

Acid-base regulation and hypercapnia: An introduction

Martin Tresguerres, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, United States

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Key points

- Provide an introduction to acid-base (A-B) physiology.
- Explain the differences in A-B regulation between fish and air-breathing animals.
- Highlight the most common A-B challenges experienced by fish, including exposure to elevated CO₂ levels (hypercapnia).
- Provide an overview of the cellular mechanisms used by fish to regulate the A-B status of their internal fluids.
- Explain the challenges associated with measuring A-B parameters in fish blood.

Glossary

Endergonic used to describe a chemical reaction or process that requires energy input to proceed.

Exergonic used to describe a chemical reaction or process that releases energy.

Endotherm an animal that uses metabolic reactions to maintain its body temperature relatively constant independently from environmental temperature, such as mammals and birds.

Hypoxia state in which oxygen levels are not enough to sustain aerobic respiration. This might happen as a result of decreased oxygen levels in the environment ("environmental hypoxia") or when oxygen supply does not meet oxygen demand during exercise ("functional hypoxia"). As such, the level of oxygen that define hypoxia depend on the species and activity level.

Law-of-mass-action it states that the rate at which a reaction or process takes place is proportional to the abundance of the substrates. For example, a protein located in the apical membrane of a gill ionocyte that excretes metabolic H⁺ in exchange for Na⁺ from the water (e.g., an NHE) will work at a faster rate when there is more H⁺ inside the ionocyte, which could happen during a metabolic acidosis.

Ectotherm an animal that cannot regulate its body temperature using metabolic reactions, resulting in relatively large temperature fluctuations that match the environment.

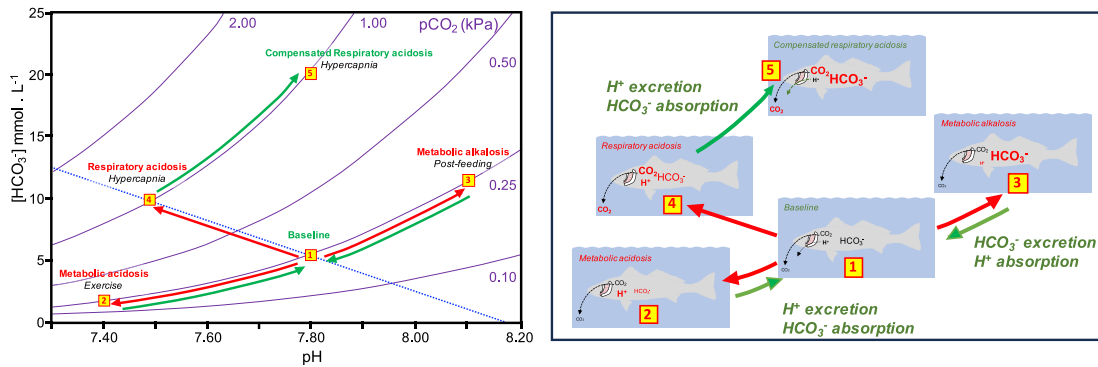
Post-translational modification a covalent change to an already synthesized protein that alters its structure, activity, localization, or combinations thereof. Common examples of post-translational modifications include phosphorylation, acetylation, glycosylation, ubiquitination, and proteolytic cleavage, but there are many more.

Abstract

All organisms must maintain the acidity levels of their internal fluids within a range that is compatible with cellular functions; the underlying homeostatic mechanisms are collectively referred to as "acid-base (A-B) regulation." In fish, the most

common A-B challenges include a metabolic acidosis resulting from intense exercise, a metabolic alkalosis related with food digestion, and a respiratory acidosis during exposure to elevated CO_2 level in the water. This latter condition is found both in natural and aquaculture settings and is known as “hypercapnia.” The gills are the main A-B regulatory organ of fish; however, species-specific differences in the cellular mechanisms can determine resilience or vulnerability to A-B stress.

Teaching slide



Introduction

The concentration of hydrogen ions ($[\text{H}^+]$) can greatly affect chemical reactions and protein function, and thus all animals must regulate the $[\text{H}^+]$ in their internal fluids. Typical $[\text{H}^+]$ vary widely among bodily fluids: from about 40 nM in the cytosol of most cells, to between six and ~ 300 nM in blood plasma; as high as 160 mM in gastric juices, and as low as one nM in pancreatic fluid. Remarkably, the difference in $[\text{H}^+]$ between the latter two is 160-million-fold! Similarly, $[\text{H}^+]$ in waters where fish live ranges from ~ 300 μM in Lake Osorezan (Japan), to 10 nM in the open ocean, to 100 pM in Lake Magadi (Kenya), which span a three-million-fold difference. Because comparisons and calculations across such a wide range is cumbersome, $[\text{H}^+]$ are usually transformed to pH values. However, the pH scale establishes an inverse logarithmic relationship with $[\text{H}^+]$, with two important implications: (1) higher $[\text{H}^+]$ (“acidity”) corresponds with lower pH values and lower $[\text{H}^+]$ (“basicity”) corresponds with higher pH values; (2) changes in $[\text{H}^+]$ are not linear throughout the pH scale (Fig. 1A). Moreover, the pH of biological fluids is intrinsically linked to the concentrations of carbon dioxide (CO_2), bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}) (Fig. 1B,C), and the underlying homeostatic processes are collectively referred to as “acid-base (A-B) regulation.”

