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COMMENTARY

# Preferential intracellular pH regulation: hypotheses and perspectives

**Ryan B. Shartau[1,*], Daniel W. Baker[2], Dane A. Crossley, II[3] and Colin J. Brauner[1,*]**

## ABSTRACT

The regulation of vertebrate acid–base balance during acute episodes of elevated internal $P_{CO_2}$ is typically characterized by extracellular pH ($pH_e$) regulation. Changes in $pH_e$ are associated with qualitatively similar changes in intracellular tissue pH ($pH_i$) as the two are typically coupled, referred to as 'coupled pH regulation'. However, not all vertebrates rely on coupled pH regulation; instead, some preferentially regulate $pH_i$ against severe and maintained reductions in $pH_e$. Preferential $pH_i$ regulation has been identified in several adult fish species and an aquatic amphibian, but never in adult amniotes. Recently, common snapping turtles were observed to preferentially regulate $pH_i$ during development; the pattern of acid–base regulation in these species shifts from preferential $pH_i$ regulation in embryos to coupled pH regulation in adults. In this Commentary, we discuss the hypothesis that preferential $pH_i$ regulation may be a general strategy employed by vertebrate embryos in order to maintain acid–base homeostasis during severe acute acid–base disturbances. In adult vertebrates, the retention or loss of preferential $pH_i$ regulation may depend on selection pressures associated with the environment inhabited and/or the severity of acid–base regulatory challenges to which they are exposed. We also consider the idea that the retention of preferential $pH_i$ regulation into adulthood may have been a key event in vertebrate evolution, with implications for the invasion of freshwater habitats, the evolution of air breathing and the transition of vertebrates from water to land.

**KEY WORDS: Acid–base regulation, Preferential $pH_i$ regulation, Fish, Amniotes, Hypercarbia, Development, Physiology**

## Introduction

Acid–base regulation is an essential physiological process that demands tight control. It is well known that absolute physiological pH values differ between species and between body compartments within species, and are affected by temperature (Rahn, 1974); however, within a given system, pH values are regulated within a relatively narrow range (Cameron, 1989; Heisler, 1984). Deviations from normal physiological pH values can affect molecular charge, altering the structure and function of proteins, lipids, carbohydrates and nucleic acids, and, ultimately, reducing whole-animal performance (Occhipinti and Boron, 2015; Putnam and Roos, 1997). The degree to which a pH change affects function depends on the system in question.

[1]Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z4. [2]Department of Fisheries and Aquaculture, Vancouver Island University, Nanaimo, British Columbia, Canada, V9R 5S5. [3]Department of Biological Sciences, University of North Texas, Denton, TX 76203-5017, USA.

*Authors for correspondence (shartau@zoology.ubc.ca; brauner@zoology.ubc.ca)

C.J.B., 0000-0002-3695-7707

Acid–base disturbances may arise from respiratory or metabolic sources (see Glossary). Respiratory acidoses occur as a result of an increase in blood $CO_2$, either from the environment (hypercarbia; see Glossary) or by retention of metabolically produced $CO_2$ (hypercapnia; see Glossary); typical arterial values of partial pressure of CO ($P_{CO_2}$) for adult water-breathing and bimodally breathing fishes, and reptiles and mammals are 0.1–0.5 (Ultsch, 1996), 0.5–3.5 (Shartau and Brauner, 2014), 1.8–4.3 (Ultsch, 1996) and 4.5–5.6 kPa $P_{CO_2}$ (Arieff et al., 1976; Malan et al., 1985; Wood and Schaefer, 1978; Yaksh and Anderson, 1987), respectively. Any increase in $P_{CO_2}$ beyond those values shifts the equilibrium of the $CO_2$ hydration reaction ($CO_2+H_2O \leftrightarrow H^+ + HCO_3^-$), promoting the formation of $H^+$ and $HCO_3^-$ and lowering pH, resulting in acidosis. Metabolic acidoses occur because of the production of metabolically generated acid, for example, following exercise or hypoxia, as a result of anaerobic metabolism, which lowers $HCO_3^-$ at a relatively constant $P_{CO_2}$ (Occhipinti and Boron, 2015).

Typically, organismal acid–base regulation in vertebrates is characterized by regulation of blood extracellular pH ($pH_e$). During an acute respiratory acidosis, reductions in $pH_e$ are associated with qualitatively similar reductions in intracellular tissue pH ($pH_i$). Following the onset of a respiratory acidosis, the recovery of $pH_i$ is often more rapid than that of $pH_e$, but complete correction of $pH_i$ generally requires $pH_e$ compensation (see Glossary) of >50% (Baker et al., 2015; Larsen et al., 1997; Wood and LeMoigne, 1991; Wood et al., 1990; Wood and Schaefer, 1978), which we refer to here as 'coupled pH regulation' (Fig. 1A,C). Coupled regulation is thought to represent the normal response to an acute persistent acidosis both *in vivo* and *in vitro*. Some vertebrates, however, do not exhibit coupled pH regulation during a severe acute acidosis, but instead preferentially and rapidly regulate $pH_i$, despite maintained reductions in $pH_e$ that can exceed 1 pH unit (Fig. 1B,D) (Baker et al., 2009; Brauner and Baker, 2009; Shartau and Brauner, 2014). Although all cells have the capacity for some degree of $pH_i$ regulation (Occhipinti and Boron, 2015; Putnam and Roos, 1997; Vaughan-Jones et al., 2009), in animals that employ coupled pH regulation, cells cannot fully compensate for changes in $pH_i$ during a large sustained reduction in $pH_e$.

Although vertebrates are the focus of this Commentary, it is worth noting that limited studies on invertebrates during acute severe hypercarbia suggest that invertebrate acid–base regulation may be characterized by coupled pH regulation, similar to vertebrates. In the few studies where both $pH_e$ and $pH_i$ have been measured during acute severe hypercarbia, reductions in $pH_e$ and $pH_i$ in a land snail (*Otala lactea*) (Barnhart and McMahon, 1988), deep sea bivalve (*Acesta excavata*) (Hammer et al., 2011), cuttlefish (*Sepia officinalis*) (Gutowska et al., 2010) and peanut worm (*Sipunculus nudus*) (Pörtner et al., 1998) have been observed. Unlike vertebrates, there is no evidence of preferential $pH_i$ regulation in invertebrates to date; however, given the dearth of

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**List of symbols and abbreviations**

| | |
|---|---|
| $pH_e$ | extracellular pH |
| $pH_i$ | intracellular tissue pH |
| $P_{CO_2}$ | partial pressure of $CO_2$ |
| $P_{O_2}$ | partial pressure of $O_2$ |
| RBC | red blood cell |

data, it is premature to ascribe a single strategy of acid–base regulation to this large and diverse group of animals.

