Patterns of Acid–Base Regulation During Exposure to Hypercarbia in Fishes

C.J. Brauner and D.W. Baker

Abstract Acid–base regulation is one of the most tightly regulated physiological processes among vertebrates, and the specific mechanisms and patterns of acid–base regulation in fish have been investigated for decades, although primarily on a few species of teleosts and elasmobranchs. The most common response observed in fish during short-term (up to 96 h) exposure to hypercarbia (elevated environmental CO_2) is that of blood pH compensation for the induced respiratory acidosis by a net increase in plasma HCO_3^- in exchange for Cl^-, predominantly through processes at the gills. Studies on hagfish indicate that this pattern of pH compensation (i.e. net plasma HCO_3^-/Cl^- exchange, driving pH recovery) probably represents the ancestral state for fishes. Due to an apparent limit to this net HCO_3^-/Cl^- exchange, most fishes examined to date exhibit incomplete pH compensation for the acidosis, in both plasma and tissues associated with CO_2 tensions greater than 10–16 mmHg; in CO_2-sensitive fishes, this may be the basis for mortality during exposure to high CO_2. A few fish species, however, are capable of tolerating $PaCO_2$s well above 10–16 mmHg; in some of these species, this tolerance appears to be associated with the ability to completely regulate intracellular pH (preferential pHi regulation) of tissues, such as brain, muscle and liver, despite a large reduction in extracellular pH. We hypothesize that: (a) preferential pHi regulation in fish evolved in the ancestors of the pleisiomorphic freshwater (non-teleost) actinopterygiians, (b) is associated with high CO_2 tolerance, and (c)was an exaptation for air-breathing. A great deal of research remains to test these hypotheses, and to elucidate the origin and ubiquity of preferential pHi regulation among fishes and the cellular and molecular mechanisms involved.

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1 Introduction

Changes in pH can alter local charges on proteins, which will affect protein function through, for example, enzyme and membrane channel properties. These changes can in turn ultimately affect cellular processes such as cell-to-cell signalling, volume regulation and gene expression, as well as whole animal performance, including that relying on muscle contractility and metabolic pathways (see review by Putnam and Roos 1997). Consequently, pH homeostasis is central to survival in most vertebrates at both the cellular and extracellular level. All cells have some capacity for intracellular pH regulation (Putnam and Roos 1997), but the degree to which they defend cytoplasmic pH during an extracellular acid–base disturbance depends upon the origin and severity of the acidosis (i.e. environmental, respiratory or metabolic), the buffering capacity of the cell, and the degree to which the pH of the blood compartment can be actively altered during the disturbance (Truchot 1987). The specific mechanisms and general pattern of acid–base regulation in fishes have been studied extensively over the last several decades (for example, Heisler 1986, 1999; Goss et al. 1998; Claiborne et al. 2002; Evans et al. 2005; Perry and Gilmour 2006). While a great deal is known about this exciting field, much remains to be discovered at the molecular, cellular and organismal levels. While this chapter will first describe current models of acid–base regulation in fishes in a very brief summary of several recent excellent reviews (Claiborne et al. 2002; Evans et al. 2005; Perry and Gilmour, 2006), it will focus mainly upon new patterns of acid–base regulation observed in response to short-term (i.e. up to several days) hypercarbia (elevated environmental CO_2) in pleisiomorphic and air-breathing fishes.

2 Regulation of pH in the Blood and Extracellular Space

In vertebrates, an acid–base disturbance can be either minimized or compensated for by the following mechanisms: (a) physicochemical buffering with bicarbonate and non-bicarbonate buffers, (b) a change in ventilation to alter PCO_2 and thus pH, via the CO_2–HCO_3^- buffer system, or (c) net transport of acid–base equivalents between the cell and the blood compartment, and/or the blood compartment and the environment (See Evans et al. 2005). In terrestrial air breathers, bicarbonate buffering through ventilatory changes in blood PCO_2 plays a principle short-term role in acid–base regulation. In water breathers, blood PCO_2 levels are low relative to air-breathers due to the high ventilatory requirement for O_2 uptake and the high CO_2 capacitance of water relative to O_2 (Dejours 1988), and changes in ventilation can only slightly alter arterial CO_2 tensions. Thus, breathing can only have moderate effects on acid–base regulation in water-breathing fish. Clearly, more research is required to understand the role of breathing on acid–base status in fish (See Gilmour 2001; Perry and Gilmour 2006); however, the current consensus is that physicochemical buffering and net transport of acid–base equivalents (a and c

above) are the predominant pathways for respectively minimizing or compensating for pH disturbances in fish.

In general, the role of buffering is restricted to early periods of acid–base disturbance, and the buffer capacity of the blood and extracellular compartment is limited (Heisler 1986). Consequently, this mechanism cannot be heavily relied upon during an acidosis, and so net transport of acid–base equivalents between the fish and environment is of great importance, and the gills, kidney and intestine all play a role. The gills have traditionally been thought to account for approximately 90% or more of the net acid–base relevant ion transport in fish during compensation for an acid–base disturbance (See Heisler 1984; Claiborne et al. 2002; Evans et al. 2005), and as a result this organ has been investigated most intensively. Even so, exactly which molecular mechanisms are responsible for pH compensation remains largely uncertain (Evans et al. 2005; Perry and Gilmour 2006). Interest in the role of the kidney and intestine in whole animal acid–base regulation has increased of late (see Evans et al. 2005; Perry and Gilmour 2006 for reviews), and they may play a greater role than previously thought; however, it appears that quantitatively these organs remain secondary to the role of the gills. Many methods have been used to investigate acid–base regulation in fish (e.g. acid or base infusion, exercise, hypoxia, environmental acidification and alkalinization); however, this review will focus predominantly upon studies employing environmental hypercarbia. Hypercarbia is of current interest because of its environmental relevance associated with natural and anthropogenic processes, and historically it has been widely used because of its convenience as a tool to study acid–base regulation, as it can be used to generate a sustained acid–base challenge while PCO_2 is held constant. The following is a discussion of the sources of environmental hypercarbia followed by how fish compensate for the induced respiratory acidosis during short-term exposure (0–96 h) to elevated CO_2 tensions.

3 Environmental Hypercarbia

Environmental hypercarbia has been used extensively by physiologists as a tool to investigate the mechanisms and patterns of acid–base regulation in fish, and the relative contributions of the gills and kidney, in particular, to pH compensation. Consequently, a fair amount is known about fish responses to environmental CO_2 tensions of 7–14 mmHg. Hypercarbia is not only an experimental tool, however, as it also represents an acid–base challenge with great relevance both historically and currently. Hypercarbia occurs in both fresh and marine waters, and in tropical fresh water systems, for example, $PwCO_2$ levels of up to 60 mmHg (20- to 30-fold increase over the arterial PCO_2 of normocapnic fish) have been observed (Heisler et al. 1982; Ultsch 1996). Contributing factors can include thick surface vegetation, poor water mixing, thermostratification, high flora or fauna biomass, and anaerobic metabolism of micro-organisms (Heisler 1999; Ultsch 1996). While the degree of hypercarbia observed naturally in the marine environment is less, levels can still

reach 5–10 mmHg, for example, at depths of 200–500 m (Heisler 1986) or in tide pools (Burggren and Roberts 1991).

In addition to occurring naturally, hypercarbia can be induced through anthropogenic activities. Within aquaculture, an increasingly popular goal is to relocate fish-holding sites from, for example, open-sea pens to closed containment and thus re-circulating systems. While supplemental oxygenation within a re-circulating system permits increased biomass, it also results in hypercarbia (Sowerbutts and Forster 1981; Colt and Orwics 1991). As a result of other anthropogenic activities, globally projected increases in atmospheric CO_2 levels over the next several centuries are hypothesized to elevate surface-water CO_2 levels 5-fold, which may reduce the pH of these waters by 0.7 pH units (Caldeira and Wickett 2003). While this predicted level of hypercarbia is relatively low compared to the environmental and aquaculture-based levels described above, sequestering atmospheric CO_2 to deep ocean sites through high-pressure injection has been proposed as a means to prevent further increases in atmospheric CO_2. This procedure would potentially create a point source for CO_2 in the marine environment, which could result in CO_2 tensions greater than any naturally occurring levels, past or present, and consequently would create a severe challenge to marine organisms (Seibel and Walsh 2001). Given the anthropogenic potential for generating such high levels of hypercarbia, there is renewed interest in assessing CO_2 tolerance and understanding the compensatory physiological responses in fish during exposure to high CO_2 levels (10–50 mmHg, Hayashi et al. 2004; Ishimatsu et al. 2004, 2005; Kikkawa et al. 2004; Portner et al. 2004).

