic ability. However, this concept may apply to teleosts in general, and it has been proposed that this mechanism for enhanced O_2 unloading may have played an important evolutionary role in the adaptive radiation of teleosts (Harter and Brauner, 2017; Randall et al., 2014; Rummer at al., 2013), which is clearly an area worthy of further investigation.

3.5 CONCLUSION

As aerobic respiration is a prerequisite for life among the fishes, it follows that selective pressures on the molecular, biochemical, physiological, and behavioural aspects of gas exchange have been of key interest among researchers. Here, we reiterate some of the fundamental principles associated with gas exchange in the fishes and highlight where previous assumptions have been tested and revised. We also note interesting caveats, knowledge gaps, and areas for future research. Representing over half of all extant vertebrates and over 400 million years of evolutionary history, the fishes, indeed, provide a fascinating group of organisms in which to investigate the array of adaptations at the level of gas exchange.

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