Preferential $pH_i$ regulation is a phenomenon that has been identified in only a few vertebrate species to date, and it represents a notably different pattern of acid–base regulation, involving complete correction of $pH_i$ in the absence of $pH_e$ recovery. Based on our recent findings (Shartau et al., 2016), we hypothesize that preferential $pH_i$ regulation may represent the basal and embryonic pattern of acid–base regulation in vertebrates, providing extreme tolerance to severe acid–base challenges. It is possible that this exceptional pattern of acid–base regulation represents an exaptation (see Glossary) associated with both the evolution of air breathing and the transition of life from water to land – events that are likely to have posed challenges to acid–base homeostasis.

This Commentary will introduce readers to preferential $pH_i$ regulation as a pattern of acid–base regulation used during acute acid–base challenges, and discuss how it differs from the more commonly accepted vertebrate pattern of coupled pH regulation. We will discuss the differences between these two means of controlling

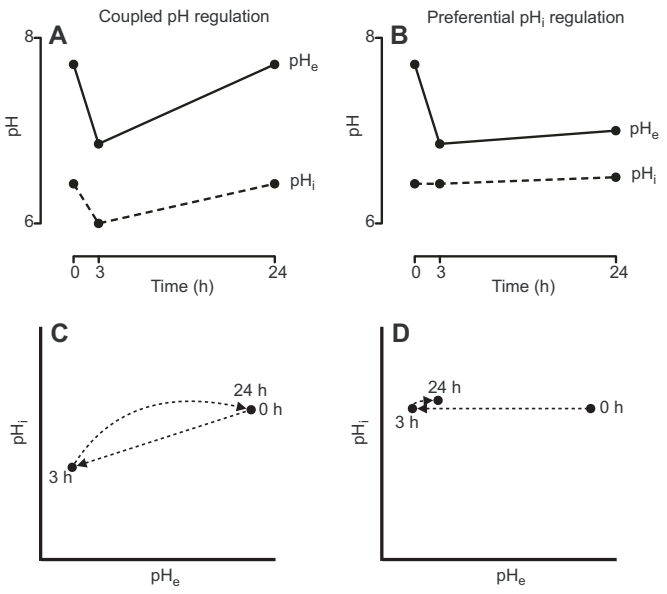


**Fig. 1. Representation of the typical response of vertebrates during acute sustained hypercapnia utilizing either coupled pH regulation or preferential intracellular pH ($pH_i$) regulation.** (A,C) In species utilizing coupled pH regulation, a hypercarbia-induced respiratory acidosis (initiated at *t*=0) leads to a rapid reduction in both extracellular pH ($pH_e$) and $pH_i$, with maximal pH depression occurring typically by 3 h or less. Recovery of pH then occurs by 24 h, but the rate of recovery depends on the severity of the pH depression and the ability for net acid excretion to the environment. $pH_i$ often recovers more rapidly than $pH_e$, but complete $pH_i$ compensation generally requires >50% of complete $pH_e$ recovery. (B,D) In species utilizing preferential $pH_i$ regulation, a hypercarbia-induced respiratory acidosis leads to a rapid reduction in $pH_e$ but no change (or even a slight increase) in $pH_i$. During hypercarbia, $pH_i$ remains independent of the sustained, uncompensated reduction of $pH_e$ for periods >24 h.

**Glossary**

**Exaptation**
An adaptation that has been co-opted for another, unrelated use.
**Hypercapnia**
Elevated internal $CO_2$.
**Hypercarbia**
Elevated environmental $CO_2$.
**Hypochloremia**
Reduced level of chloride ions in the blood.
**Metabolic acidosis**
Reduced pH because of a reduction in $HCO_3^-$ at a constant $P_{CO_2}$.
**pH compensation**
A return of pH to its normal value following an acid–base disturbance.
**Respiratory acidosis**
Reduced pH because of increased blood $CO_2$ from an environmental or internal source.

acid–base balance. Next, we will show that preferential $pH_i$ regulation is more prevalent than previously thought by discussing the increasing number of species that display this trait, considering both embryonic and adult stages. Following that, we will discuss our hypothesis that preferential $pH_i$ regulation is prevalent in embryonic vertebrates but is lost during the course of development in most species. Finally, ideas concerning the selective pressure for preferential $pH_i$ regulation and its potential role in vertebrate evolution will be examined.

## Coupled pH regulation

Findings from *in vivo* studies conducted on a relatively small selection of juvenile and adult mammals, reptiles, amphibians and fishes have established that vertebrates utilize coupled pH regulation during compensation for respiratory acid–base disturbances (Brauner and Baker, 2009; Cameron, 1989; Occhipinti and Boron, 2015; Shartau and Brauner, 2014). Likewise, *in vitro* cell culture studies have demonstrated that most vertebrate cells are unable to completely compensate for an acute respiratory acidosis while $pH_e$ remains reduced, indicating that complete $pH_i$ recovery is contingent on significant recovery of $pH_e$. Coupled pH regulation has formed the dogma of vertebrate acid–base homeostasis for decades (Albers, 1970; Cameron, 1989; Heisler, 1984; Occhipinti and Boron, 2015; Roos and Boron, 1981). Visual representation of coupled pH regulation (Fig. 1A,C) illustrates that as $pH_e$ is reduced, $pH_i$ falls in a qualitatively similar manner. Although the recovery of $pH_i$ generally occurs more rapidly than that of $pH_e$, complete $pH_i$ compensation has typically been associated with $pH_e$ recovery of ∼50% or greater (Baker et al., 2015; Larsen et al., 1997; Wood and LeMoigne, 1991; Wood et al., 1990; Wood and Schaefer, 1978). This limit to $pH_i$ correction is in clear contrast to the recovery seen in vertebrates exhibiting preferential $pH_i$ regulation. These species can tolerate severe reductions in $pH_e$ with no change in $pH_i$ – in some cases $pH_i$ actually increases (Fig. 1B,D). The pattern of coupled pH regulation has been thoroughly described in a number of reviews (see Brauner and Baker, 2009; Cameron, 1989; Heisler, 1984; Occhipinti and Boron, 2015), so will only briefly be discussed here.