4 Acid–Base Compensation During Exposure to Hypercarbia

Studies on acid–base regulatory responses to hypercarbia in fishes are limited to a relatively small number of fish species, which are either teleosts or elasmobranchs (e.g. rainbow trout, *Oncoryhnchus mykiss*, Lloyd and White 1967; Wood and LeMoigne 1991; Larsen and Jensen 1997; Hyde and Perry 1989; Goss and Perry 1994; Bernier and Randall 1998; common carp, *Cyprinus carpio*, Claiborne and Heisler 1984; brown bullhead, *Ictalurus nebulosus*, Goss et al. 1992; *Anguilla anguilla*, McKenzie et al. 2002; *Conger Conger*, Toews et al. 1983; *Fundulus heteroclites*, Edwards et al. 2005; cod, *Gadus morhua*, Larsen et al. 1997; Tench, *Tinca tinca*, Jensen and Weber 1985; little skate, *Raja ocellata*, Graham et al. 1990; Wood et al. 1990; dogfish, *Scyliorhinus stellaris*, Heisler et al. 1988, *Squalus acanthias*, Claiborne and Evans 1992, Japanese amberjack, *Seriola quinqueradiata*, Ishimatsu et al. 2004; the bastard halibut, *Paralichthys olivaceus,* Hayashi et al. 2004). In general, the 'typical' acid–base regulatory response in these fishes consists of a respiratory acidosis followed by pH recovery over the following 24–96 h. This initial acidosis is rapid, where arterial PCO_2 equilibrates with water PCO_2 within minutes, and the pH of blood (pHe) and tissues (pHi) decreases as a function of both the newly-equilibrated CO_2 tension and non-bicarbonate (i.e. intrinsic) buffer value

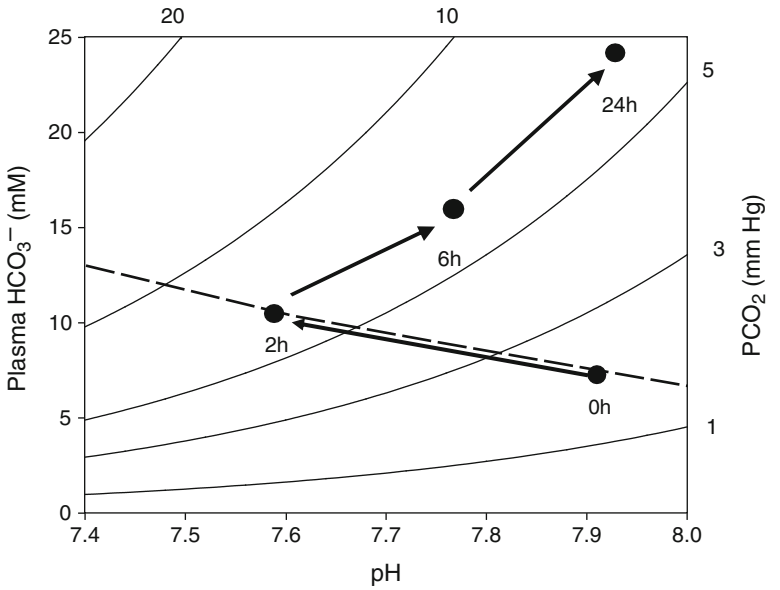

Fig. 1 The effect of sustained exposure to a $PwCO_2$ of 7 mm Hg on blood pH and plasma HCO_3^- in rainbow trout as represented on a $pH/HCO_3^-/CO_2$ plot. Isopleths are calculated based on previous pK' and solubility coefficients for CO_2 as reported by Boutilier and colleagues (1984). *Numbers* proximal to each data point represent exposure time; *arrows* indicate temporal pattern of change in blood pH and plasma HCO_3^-. The *dotted line* indicates the non-bicarbonate (i.e. intrinsic) buffer value of whole blood as reported by Wood and LeMoigne (1991) (see text for more details). Data from Larsen and Jensen (1997)

of the respective compartment. As a visual aid, this acidification is represented on a $pH/HCO_3^-/CO_2$ plot for rainbow trout, *O. mykiss*, in Fig. 1 (between 0 and 2 h, data are taken from Larsen and Jensen 1997). The dotted line on this and all subsequent $pH/HCO_3^-/CO_2$ plots (i.e. Figs. 1, 3, 4, and 6), represents the non-bicarbonate (i.e. intrinsic) buffering for whole blood. As tissues have greater intrinsic buffering than the blood, the initial intracellular acidosis during hypercarbia is less severe than in the blood, yielding a $\Delta pHi/\Delta pHe$ of 0.3–0.7 that is both tissue and species specific.

However, few studies have investigated this relationship between pHe and pHi in fish. In those few, pHi changes in a qualitatively similar pattern to pHe during a respiratory acidosis. This is the case for red blood cells, liver, and muscle in rainbow trout in vivo (although not the gill) (Wood and LeMoigne 1991; see Fig. 2). In vitro, isolated hepatocytes, when exposed to 1% CO_2 (7.5 mmHg), exhibited a decrease in pHi which stabilized within minutes, and did not change further over the following 60 min incubation. The resulting $\Delta pHi/\Delta pHe$ was 0.61 (Walsh et al. 1988) with a similar relationship observed in isolated hepatocytes during an isocapnic acidosis (Walsh et al. 1988). In primary hepatocyte isolations from the Antarctic eelpout (*Pachycara brachycephalum*) incubated in normoxia or 1% CO_2 (7.5 mmHg), depression of pHe through HCl addition at constant gas tension resulted in a depression in pHi, with a slope of $\Delta pHi/\Delta pHe$ of 0.4 after 50 min of

