

CO₂ AND ACID-BASE SENSING

MARTIN TRESGUERRES^{*,1}

WILLIAM K. MILSOM[†]

STEVE F. PERRY[‡]

*Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, San Diego, CA, United States

†Department of Zoology, University of British Columbia, Vancouver, BC, Canada

‡Department of Biology, University of Ottawa, Ottawa, ON, Canada

¹Corresponding author: mtresguerres@ucsd.edu

1. Introduction
 2. Need for Acid/Base Sensing
 3. Physiologically Relevant Sites of Acid-Base Sensing
 - 3.1. Peripheral CO₂ Sensing
 - 3.2. Central CO₂/H⁺ Sensing
 - 3.3. Cellular Acid-Base Sensing
 4. Molecular A/B Sensors
 - 4.1. CO₂/pH/HCO₃⁻ Sensing sAC
 - 4.2. H⁺ Sensing GPCRs
 5. Conclusions and Future Directions
- Acknowledgments
- References

Carbon dioxide (CO₂) and its hydration products hydrogen (H⁺), bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions collectively contribute to the acid-base status of aqueous solutions, and have major effects on the physiology of organisms. Correspondingly, organisms have developed the ability to sense specific acid-base disturbances that routinely arise from metabolic and environmental sources, and to coordinate a variety of homeostatic responses. A common requirement for all homeostatic mechanisms is the ability to sense specific acid-base disturbances and to coordinate appropriate responses. This chapter synthesizes our knowledge concerning the sensory pathways that allow fish to

sense acid-base disturbances of both metabolic and environmental origin and the ensuing downstream physiological responses that promote homeostasis in different organs. We focus largely on the peripheral, and to a lesser extent, the central sites of CO_2/H^+ detection, emphasizing the cellular sites and molecular mechanisms of acid-base sensing.

1. INTRODUCTION

In aqueous solutions, carbon dioxide (CO_2) establishes a reversible equilibrium with hydrogen (H^+), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. Together with non-bicarbonate buffering, the levels of these molecules define the acid-base status of the fluid. Maintaining acid-base homeostasis in physiological fluids is essential for life because the concentration of $[\text{H}^+]$ ($\sim\text{pH}$) greatly affects protein folding and function. Additionally, acid-base conditions affect (and are affected by) various physiological processes such as metabolism, pH buffering, biomineralization, neurotransmission, oxygen (O_2) delivery, and feeding and digestion. Fish acid-base status can also be affected by environmental factors, the most prominent being elevated CO_2 (hypercapnia) and the associated reduction in pH. Environmental hypercapnia can be found at night in densely populated environments such as kelp forest, coral reefs, mangroves, estuaries and tide pools (Duarte et al., 2013; Hofmann et al., 2011; Kline et al., 2012; Truchot and Duhamel-Jouve, 1980) due to organismal aerobic respiration that produces CO_2 as it depletes O_2 . Thus, environmental hypercapnia often is associated with hypoxia. Indeed, fish might also experience environmental hypercapnia and hypoxia during upwelling events (Frieder et al., 2012). Chronic environmental hypercapnia may occur in recirculating aquaculture systems (Ellis et al., 2017), and at much lower levels, as ocean acidification develops (Duarte et al., 2013; Raven et al., 2005; Sabine et al., 2004).

2. NEED FOR ACID/BASE SENSING

Because virtually every physiological process is affected by the acid-base status, organisms have developed a variety of homeostatic responses to compensate for acid-base disturbances that arise from metabolic and environmental sources. A common requirement for all homeostatic responses is the ability to sense acid-base disturbances in the first place. In a broad sense, this means sensing acidosis and alkalosis from a specific set point. In addition,

an acid-base sensing mechanism must be able to differentiate between acid-base disturbances of metabolic or respiratory origin, and if those are from environmental or internal sources. Furthermore, the acid-base set point can differ between subcellular compartments, cell types and organs, as well as between fish living in different environments, or having different metabolic capacities and breathing modes. Another consideration is that acid-base set points may change as a function of temperature according to the “alphastat hypothesis” (reviewed in [Somero, 1986](#)), and have the potential to dynamically adjust upon prolonged exposure to change A/B conditions. Clearly, acid-base sensing is a complex process involving multiple molecular sensors and feedback loops, much of which remains unexplored.