### *In vivo* studies

Marine and freshwater fishes experiencing a respiratory acidosis that is due to acute hypercarbia exposure exhibit coupled pH regulation, which has been observed in agnathans (Baker et al., 2015), elasmobranchs (Heisler et al., 1988; Wood et al., 1990) and many teleosts (e.g. Brauner and Baker, 2009; Hayashi et al., 2004; Larsen et al., 1997; Wood and LeMoigne, 1991). Coupled pH regulation is

driven primarily by acid extrusion and base uptake at the gills; although the kidneys are the primary site of acid–base regulation in air breathers, they probably play only a minor role in acid–base exchange in water breathers but are important for base retention (Brauner and Baker, 2009; Cameron, 1989). In fishes that exhibit coupled pH regulation, compensation of $pH_e$ and $pH_i$ occurs over the initial 24–72 h during sustained hypercarbia exposure (Fig. 1A,C), with $pH_i$ recovery usually preceding that of $pH_e$, partly because intracellular fluids typically display a lower pH than the extracellular blood environment, which places the pK' (the apparent dissociation constant) of the $CO_2$–$HCO_3^-$ reaction closer to $pH_i$. Thus, relatively less $HCO_3^-$ is required to compensate $pH_i$ compared with $pH_e$. This recovery is further aided by the greater buffering capacity of intracellular fluids, which moderates the initial pH disturbance (Brauner et al., 2004; Ultsch, 1996).

Coupled pH regulation during acute hypercarbia is accomplished by a net increase in plasma $HCO_3^-$ associated with an equimolar loss of $Cl^-$. The extent of this $HCO_3^-$ elevation, however, appears to be limited, in that plasma [$HCO_3^-$] rarely exceeds 27–33 mmol $l^{-1}$ during exposure to acute hypercapnia – this has been previously referred to as the 'apparent bicarbonate concentration threshold' (Heisler, 1984). This threshold is associated with a ceiling of complete $pH_e$ compensation in most fish during acute exposure to $CO_2$ tensions below 2–2.5 kPa $P_{CO_2}$ (Baker et al., 2009; Brauner and Baker, 2009). Although the basis of this threshold is unknown, recent work has supported the hypothesis that $pH_e$ compensation during acute hypercarbia may be limited by the relative decrease in plasma $Cl^-$ levels to avoid hypochloremia (see Glossary; Baker et al., 2015). The osmo- and iono-conforming Pacific hagfish (*Eptatretus stoutii*), a coupled pH regulator, has a plasma [$Cl^-$] of ~458 mmol $l^{-1}$, which is three to four times higher than that of typical teleosts, and perhaps as a result, hagfish are able to increase plasma [$HCO_3^-$] to >80 mmol $l^{-1}$, driving $pH_e$ and $pH_i$ recovery during exposure to severe hypercarbia ($P_{CO_2}$ of ~6.5 kPa) (Baker et al., 2015). In contrast to acute hypercarbia, chronic $CO_2$ exposure allows some teleosts to elevate [$HCO_3^-$] well beyond this threshold, aiding $pH_e$ recovery. Rainbow trout (*Oncorhynchus mykiss*) subjected to increasing hypercarbia over 3 days to reach 3.47 kPa $P_{CO_2}$, and maintained at this level for an additional 3 days, had a blood [$HCO_3^-$] of 66 mmol $l^{-1}$ (Dimberg, 1988). Similarly, European eels (*Anguilla anguilla*) gradually exposed to and maintained at 6 kPa $P_{CO_2}$ for 6 weeks had plasma [$HCO_3^-$] of 73 mmol $l^{-1}$ (McKenzie et al., 2003). The degree and speed of $pH_e$ compensation in aquatic coupled pH regulators during acute and chronic hypercarbia exposure is affected by the physicochemical characteristics of the surrounding water, such as the levels of acid–base relevant counter-ions (Larsen and Jensen, 1997).

In adult air-breathing tetrapods, coupled pH regulation is observed in all taxa where $pH_e$ and $pH_i$ have been measured during an acute respiratory acidosis; furthermore, there is no evidence to date for preferential $pH_i$ regulation in these adult animals. Exposure of the cane toad (*Bufo marinus*) (Snyder and Nestler, 1991; Toews and Heisler, 1982), knight anole (*Anolis equestris*) and desert iguana (*Dipsosaurus dorsalis*) (Snyder et al., 1995) to 5 kPa $P_{CO_2}$ for 1 h resulted in a respiratory acidosis with severe reductions in $pH_e$ and $pH_i$; reductions in $pH_e$ and $pH_i$ were observed in western painted turtles (*Chrysemys picta bellii*) for up to 6 h during a severe acute respiratory acidosis associated with diving (Wasser et al., 1991) (see Table 1 for further detail). Coupled pH regulation also occurs during an acute metabolic acidosis following exhaustive exercise in the saltwater crocodile (*Crocodylus porosus*) (Baldwin et al., 1995), strongly supporting speculation that coupled pH regulation is a general pattern for all pH disturbances in adult air-breathing vertebrates. Further support for this conclusion is the substantial evidence that mammals subjected to an acute respiratory acidosis display coupled pH regulation. Adult dog (Arieff et al., 1976) and cat (Yaksh and Anderson, 1987) $pH_e$ and $pH_i$ were reduced following exposure to ≥8 kPa $P_{CO_2}$ for 3 h and 10 min, respectively. In guinea pigs exposed to 15 kPa $P_{CO_2}$, there was an uncompensated reduction in $pH_e$ and $pH_i$ of lung, kidney, heart and muscle between 2 and 8 h of exposure, but at 7 days, $pH_e$ and $pH_i$ exhibited compensation of 68% and 80–106%, respectively; a response indicative of coupled pH regulation (Wood and Schaefer, 1978). This pattern has also been corroborated in rats (Gonzalez and Clancy, 1986; Litt et al., 1985) and hamsters (Malan et al., 1985) during acute hypercarbia exposure (Table 1). Thus, in all adult amniotes investigated to date *in vivo* (dog, cat, rat, hamster, guinea pig, western painted turtle, knight anole and desert iguana), an acute respiratory acidosis results in reduced $pH_e$ and $pH_i$, and recovery occurs through coupled pH regulation, as has been observed for a relatively small number of fish species that have been examined to date.

### *In vitro* studies

There are many cell culture studies that have examined $pH_i$ regulation in cells following transitory reductions in $pH_e$ (e.g. Bouyer et al., 2004; Filosa et al., 2002; Furimsky et al., 1999; Goldstein et al., 2000; Huynh et al., 2011b; Liu et al., 1990; Nottingham et al., 2001; Ritucci, 2005; Salameh et al., 2014). Only a few of these have been conducted in the presence of sustained and elevated $CO_2$; however, other studies using metabolic acid–base challenges are informative about the relationship between $pH_i$ and $pH_e$. The general pattern shown in these studies is that (1) reducing $pH_e$ causes $pH_i$ to be reduced and (2) complete recovery of $pH_i$ does not occur until the starting $pH_e$ is re-established (Occhipinti and Boron, 2015; Putnam and Roos, 1997; Vaughan-Jones et al., 2009). For example, when a metabolic acidosis was induced in a variety of mouse cell types (hippocampal neurons, astrocytes, medullary raphe neurons, colon cancer cells, skeletal muscle cells, macrophages, dendritic cells, melanocytes and keratinocytes) by lowering the external fluid [$HCO_3^-$] to reduce $pH_e$, the $pH_i$ of all cell types was reduced and only fully recovered following the return of $pH_e$ to control values (Salameh et al., 2014). Generally, *in vitro* studies indicate that acute changes in external or environmental pH will rapidly affect $pH_i$. Although $pH_i$ recovers in all cells once the source of the external acidosis is removed, most vertebrate cells are unable to avoid acute $pH_i$ reduction when $pH_e$ is reduced in a cell culture environment, characteristic of coupled pH regulation. Of course, $pH_i$ responses recorded *in vitro* may not necessarily be representative of patterns of whole-animal acid–base regulation; however, in white sturgeon exposed to hypercarbia, the $pH_i$ protective response observed in liver tissue *in vivo* (Baker et al., 2009) was also observed to some degree in isolated hepatocytes exposed to hypercarbia that reduced $pH_e$ to a similar magnitude (Huynh et al., 2011a).