Differences between air-breathing and water-breathing animals

Animals with a circulatory system have developed specialized organs that maintain blood pH relatively constant despite internal and external A-B challenges. This systemic A-B regulation provides stable conditions for all the cells throughout the body, which decreases their energetic cost associated with intracellular pH (pH_i) regulation and affords them more energy for other physiological processes. However, there are important differences in the systemic A-B regulatory strategies used by air-breathing vertebrates and those used by water-breathing fishes. The former largely regulate blood pH by adjusting their ventilatory rate to blow off or retain more CO_2 , which respectively help increase or decrease blood pH. Additionally, their kidneys can modulate the excretion of metabolic H^+ and HCO_3^- into the urine and their reabsorption into the blood (Fig. 2). In contrast, water-breathing animals cannot rely on ventilatory adjustments to regulate blood A-B status because CO_2 is much more soluble in water than O_2 , so they do not have much scope for slowing down ventilation (because they would not be able to take up enough O_2) or for increasing it (because most metabolic CO_2 diffuses out to the water as the blood passes through the gills even during baseline conditions).¹ In turn, the high CO_2 solubility in water determines lower pCO_2 and $[\text{HCO}_3^-]$ in fish internal fluids that result in a lower blood buffering capacity compared to air-breathers. Furthermore, fish use their gills as the main organ for blood A-B regulation (Fig. 2), which directly excrete metabolic H^+ and HCO_3^- into the surrounding water using cellular mechanisms that are conceptually similar to those in the mammalian kidney. However, the physicochemical properties of environmental waters are much more variable than that of mammalian bodily fluids, and thus fish gill A-B regulatory mechanisms are both more diverse and more flexible, and shape to species- and environment-specific patterns. For example, ectotherms (including fish) (see Glossary) living in warm water have higher blood CO_2 partial pressure (pCO_2) and $[\text{H}^+]$ (i.e., lower pH) than those living in cold water. As a result, the baseline blood acid-base parameters of fish are much more variable than in endotherms (i.e., birds and mammals) (see Glossary) (Fig. 2). The pCO_2 in fish arterial blood can range between ~ 0.1 and 0.6 kPa (~ 0.8 – 5.0 mmHg—*torr*, ~ 1000 and ~ 6000 μatm), and pH

¹However, see (Brauner et al., 2019) for some exceptions.