3. PHYSIOLOGICALLY RELEVANT SITES OF ACID-BASE SENSING

3.1. Peripheral CO₂ Sensing

3.1.1. CARDIORESPIRATORY REFLEXES

The most commonly reported cardiorespiratory response to elevated ambient CO₂ levels in adult fish is hyperventilation, an increase in the volume of water flowing over the gills ([Dejours, 1973](#)). Hypercapnic hyperventilation has been reported in agnathans (Pacific hagfish *Eptatretus stoutii*, [Perry et al., 2009b](#)), chondrichthyans (spotted dogfish *Scyliorhinus stellaris*, [Randall et al., 1976](#); Atlantic big skate *Raja ocellata*, [Graham et al., 1990](#); spiny dogfish *Squalus suckleyi*, [Perry and Gilmour, 1996](#)) and a variety of actinopterygians including holosteans (spotted gar *Lepisosteus oculatus*, [Smatresk and Cameron, 1982](#)), chondrosteans (white sturgeon *Acipenser transmontanus*, [Crocker et al., 2000](#)) and teleosts (e.g., rainbow trout *Oncorhynchus mykiss*, [Janssen and Randall, 1975](#); [Smith and Jones, 1982](#); Atlantic salmon *Salmo salar*, [Perry and McKendry, 2001](#); common carp *Cyprinus carpio*, [Soncini and Glass, 2000](#)) and zebrafish *Danio rerio* ([Vulesevic et al., 2006](#)). Increases in ventilation are mediated by adjustments to ventilation frequency (f_V) and/or amplitude (a determinant of respiratory stroke volume, which is analogous to tidal volume in air-breathers). Although relatively few species have been examined, hypercapnic hyperventilation typically is associated with increases in breathing amplitude (see [Gilmour and Perry, 2007](#)). However, the response patterns are highly variable with some fish (e.g., zebrafish) responding to elevated CO₂ by increasing ventilation amplitude exclusively ([Vulesevic and Perry, 2006](#)) with others (e.g., tambaqui) solely adjusting f_V ([Sundin et al., 2000](#)). From an energetics perspective, hyperventilatory responses mediated largely by increases in ventilation amplitude are thought to be more efficient ([Perry and Wood, 1989](#)). Minimizing the costs associated

with hyperventilation is particularly important in water-breathers given the high metabolic costs associated with moving water across the gills (Jones and Schwarzfeld, 1974). It was shown recently in air-breathing *Pangasius hypophthalmus* that intra-arterial injections of lactate produce dose-dependent increases in gill ventilation amplitude and frequency, and at higher doses, stimulate air breathing (Thomsen et al., 2017). These responses, however, were independent of changes in pH.

Although less well-studied, hypercapnia may also initiate cardiovascular reflexes in adult fish including a reduction in heart rate (bradycardia) and an elevation of blood pressure (reviewed by Gilmour and Perry, 2007). In rainbow trout, the bradycardia is mediated by increased parasympathetic input to the heart while the elevated blood pressure is a consequence of increased systemic vascular resistance owing to neuronal mediated sympathetic peripheral vasoconstriction linked to α -adrenergic receptor stimulation (Perry et al., 1999). Despite bradycardia, cardiac output generally remains constant or may even increase slightly in the few species that have been examined (see table 3.2 in Gilmour and Perry, 2007) owing to increasing stroke volume. The specific mechanisms underlying the rise in stroke volume during hypercapnia are unclear but may involve the stimulatory effects of circulating catecholamines (e.g., Perry et al., 1987), increased sympathetic nerve activity (Perry et al., 2009a), elevated central venous pressure (Perry et al., 2009a) and the increased filling time associated with the bradycardia (Starling's law of the heart).

Of the three principal cardiorespiratory reflexes evoked by hypercapnia (hyperventilation, bradycardia and increased systemic vascular resistance), only the hyperventilatory response appears to impart obvious physiological benefit to gas transfer across the gill. By analogy to branchial O_2 transfer (Perry et al., 2009a), increasing water flow over the gill when metabolically produced CO_2 is elevated will increase the blood-to-water diffusion gradient and cause arterial blood PCO_2 to be lower than it otherwise would be without hyperventilation (Gilmour, 2001; Perry and Wood, 1989). The underlying mechanism facilitating the lowering of arterial PCO_2 during hyperventilation is a reduction in the residence time during which inspired water is in contact with the respiratory surfaces, thereby decreasing the accumulation of CO_2 into the inspired water during gill passage and lowering the average PCO_2 in the ventilatory flow. Clearly, respiratory acidosis is unavoidable during exposure to hypercapnic water but theory predicts that hyperventilation will lessen the extent of the acidosis by removing metabolically produced CO_2 and hence lowering arterial PCO_2 . It has long been held that bradycardia, with or without an accompanying fall in cardiac output, increases branchial gas transfer efficiency (Randall and Daxboeck, 1984). With a single exception, however (Short et al., 1979), previous studies have failed to provide any empirical evidence in support of a beneficial role of bradycardia on gas transfer during