### Preferential $pH_i$ regulation in adult vertebrates

Adult fishes make up the majority of species where preferential $pH_i$ regulation has been identified thus far. The white sturgeon (an acipenseriform) is presently the most basal actinopterygian in which preferential $pH_i$ regulation has been observed (Fig. 2). Exposure of white sturgeon for 48 h to 6 kPa $P_{CO_2}$ reduced $pH_e$ by ~0.6 pH units, but no reductions in $pH_i$ were observed in heart, liver, brain or white muscle (Baker et al., 2009); following 6 h of exposure to 12 kPa $P_{CO_2}$, $pH_e$ fell by ~1.0 pH unit, but liver $pH_i$ increased by

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**Table 1. Examples of the changes in blood and tissue pH ($pH_e$ and $pH_i$, respectively) following a severe acute hypercapnia-induced respiratory acidosis in selected vertebrates**

| Species | Source of acidosis | $P_{CO_2}$ (kPa) | Sampling time (h) | $pH_e$ change | Tissue | $pH_i$ change | References |
|---|---|---|---|---|---|---|---|
| Rat (*Rattus norvegicus*) | Hypercarbia | 7–8 | 4 | ↓ | Heart | ↓ | Gonzalez and Clancy, 1986 |
| | | | | | Muscle | ↓ | |
| | Hypercarbia | 70 | 0.25 | ↓ | Brain | ↓ | Litt et al., 1985 |
| Hamster (*Cricetus cricetus*) | Hypercarbia | 12 | 1.5 | ↓ | Brain | ↓ | Malan et al., 1985 |
| | | | | | Liver | ↓ | |
| | | | | | Heart | ↓ | |
| | | | | | Muscle | ↓ | |
| Guinea pig (*Cavia porcellus*) | Hypercarbia | 15 | 2 and 8 | ↓ | Lung | ↓ | Wood and Schaefer, 1978 |
| | | | | | Kidney | ↓ | |
| | | | | | Heart | ↓ | |
| | | | | | Muscle | ↓ | |
| Dog (*Canis lupus familiaris*) | Hypercarbia | 8–14 | 1 | ↓ | Brain | ↓ | Arieff et al., 1976 |
| | | | | | Muscle | ↓ | |
| Cat (*Felis catus*) | Hypercarbia | 8 | 0.17 | ↓ | Brain | ↓ | Yaksh and Anderson, 1987 |
| Common snapping turtle (*Chelydra serpentina*) 70% to hatch | Hypercarbia +hypoxia (9 kPa $P_{O_2}$) | 13 | 1 | ↓ | Heart | ↔ | Shartau et al., 2016 |
| | | | | | Brain | ↑ | |
| | | | | | Liver | ↔ | |
| | | | | | Lung | ↑ | |
| | | | | | Kidney | ↔ | |
| | | | | | Muscle | ↑ | |
| 90% to hatch | Hypercarbia +hypoxia (9 kPa $P_{O_2}$) | 13 | 1 | ↓ | Heart | ↑ | Shartau et al., 2016 |
| | | | | | Brain | ↔ | |
| | | | | | Liver | ↔ | |
| | | | | | Lung | ↔ | |
| | | | | | Kidney | ↔ | |
| | | | | | Muscle | ↔ | |
| Yearling | Hypercarbia +hypoxia (9 kPa $P_{O_2}$) | 13 | 1 | ↓ | Heart | ↔ | Shartau et al., 2016 |
| | | | | | Brain | ↔ | |
| | | | | | Liver | ↔ | |
| | | | | | Lung | ↔ | |
| | | | | | Kidney | ↔ | |
| | | | | | Muscle | ↔ | |
| Western painted turtle (*Chrysemys picta bellii*) | Submergence | ~6.5 | 1 | ↓ | Heart | ↓ | Wasser et al., 1991 |
| | | | | | Liver | ↓ | |
| | | | | | Brain | ↓ | |
| | | | | | Muscle | ↓ | |
| Knight anole (*Anolis equestris*) | Hypercarbia | 5 | 1 | ↓ | Brain | ↓ | Snyder et al., 1995 |
| | | | | | Muscle | ↓ | |
| Desert iguana (*Dipsosaurus dorsalis*) | Hypercarbia | 5 | 1 | ↓ | Brain | ↓ | Snyder et al., 1995 |
| | | | | | Muscle | ↓ | |
| Cane toad (*Bufo marinus*) | Hypercarbia | 5 | 1 | ↓ | Muscle | ↓ | Snyder and Nestler, 1991; Toews and Heisler, 1982 |
| | | | | | Heart | ↓ | |
| | | | | | Brain | ↓ | |
| | | | | | Liver | ↓ | |
| | | | | | Skin | ↓ | |
| Greater siren (*Siren lacertina*) | Hypercarbia | 6.3 | 72–80 | ↓ | Heart | ↔ | Heisler et al., 1982 |
| | | | | | Muscle | ↔ | |
| American bullfrog (*Rana catesbeiana*) tadpoles | Hypercarbia | 5 | 2 | ↓ | Liver | ↓ | Busk et al., 1997 |
| | | | | | Muscle | ↓ | |
| Pacific hagfish (*Eptatretus stoutii*) | Hypercarbia | 6 | 3 | ↓ | RBC | ↓ | Baker et al., 2015 |
| | | | | | Heart | ↓ | |
| | | | | | Liver | ↓ | |
| | | | | | Muscle | ↓ | |
| Atlantic big skate (*Raja ocellata*) | Hypercarbia | 1 | 0.5 and 2 | ↓ | RBC | ↓ | Wood et al., 1990 |
| | | | | | Heart | ↓ | |
| | | | | | Brain | ↓ | |
| | | | | | Muscle | ↓ | |
| White sturgeon (*Acipenser transmontanus*) | Hypercarbia | 6 | 3 | ↓ | RBC | ↓ | Baker et al., 2009 |
| | | | | | Heart | ↑ | |
| | | | | | Liver | ↔ | |
| | | | | | Brain | ↑ | |
| | | | | | Muscle | ↔ | |
| Armoured catfish (*Pterygoplichthys pardalis*) | Hypercarbia | 4.3 | 3 | ↓ | RBC | ↓ | (Brauner et al., 2004) |
| | | | | | Heart | ↔ | |