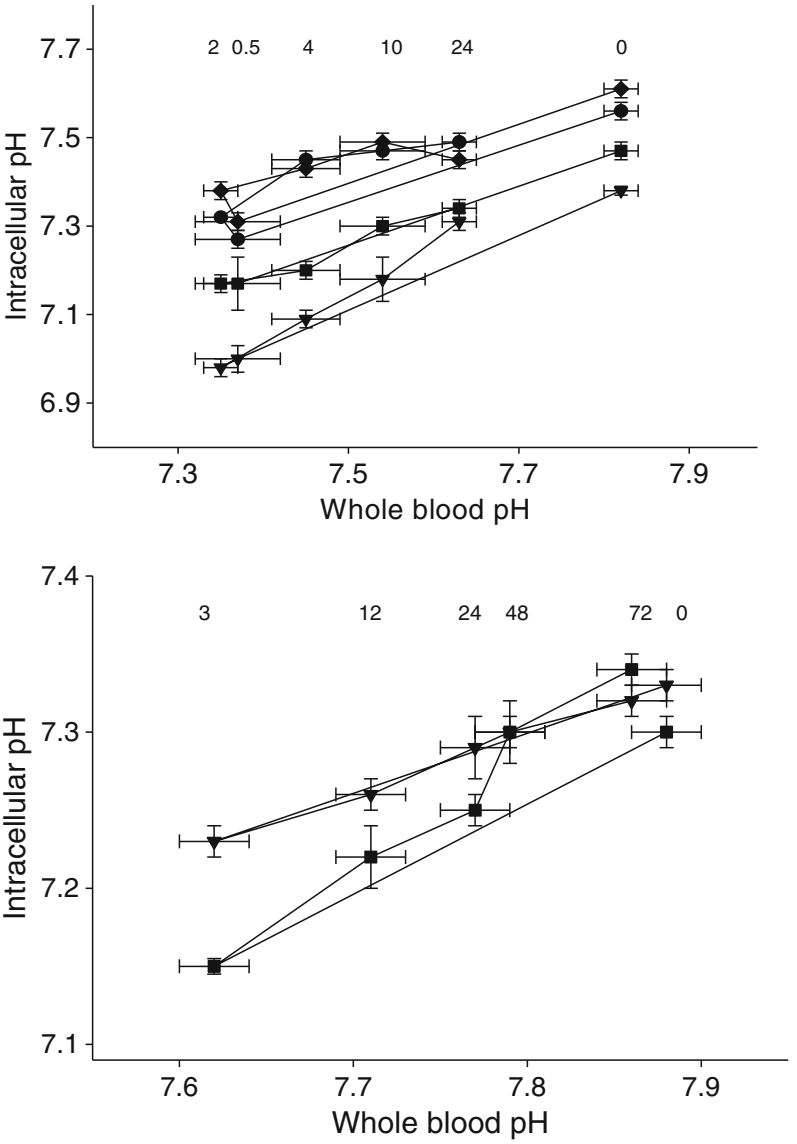

Fig. 2 The relationship between the pH of blood (pHe) and intracellular pH (pHi) of red blood cells (*inverted triangle*), brain (*circle*), heart (*diamond*), and white muscle (*square*) during exposure to short term hypercarbia in (*upper panel*) little skate, *R. ocellata*, and (*lower panel*) hyperoxia-induced hypercapnia in rainbow trout, *O. mykiss*. Note not all tissues are present in each panel. Time course (h) is indicated by *numbers* located directly above vertically oriented groupings. Note reversal of early time points (0.5 and 2 h) in *upper panel*. While pHi can recover more rapidly than pHe in tissues such as the brain and heart of skate, in all tissues presented here, pHi remains depressed if pHe does not recover. Mean values and SEM error bars approximated from Wood et al. (1990) for skate and Wood and LeMoigne (1991) for trout

incubation (Langenbuch and Portner 2003). In skate exposed to hypercarbia in vivo, a depression of pHe resulted in a reduction of pHi in red blood cell, heart, brain, and muscle consistent with that observed in trout; however, the brain and heart exhibited slightly more rapid pHi recovery than blood or other tissues (Fig. 2, data from Wood et al. 1990). Even so, the pattern of concurrent pH changes between pHe and pHi is a common pattern in the few studies published to date: despite this, some fish are able to defend pHi during large reductions in pHe (Heisler 1982; Brauner et al. 2004; Baker et al. in press) and these are described below.

Following a respiratory acidosis, pH recovery of the blood is associated with branchial acid–base relevant ion transfer, which over hours to days (Larsen and Jensen 1997), returns blood pH to normocapnic levels, through non-respiratory accumulation of HCO_3^-, which drives pH along the respective PCO_2 isopleth during sustained hypercarbia and is shown for rainbow trout in Fig. 1 (between 2 and 24 h). The elevation in plasma $[HCO_3^-]$ in most fishes studied to date is matched by an equimolar decrease in $[Cl^-]$ (Goss et al. 1998; Claiborne et al. 2002). While direct evidence for the specific cellular and molecular mechanisms associated with branchial acid–base relevant ion transport is largely lacking (Claiborne et al. 2002; Evans et al. 2005; Perry and Gilmour 2006), there are many studies that provide indirect evidence through, for example, changes in mRNA expression patterns and protein levels of putative transporters in the gills [i.e. Na^+/H^+ exchangers (NHE's), V-type H^+-ATPases coupled to apical membrane Na^+ channels (ENaC), HCO_3^-/Cl^- exchange via transporters belonging to the SLC26 and 24 multi-gene families] in response to acid- or base-loading events (Claiborne et al. 2002; Evans et al. 2005; Perry and Gilmour 2006). Further support for transport mechanisms comes from the experimentally observed morphological alterations of specific cell types in the gill epithelium (as described below) during exposure to hypercarbia and other acid–base disturbances (see Goss et al. 1998; Claiborne et al. 2002; Perry et al. 2003; Evans et al. 2005; Perry and Gilmour 2006 for excellent reviews).

While pH compensation for the respiratory acidosis during exposure to hypercarbia can be initiated quickly (within an hour), as indicated from the onset of increases in net H^+ efflux (for example, Wheatley et al. 1984; Edwards et al. 2005), a less rapid (hours to days) but extensive gill remodelling has been observed in a number of fish species, and is thought to play a role in pH recovery as well. The mitochondria rich cells (MRCS, also called chloride cells and most recently PNA+ MRCs) are thought to be the predominant site for Cl^- uptake and base secretion, while pavements cells are hypothesized to be the site of proton extrusion (see Perry 1997; Goss et al. 1998; Evans et al. 2005; Perry and Gilmour 2006). In rainbow trout and the brown bullhead, exposure to hypercarbia results in an increase in apical surface area of the acid-excreting PVCs (through proliferation of microridges) and a decrease in the fractional surface area of the base-secreting chloride cells (by physical covering by PVCs; Goss et al. 1994, 1995, 1998). By altering the cell surface area exposed to the environment, and thus sites for ion transport in the respective cell types, these morphological changes potentially aid in either increasing acid efflux or limiting base efflux during exposure to hypercarbia.

These responses described above (net HCO_3^-/Cl^- exchange and gill remodelling) together are effective in driving pH recovery during hypercarbia, and, albeit with modification, represent the paradigm for acid–base regulation during hypercarbia in water-breathing fish (Evans et al. 2005; Perry and Gilmour 2006). However, there are limitations to the use of this response to compensate for hypercarbia, which is the focus of the next section.

5 Limitations to Extracellular pH Compensation During Hypercapnia

As hypercapnia and the resulting acidosis increase in severity, the capacity of the fish to achieve complete pHe recovery through net HCO_3^-/Cl^- exchange becomes reduced. By extrapolation along the respective PCO_2 isopleth in Fig. 3 (see figure caption for more details), it is clear that a fish exposed to a PCO_2 of 30–50 mmHg would have insufficient Cl^- in the plasma to match the HCO_3^- accumulation necessary (i.e. $>$100–120 mmol l^{-1}) for complete pH compensation for the respiratory

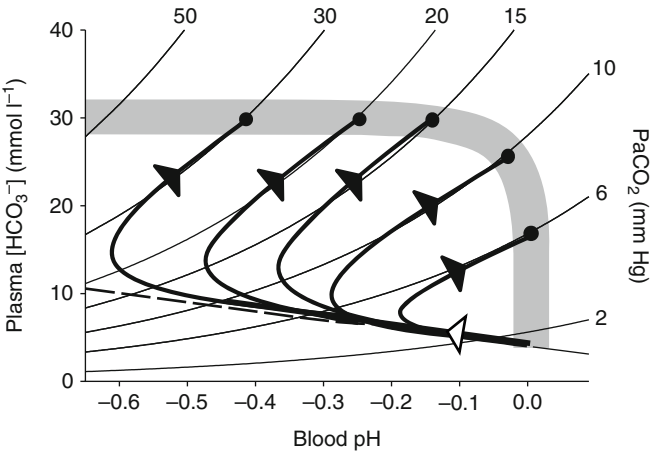

Fig. 3 A theoretical representation of the typical temporal response to short-term (less than 5 days) hypercarbia in fish. Upon transfer from normocarbia to hypercarbia, blood pH rapidly falls along the non-bicarbonate buffer line of the blood as indicated by *black open arrowhead*, and pH recovers along a given PCO_2 isopleth through a net increase in HCO_3^- in exchange for Cl^- as indicated by *black filled arrowheads*. *Black filled circles* represent final pHe values that would be achieved based upon limits to net HCO_3^- accumulation within 24–96 h of exposure to hypercarbia (see text for more details). *Shaded bar* indicates maximal pH compensation limited by the 'bicarbonate concentration threshold', as most fish do not increase plasma HCO_3^- beyond 25–35 mM (modified from Heisler 1986, 1999). Thus, compensation for a respiratory acidosis (within 48–96 h) during exposure to hypercarbia is incomplete above a PCO_2 of 10–15 mm Hg in most water-breathing fishes. Note that CO_2 isopleths are dependent on both temperature and osmolarity, and that the isopleths represented here are plotted for clarity purposes only

acidosis induced, and thus, ultimately, net HCO_3^-/Cl^- exchange has limits based on Cl^- availability; however, net HCO_3^- accumulation plateaus at HCO_3^- and corresponding PCO_2 levels much lower than this absolute limit. This 'apparent' limit has been referred to as 'the bicarbonate concentration threshold' (Heisler 1986, 1984). Because this bicarbonate concentration threshold (between approximately 27 and 33 mmol l^{-1}) is rarely surpassed (for the few exceptions, see Heisler 1986, 1999; Ishimatsu et al. 2005) during short-term exposure (<96 h) to hypercarbia, the acidosis induced by $PaCO_2$ tensions greater than 10–16 mmHg (resulting in an acidosis of approximately 0.25–0.4 pH units) cannot be fully compensated for in the blood of water-breathing fishes. This threshold was described over 20 years ago, yet the source of the limitation remains unresolved; however, there is evidence for the contribution of several factors.