hypoxia (Iversen et al., 2010; McKenzie et al., 2009; Perry and Desforges, 2006; Taylor and Barrett, 1985) or hypercapnia (Perry and Desforges, 2006). In rainbow trout, the increase in blood pressure during hypercapnia has no obvious benefit on branchial CO₂ transfer (Perry and Desforges, 2006) despite theoretical arguments linking elevated blood pressure to increased diffusive conductance (Farrell et al., 1980). Thus, it remains to be determined whether fish derive any benefit from the cardiovascular responses associated with hypercapnia.

Unlike for hypoxia, little is known about the cardiorespiratory reflexes associated with hypercapnia in developing (larval) fish. Indeed, to our knowledge, the only species that has been studied during larval stages is zebrafish. Unlike the majority of species that have been examined as adults, the larval zebrafish (5–7 days post-fertilization) experiences tachycardia when exposed to hypercapnia (Miller et al., 2014). The hypercapnic tachycardia appears to be mediated by activation of cardiac β_1 -adrenergic receptors by catecholamines originating from sympathetic neurons (Miller et al., 2014). Although data are lacking, it is possible that the cardiac response to hypercapnia in adult zebrafish is bradycardia, similar to other species (see above). Unlike adults, which increase gill ventilation during hypercapnia exclusively by adjusting ventilation amplitude (see above), larval zebrafish hyperventilate via marked increases in breathing frequency when exposed to elevated ambient CO₂ (Koudrina, 2017; Fig. 1). Currently, it is not possible to measure ventilation amplitude in zebrafish larvae.

3.1.2. SITES OF CO₂ CHEMORECEPTION

It was originally held that the cardiorespiratory reflexes initiated by hypercapnia were mediated indirectly via hypoxemia (Randall, 1982; Smith and Jones, 1982) arising from the adverse effects of respiratory acidosis on lowering blood O₂ carrying capacity (the Root effect). However, it is now well established that increased ambient CO₂ can be sensed directly via peripheral chemoreceptors (Abdallah et al., 2012, 2015; Perry and Abdallah, 2012; Qin et al., 2010). Based largely on indirect in vivo evidence gathered from numerous adult species, these peripheral chemoreceptors appear to be activated by changes in water CO₂ tension rather than by accompanying changes in water pH (Gilmour et al., 2005; Miller et al., 2014; Perry and McKendry et al., 2001; Reid et al., 2000; Sundin et al., 2000). The CO₂ receptors are localized predominantly to the gills but occasionally may also reside on surfaces of the orobranchial cavity or other peripheral sites (Burlison and Smatresk, 2000; Florindo et al., 2004; McKendry et al., 2001; Milsom et al., 2002; Perry and Reid, 2002; Reid et al., 2000; Sundin et al., 2000). In some species (e.g., rainbow trout), the cardiorespiratory responses appear to be mediated largely by CO₂ chemoreceptors associated with the first gill arch (Perry and