Continued



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**Table 1. Continued**

| Species | Source of acidosis | $P_{CO_2}$ (kPa) | Sampling time (h) | $pH_e$ change | Tissue | $pH_i$ change | References |
|---|---|---|---|---|---|---|---|
| | | | | | Liver | ↔ | |
| | | | | | Muscle | ↔ | |
| Channel catfish (*Ictalurus punctatus*) | Hypercarbia | 1.5 | 2 | ↓ | Whole body | ↓ | (Cameron, 1980) |
| Marbled swamp eel (*Synbranchus marmoratus*) | Air exposure | 3.5 | 96 | ↓ | Heart | ↔ | (Heisler, 1982) |
| | | | | | Muscle | ↔ | |
| Lemon sole (*Parophrys vetulus*) | Hypercarbia | 1 | 0.5 | ↓ | RBC | ↓ | (Wright et al., 1988) |
| | | | | | Heart | ↓ | |
| | | | | | Brain | ↓ | |
| | | | | | Muscle | ↓ | |
| Cod (*Gadus morhua*) | Hypercarbia | 1 | 3 | ↓ | Heart | ↔ | (Larsen et al., 1997) |
| | | | | | Liver | ↓ | |
| | | | | | Muscle | ↓ | |
| Rainbow trout (*Oncorhynchus mykiss*) | Hyperoxia | ~1 | 3 | ↓ | RBC | ↓ | (Wood and LeMoigne, 1991) |
| | | | | | Brain | ↓ | |
| | | | | | Muscle | ↓ | |
| | | | | | Gill | ↔ | |

Studies used adult animals unless indicated otherwise. $P_{CO_2}$ tensions indicate the level of environmental $CO_2$ exposure, except in western painted turtle, marbled swamp eel and rainbow trout, where the values indicate arterial $P_{CO_2}$. RBC, red blood cell. ↓, pH decrease; ↔, no pH change; ↑, pH increase.

0.2 pH units (Baker and Brauner, 2012). Protection of $pH_i$ during exposure to hypercarbia in white sturgeon appears to be nearly instantaneous. When heart $pH_i$ was measured in real time at 2-min intervals using magnetic resonance imaging, there was no evidence for heart muscle $pH_i$ ever decreasing (D. W. Baker, Physiological responses associated with aquatic hypercarbia in the $CO_2$-tolerant white sturgeon *Acipenser transmontanus*, PhD thesis, University of British Columbia, 2010).

All other adult species in which preferential $pH_i$ regulation has been observed are bimodal breathers. The armoured catfish (*Pterygoplichthys pardalis*), a tropical freshwater teleost, preferentially regulates $pH_i$ of heart, brain, liver and white muscle during acid–base disturbances of different origins, including respiratory acidosis (hypercarbia) (Brauner et al., 2004), metabolic acidosis (exhaustive exercise and anoxia) and intravenous base addition ($HCO_3^-$ injection) (Harter et al., 2014). Similarly, another tropical freshwater teleost, the marbled swamp eel (*Synbranchus marmoratus*), is able to preferentially regulate heart and white muscle $pH_i$ during exposure to 3.5 kPa $P_{CO_2}$ for 96 h, despite $pH_e$ being reduced by ~0.6 pH units and remaining uncompensated throughout the exposure period (Heisler, 1982). Recently, preferential $pH_i$ regulation has been observed in striped catfish (*Pangasianodon hypophthalmus*; R.B.S., M. Sackville, C. Damsgaard, L. M. Phuong, M. Hvas, T. Wang, M. Bayley, D. T. T. Huong, N. T. Phuong and C.J.B., unpublished observations), spotted gar (*Lepisosteus oculatus*), longnose gar (*L. osseus*) and alligator gar (*Atractosteus spatula*) (Shartau and Brauner, 2014) during severe acute hypercarbia. There is indirect evidence for preferential $pH_i$ regulation in bowfin (*Amia calva*) (Brauner and Baker, 2009) and South American lungfish (*Lepidosiren paradoxa*) (Brauner and Baker, 2009; Sanchez et al., 2005; Shartau and Brauner, 2014), based upon the presence of severe uncompensated $pH_e$ reductions, which are highly characteristic of preferential $pH_i$ regulation; however, further studies are required to validate the pattern of acid–base regulation in these species.

To date, only one adult tetrapod is known to preferentially regulate $pH_i$ – an aquatic bimodally breathing amphibian, the greater siren (*Siren lacertina*). Exposing the greater siren to aquatic hypercarbia of 6.3 kPa $P_{CO_2}$ resulted in an uncompensated reduction in $pH_e$, but heart and white muscle $pH_i$ were unchanged from values recorded under normocapnic conditions following 32 h of exposure (Heisler et al., 1982), the earliest measure taken in that study. As it stands, the lack of evidence for preferential $pH_i$ regulation in adult amniotes strongly suggests that these animals rely on coupled pH regulation (see previous section). Intriguingly, recent studies indicate that acid–base regulation in some embryonic amniotes may differ from that of adults – this is discussed in the next section.

### Preferential $pH_i$ regulation during development

Characterizing $pH_i$ regulation in early development is challenging because of the difficulty of measuring $pH_i$ in single cells *in vivo*; consequently, evidence supporting or refuting preferential $pH_i$ regulation at early developmental stages is severely lacking. A number of studies have measured $pH_i$ in early-stage embryos, demonstrating that, early in development, cells are capable of pH regulation just after fertilization and are able to compensate for an intracellular acid–base disturbance of almost 1 pH unit (FitzHarris and Baltz, 2009; Lane, 1999). Work on fish has also showed this pattern, as early-stage zebrafish embryos exposed to 3.3 kPa $P_{CO_2}$ for 2 h *in vitro* display a respiratory acidosis, but are still able to restore $pH_i$ to pre-hypercarbic values (Molich and Heisler, 2005). These studies indicate that during an acute acidosis, cells have the capacity to compensate $pH_i$ at the earliest developmental time points. It is unknown for how long embryos retain this capacity for $pH_i$ regulation, as it may be reduced or enhanced following the appearance of the extracellular space and the growth of organs involved in regulating $pH_e$; additionally, the above findings are from *in vitro* studies and, as indicated previously, these may or may not be representative of *in vivo* responses during an acid–base disturbance.