For example, water conditions have been experimentally demonstrated to play a role in affecting the rate and degree of pH recovery during hypercarbia. Increasing water hardness (as measured by $CaCO_3$) increased the rate of normocapnic pH recovery in rainbow trout acclimated to the respective water types, so that compensation occurred earlier and was more complete than in softer water (Larsen and Jensen 1997). Carp acclimated to water with higher $[Ca^+]$ exhibited an increased rate of net bicarbonate influx into the plasma (Heisler 1999). Heisler proposed that increased calcium led to less permeable paracellular junctions, and reduced the rate of diffusive loss of HCO_3^- at the gill, allowing for greater net influx rates; this theory is yet to be verified experimentally.

It has also been suggested that increased salinity mediates faster pH recovery in fish exposed to short-term hypercarbia. Most support for this premise appears to be attributable to a perceived faster pH recovery in marine fishes (including elasmobranchs). Iwama and Heisler (1991) examined the rate of pH recovery during hypercarbia at two salinities (30 and 300 mOsm l^{-1}) in rainbow trout, and observed minor increases in the rate of pH recovery as environmental $[Na^+]$ and $[Cl^-]$ ions, (the only two ions manipulated in this study) increased. However, a review that examines pH recovery rates between or within a species has yet to be done, and attributing differences to environmental salinity is confounded by both the small sample sizes and species differences used in freshwater and seawater studies.

Interestingly, the effect of water pH on extracellular pH compensation during hypercarbia remains relatively unexplored, despite the fact that as CO_2 levels increase water pH decreases. To complicate the effect of water pH on net HCO_3^- accumulation, water $[HCO_3^-]$ is dependent on pH when PCO_2 is held constant: that is, more acidic water will have lower $[HCO_3^-]$ at the same $PwCO_2$ tensions. Furthermore, water pH may affect the rate of net H^+ efflux from branchial tissue; for example, the relative activity of V-type H^+ ATPase, which may play an important role in blood pH compensation during hypercapnia (Heisler 1999; Perry and Gilmour 2006), decreases as water pH decreases in rainbow trout (Lin and Randall 1990). In both dogfish and carp, pH recovery was severely compromised when water pH and $[HCO_3^-]$ were decreased at a constant $PwCO_2$ (Heisler 1986, 1999).

In summary, the rate and degree of pH compensation are influenced by ionic composition of the water. Furthermore, net HCO_3^- uptake in exchange for Cl^- for

complete pHe compensation appears limited as a viable response to CO_2 levels below 10–15 mmHg (see Fig. 3). Some species of fish do not survive CO_2 levels above this. For example, when exposed to $PaCO_2$ of 37.5 mmHg, the Japanese amberjack (*Seriola quinqueradiata*) died within 8 h. At the same tension, the bastard halibut (*Paralichthys olivaceus*) did not survive 48 h, and 17% had died by 8 h (Ishimatsu et al. 2004). Rainbow trout exposed to a PCO_2 of 30 mmHg did not survive even 12 h (Baker and Brauner, personal observation). The sensitivity of these species to hypercarbia is probably associated with exceeding the capacity to compensate for the respiratory acidosis. As environmental hypercarbia does occur at levels higher than 15 mmHg, it is no surprise that a few fish species have demonstrated the ability to tolerate CO_2 tensions well beyond the proposed limit of pH_e compensation. The strategies and mechanisms associated with this high CO_2 tolerance in these species will constitute the discussion of the remainder of this chapter; to facilitate conceptual ideas regarding the evolution of acid–base regulation, the following fish groups are discussed in order of phylogenetic progression.

6 Novel Patterns of pH Regulation in Response to Hypercarbia in Pleisiomorphic and Air-Breathing Fishes

6.1 Pacific Hagfish

Hagfish are the most basal extant craniate and many aspects of their physiology may be representative of the common ancestor of vertebrates (Holland and Chen 2001; Janvier 2007). Consequently, there is considerable interest in understanding their physiology. Hagfish are the only craniates that are ionoconformers; that is, they do not actively regulate Na^+ or Cl^- at levels much different from their environment (Morris 1965, Sardella et al. 2009); this strategy may represent the ancestral vertebrate state. Hagfish are burrowing animals, and this behaviour may include burrowing into mud or into a carcass during feeding for extended periods (Strahan 1963), probably severely limiting gill:water interaction and gas transfer. Therefore, it is likely that hagfish experience major disturbances to whole animal acid–base balance; however, this remains to be determined experimentally.

Hagfish possess MRCs in gills (Mallat and Paulsen 1986) which, it has been proposed, play a role in acid–base regulation, given that hagfish are ionoconformers (Evans 1984; Mallatt et al. 1987). In a few studies hagfish have been shown to be effective at whole animal acid–base regulation; both acid and base loads can be well-tolerated and compensated for (McDonald et al. 1991; Tresguerres et al. 2006). The gills of hagfish possess the ion-transporting proteins V–H^+-ATPase, Na^+, K^+-ATPase and NHE2, which are thought to be involved in acid–base regulation in teleosts (see above, and Evans et al. 2005; Perry and Gilmour 2006). Curiously, in hagfish all three transporters are localized to a single cell rather than distributed among two cell types, as seen in elasmobranchs and teleosts, where there are

different acid- and base-secreting cells (Tresguerres et al. 2006). Thus, hagfish represent the only water-breathing craniate with a single cell type for both acid and base excretion; no vertebrate studied to date exhibits this pattern. Concluding that the hagfish condition represents an ancestral state would be premature, but it remains an interesting and plausible possibility.

The CO_2 tolerance of Pacific hagfish (*Eptatratus stoutii*) is impressive in that they survive exposures of up to 50 mmHg for 96 h. This level of CO_2 induces a massive respiratory acidosis in the blood (greater than 1 pH unit within 3 h), which is reflected in the tissues. Remarkably, most of this pH decrease ($>$70% or 0.75 pH units) is compensated for within 96 h, representing the greatest extracellular pH compensatory capacity in a water-breathing animal studied to date (Baker, Sardella, Rummer and Brauner, unpublished). During the initial acidosis and recovery, the $\Delta pHi/\Delta pHe$ in the red blood cell was 0.65, and that in the muscle, liver and heart varied from 0.35–0.6, similar to that described above for trout and skate. Plasma pH compensation in hagfish is associated with net bicarbonate accumulation as high as 100 mmol l^{-1} (mean values of approximately 75 mmol l^{-1}; Baker, Sardella, Rummer and Brauner, unpublished data), well above the previously described 'bicarbonate concentration threshold' observed in other fishes (refer to Fig. 3). Plasma HCO_3^- accumulation is associated with an equimolar reduction in plasma Cl^-, indicating that pH compensatory ion exchange in hagfish is consistent with that of the typical response described above (i.e. net HCO_3^-/Cl^- exchange), but with an approximately threefold greater capacity. While the capacity for compensation in hagfish is exceptional relative to water-breathing fishes, net non-respiratory HCO_3^- accumulation expressed as a proportion of normocarbic plasma Cl^- is slightly greater than 15% (66–86 mM $[HCO_3^-]$/455 mM $[Cl^-]$), very close to the proportion observed in other fishes (about 20%, 25–30 mM $[HCO_3^-]$/120 mM $[Cl^-]$). While hagfish differ from water-breathing fishes in many ways (e.g. gill morphology), which could be the basis for their tremendous acid–base regulatory ability, it is intriguing to think that absolute plasma Cl^- levels may set the limits of net HCO_3^-/Cl^- exchange associated with pH compensation. While difficult to conclude or address, the tremendous increase in the capacity for acid–base regulation that this would confer to a seawater ionoconfomer may represent an important advantage to what is otherwise just thought of as the ancestral state (i.e. ionoconforming) in vertebrates.