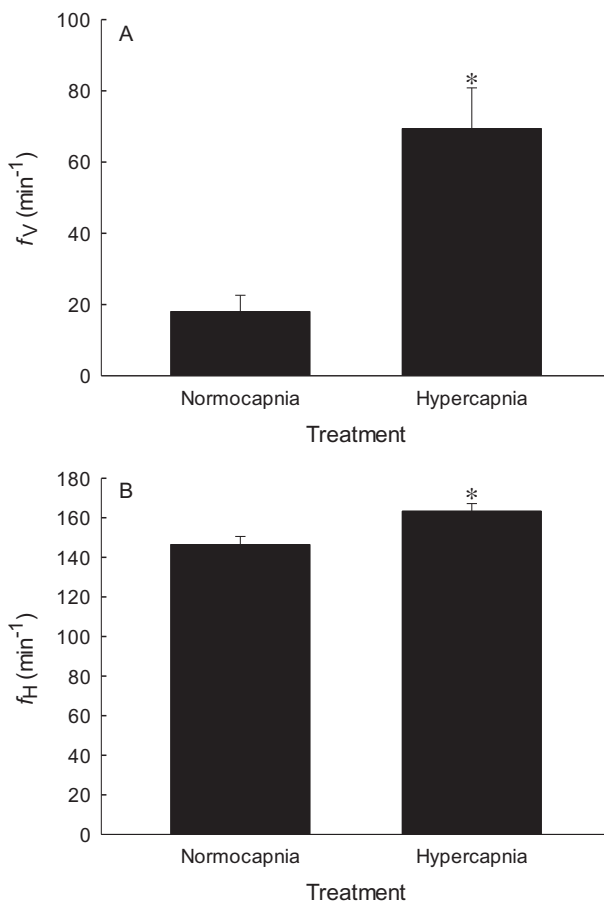


Fig. 1. The effect of acute (30min) hypercapnia (1.5% CO_2 in air; $\text{PCO}_2 = 11.25 \text{ mmHg}$) on (A) ventilation frequency (f_V) and (B) cardiac frequency (f_H) in larval zebrafish (*Danio rerio*; $N = 10$) at 4 days post-fertilization. Each larva was exposed to normoxic, normocapnic conditions for a 20 min acclimation/control period. Baseline f_V and f_H measurements were collected for the last 2 min of this normocapnic period. To evaluate the cardiorespiratory responses to hypercapnia, f_V and f_H were assessed for each 2 min interval during the 30 min exposure period, and the peak (maximum) response was identified. Significant differences from the normocapnic period are indicated by asterisks ($P < 0.05$; paired Student's t -test). Previously unpublished data from Koudrina (2017).

Reid, 2002) while in others, the receptors contributing to the reflex responses are distributed across all four gill arches (Reid et al., 2000; Sundin et al., 2000; reviewed by Milsom, 2012).

The bulk of available evidence suggests that the branchial CO_2 chemoreceptors respond preferentially to external changes in CO_2 tensions and are

largely insensitive to changes in internal (blood) CO₂ levels (Gilmour et al., 2005; reviewed by Gilmour and Perry, 2007). Thus, it has been suggested that the branchial CO₂ chemoreceptors are oriented in such a manner as to exclusively detect changes in ambient CO₂ levels (Gilmour and Perry, 2007). Despite the predominance of evidence implicating external sites of CO₂ sensing in fish, there are data, albeit equivocal, to support the existence of CO₂ receptors able to monitor internal CO₂ levels or acid-base status in teleosts (Wood and Munger, 1994) and elasmobranchs (Graham et al., 1990; Wood et al., 1990). The results of intra-arterial injections of acid, bicarbonate or CO₂-equilibrated solutions have yielded conflicting results. Most frequently such injections have been without effect (Burleson and Smatresk, 2000; Florindo et al., 2004; Gilmour and Perry, 1996; Gilmour et al., 2007; Lopes et al., 2010; McKendry et al., 2001; Reid et al., 2000; Sundin et al., 2000). There have been some studies, however, where such injections in rainbow trout did stimulate ventilation (Aota et al., 1990; Gilmour and Perry, 1996; Janssen and Randall, 1975; McKenzie et al., 1991, 1993; Reid et al., 2000). Although these data suggest the existence of internal receptors that monitor changes in arterial CO₂/H⁺ levels, the sites remain unknown and may even be centrally located (i.e., within the brain) (see Section 3.2). In rainbow trout following exhaustive exercise in normoxic water, ventilation remains elevated even though arterial PO₂ is normal. CO₂ that has accumulated during the exhaustive exercise takes longer to clear and hence arterial PCO₂ remains elevated, and pH reduced at this time (Perry and Wood, 1989; Tufts and Perry, 1998; Wood, 1991; Wood and Munger, 1994; Wood and Perry, 1985). Administering carbonic anhydrase (CA) to reduce the post-exercise acidosis and enhance CO₂ clearance reduced this hyperventilation (Wood and Munger, 1994). Similarly, when dogfish (*S. stellaris*) were exposed to hyperoxia the hypoventilatory response appeared to be fine-tuned by blood acid-base status (Heisler et al., 1988). Interestingly, in a study designed to examine the possible role of central acid-base stimuli in the increase in ventilation induced by hypercapnia in the skate, there was a better correlation between the changes in ventilation and changes in arterial pH rather than with cerebral-spinal fluid pH, the intracellular pH (pHi) of brain tissue, or arterial PCO₂ (Wood et al., 1990).