Beyond the earliest developmental stages, acid–base regulation has been poorly studied in embryonic vertebrates; however, several studies to date have examined the response of late-stage chicken (*Gallus gallus*) embryos to respiratory and metabolic acidoses (Everaert et al., 2011). Exposing chicken embryos *in vivo* to severe acute respiratory or metabolic acidosis for up to 24 h results in large reductions in $pH_e$ that are not fully compensated, but the embryos survive (Burggren et al., 2012; Mueller et al., 2014) – a pattern that is consistent with the $pH_e$ changes observed in fish that preferentially regulate $pH_i$ (Brauner and Baker, 2009). These studies did not

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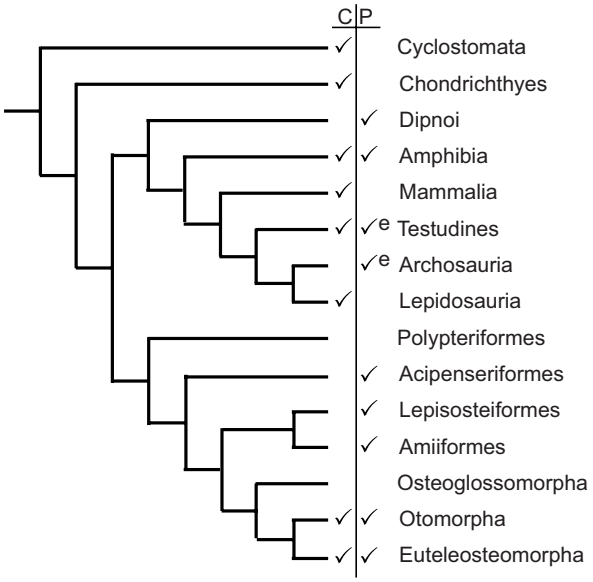


**Fig. 2. Prevalence of preferential $pH_i$ regulation amongst vertebrates exposed to an acute (<48 h) respiratory acidosis of ≥1 kPa blood $P_{CO_2}$.** Groups containing species in which either coupled (C) or preferential (P) $pH_i$ regulation has been observed in adults are indicated by ✓; embryos are indicated by ✓$^e$. Table 2 contains the information on the species in which this has been investigated, along with the relevant references.

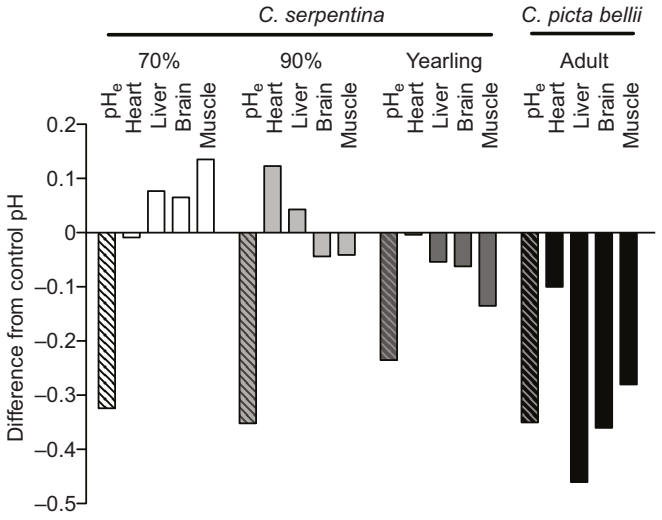


**Fig. 3. Difference in blood and tissue pH during development in turtles.** Difference in blood pH ($pH_e$) and tissue pH ($pH_i$) during development is shown following exposure to hypercarbia relative to normocarbia in common snapping turtles [*Chelydra serpentina*; at 70% (open bars) and 90% (shaded bars) to hatch, and in yearlings (dark shaded bars) (Shartau et al., 2016)] and adult western painted turtles [*Chrysemys picta bellii*, black bars (Wasser et al., 1991)]. Control pH values for $pH_e$, and $pH_i$ of heart, liver, brain and muscle were subtracted from the values determined following either 1 h exposure to 13 kPa $P_{CO_2}$, 9 kPa $P_{O_2}$ in common snapping turtles or 1 h exposure to 6.5 kPa arterial $P_{CO_2}$ in western painted turtles. A value ≥0 is indicative of preferential $pH_i$ regulation. Bars for $pH_e$ are hatched.

measure $pH_i$; however, based on these data, we hypothesize that embryonic chickens may preferentially regulate $pH_i$, making this a potential avian model that is clearly worthy of study.

The only simultaneous measurements of $pH_e$ and $pH_i$ that have been made in later-stage developing vertebrate embryos during acute acid–base disturbances were in the common snapping turtle (*Chelydra serpentina*) (Shartau et al., 2016) and American alligator (*Alligator mississippiensis*) (R.B.S., D.A.C., Z. F. Kohl, R. M. Elsey and C.J.B., unpublished observations), where preferential $pH_i$ regulation was observed for the first time in amniotes. Turtles at three developmental stages (two embryonic and one post-hatch stage) were exposed to hypercarbic hypoxia (13 kPa $P_{CO_2}$, 9 kPa $P_{O_2}$) for 1 h to create a severe acute respiratory metabolic acidosis, similar to the above studies on embryonic chickens. It was observed that the pattern of acid–base regulation changes throughout development. Younger turtle embryos appeared to exhibit a more robust capacity for preferential $pH_i$ regulation than turtles at post-hatch stages. Turtle embryos at 70% to hatch displayed a $pH_e$ reduction of ∼0.3 pH units, while $pH_i$ increased in brain, lung and white muscle; no changes in $pH_i$ were observed in heart, liver or kidney (Fig. 3). Embryos at 90% to hatch experienced a similar $pH_e$ reduction, accompanied by an increase in heart $pH_i$, with no significant reductions in $pH_i$ of the other tissues. Post-hatch yearling turtles exhibited a slightly smaller $pH_e$ reduction (∼0.25 pH units) and also displayed no significant decreases in $pH_i$ of any tissue (Fig. 3) (Shartau et al., 2016). By our definition, all three of these stages exhibit preferential $pH_i$ regulation (see Fig. 1), but interestingly, as development proceeded, there were fewer tissues in which $pH_i$ was regulated above control values, which suggests a reduced capacity for preferential $pH_i$ regulation later in development. This would potentially culminate with coupled pH regulation in adults, which is supported by data showing that $pH_i$ in adult western painted turtles closely follows $pH_e$ reductions during hypercapnia associated with diving (Fig. 3) (Wasser et al., 1991).