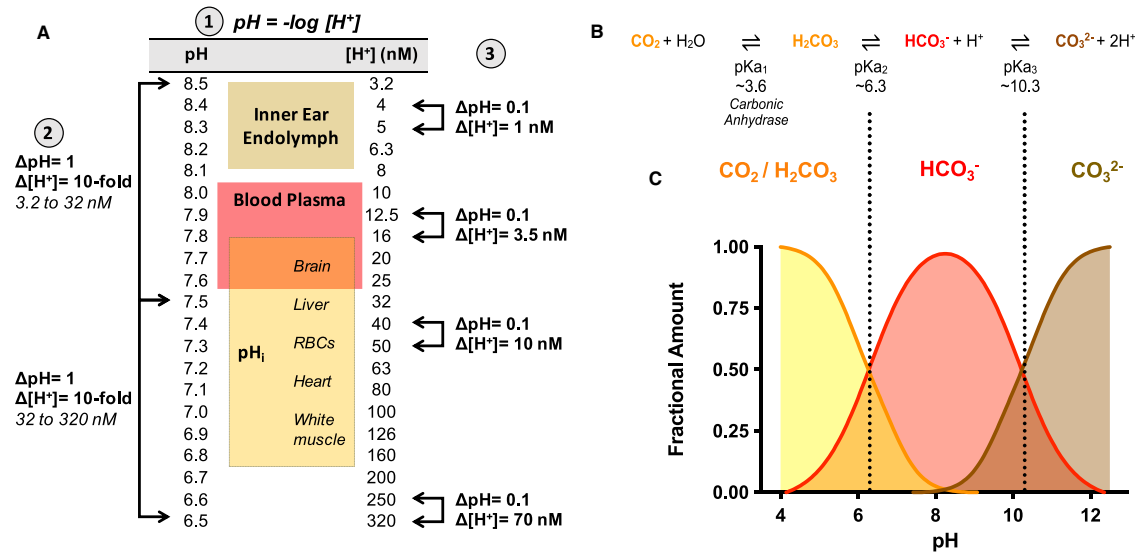


Fig. 1 Fundamental pH and acid-base concepts. (A1) The pH scale establishes an inverse logarithmic relationship with the concentration of hydrogen ions ($[H^+]$) whereby higher $[H^+]$ (“acidity”) corresponds with lower pH values, and lower $[H^+]$ (“basicity”) corresponds with higher pH values. In addition, a given change in pH does not represent the same change in $[H^+]$ throughout the pH scale. For examples, (A2) a change in one pH unit reflects a 10-fold change in $[H^+]$ at any position in the pH scale, and (A3) a change in 0.1 pH units reflects a difference of 1 nM in $[H^+]$ between pH 8.4 and 8.5, of 3.5 nM $[H^+]$ between pH 7.8 and 7.9, of 10 nM $[H^+]$ between pH 7.3 and 7.4, and of 70 nM $[H^+]$ between pH 6.5 and 6.6. Typical ranges for some fish bodily fluids are provided; pH_i = intracellular pH; RBC = red blood cell. (B) CO_2 combines with water in a series of reactions that yield H_2CO_3 , HCO_3^- , CO_3^{2-} and H^+ in a pH-dependent manner according to the dissociation constants (pK_a 's) listed in the figure. The hydration of CO_2 into H_2CO_3 happens at a relatively slow rate; however, enzymatic catalysis by carbonic anhydrases that are present in many biological fluids makes it almost instantaneous. (C) pH-dependent CO_2 , HCO_3^- and CO_3^{2-} equilibria that help determine acid-base status in biological fluids. Notice that HCO_3^- is the predominant species in the typical pH range for blood and cell cytoplasm, while CO_2 and CO_3^{2-} are favored at more acidic and basic pH, respectively.

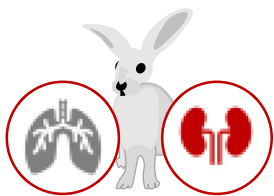
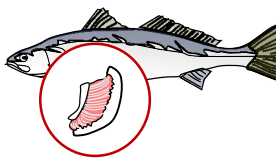
Main Blood A-B regulatory organ(s)		
Temperature	36 to 40 °C	-2 to 45 °C
Arterial pCO_2	4.7 to 6.0 kPa	0.1 to 0.6 kPa
Arterial $[H^+]$ and pH	35 to 45 nM H^+ (7.45 to 7.35 pH units)	8 to 15 nM H^+ (8.10 to 7.60 pH units)
Arterial $[HCO_3^-]$	22 to 28 mM	2 to 10 mM

Fig. 2 Acid-base (A-B) physiology of mammals and fish.

can range between ~ 8.10 and ~ 7.50 pH units. In contrast, plasma $[HCO_3^-]$ decreases with temperature and ranges between ~ 2 and ~ 10 mM (Ultsch and Jackson, 1996). These variations in A-B chemistry help preserve the net charge of proteins despite temperature-dependent changes in ionization and pH neutrality (i.e., when $[H^+] = [OH^-]$) according to the alpha-stat hypothesis (Burton, 2002a). In addition, freshwater fishes typically have higher blood pCO_2 , pH, and $[HCO_3^-]$ than marine fishes at the same temperature, which may be related to their lower $[Na^+]$ in blood (Burton, 2002b).

Common A-B disturbances and A-B stress in fish

Baseline metabolism produces H^+ and CO_2 and thus just being alive generates a continuous acidic load. In addition, A-B homeostasis is routinely challenged by both metabolic and environmental factors. The two most common metabolic A-B challenges are an acidosis that is induced upon exhaustive exercise and an alkalosis that occurs in the post-feeding period in fishes with an acidic

stomach. Among the environmental factors that challenge blood A-B, an acidosis resulting from elevated $p\text{CO}_2$ in the water is arguably the most common one; this condition is known as “hypercapnia.” Because it resembles the A-B disturbances associated with hypoventilation and with respiratory diseases that impair the clearance of CO_2 from the blood in air-breathing animals, hypercapnia is considered a type of respiratory acidosis.

Baseline metabolic H^+ and CO_2 production

The free energy required to drive the biochemical reactions that sustain life largely comes from the hydrolysis of ATP. This includes endergonic reactions such as neuronal action potentials and muscular contraction, as well as exergonic reactions that require activation energy to proceed at biologically appropriate rates such as the initial steps of glycolysis. Because the hydrolysis of ATP releases H^+ in equimolar amounts, cells are continuously being acidified from within at a rate that is proportional to their metabolic activity. Indeed, an average cell consumes several million ATP molecules per second (Flamholz et al., 2014) and, in the absence of pH regulatory processes, the resulting H^+ would rapidly acidify the cell to levels that are incompatible with life. However, this is prevented by the mitochondrial sink, intracellular buffers, and active extrusion of H^+ to the surrounding extracellular fluid and subsequently to the blood (see Section **Intracellular pH regulation**). Additionally, mitochondrial respiration produces CO_2 that, upon hydration, can generate H^+ and have an acidifying effect (Fig. 1B); however, CO_2 largely diffuses toward the blood. As the blood passes through the capillaries, it loads up metabolic waste from the tissues resulting in venous blood $p\text{CO}_2$, $[\text{H}^+]$ and $[\text{HCO}_3^-]$ that are always higher (and pH is lower) than those in arterial blood. Of course, venous blood also has a lower $p\text{O}_2$ than arterial blood due to offloading from red blood cells (RBCs) to the tissues to sustain aerobic respiration. As the blood passes through the gills, these processes are reversed: O_2 is loaded into the RBCs, CO_2 diffuses out to the water, and remaining excess H^+ are actively excreted by specialized ion-transporting cells (“ionocytes”). During baseline conditions, ATP consumption (and therefore, H^+ production) matches CO_2 and H^+ excretion across the gills and the fish is in A-B homeostasis. Evidently, changes to any of these steps can induce an acidosis or an alkalosis.