It is important to note that although several studies reported cardiorespiratory changes (bradycardia, hypertension, and hyperventilation) during experimentally evoked internal acidosis (see above), blood O₂ status was not monitored. Therefore, activation of blood-oriented O₂-chemoreceptor reflexes owing to acid-induced hypoxemia (Root effect) cannot be ruled out.

As stated by Gilmour and Perry (2007), “the role of arterial acid-base status in regulating cardiorespiratory function remains uncertain as do the location and stimulus specificity of any chemoreceptors involved in mediating such responses.”

3.1.3. NEUROEPITHELIAL CELLS

Although direct data exist only for a limited number of species (zebrafish, rainbow trout, and channel catfish *Ictalurus punctatus*), it is widely accepted that a single cell type, the neuroepithelial cell (NEC) is the predominant peripheral chemoreceptor in fish (see reviews by Jonz, 2018; Jonz and Nurse, 2008; Jonz et al., 2015, 2016; Milsom, 2012; Perry and Tzaneva, 2016; Porteus et al., 2012; Zachar and Jonz, 2012). Originally described in a variety of fish species and proposed as O₂ chemoreceptors in rainbow trout gill >35 years ago (Dunel-Erb et al., 1982), it was not until 2004 that the gill filament NECs of zebrafish were characterized definitively as O₂ sensors (Jonz et al., 2004). Shortly thereafter, an O₂ sensory function was attributed to the NEC's of channel catfish gills (Burleson et al., 2006). In rainbow trout, the NEC's detect elevated ammonia levels (Zhang et al., 2011) and their stimulation by elevation of internal ammonia is thought to contribute to hyperventilation, which aids in whole body ammonia clearance. It is likely that the ventilatory responses to lactate mentioned earlier in *Pangasius* also arise from a sub-population of NECs distributed throughout the gills. Several NEC types have been identified that respond to different stimuli or groups of stimuli, including O₂, CO₂, H⁺, NH₄⁺, CO, NO and H₂S (Perry and Tzaneva, 2016).

Thus in zebrafish, a subset of the gill filament NEC's are dual sensors of O₂ and CO₂ (Abdallah et al., 2015; Qin et al., 2010) and appear to be functionally analogous to the chemosensory cells of the mammalian carotid body, the glomus (Type I) cells, which are also known to be bimodal sensors of O₂ and CO₂. Although functionally analogous to the carotid body glomus cells, the zebrafish NECs may not be homologous to the glomus cells of mammals (Hockman et al., 2017). Instead, the NECs of zebrafish appear to arise from embryonic endoderm whereas the glomus cells are derived from embryonic ectoderm cells of the neural crest (Hockman et al., 2017). Therefore, contrary to our earlier understanding of the evolution of vertebrate peripheral chemoreceptors (Milsom and Burleson, 2007), it is possible that the fish gill NECs are not the evolutionary precursors of the glomus cells of the mammalian carotid body as thought previously. Rather, it is possible that the NEC's of fish are the evolutionary precursors of the pulmonary neuroendocrine cells of the lung airways of higher vertebrates (reviewed by Cutz and Jackson, 1999), which also are derived from endoderm during development.

The number of adult species in which NECs have been identified in the gill of water-breathers or the skin of amphibious fishes is increasing steadily (Coolidge et al., 2008; Dunel-Erb et al., 1982; Porteus et al., 2012, 2014; Regan et al., 2011; Saltys et al., 2006; Zaccone et al., 2006, 2017). Indeed, NEC's may be ubiquitous in fish although their location may vary between the gill in water-breathers and the skin or other air-breathing organs in