Similarly, when American alligator embryos at 70% to hatch were exposed to 1 h hypercarbic hypoxia (13 kPa $P_{CO_2}$, 9 kPa $P_{O_2}$), they preferentially regulated $pH_i$. A $pH_e$ reduction of ∼0.4 pH units corresponded to significant increases in heart and brain $pH_i$; no $pH_i$ changes occurred in other tissues (i.e. no acidification; R.B.S., D.A.C., Z. F. Kohl, R. M. Elsey and C.J.B., unpublished observations). These findings indicate that embryonic American alligators also preferentially regulate $pH_i$ at this stage of development and thus utilize a different acid–base regulatory strategy than adults, consistent with turtles, as described above. Although snapping turtle and American alligator embryos preferentially regulate $pH_i$ during hypercarbia hypoxia, other studies observing preferential $pH_i$ regulation in adult fishes have used hypercarbia alone. The response to hypercarbia alone in amniote embryos is unknown; consequently, the possible differences that are due to the effects of hypoxia on $pH_e$ and $pH_i$ during hypercarbia should be considered.

It is intriguing to hypothesize that vertebrate embryos in general may rely on preferential $pH_i$ regulation to protect $pH_i$ against sustained extracellular acid–base changes and that this ability is lost at some point post-hatch in amniotes and in certain amphibians and fishes, where coupled pH regulation becomes the dominant strategy for acid–base regulation. Clearly, more work needs to be conducted on a range of embryonic vertebrates during exposure to hypercarbia, as well as other acid–base disturbances, to determine whether preferential $pH_i$ regulation is a general strategy of acid–base regulation during embryonic development.

## Preferential $pH_i$ regulation: a potential developmental and evolutionary strategy to cope with acute acid–base disturbances

As mentioned above, we hypothesize that preferential $pH_i$ regulation may represent the basal pattern of acid–base regulation in vertebrates as a strategy during development, with adults either

retaining or losing this trait. The mechanisms responsible for preferential $pH_i$ regulation are unknown, but allow embryos to compensate for acid–base challenges to $pH_i$, despite the incomplete formation in embryos of the extracellular compartment and associated structures that are required for coupled pH regulation. These challenges apply to all embryos, and acid–base challenges may arise from hypoxia and hypercarbia or increased metabolic activity throughout development (Tazawa et al., 1983). In the specific examples described above, additional acid–base challenges may be experienced in the form of hypoxia or hypercarbia, conditions associated with nests or egg masses (Booth, 1998; Grigg et al., 2010; Lutz and Dunbar-Cooper, 1984). Furthermore, embryos encapsulated within extra-embryonic structures (e.g. eggshells) face additional restrictions on the diffusion of $O_2$ and $CO_2$ (Goldberg et al., 2008; Tazawa, 1980), which makes the exchange of gases and acid–base equivalents with the environment challenging and limits the ability of the embryo to undergo coupled pH regulation (Erasmus et al., 1971).

Although turtles and alligators appear to lose the capacity for preferential $pH_i$ regulation as adults, a number of adult fishes and an aquatic salamander preferentially regulate $pH_i$ (Fig. 2, Table 2), perhaps retaining this ability from embryonic development (although developmental studies are largely lacking and clearly remain worthy of investigation). This retention of preferential $pH_i$ regulation may be correlated with environmental conditions. For example, fish may lack the ability for coupled pH regulation because of the limited availability of acid–base equivalents in the water they inhabit. Tropical freshwater environments that are high in $CO_2$ and ion-poor, such as the Amazon River, where water $P_{CO_2}$ can reach 8 kPa during diurnal fluctuations (Brauner et al., 2004; Heisler, 1984), may pose challenges for acid–base regulation and compensation through coupled pH regulation. Consequently, retention of preferential $pH_i$ regulation in adults could play a crucial role in acid–base regulation in tropical freshwater fishes; indeed, a number of fish species known to preferentially regulate $pH_i$ are found in the Amazon. It is possible that the first vertebrates to retain preferential $pH_i$ regulation as adults included species making the transition from ion-rich environments (e.g. marine waters) to ion-poor environments (e.g. tropical freshwaters) – similar to current tropical systems such as the Amazon Basin – where their capacity for coupled pH regulation was diminished and thus preferential $pH_i$ regulation may have protected $pH_i$ against severe acidoses, including those induced by high $CO_2$ (Brauner and Baker, 2009; Shartau and Brauner, 2014). Vertebrates in ion-rich environments are likely to be able to use coupled pH regulation as there are sufficient acid–base equivalents for $pH_e$ compensation during severe acidoses, including those resulting from high $CO_2$; this is observed in a number of marine species exposed to severe hypercarbia, including hagfish (Baker et al., 2015) and teleosts (Hayashi et al., 2004).

In adult bimodally breathing fishes, the retention of preferential $pH_i$ regulation may provide a means of coping with acid–base challenges associated with air breathing (Shartau and Brauner, 2014). These fishes are typically capable of $O_2$ uptake from water or air, but excrete the majority of $CO_2$ to the water. Consequently, in bimodally breathing fishes, air breathing may lead to a rapid increase in blood $P_{CO_2}$, as $CO_2$ excretion rates at the gills are reduced as a result of emersion or reduced gill blood flow and/or gill ventilation. Depending on the species and conditions, blood $P_{CO_2}$ can increase to >3 kPa during an air-breathing episode (Shartau and Brauner, 2014). All fish species (with the exception of one, the white sturgeon) currently known to use preferential $pH_i$ regulation as adults are bimodal breathers. Air-breathing in fishes is thought to have evolved in more tropical environments that likely experience both hypoxia and hypercapnia; thus, these fishes may have already been subjected to selection pressures to retain preferential $pH_i$ regulation, which could then serve as an exaptation for air breathing (Brauner and Baker, 2009; Shartau and Brauner, 2014).

We hypothesize that preferential $pH_i$ regulation may have been an exaptation associated with niche expansion from water to land in the vertebrate lineage. This pattern of acid–base regulation possibly allowed these transitioning vertebrates, which likely possessed acid–base characteristics similar to those of present-day water breathers (high $pH_e$, low arterial $P_{CO_2}$ and low plasma $[HCO_3^-]$) and still relied on the gills for exchange of acid–base ion equivalents (Ultsch, 1996, 2012; Witzmann, 2015), to protect $pH_i$ during terrestrial excursions. Gradually, coupled pH regulation may have replaced preferential $pH_i$ regulation as the strategy to maintain whole-body pH as (1) these terrestrial excursions became longer, (2) the animals' blood acid–base status became more similar to that of terrestrial air breathers (higher arterial $P_{CO_2}$, higher plasma $[HCO_3^-]$ and lower $pH_e$) and (3) the kidneys replaced the gills as the dominant organ for acid–base regulation.