Metabolic acidosis

The contraction of cardiac and skeletal muscle consumes ATP at a significant rate even when an organism is at rest. During exercise, the ATP consumption rate goes up in proportional manner, and so does H^+ production inside the muscle cells (“myocytes”). An ensuing intracellular acidification is initially prevented by the mitochondrial H^+ sink and by intracellular buffering. Indeed, myocytes typically have a large number of mitochondria, a high intracellular buffering capacity, or both, which helps prevent acidosis-mediated muscle fatigue and contractile failure (Jarvis et al., 2018). However, during intense and prolonged exercise, O_2 supply cannot meet O_2 demand, a situation known as functional hypoxia (see **Glossary**). Although muscle is well equipped to hydrolyze ATP fermentatively to continue working, the resulting H^+ cannot be consumed in the mitochondrial sink, overwhelm intracellular buffers, and are excreted into the blood together with the fermentative end-product, lactate. The ensuing blood acidification is the most common type of metabolic acidosis in fish.

Fish have a large white muscle mass, which has greater fermentative capacity and lower mitochondrial density than those of red muscle. Although white muscle also has a larger intracellular buffering capacity (see Section **Intracellular pH regulation**), white muscle activity can conduce to pronounced intracellular and blood metabolic acidosis. In nature, fish may experience short episodes of metabolic acidosis due to swim bursts during predator-prey chases, as well as longer and more intense episodes during upstream reproductive migrations. Fish correct this metabolic acidosis by increasing the rates of H^+ excretion and HCO_3^- reabsorption across the gills. Additionally, a blood metabolic acidosis occurs as the fish struggles on a fisherman’s or scientist’s hook or net. As described in **Measuring and interpreting fish blood A-B status and regulation** section, this has important implications for the collection of blood for A-B measurements.

Metabolic alkalosis

When fish excrete H^+ into their stomach to aid in digestion, an equimolar amount of HCO_3^- is simultaneously absorbed into the blood that induces a metabolic alkalosis in the blood and is known as the alkaline tide. This phenomenon was originally described in humans (reviewed in (Niv and Fraser, 2002)) and thoroughly studied in snakes and other reptiles that consume large meals (Wang et al., 2001). In fish, it was first identified in dogfish shark (Wood et al., 2005) and subsequently in rainbow trout (Buckling and Wood, 2008; Cooper and Wilson, 2008) and catfish (Li et al., 2010). Air-breathing animals correct for the post-prandial metabolic alkalosis by retaining CO_2 through hypo-ventilating and by HCO_3^- excretion by the kidneys into the urine and by the pancreas into the duodenum. In contrast, sharks excrete HCO_3^- to the surrounding water across the gills, and bony fishes use a combination of branchial and intestinal HCO_3^- excretion to correct the postprandial alkalosis (Wood, 2019). Because a larger meal requires a larger amount of H^+ to be digested, the magnitude and duration of the alkaline tide is proportional to the size of the meal.

However, not all fish species experience a post-prandial alkaline tide. For example, killifish do not have a stomach and use alkaline food digestion and undergo a postprandial acidic tide due to HCO_3^- excretion into the intestine and H^+ absorption into the blood (Wood et al., 2010).

Respiratory acidosis (hypercapnia)

The combined CO_2 respiratory output of organisms can elevate pCO_2 in the water, a condition commonly referred to as hypercapnia (hyper = abnormally great; *kpnos* = Greek term for smoke, which contains much CO_2). The magnitude of the hypercapnia will be proportional to organismal density and inversely proportional to water mixing, and it will be especially pronounced at night because plants and algae cannot remove CO_2 by photosynthetic carbon fixation (Burnett, 1997). Thus, some habitats that are prone to developing hypercapnia include coral reefs, mangroves, kelp forests, tidal pools, lakes, and swamps (reviewed in Tresguerres et al., 2020). In addition, hypercapnia can manifest inside decomposing carcasses (Clifford et al., 2022), in aquaculture settings (Kwan et al., 2021), during algal blooms (Skelton et al., in press) and upwelling (Frieder et al., 2012) events, and as a result of absorption of anthropogenic CO_2 from the atmosphere. This latter situation is known as aquatic acidification (which includes both ocean and freshwater acidifications). However, not all hypercapnia situations are equal, and the effect on fish will depend on the magnitude of the CO_2 elevation, its duration, and whether there are any associated changes in environmental pO_2 .