amphibious or air-breathing species (Zaccone et al., 2006). Moreover, the location of NECs may differ depending on species and developmental age. For example, in zebrafish larvae, the NECs initially are situated on the skin (Jonz and Nurse, 2005) but as the gills develop, the numbers of cutaneous NECs decline while the numbers in the gills increase, eventually becoming the predominant site of NEC localization at about 2 weeks post-fertilization (Coccimiglio and Jonz, 2012; Jonz and Nurse, 2003). In adult fish, the branchial NECs are found preferentially within the distal region of filaments where they lie in close proximity to the efferent filament artery (the blood vessel that exits the gill filament) and thus on the leading edge of the filament incident to the inspired water current flowing over the gills (Hughes, 1966; Hughes and Morgan, 1973). As such, the filament NECs are situated ideally to sense respiratory gases both from the external environment (inspired water) and the blood leaving the gill (Jonz and Nurse, 2003). In several species examined to date, the NECs also are found scattered on the respiratory lamellae (Bailly et al., 1992; Coolidge et al., 2008; Dunel-Erb et al., 1982; Jonz and Nurse, 2003; Tzaneva and Perry, 2010) (Table 1). Lamellar NECs are smaller and appear more spherical than filament NECs (Jonz and Nurse, 2003). On the lamellae, the NECs are usually exposed to the external environment making them suitable for sensing external changes in respiratory gases (Jonz and Nurse, 2003; Tzaneva and Perry, 2010).

3.1.4. CELLULAR MECHANISMS OF CO₂ SENSING BY NECs

In zebrafish NECs, the cellular responses to hypercapnia and hypoxia are similar, consisting of membrane depolarization (Qin et al., 2010) and an associated increase in the levels of intracellular free Ca²⁺ ([Ca²⁺]_i) (Abdallah et al., 2015). Unlike in mammalian glomus cells, however, the increase in [Ca²⁺]_i in zebrafish NECs exposed to high CO₂, is derived exclusively from intracellular storage compartments with no contribution from extracellular stores (Abdallah et al., 2015). Similar to hypoxia, the membrane depolarization associated with hypercapnia is derived from an inhibition of K⁺ flux through background K⁺ channels (Qin et al., 2010) that are influenced by changes in pHi and possibly extracellular pH (pHe). Thus, Qin et al. (2010) demonstrated that membrane depolarization persisted during isohydric hypercapnia (i.e., increased PCO₂ at constant pH) and that its rate of development and final magnitude were decreased by CA inhibition using acetazolamide (Maren, 1967).

Interestingly, the sensing mechanisms underlying membrane depolarization (Qin et al., 2010) and the increase in [Ca²⁺]_i (Abdallah et al., 2015) are dissimilar. For example, while the membrane depolarization appears to be linked to changes in pHi, the increase in [Ca²⁺]_i is associated with changes in pHe. Abdallah et al. (2015) observed that isohydric hypercapnia or

Table 1

A summary of neuroepithelial cell distribution and their intracellular neurochemicals in adult and larval fish

Species and developmental stage	NEC location	Neurochemicals	References
Lesser spotted dogfish (<i>Scyliorhinus canicula</i>); adult	Gill filament	Serotonin	Dunel-Erb et al. (1982)
European eel (<i>Anguilla anguilla</i>); adult	Gill filament	Serotonin	Dunel-Erb et al. (1982)
European perch (<i>Perca fluviatilis</i>); adult	Gill filament	Serotonin	Dunel-Erb et al. (1982)
Smallmouth bass (<i>Micropterus dolomieu</i>); adult	Gill filament	Serotonin	Dunel-Erb et al. (1982)
Black bullhead catfish (<i>Ictalurus melas</i>); adult	Gill filament	Serotonin	Dunel-Erb et al. (1982)
Rainbow trout (<i>Oncorhynchus mykiss</i>); adult	Gill filament	Serotonin	Dunel-Erb et al. (1982)
Pikeperch (<i>Sander lucioperca</i>); adult	Gill filament	Serotonin	Dunel-Erb et al. (1982)
Zebrafish (<i>Danio rerio</i>); adult	Gill filament, lamella	Serotonin	Jonz and Nurse (2003)
Zebrafish (<i>Danio rerio</i>); larva	Skin	Serotonin	Jonz and Nurse (2005)
Zebrafish (<i>Danio rerio</i>); larva	Skin	Hydrogen sulfide	Porteus et al. (2015)
Zebrafish (<i>Danio rerio</i>); adult, larva	Skin, gill filament	Serotonin, nitric oxide	Porteus et al. (2015)
Goldfish (<i>Carassius auratus</i>); adult	Gill filament, lamella	Serotonin	Saltys et al. (2006)
Trairão (<i>Hoplias lacerdae</i>), adult	Gill filament, lamella	Serotonin	Coolidge et al. (2008)
Taira (<i>Hoplias malabaricus</i>), adult	Gill filament, lamella	Serotonin	Coolidge et al. (2008)
Mangrove rivulus (<i>Kryptolebias marmoratus</i>), adult	Gill filament, skin	Serotonin, acetylcholine?	Regan et al. (2011)
Sockeye salmon (<i>Oncorhynchus nerka</i>), adult	Gill filament	Serotonin	Porteus et al. (2012)
Medaka (<i>Oryzias latipes</i>), adult	Gill filament	Serotonin	Porteus et al. (2012)
Mudskipper, (<i>Periophthalmodon schlosseri</i>), adult	Gill filament, lamella	Serotonin, acetylcholine?, catecholamines?	Zaccone et al. (2017)
Brown trout (<i>Salmo trutta</i>)	Gill filament	Serotonin	Zaccone et al. (1992)
Brown bullhead catfish (<i>Ictalurus nebulosus</i>), adult	Gill filament	Serotonin	Zaccone et al. (1992)
African lungfish (<i>Protopterus annectens</i>), adult	Gill lamella	Serotonin	Zaccone et al. (1992)
Bowfin (<i>Amia calva</i>), adult	Gill filament, lamella	Serotonin, acetylcholine?	Porteus et al. (2014)