6.2 Sturgeon

Sturgeons are another group of fishes that are very hypercarbia tolerant. They represent an ancient chondrostean family of fishes over 250 million years old, and have enormous value for studying vertebrate evolution, including physiological adaptations to the environment (Cech and Doroshov 2004). White sturgeon, *Acipenser transmontanus*, exhibit a biphasic response during exposure to hypercarbia. When exposed to CO_2 tensions below 14 mmHg, levels within the capacity for pHe compensation (see Figs. 1, 3), blood pH recovery is relatively rapid (within 24 h)

and similar to that described above as the typical response in fishes (Baker and Brauner, unpublished observations). Blood HCO_3^- is elevated to 25–30 mmol l^{-1} in exchange for Cl^-, and changes in gill morphology occur that are similar to those described for trout and catfish (Goss et al. 1994, 1995, 1998), that is, there is a reduction in apical surface area of mitochondria-rich cells and an increase in the apical surface area of pavement cell in the gills (Baker et al. in press). However, at higher CO_2 tensions, the response to hypercarbia is quite different. White sturgeon, *A. transmontanus*, can tolerate hypercarbia of 30 mm Hg for days, despite an extended acidosis in arterial blood (Crocker and Cech 1998) and both juveniles and adults can tolerate a PCO_2 of over 45 mm Hg CO_2 for days without mortality (Baker and Brauner, unpublished data). During exposure to a PCO_2 of 30 and 45 mmHg, net bicarbonate accumulation (and associated Cl^- loss) is almost absent, and consequently pHe compensation is minimal during exposure to these high CO_2 levels (Fig. 4). Although a depression in pHe of this magnitude would be expected to greatly reduce pHi in some fishes (Fig. 2 and discussion above) complete intracellular pH protection is achieved in heart, brain, liver and white muscle during both transient (i.e. 6 h, 11 mm Hg CO_2) and extended (i.e. 48 h, 23 and 45 mm Hg CO_2) pHe depression in sturgeon (Baker et al. in press). This tremendous capacity to protect intracellular pH, which will be referred to as preferential pHi regulation from this point forward (and is defined as a $\Delta pHi/\Delta pHe = 0$ within 3–6 h

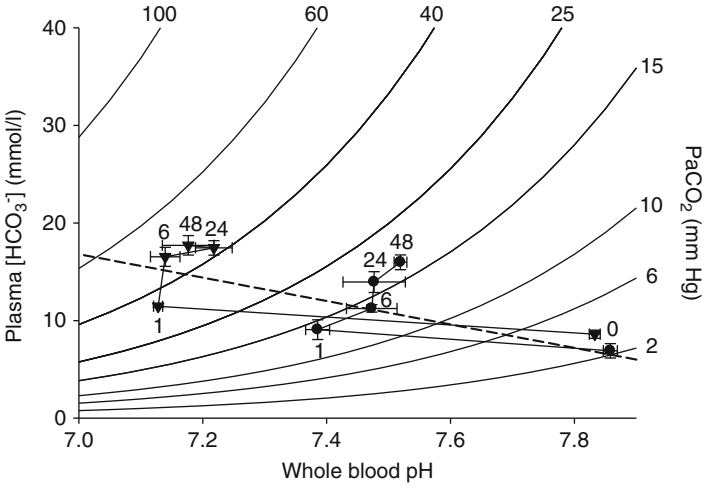

Fig. 4 The effect of sustained exposure at PCO_2 of 23 (*circles*) and 45 (*inverted triangles*) mm Hg on blood pH and plasma HCO_3^- in cannulated white sturgeon, *A. transmontanus*. Numbers proximal to data points indicate time (h) after transfer. The *dashed line* indicates the non-bicarbonate buffer value of whole blood as measured experimentally. Note that during exposure to both CO_2 tensions, blood pH drops below the blood buffer line, indicating preferential HCO_3^- transport into the tissues (Heisler 1982). Although sturgeon can accumulate up to 28 mM HCO_3^- during exposure to a PCO_2 of 11 mm Hg (see text), at higher CO_2 tensions net non-respiratory HCO_3^- accumulation does not exceed 5 mM in the plasma, and thus does not approach the proposed bicarbonate concentration threshold. Modified from Baker et al. in press

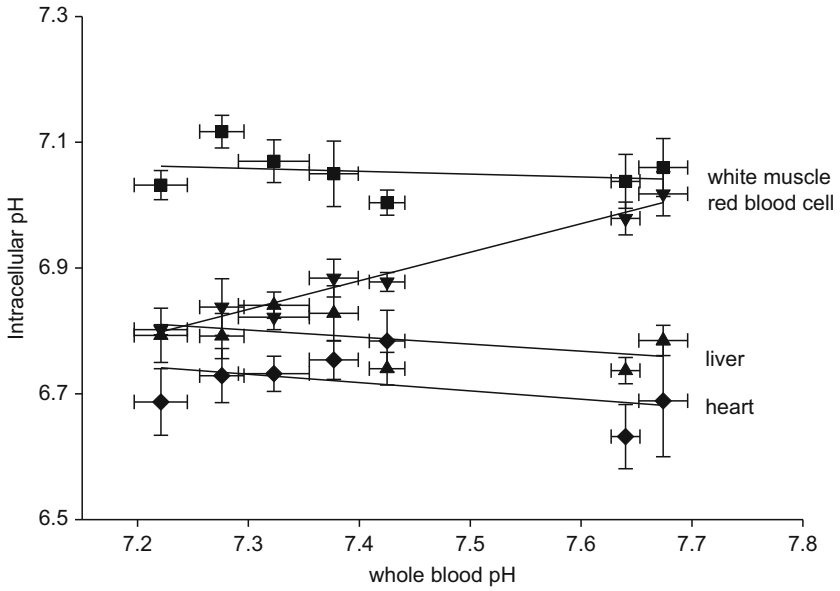

Fig. 5 The relationship between the pH of blood (pHe) and intracellular pH (pHi) of red blood cells (*inverted triangle*), heart (*diamond*), white muscle (*square*) and liver (*upright triangle*) following transfer to hypercarbia in the armoured catfish, *L. pardalis*. Armoured catfish were exposed to 14 or 28 mm Hg for up to 48 h (for a more detailed description of time course, see Brauner et al. 2004). Note that although pHe is greatly depressed, pHi is maintained at a constant value that did not differ between 6 and 48 h. Data for the armoured catfish is from Brauner et al. 2004

following exposure to hypercarbia) is not due to inherent buffering characteristics of the tissues, as they are not higher than that found in trout (Baker et al. in press), but probably reflect activation of cellular acid-extruding mechanisms, as indicated by the depression of blood HCO_3^- below the blood buffer line during exposure to hypercarbia (Fig. 4). The latter is indicative of HCO_3^- uptake into the tissues associated with maintenance of pHi (Heisler 1982). Preferential pHi regulation during hypercarbia has also been found in other fishes: however, all examples of this magnitude of pHi regulation to date have been observed in air-breathers. Consequently, this finding in white sturgeon is novel in that it is both the most basal vertebrate and the first non-air-breathing fish to exhibit this capacity for preferential pHi regulation. Furthermore, it seems entirely plausible that this may be the basis for the high tolerance of white sturgeon to hypercarbia and other acid-loading events.