Let us consider a fish with an arterial blood pCO_2 hovering around 0.25 kPa and placed in a tank with water equilibrated with the atmosphere at 0.05 kPa. As the blood passes through the gills, CO_2 will diffuse out to the water following a pCO_2 difference (ΔpCO_2) of 0.20 kPa (Fig. 3A). If CO_2 is bubbled into the tank until pCO_2 reaches 0.10 kPa, the pCO_2 in arterial blood will remain higher than in the water, but the new ΔpCO_2 will be 0.15 kPa. Because gases can only move across an epithelium by diffusion and based on differences in partial pressure, outward CO_2 diffusion will initially slow down and arterial pCO_2 will increase until the ΔpCO_2 between the blood and the water is restored. Thus, arterial pCO_2 will increase and pH will decrease in proportion following the equation shown in Fig. 1B. To correct this respiratory acidosis, the fish will excrete the excess H^+ through specialized gill ionocytes and will absorb HCO_3^- in the blood using the cellular mechanisms described in Blood A-B regulation Section. During this mild hypercapnia, the new steady state in arterial blood will show a pCO_2 of 0.30 kPa, a slightly elevated $[\text{HCO}_3^-]$, and a compensated pH (Fig. 3B). These processes will magnify during exposure to a moderate hypercapnia of 0.20 kPa pCO_2 : as ΔpCO_2 between blood and water decreases, the diffusive excretion of CO_2 will slow down leading to an increase in arterial pCO_2 until ΔpCO_2 is restored, in turn resulting in a more pronounced respiratory acidosis that will be corrected by excreting H^+ at a higher rate to restore blood pH, and by accumulating more HCO_3^- (Fig. 3C). If CO_2 continues to be bubbled into the water to generate an extreme hypercapnia, such as 1 kPa, the ΔpCO_2 will reverse and CO_2 will diffuse from the water into the fish (Fig. 3D). At the new steady state, arterial pCO_2 will equilibrate at around 1.00 kPa. The gills will excrete H^+ to correct the respiratory acidosis, but species-specific differences are seen whereby not all species are able to fully restore blood pH. In any case, the blood $[\text{HCO}_3^-]$ will be greatly elevated.

Fish generally have an excellent ability to regulate blood pH and most species are tolerant to extreme hypercapnia. But while extreme hypercapnic levels that reverse the ΔpCO_2 gradient used to be standard in A-B physiology research a few decades ago (Ishimatsu et al., 2008) and may be found in intense aquaculture facilities (Ellis et al., 2017), they are seldomly found in nature. In addition, the accumulation of HCO_3^- that is associated with the compensation of respiratory acidosis caused by chronic hypercapnia can potentially affect various aspects of fish physiology, even if pH is restored to baseline levels. Some examples include otolith overgrowth (Section A-B regulation of other bodily fluids) and neurobehavioral alterations (Tresguerres and Hamilton,

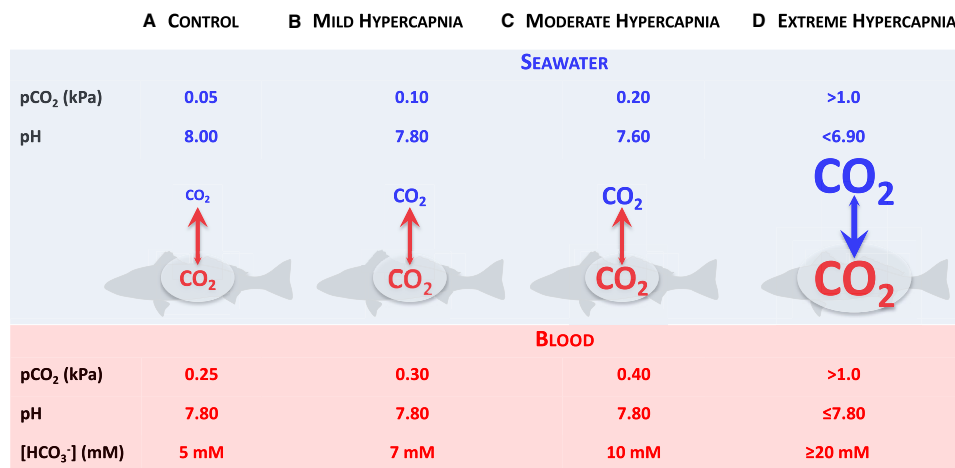


Fig. 3 Carbon dioxide diffusive fluxes between fish and seawater. (A) Control conditions of 0.05 kPa CO_2 (ca. 490 μatm , 0.38 torr, 0.5%); (B) Mild hypercapnia of 0.10 kPa CO_2 (ca. 1000 μatm , 0.75 torr, 0.1%); these levels might routinely be experienced by fish in densely populated areas at night or during upwelling events and are predicted for the open ocean for the end of this century as a result of anthropogenic ocean acidification (OA). (C) Moderate hypercapnia of 0.20 kPa CO_2 (ca. 2000 μatm , 1.5 torr, 0.2%); these levels might also be found in the natural situations described earlier and in aquaculture settings and are predicted for the open ocean for the year 2300 due to OA. (D) Extreme hypercapnia of 1.0 kPa CO_2 (ca. 10,000 μatm , 7.5 torr, 1%); this condition may be occasionally experienced in nature during brief periods, or more chronically in intense aquaculture settings. Diffusive CO_2 fluxes and blood pH regulation and HCO_3^- accumulation are qualitatively the same in freshwater; however, freshwater has a lower buffering capacity than seawater and thus addition of CO_2 has a greater acidifying effect.