intracellular acidification (without a change in pHe) did not affect cytosolic Ca²⁺ levels. Moreover, the rise in [Ca²⁺]_i was unaffected by CA inhibition. Therefore, in zebrafish NECs, there can be uncoupling between membrane depolarization and [Ca²⁺]_i changes. The results also suggest that several categories of K⁺ channels are present in NECs with some responding to changes in pHi and others responding to changes in pHe. The channels being controlled by pHe are likely members of the Twik-related acid-sensitive K⁺ channel (TASK) family (Peña-Munzenmayer et al., 2014). Zebrafish have two specific TASK-2 channel paralogs encoded by *kcnk5a* (TASK-2) and *kcnk5b* (TASK2b). Using antisense gene silencing (morpholinos), it was demonstrated that the increases in cardiac and breathing frequencies associated with hypercapnia in zebrafish larvae were blunted in fish experiencing either TASK-2 and/or TASK-2b knockdown (Koudrina, 2017).

The NECs of adult zebrafish gill (Qin et al., 2010) and skin of 4dpf larvae (Miller et al., 2014) exhibit CA immunoreactivity. The slowing of CO₂-mediated intracellular acidification and attenuation of the membrane depolarization in zebrafish NECs experiencing inhibition of CA activity is reflected by a decreased reflex cardiovascular response to hypercapnia in larvae in vivo. For example, Miller et al. (2014) demonstrated that inhibition of CA activity using acetazolamide or following gene knockdown of the cytosolic CA isoform (CAc; Esbaugh et al., 2005) led to a blunting of the tachycardia that is typically observed in zebrafish larvae exposed to hypercapnia. No studies, however, have yet addressed the role of CA in the ventilatory response of fish to hypercapnia.

Although direct data are lacking, it is believed that the increased levels of [Ca²⁺]_i accompanying hypercapnic activation of NECs leads to the exocytosis of neurotransmitter(s) and activation of afferent neurons that ultimately promotes the ensuing downstream cardiorespiratory reflex responses. The specific neurotransmitter(s) secreted by NECs during hypercapnic stimulation is unknown but the most likely candidate is serotonin (5-HT) given that it is the principal neurochemical contained within serotonergic NECs of the gill and skin (reviewed by Porteus et al., 2012; see Table 1).

We must emphasize that although it is largely assumed based on in vitro studies and indirect correlative data from intact fish (Miller et al., 2014) that the NECs are functioning in vivo as CO₂ chemoreceptors at least in zebrafish, there is little, if any, direct evidence to support this view. The absence of direct in vivo data, in part, reflects the technical limitations associated with conducting electrophysiological recordings on NECs in situ and the absence of a viable loss-of-function model whereby cardiorespiratory responses to elevated CO₂ can be assessed in the absence of functional NECs.