6.3 Air-Breathing Fish

In bimodal breathers, the transition from water to air breathing is associated with a respiratory acidosis (Dejours 1981), and thus bimodal air-breathers may be quite tolerant to hypercarbia; this hypothesis has not been explicitly investigated. The

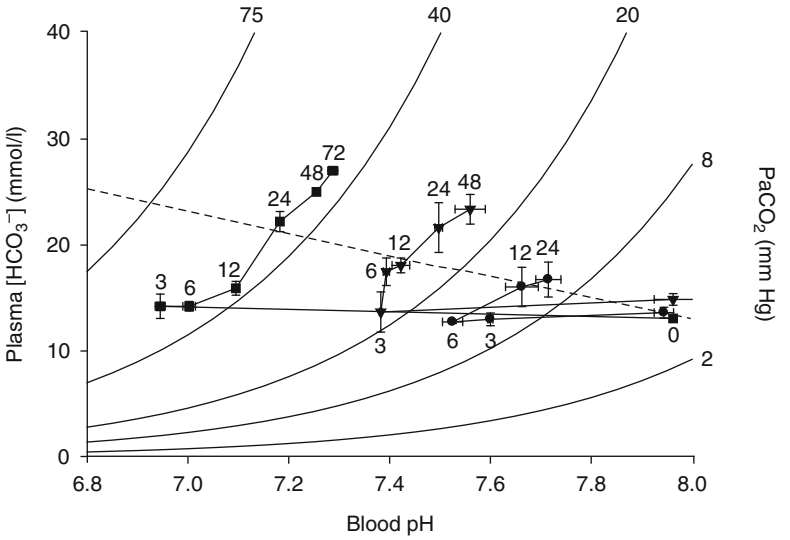

Fig. 6 The effect of sustained exposure to a PCO_2 of 11 (*circles*) and 23 (*inverted triangles*) or 45 (*squares*) mm Hg on blood pH and plasma HCO_3^- in the bowfin, *Amia calva*. *Numbers* proximal to data points indicate time (h) after transfer. The *dashed line* indicates the non-bicarbonate buffer value of whole blood. Note that up to 12 h following transfer to any of the hypercarbic treatments, blood pH is below the blood buffer line, suggesting preferential HCO_3^- transport into the tissues (Heisler 1986). Note that net non-respiratory HCO_3^- accumulation is almost negligible after 24 h at 11 mm Hg, and very similar between 23 and 45 mm Hg after 48 and 72 h respectively. Data are from Baker and Brauner, unpublished observations

pleisiomorphic facultative air-breather *Amia calva*, (common name bowfin) is very tolerant to hypercarbia. During exposure to a PCO_2 of 11, 23 and 45 mmHg, *A. calva* experience a respiratory acidosis in the blood that is largely uncompensated over 24 and 48 h respectively. Blood HCO_3^- levels during this time drop below the blood buffer line, indicating possible HCO_3^- transport into the tissues from the blood, as discussed for sturgeon above (Heisler 1982; Fig. 6). Intracellular pH of tissues were not measured in this study, but similarities to the patterns and tolerance of *A. transmontanus*, as discussed above, suggest that *A. calva* may use a similar strategy to sturgeon in tolerating hypercarbia, and are good candidates for preferential pHi regulation under these conditions.

In the facultative air-breathing teleost, the marbled swamp eel, *Synbranchus marmoratus*, aquatic hypoxia ($PO_2 = 16$ mmHg) induces air-breathing which results in a respiratory acidosis (Heisler 1982). Blood PCO_2 increased from 6 to 26 mmHg, and blood pH fell from 8.15 to 7.55 over 10 h, with little or no compensation in pHe over the following 4–5 days of maintained aquatic hypoxia. While air-breathing, the fish 'filled its buccal cavity with air and floated with its head on the water surface, with the rostral half of it in air'. During this time, there would not have been continuous contact between the gills and environmental water, and thus it is not surprising that it could not correct for the respiratory acidosis, given that the gills are the primary

site for acid–base compensation in other fishes. Despite the severe, uncompensated blood respiratory acidosis, pHi of skeletal and heart muscle following 4–5 days of air-breathing (the only time point measured) was not significantly different from water-breathing controls. This was the first observation of what appears to be preferential pHi regulation in tissues in fish. This regulation of pHi was associated with net transfer of HCO_3^- (presumably in exchange with Cl^-) from the plasma to the intracellular compartment, as indicated by blood HCO_3^- levels dropping below the blood buffer line, which was observed in white sturgeon and *A. calva* above. Thus, during an air-breathing-induced respiratory acidosis in *S. marmoratus*, pHi is preferentially regulated over pHe. Further investigation into the time course over which preferential pHi regulation may be occurring during air-breathing in *S. marmoratus* might prove extremely interesting, as might the acid–base regulatory response of these fish to aquatic hypercarbia; both of these remain to be investigated.

Another facultative air-breathing teleost, the armoured catfish, *Liposarcus pardalis*, is remarkably tolerant of aquatic hypercarbia. During exposure to a PCO_2 of 42 mmHg, blood pH dropped from 7.90 to 6.99 within 2 h, and there was minimal compensation over the following 96 h (Brauner et al. 2004). At 6, 24 and 72 h of exposure to hypercarbia (14 and 32 mmHg) there was no significant reduction in pHi of heart, liver, or white muscle, despite a largely uncompensated respiratory acidosis, again due to net intracellular uptake of HCO_3^- from the plasma (Fig. 5b). Thus, *L. pardalis* also has a tremendous ability to regulate intracellular pH in the presence of a large, almost completely uncompensated extracellular acidosis. In *L. pardalis* exposed to anoxia for 2 h, or following exhaustive exercise, a severe metabolic acidosis was observed in the blood (reduction in pHe of 0.4 and 0.7 pH units, respectively) but there was no significant effect on pHi of brain, heart or liver, indicating that intracellular pH is tightly regulated regardless of the source of the acidosis (Brauner, Baker, Hanson, Kuchel, Jackson and Val, unpublished).

It is not known whether preferential pHi regulation is a common characteristic of all facultative air-breathing fishes, as these are the only facultative air-breathing fishes in which it has been investigated to date. Clearly, more studies are needed to determine the ubiquity of preferential pHi regulation of this magnitude. Ultsch (1996) first pointed out that freshwater hypercarbia has been overlooked as a parameter influencing the transition of life from water to land. Adjustment of net plasma HCO_3^-/Cl^- exchange appears to be effective only in compensating for a respiratory acidosis at a PCO_2 below 10–16 mmHg, far lower than naturally occurring levels in tropical freshwaters, which, as mentioned earlier, can reach up to 60 mmHg (Heisler et al. 1982; Ultsch 1996). Preferential pHi regulation may have evolved as an adaptation to these high CO_2 tensions; and if it evolved in freshwater pleisiomorphic actinopterygiians, at a time when average global water temperatures, eutrophication and water CO_2 tensions were considerably higher than today (Clack 2007), preferential pHi regulation may have been an exaptation to air-breathing by providing a mechanism for dealing with the air-breathing-induced respiratory acidosis. Hypoxia would still represent the driving force for air-breathing in fishes (Graham 1997), but preferential pHi regulation would minimize the acid–base disturbances at the cellular level associated

with air-breathing. This intriguing and speculative hypothesis remains in need of experimental and conceptual support through further study.

7 Conclusions and Speculations

Acid–base regulation is one of the most tightly regulated physiological processes among vertebrates (Boron and De Weer 1976; Heisler 1986), and the specific mechanisms and patterns of acid–base regulation in fish (predominantly teleosts and a few elasmobranchs) have been investigated for decades (Heisler 1984, 1993, 1999; Claiborne et al. 2002; Evans et al. 2005; Perry and Gilmour 2006). The most generally described response in fish during short-term exposure to hypercarbia is to compensate for the induced respiratory acidosis by a net increase in plasma HCO_3^- in exchange for Cl^-, mediated through processes at the gills (Perry and Gilmour 2006). Most fishes investigated to date cannot completely compensate for the blood acidosis induced by CO_2 tensions greater than 10–16 mmHg, due to some apparent limit for net HCO_3^-/Cl^- exchange. Exposure to CO_2 tensions greater than this results in an uncompensated respiratory acidosis in the plasma, which in CO_2 sensitive fishes may be the basis for mortality. However, several fish species are capable of tolerating CO_2 tensions well beyond this limit, and studies on pleisiomorphic and air-breathing fishes are providing interesting insight into mechanisms associated with CO_2 tolerance.

The Pacific hagfish can tolerate direct transfer to a PCO_2 of greater than 50 mmHg, during which time blood HCO_3^- levels can reach almost 100 mM within 96 h, with an equivalent decrease in plasma Cl^-. Whether this exceptional ability to accumulate HCO_3^- in the plasma is associated with high Cl^- levels, the result of being a marine osmo- and ionoconformer, is unknown, but remains an interesting possibility. The hagfish is the most pleisiomorphic extant craniate; if its physiology is assumed to represent the ancestral state, then compensation for an acid–base disturbance through net HCO_3^-/Cl^- exchange at the gills is probably the ancestral condition in vertebrates. If the ancestral vertebrate which evolved in seawater (Bray 1985; Halstead 1985) was an osmoconformer, this may have conferred a tremendous capacity for acid–base regulation by virtue of high plasma Cl^- and potential for net HCO_3^-/Cl^- exchange. An addendum to this possibility is that the reduction in plasma ion levels in all other fish, including elasmobranchs to some degree, may have severely limited acid–base regulation when relying on net plasma HCO_3^-/Cl^- exchange at the gills.