2017). It is important to keep in mind that the magnitude of the effect will be proportional to the magnitude of the disturbance. For example, exposure to 0.10 kPa CO₂ and to 1 kPa CO₂ (~1000 and ~10,000 µatm CO₂, respectively) both induce a respiratory acidosis; however, the latter is much more pronounced resulting in much greater compensatory accumulation of HCO₃⁻ that could have much greater physiological side effects. While this may sound trivial, the literature is full of examples that reach careless conclusions about the effects of mild hypercapnia on fish (and other organisms) based on the reported effects of much greater levels of hypercapnia, only because both situations induce a respiratory acidosis. This unfortunate scenario is especially prevalent in ocean acidification research.

Importantly, the hypercapnia that takes place at night in densely populated environments, inside a decomposing carcass, and during upwelling events are associated with a decrease in environmental pO₂ that can reach hypoxic levels (see [Glossary](#)). This is different from ocean acidification or aquaculture settings, during which pO₂ levels remain unchanged or can even be elevated due to increased photosynthetic production and artificial aeration, respectively. This is very important because hypoxia impairs aerobic respiration, potentially resulting in decreased metabolic ATP and CO₂ production, increased fermentative ATP utilization and ensuing metabolic acidosis, or combinations. Thus, the effects of hypercapnia and hypoxia are not necessarily additive.

Cellular mechanisms for A-B regulation in fish

While every cell can regulate its p*H*_i to some extent, the gills maintain blood A-B homeostasis and therefore provide a stable environment for the other cells in the body and alleviate the energetic cost of p*H*_i. Additionally, some physiological processes require p*H* levels that are different from systemic conditions and engage organ-specific A-B regulatory mechanisms.

Intracellular pH regulation

Under aerobic conditions, most of the metabolically produced H⁺ are consumed by oxidative phosphorylation in mitochondria ([Hochachka and Mommsen, 1983](#)), a phenomenon known as the mitochondria H⁺ sink. In addition, cells contain proteins, histidine-containing dipeptides, and phosphates that can buffer p*H* in the physiological range ([Tresguerres et al., 2020](#)). These two systems are complemented by energy-dependent mechanisms that can excrete or take up H⁺ and HCO₃⁻ and other ions to actively regulate p*H*_i and gain importance when the mitochondrial sink and intracellular buffering are overwhelmed. The molecular mechanisms for p*H*_i regulation in fish seem to be similar to those in mammals, which have been thoroughly studied ([Casey et al., 2010](#); [Putnam and Roos, 1997](#)). The general concept is the same as for blood A-B regulation: an intracellular acidosis is compensated by excreting H⁺ and accumulating HCO₃⁻, and vice-versa for an alkalosis ([Tresguerres et al., 2020](#)).

Active cells, such as neurons and myocytes, tend to have a better p*H*_i regulatory capacity than other cell types because metabolic activity is directly related to the rate of ATP hydrolysis, which in turn determines p*H*_i acidification. For example, the myocytes in fish white muscle fibers have a great intracellular buffering capacity due to the accumulation of large amounts of the histidine-containing dipeptides carnosine, anserine and balenine ([Abe, 2000](#)).

Blood A-B regulation

Much of the metabolic acid produced by fish is blown off as CO₂ into the water as the blood passes through the gills. When a blood acidosis or alkalosis develops, they are largely corrected by active excretion and absorption of H⁺ and HCO₃⁻ by gill A-B regulatory ionocytes, a type of ion-transporting cell. For the purpose of A-B regulation, H⁺ and HCO₃⁻ are generally transported in opposite directions: a blood acidosis is corrected by excreting H⁺ into the water and absorbing HCO₃⁻ into the blood, and a blood alkalosis is corrected by excreting HCO₃⁻ into the water and absorbing H⁺ into the blood. To maintain electrical neutrality, H⁺ excretion is associated with Na⁺ uptake, while HCO₃⁻ excretion is associated Cl⁻ uptake. This trait determines an intrinsic link between the molecular mechanisms of gill A-B regulatory ionocytes and environmental salinity.

Generally, hagfish gills have one ionocyte subtype that alternatively performs H⁺ or HCO₃⁻ excretion, elasmobranch gills have an ionocyte subtype specialized for H⁺ excretion and another ionocyte subtype specialized for HCO₃⁻ excretion, and bony fishes have multiple ionocyte subtypes that vary in environment- and species-specific manner. During routine A-B challenges, the pre-existing A-B machinery becomes activated due to law-of-mass action or *via* post-translational modifications (see [Glossary](#)) to increase the rate of H⁺ or HCO₃⁻ excretion and maintain blood A-B homeostasis. More intense or extended A-B challenges might induce an upregulation in the synthesis of proteins of the ionocyte A-B regulatory machinery (for reviews, see [Tresguerres, 2014](#); [Tresguerres et al., 2023, 2020](#)).