The retention of preferential $pH_i$ regulation beyond development may be associated with survival during severe acute acid–base disturbances; however, this pattern of acid–base regulation is diminished and ultimately lost in many adult vertebrates. Why preferential $pH_i$ regulation was not retained more broadly can only be speculated upon at this time. Although preferential $pH_i$ regulation may be metabolically less costly than coupled pH regulation (Baker and Brauner, 2012) and protects cardiac performance during acute hypercarbia exposure (Baker et al., 2011; Hanson et al., 2009; Shartau et al., 2016), there may be unquantified long-term costs associated with preferential $pH_i$ regulation. This premise remains to be investigated. Ultimately, the benefit of preferential $pH_i$ regulation over coupled pH regulation is that vertebrate embryos and adults can completely protect $pH_i$ even if $pH_e$ reductions are uncompensated, as occurs during severe acute respiratory acidoses; limited data suggest that preferential $pH_i$ regulation may function to protect $pH_i$ during other types of acid–base disturbances (Harter et al., 2014; Shartau et al., 2016).

## Conclusions and future perspectives

Preferential $pH_i$ regulation is a strategy of acid–base regulation that allows vertebrates to maintain pH homeostasis during severe acute acid–base disturbances under conditions in which compensation through coupled pH regulation would be limited. It has been observed in embryos of two amniote species, as well as in several adult vertebrates (Fig. 2). Based on recent findings in embryonic amniotes, we hypothesize that preferential $pH_i$ regulation may represent a general pattern of acid–base regulation in vertebrate embryos that is either retained or lost during development, likely depending upon acid–base challenges that these groups have experienced during adaptation to their environment. Consequently, we propose that many other vertebrates, including those that exhibit coupled pH regulation as adults, may rely exclusively on preferential $pH_i$ regulation during development; an area clearly worthy of further investigation. Finally, we hypothesize that the retention of preferential $pH_i$ regulation may have been an exaptation for a number of important evolutionary transitions in vertebrates, including the invasion of freshwater, the evolution of air breathing and the transition to land, events where acid–base homeostasis would otherwise be impaired (Brauner and Baker,

**Table 2. Pattern of vertebrate pH regulation following exposure to an acute (<48 h) respiratory acidosis of ≥1 kPa blood $P_{CO_2}$**

| Group | Species | Pattern of pH regulation | Comments | References |
|---|---|---|---|---|
| Cyclostomata | Pacific hagfish (*Eptatretus stoutii*) | Coupled pH | | Baker et al., 2015 |
| Chondrichthyes | Atlantic big skate (*Raja ocellata*) | Coupled pH | | Wood et al., 1990 |
| Dipnoi | South American lungfish (*Lepidosiren paradoxa*) | Preferential $pH_i$ | Indirect determination based on $CO_2$ tolerance and $pH_e$ measurement | Sanchez et al., 2005; Shartau and Brauner, 2014 |
| Amphibia | Cane toad (*Bufo marinus*) | Coupled pH | | Snyder and Nestler, 1991 |
| | Greater siren (*Siren lacertina*) | Preferential $pH_i$ | | Heisler et al., 1982 |
| Mammalia | Rat (*Rattus norvegicus*) | Coupled pH | | Gonzalez and Clancy, 1986; Litt et al., 1985 |
| | Hamster (*Cricetus cricetus*) | Coupled pH | | Malan et al., 1985 |
| | Guinea pig (*Cavia porcellus*) | Coupled pH | | Wood and Schaefer, 1978 |
| | Dog (*Canis lupus familiaris*) | Coupled pH | Newborn | Nattie and Edwards, 1981 |
| | Dog (*Canis lupus familiaris*) | Coupled pH | | Arieff et al., 1976 |
| | Cat (*Felis catus*) | Coupled pH | | Yaksh and Anderson, 1987 |
| Testudines | Common snapping turtle (*Chelydra serpentina*) | Preferential $pH_i$ | Embryo (70 and 90% to hatch) and yearling | Shartau et al., 2016 |
| | Western painted turtle (*Chrysemys picta bellii*) | Coupled pH | | Wasser et al., 1991 |
| Archosauria | American alligator (*Alligator mississippiensis*) | Preferential $pH_i$ | Embryo (70% to hatch) | R.B.S., D.A.C., Z. F. Kohl, R. M. Elsey and C.J.B., unpublished |
| Lepidosauria | Knight anole (*Anolis equestris*) | Coupled pH | | Snyder et al., 1995 |
| | Desert iguana (*Dipsosaurus dorsalis*) | Coupled pH | | Snyder et al., 1995 |
| Acipenseriformes | White sturgeon (*Acipenser transmontanus*) | Preferential $pH_i$ | | Baker et al., 2009 |
| Lepisosteiformes | Spotted gar (*Lepisosteus oculatus*) | Preferential $pH_i$ | | Shartau and Brauner, 2014 |
| | Longnose gar (*L. osseus*) | Preferential $pH_i$ | | Shartau and Brauner, 2014 |
| | Alligator gar (*Atractosteus spatula*) | Preferential $pH_i$ | | Shartau and Brauner, 2014 |
| Amiiformes | Bowfin (*Amia calva*) | Preferential $pH_i$ | Indirect determination based on $CO_2$ tolerance and $pH_e$ measurement | Brauner and Baker, 2009 |
| Otomorpha | Armoured catfish (*Pterygoplichthys pardalis*) | Preferential $pH_i$ | | Brauner et al., 2004 |
| | Striped catfish (*Pangasianodon hypophthalmus*) | Preferential $pH_i$ | Hypercarbia in low pH water (4.5 pH units) | R.B.S., M. Sackville, C. Damsgaard, L. M. Phuong, M. Hvas, T. Wang, M. Bayley, D. T.T. Huong, N. T. Phuong and C.J.B., unpublished |
| | Channel catfish (*Ictalurus punctatus*) | Coupled pH | | Cameron, 1980 |
| Euteleosteomorpha | Marbled swamp eel (*Synbranchus marmoratus*) | Preferential $pH_i$ | | Heisler, 1982 |
| | Lemon sole (*Parophrys vetulus*) | Coupled pH | | Wright et al., 1988 |
| | Cod (*Gadus morhua*) | Coupled pH | | Larsen et al., 1997 |
| | Trout (*Oncorhynchus mykiss*) | Coupled pH | | Wood and LeMoigne, 1991 |

Studies used adult animals unless indicated otherwise.

2009; Janis et al., 2012; Shartau and Brauner, 2014). The specific cellular and molecular mechanisms of preferential $pH_i$ regulation are currently unknown and represent a fascinating opportunity for further investigation. The role of preferential $pH_i$ regulation during development and vertebrate evolution are areas for research that remain largely unexplored, and experiments addressing these issues will likely provide valuable insight into what may represent a basal pattern of acid–base regulation in vertebrates.



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**Author contributions**
R.B.S. wrote the manuscript. All authors discussed and edited the manuscript.

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