In the more derived CO_2 -tolerant fishes studied to date, exposure to, and tolerance of, high CO_2 tensions appear to be associated with a combination of net HCO_3^-/Cl^- exchange (in sturgeon and *A. calva*) and tight pHi regulation, despite a large reduction in pHe. Sturgeon exposed to lower CO_2 tensions (10 mmHg) exhibit the typical response to hypercarbia, whereby they compensate for a respiratory acidosis with similar physiological and anatomical changes to those seen in teleosts. However, at CO_2 tensions of approximately 23 and 45 mmHg, which, as

mentioned above, precludes complete pH recovery by net HCO_3^-/Cl^- exchange at the gills (Fig. 3), this strategy is abandoned (i.e. there is virtually no pHe compensation), but there is complete regulation of pHi of heart, liver, brain and muscle. The exceptional CO_2 tolerance of sturgeon may be associated with the ability to regulate pHi tightly and preferentially despite a large reduction in pHe. To date, the only other fishes which have been demonstrated to regulate pHi preferentially despite a large and predominantly uncompensated respiratory acidosis are, possibly, *A. calva* (another pleisiomorphic actinopterygiian) and two teleost facultative air-breathers, *S. marmoratus* and *L. pardalis*. The other extant non-teleost actinopterygiian groups (reedfish and bichirs, paddlefish and osteoglossomorphs) are in general extremely hardy, largely facultative air-breathers (Brauner and Berenbrink 2007; Ilves and Randall 2007), and are promising candidates for preferential pHi regulation, however, this remains to be determined.

We hypothesize that preferential pHi regulation in vertebrates evolved in the pleisiomorphic (non-teleost) actinopterygiians, as it does not exist in hagfish or elasmobranchs (but remains to be investigated in lampreys), may be associated with high CO_2 tolerance, and may have been an exaptation to air-breathing. Clearly, a great deal of work remains to test these hypotheses; however, if validated, these ideas may support Ultsch's (1996) proposal that freshwater hypercarbia has been overlooked as a parameter influencing the transition of life from water to land. Finally, almost nothing is known about the cellular/molecular basis for preferential pHi regulation, and consequently, it remains an exciting area for further research.

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References

Baker DW, Matey V, Huynh K, Wilson J, Morgan, JD, Brauner CJ (in press) Complete intracellular pH protection during extracellular pH depression is associated with hypercarbia tolerance in white sturgeon, *Acipenser transmontanus*. American Journal of Physiology

Bernier NJ, Randall DJ (1998) Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment. Journal of Fish Biology 52:621–637

Boron WF, De Weer P (1976) Intracellular pH transients in squid giant axons caused by CO_2, NH_3, and metabolic inhibitors. The Journal of General Physiology 67:91–112

Boutilier RG, Heming TA, Iwama GK (1984) Appendix: physicochemical parameters for use in fish respiratory physiology. In: Hoar WS, Randall DJ (eds) Fish Physiology, Vol 10A. Academic, New York, pp 403–430

Brauner CJ, Berenbrink M (2007) Gas Exchange. In: McKenzie DJ, Farrell AP, Brauner CJ (eds) Primitive Fishes, Fish Physiology, Vol. 26. Elsevier, New York, pp 213–282

Brauner CJ, Wang T, Wang Y, Richards JG, Gonzalez RJ, Bernier NJ, Xi W, Patrick M, Val AL (2004) Limited extracellular but complete intracellular acid-base regulation during short-term environmental hypercapnia in the armoured catfish, *Liposarcus pardalis*. Journal of Experimental Biology 207:3381–3390

Bray AA (1985) The evolution of the terrestrial vertebrates: environmental and physiological considerations. Philosophical Transactions of the Royal Society of London B 309:289–322

Burggren WW, Roberts JL (1991) Respiration and metabolism. In: Prosser CL (ed) Environmental and Metabolic Animal Physiology. Wiley–Liss, New York, pp 354–436

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365

Cech JJ, Doroshov SI (2004) Environmental requirements, preferences, and tolerance limits of North American sturgeons. In: LeBreton GTO, Beamish FWH, McKinley RS (eds) Sturgeons and paddlefish of North America. Kluwer, Dordrecht, pp 73–86

Clack JA (2007) Devonian climate change, breathing, and the origin of the tetrapod stem group. Integrative and Comparative Biology 47(4):510–523

Claiborne JB, Evans DH (1992) Acid-base balance and ion transfers in the Spiny Dogfish (*Squalus acanthias*) during hypercapnia: a role for ammonia excretion. The Journal of Experimental Zoology 261:9–17

Claiborne J, Heisler N (1984) Acid-base regulation and ion transfers in the carp (*Cyprinus Carpio*) during and after exposure to environmental hypercapnia. Journal of Experimental Biology 108:25–43

Claiborne J, Edwards S, Morrison-Shetlar A (2002) Acid-base regulation in fishes: cellular and molecular mechanisms. Journal of Experimental Zoology 293:302–319

Colt J, Orwics K (1991) Modeling production capacity of aquatic culture systems under freshwater conditions. Aquacultural Engineering 10:1–29

Crocker CE, Cech JJ (1998) Effects of hypercapnia on blood-gas and acid-base status in the white sturgeon, *Acipenser transmontanus*. Journal of Comparative Physiology – Part B: Biochemical, Systemic, and Environmental Physiology 168:50–60

Dejours P (1981) Principles of comparative respiratory physiology, 2nd edn. Elsevier/North-Holland Biomedical, New York

Dejours P (1988) Respiration in water and air: adaptations, regulation, evolution. Elsevier, Amsterdam

Edwards SL, Wall BP, Morrison-Shetlar A, Sligh S, Weakley JC, Claiborne JB (2005) The effect of environmental hypercapnia and salinity on the expression of NHE-like isoforms in the gills of a euryhaline fish (*Fundulus heteroclitus*). Journal of Experimental Zoology – Part A: Comparative Experimental Biology 303A:464–475

Evans DH (1984) Gill Na^+/H^+ and Cl^-/HCO_3^- exchange systems evolved before the vertebrates entered fresh water. Journal of Experimental Biology 113:465–469

Evans DH, Piermarini PM, Choe KP (2005) The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiological Reviews 85:97–177

Gilmour K (2001) The CO_2/pH ventilatory drive in fish. Comparative Biochemistry and Physiology 130:219–240

Goss GG, Perry SF (1994) Different mechanisms of acid-base regulation in rainbow trout (*Oncorhynchus mykiss*) and American eel (*Anguilla rostrata*) during $NaHCO_3$ infusion. Physiological Zoology 67:381–406

Goss GG, Perry SF, Wood CM, Laurent P (1992) Mechanisms of ion and acid-base regulation at the gills of freshwater fish. Journal of Experimental Zoology 263:143–159

Goss GG, Wood CM, Laurent P, Perry SF (1994) Morphological responses of the rainbow trout (*Oncorhynchus mykiss*) gill to hyperoxia, base ($NaHCO_3$) and acid (HCl) infusions. Fish Physiology and Biochemistry 12:465–477

Goss G, Perry S, Laurent P (1995) Ultrastructural and morphometric studies on ion and acid-base transport processes in freshwater fish. In: Wood CM, Shuttleworth TJ (eds) Fish physiology: cellular and molecular approaches to fish ionic regulation. Academic, New York, pp 257–284

Goss GG, Perry SF, Fryer JN, Laurent P (1998) Gill morphology and acid-base regulation in freshwater fishes. Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology 119:107–115