A-B regulation of other bodily fluids

The p*H*s of some bodily fluids are regulated at different set points than arterial blood. For example, the endolymph inside the fish inner ear has higher p*H*, [HCO₃⁻] and [CO₃²⁻] and lower pCO₂ ([Kwan and Tresguerres, 2022](#)); this promotes the biomineralization of the otoliths, which are essential for hearing and maintaining equilibrium. The cerebrospinal and brain interstitial fluids (CS-BIFs) of vertebrate animals have higher pCO₂ and a lower p*H*, resulting from their lower protein-buffering capacity and the high metabolic production of CO₂ by neurons and astrocytes ([Caldwell et al., 2021](#)).

The A-B status of the endolymph is regulated by ionocytes in the inner ear epithelium; however, these have received much less attention than the ionocytes in the gills and many aspects remain unknown. The A-B status of CS-BIFs is regulated by the blood-brain barrier and the blood-cerebrospinal fluid barrier. Many aspects of these mechanisms are unresolved even in mammals, and are almost completely unknown in fish, in large part due to the small fluid volume and tissue size that constitutes a technical challenge.

Although the A-B statuses of the endolymph and the CS-BIFs are regulated by specialized tissues, they remain intrinsically linked to that of the blood, and together they determine the A-B compensatory responses during A-B challenges. For example, the reported otolith overgrowth in fish exposed to ocean acidification results from blood and endolymph pH regulation at different set points that accelerates the rate of CO₂ diffusion into the endolymph, leading to increased [HCO₃⁻] and [CO₃²⁻] in that fluid (Kwan and Tresguerres, 2022).

Measuring and interpreting fish blood A-B status and regulation

The simplest way to take a blood sample from a fish involves immobilizing the fish and inserting a syringe into a blood vessel for blood collection. Unfortunately, this procedure induces a significant blood acidosis due to anaerobic metabolism from white muscle contraction while the fish struggles, CO₂ accumulation due to gill collapse, and catecholamine-induced H⁺ extrusion from RBCs. Consequently, taking blood samples for meaningful measurements of fish blood A-B parameters is not a trivial task. Traditionally, this challenge has been overcome by surgically implanting a cannula into an artery or a vein, which allows blood sampling while the fish is still submerged and with minimum disturbance of A-B status. More recently, a novel method termed *in-situ*-anesthesia was developed, which enables meaningful fish blood A-B measurements while avoiding the logistics associated with surgical cannulation (Davison et al., 2023). The *in-situ*-anesthesia blood sampling technique has the additional advantage of being suitable for small fish that cannot be fitted with a cannula (Davison et al., 2023) and for fish with non-standard vascular anatomy (Kwan and Tresguerres, 2022). Nevertheless, both cannulation and *in-situ*-anesthesia require infrastructure that make them unsuitable for field studies as well as specialized training on fish blood sampling, plasma pH and total CO₂ measurements, and [HCO₃⁻] calculations. This combination is a major reason for the relatively low number of studies reporting meaningful fish blood A-B parameters.

Once accurate blood A-B measurements are taken, the magnitude and type of an A-B challenge as well as the ensuing regulatory responses can be gauged by means of the Davenport diagram. This type of graph allows the simultaneous plotting of pCO₂, pH and [HCO₃⁻] over time, while also considering the blood non-HCO₃⁻ buffering capacity of the fish species (Fig. 4). The Davenport diagram can also be used to study A-B regulation of other fluids, both extracellular and intracellular, provided that their pCO₂, pH, [HCO₃⁻] and non-HCO₃⁻ buffering capacity are considered.

In the Davenport diagram, A-B disturbances that induce metabolic acidosis and alkalosis are visualized as changes in pH that take place at constant CO₂ ("isopleths") and are associated with a decrease or increase in [HCO₃⁻], respectively (Fig. 4, red line in examples 2 and 3). On the other hand, hypercapnia is visualized as a reduction in pH along the non-bicarbonate buffering capacity until the value reaches the new CO₂ isopleth (Fig. 4, example 4). In all cases, the magnitude of the A-B challenge will be proportional to the changes in pH and the interrelated A-B parameters pCO₂ and [HCO₃⁻].

The ventilatory and A-B properties of water-breathing animals constraints fish A-B regulation to excreting excess H⁺ and accumulating HCO₃⁻ to compensate for an acidosis, and to excreting excess HCO₃⁻ and accumulating H⁺ to compensate for an alkalosis. In the case of metabolic acidosis and alkalosis, A-B regulation is visualized as a return toward the baseline pH value along the CO₂ isopleth over time (Fig. 4, green lines in examples 2 and 3). In the case of respiratory acidosis, pH will be corrected by "climbing" along the new pCO₂ isopleth; importantly, this regulatory mechanism restores baseline pH but it elevates blood HCO₃⁻ compared to baseline levels (Fig. 4, example 5).

Conclusion

The main A-B challenges experienced by fish are continued acid load due to routine metabolism, a metabolic acidosis during exhaustive exercise, a metabolic alkalosis in the post-feeding period, and a respiratory acidosis during exposure to environmental hypercapnia. Fish are usually able to restore baseline pH by excreting and absorbing H⁺ and HCO₃⁻ across the gills; however, A-B regulation during chronic exposure to hypercapnia results in an accumulation of HCO₃⁻ in blood and other bodily fluids that may affect biomineralization, neurobehavior, and other processes.

The A-B regulatory mechanisms and A-B regulatory capacity of fishes are shaped by the metabolic and dietary characteristics of each fish: active fishes produce more metabolic H⁺ than sluggish fishes during locomotion and thus generally have a better capacity for A-B regulation, and fishes with an acidic stomach may be better equipped to dealing with a blood alkalosis than those that have an alkaline intestinal tract. Similarly, a fish that lives in a kelp forest or in the intertidal zone is more prone to naturally experience A-B and O₂ challenges than a pelagic fish that lives in the open ocean or in a large lake, so the former might count with more robust and dynamic mechanisms to regulate blood A-B.

While the gills are the main A-B regulatory organ in all fishes, some species may also utilize the intestine, or to an even lesser extent the kidneys, to help correct for certain A-B challenges. Indeed, much of the evolutionary success of fishes is rooted in their adaptive physiological versatility, which is associated with a variety of cellular and molecular A-B regulatory mechanisms.

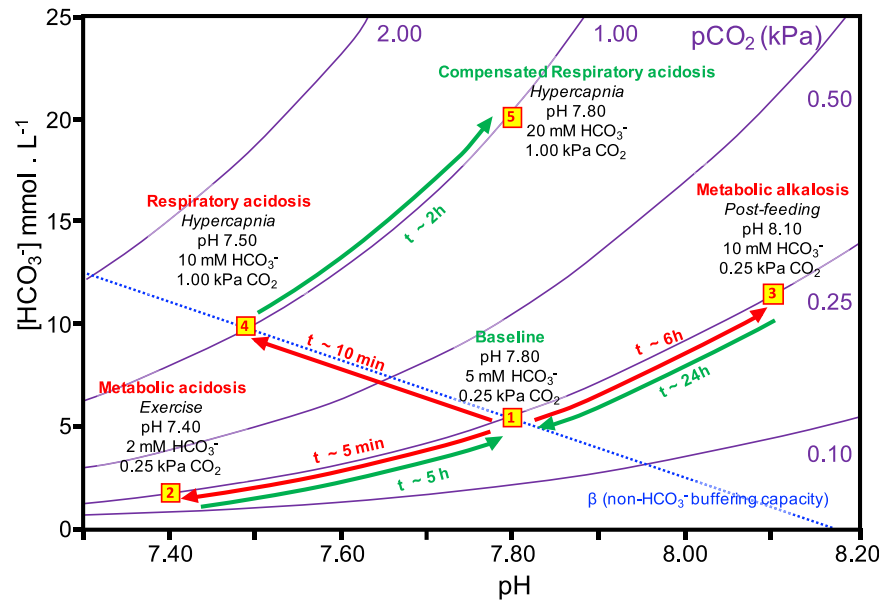


Fig. 4 Davenport diagram illustrating baseline and most common acid-base (A-B) disturbances and compensation in fish blood. The red arrows indicate the A-B disturbances, and the green arrows indicate the respective compensatory responses. (1) Indicates the baseline condition of a resting fish; (2) reflects a metabolic acidosis resulting from intense exercise in rainbow trout (Wood, 1991); (3) is a case of metabolic alkalosis in the post-feeding period in dogfish shark (Wood, 2019); (4) illustrates a respiratory acidosis upon exposure to hypercapnia (1 kPa $p\text{CO}_2$, sometimes indicated as $\sim 10,000 \mu\text{atm}$, $\sim 7.5 \text{ torr}$, or $\sim 1\%$ CO_2) in European seabass (Montgomery et al., 2022); (5) shows the compensatory response to hypercapnia, notice that baseline pH is totally restored but $p\text{CO}_2$ and $[\text{HCO}_3^-]$ are elevated compared to the baseline condition; pH compensation may take longer in other fish species, or it may not be complete. In all cases, the change in A-B parameters will be proportional to the magnitude of the A-B disturbance. For example, less intense exercise, smaller meals, and exposure to lower $p\text{CO}_2$ will respectively induce less pronounced metabolic acidosis, metabolic alkalosis, and respiratory acidosis, as well as milder compensatory HCO_3^- accumulation. Conversely, more intense exercise, larger meals, and exposure to greater $p\text{CO}_2$ will respectively induce more pronounced metabolic acidosis, metabolic alkalosis, and respiratory acidosis, as well as greater compensatory HCO_3^- accumulation.

See Also: Carbon dioxide transport and excretion; Integrative responses to hypercapnia; Multiple stressors- physiological responses to multivariate environments; The hormonal control of osmoregulation in fish; The physiology of ocean acidification; The ventilatory response to CO_2/H^+ .

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