Graham JB (1997) Air-breathing fishes: evolution, diversity and adaptation. Academic, San Diego
Graham MS, Turner JD, Wood CM (1990) Control of ventilation in the hypercapnic skate Raja ocellata: I. Blood and extradural fluid. Respiration Physiology 80:259–277
Halstead LB (1985) The vertebrate invasion of fresh water. Philosophical Transactions of the Royal Society of London B 309:243–258
Hayashi M, Kita J, Ishimatsu A (2004) Acid-base responses to lethal aquatic hypercapnia in three marine fishes. Marine Biology 144:153–160
Heisler N (1982) Intracellular and extracellular acid-base regulation in the tropical fresh-water teleost fish Synbranchus marmoratus in response to the transition from water breathing to air breathing. Journal of Experimental Biology 99:9–28
Heisler N (1984) Acid-base regulation in fishes. In: Hoar WS, Randall DJ (eds) Fish physiology, Vol. 10. Academic, San Diego, pp 315–401
Heisler N (1986) Comparative aspects of acid-base regulation. In: Heisler N (ed) Acid-base regulation in animals. Elsevier, Amsterdam, pp 395–450
Heisler N (1993) Acid-base regulation. In: Evans DH (ed) The physiology of fishes. CRC, Boca Raton, pp 343–378
Heisler N (1999) Limiting factors for acid-base regulation in fish: branchial transfer capacity versus diffusive loss of acid-base relevant ions. In: Egginton S, Taylor EW, Raven JA (eds) Regulation of acid-base status in animals and plants. Cambridge University Press, Cambridge, pp 125–154
Heisler N, Forcht G, Ultsch GR, Anderson JF (1982) Acid-base regulation in response to environmental hypercapnia in two aquatic salamanders, Siren lacertina and Amphiuma means. Respiration Physiology 49:141–158
Heisler N, Toews DP, Holeton GF (1988) Regulation of ventilation and acid-base status in the elasmobranch *Scyliorhinus stellaris* during hyperoxia-induced hypercapnia. Respiration Physiology 71:227–246
Holland NH, Chen J (2001) Origin and early evolution of the vertebrates: new insights from advances in molecular biology, anatomy, and palaeontology. BioEssays 23:142–151
Hyde DA, Perry SF (1989) Differential approaches to blood acid-base regulation during exposure to prolonged hypercapnia in two freshwater teleosts: the rainbow trout *Salmo gairdneri* and the American eel *Anguilla rostrata*. Physiological Zoology 62:1164–1186
Ilves KL, Randall DJ (2007) Why have primitive fish survived? In: McKenzie DJ, Farrell AP, Brauner CJ (eds) Primitive Fishes, Fish Physiology, Vol. 26. Academic, San Diego, pp 213–282
Ishimatsu A, Kikkawa T, Hayashi M, Lee KS, Kita J (2004) Effects of CO_2 on marine fish: larvae and adults. Journal of Oceanography 60:731–741
Ishimatsu A, Hayashi M, Lee K, Kikkawa T, Kita J (2005) Physiological effects on fishes in a high-CO_2 world. Journal of Geophysical Research-Oceans 110:C9
Iwama GK, Heisler N (1991) Effect of environmental water salinity on acid-base regulation during environmental hypercapnia in the rainbow trout (*Oncorhynchus mykiss*). Journal of Experimental Biology 158:1–18
Janvier P (2007) Living primitive fishes and fishes from deep time. In: McKenzie D, Farrell A, Brauner C (eds) Primitive Fishes, Fish Physiology, Vol. 26. Academic, San Diego, pp 1–51
Jensen FB, Weber RE (1985) Kinetics of the acclimational responses of tench to combined hypoxia and hypercapnia. Journal of Comparative Physiology 156:197–203
Kikkawa T, Kita J, Ishimatsu A (2004) Comparison of the lethal effect of CO_2 and acidification on red sea bream (*Pagrus major*) during the early developmental stages. Marine Pollution Bulletin 48:108–110
Langenbuch M, Portner HO (2003) Energy budget of hepatocytes from Antarctic fish (*Pachycara brachycephalum* and *Lepidonotothen kempi*) as a function of ambient CO_2: pH-dependent limitations of cellular protein biosynthesis? The Journal of Experimental Biology 206:3895–3903
Larsen BK, Jensen FB (1997) Influence of ionic composition on acid-base regulation in rainbow trout (Oncorhynchus mykiss) exposed to environmental hypercapnia. Fish Physiology and Biochemistry 16:157–170

Larsen BK, Portner HO, Jensen FB (1997) Extra- and intracellular acid-base balance and ionic regulation in cod (*Gadus morhua*) during combined and isolated exposures to hypercapnia and copper. Marine Biology 128:337–346

Lin H, Randall DJ (1990) The effect of varying water pH on the acidification of expired water in rainbow trout. Journal of Experimental Biology 149:149–160

Lloyd R, White WR (1967) Effect of high concentration of carbon dioxide on the ionic composition of rainbow trout blood. Nature 216:1341–1342

Mallat J, Paulsen C (1986) Gill ultrastructure of the Pacific hagfish *Epatretus stouti*. American Journal of Anatomy 177:243–269

Mallatt J, Conley DM, Ridgway RL (1987) Why do hagfish have gill chloride cells when they need not regulate plasma sodium chloride concentration? Canadian Journal of Zoology 65: 1956–1965

McDonald DG, Cavdek V, Calvert L, Milligan CL (1991) Acid-base regulation in the Atlantic hagfish *Myxine glutinosa*. Journal of Experimental Biology 161:201–215

McKenzie DJ, Taylor EW, Dalla Valle A, Steffensen JF (2002) Tolerance of acute hypercapnic acidosis by the European eel (*Anguilla anguilla*). Journal of Comparative Physiology 172B:339–346

Morris R (1965) Studies on salt and water balance in *Myxine glutionosa* (L.). Journal of Experimental Biology 42:359–371

Perry SF (1997) The chloride cell: structure and function in the gills of freshwater fishes. Annual Review of Physiology 59:325–347

Perry SF, Gilmour KM (2006) Acid-base balance and CO_2 excretion in fish: unanswered questions and emerging models. Respiratory Physiology and Neurobiology 154:199–215

Perry SF, Shahsavarani A, Georgalis T, Bayaa M, Furimsky M, Thomas SLY (2003) Channels, pumps, and exchangers in the gill and kidney of freshwater fishes: their role in ionic and acid-base regulation. Journal of Experimental Zoology Part A: Comparative Experimental Biology 300A:53–62

Portner HO, Langenbuch M, Reipschlager A (2004) Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. Journal of Oceanography 60:705–718

Putnam R, Roos A (1997) Intracellular pH. In: Hoffman J, Jamieson, J (eds) Handbook of physiology, vol 14, Cell physiology. Oxford University Press, Oxford, pp 389–440

Sardella BA, Baker DW, Brauner CJ (2009) The effects of variable water salinity and ionic composition on the plasma status of the Pacific Hagfish (Eptatretus stoutii). Journal of Comparative Physiology (In Press)

Seibel BA, Walsh PJ (2001) Potential impacts of CO_2 injection on deep-sea biota. Science 294:319–320

Sowerbutts BJ, Forster JRM (1981) Gas exchange and reoxygenation. In: Tiews K (ed) Aquaculture in heated effluents and recirculation systems, vol 1. Heenemann GmbH, Berlin, pp 199–217

Strahan R (1963) The behaviour of myxinoids. Acta Zoologica 44:73–102

Toews DP, Holeton GF, Heisler N (1983) Regulation of the acid-base status during environmental hypercapnia in the marine teleost fish *Conger conger*. Journal of Experimental Biology 107: 9–20

Tresguerres M, Parks SK, Goss GG (2006) V-HH^+-ATPase, Na^+/K^+-ATPase and NHE2 immunoreactivity in the gill epithelium of the Pacific hagfish (*Epatretus stoutii*). Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology 145:312–321

Truchot JP (1987) Comparative aspects of extracellular acid-base balance. Springer, Berlin

Ultsch GR (1996) Gas exchange, hypercarbia and acid-base balance, paleoecology, and the evolutionary transition from water-breathing to air-breathing among vertebrates. Palaeogeography, Palaeoclimatology, Palaeoecology 12:1–27

Walsh PJ, Mommsen TP, Moon TW, Perry SF (1988) Effects of acid-base variables on in vitro hepatic metabolism in rainbow trout. Journal of Experimental Biology 135:231–241

Wheatley MGH, Hobe H, Wood CM (1984) The mechanisms of acid-base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. II. The role of the kidney. Respiration Physiology 55(2):155–173

Wood CM, LeMoigne J (1991) Intracellular acid-base responses to environmental hyperoxia and normoxic recovery in rainbow trout. Respiration Physiology 86:91–113

Wood CM, Turner JD, Munger RS, Graham MS (1990) Control of ventilation in the hypercapnic skate *Raja ocellata*: II. Cerebrospinal fluid and intracellular pH in the brain and other tissues. Respiration Physiology 80:279–298