3.2. Central CO₂/H⁺ Sensing

3.2.1. CARDIORESPIRATORY REFLEXES

The evidence suggests that for most fish, CO₂-initiated cardiorespiratory reflexes arise exclusively from stimulation of branchial chemoreceptors sensitive primarily to changes in the CO₂ tension of water flowing over the gills, and not in blood perfusing the gills (see [Section 3.1](#)). There are data, however, that also suggest that changes in arterial levels of CO₂/pH contribute to cardiorespiratory reflexes, particularly in air-breathing fish. Much of the data is equivocal and remains open to interpretation.

3.2.2. HAGFISH AND LAMPREYS

Exposure to acute hypercapnia causes an increase in ventilation in hagfish ([Perry et al., 2009b](#)). There are no studies, however, specifically addressing whether the sites of respiratory chemoreception are peripheral or central in this group. In studies using the isolated brainstem-spinal cord preparation of lamprey (*Lampetra fluviatilis*), the periodic respiratory discharge indicative of 'fictive' breathing (discharge in respiratory motoneurons) decreased when the bicarbonate concentration of the bathing medium was increased in 4 of 7 preparations (concentrations not given). It also increased in 3 of 8 preparations when half the bicarbonate was titrated with HCl ([Rovainen, 1977](#)). The data were sufficiently equivocal that it was difficult to draw any firm conclusion. However, the data do suggest that, while there were pH-sensitive cells in the isolated lamprey brain that could induce respiratory discharge, they most likely did not account for the hypercapnic ventilatory responses seen in intact lamprey.

3.2.3. WATER BREATHING BONY FISHES

Studies have been carried out in several species where the PCO₂/pH of the extradural fluid surrounding the brain or within the fourth ventricle was manipulated, yielding equivocal results. In studies on tench (*Tinca tinca*) the injection of small volumes (0.2–1.0 µL) of pH 7.6 solution into areas of the posterior medulla from which respiratory related activity could be recorded, increased the amplitude of respiratory movements in some instances, and caused cessation of respiratory movements in others ([Hughes and Shelton, 1962](#)). In rainbow trout and tambaqui (*Colossoma macropomum*), such perfusions were completely without effect ([Burlleson et al., 1992](#); [Reid et al., 2000](#)).

The sum of these data suggests that central respiratory chemoreceptors sensitive to changes in CO₂/H⁺ are unlikely in strictly water breathing fish.

3.2.4. AIR-BREATHING BONY FISHES

The evidence for the existence of central CO₂/pH chemoreceptors in air-breathing teleost fish is also equivocal. In many of these species, gill ventilation initially is stimulated by low levels of environmental CO₂ but progressive hypercapnia leads to an inhibition of gill ventilation and a stimulation of air breathing (Johansen, 1970; Shelton et al., 1986; Smatresk, 1988). In other species, aquatic hypercapnia fails to produce any changes in gill ventilation (Johansen, 1966; McMahon and Burggren, 1987; Thomsen et al., 2017) or in air-breathing frequency (Lomholt and Johansen, 1974). In clown knifefish (*Chitala ornata*), aquatic hypercapnia induced a significant increase in air-breathing frequency without having any effect on gill ventilation (Perry et al., 2008), as did injection of CO₂-enriched gas into the air-breathing organ (Tuong et al., 2018, 2019).

Similar to the rainbow trout described above (Peripheral Chemoreception—Sites of Chemoreception), during recovery from the acidosis incurred as a result of exhaustive exercise in spotted gar, both branchial ventilation and air-breathing remained elevated and the prolonged recovery (4–8 h) was tightly correlated to removal of the post-exercise acidosis (Burleson et al., 1998).

In the jeju (*Hoplerythrinus unitaeniatus*), complete branchial denervation eliminated all ventilatory responses (gill and air breathing) to hypercapnia (Boijink et al., 2010) indicating that CO₂/H⁺ sensitive receptors reside exclusively in the gills. In the clown knifefish, however, exposure to both hypercapnia and acetazolamide (to increase CO₂ retention and elevate arterial PCO₂) post-denervation of the gills still produced significant air-breathing responses (Tuong et al., 2019).

The existence of central CO₂/H⁺ chemoreceptors in bowfin (*Amia calva*) has also been investigated obtained by perfusing mock extradural fluid containing elevated levels of CO₂ or [H⁺] through the cranial space in the medullary region of conscious animals. However, these perfusions were without effect on air breathing or gill ventilation (Hedrick et al., 1991). It has also been shown that superfusion of the isolated brainstem-spinal cord preparation from the Alaska blackfish (*Dallia pectoralis*) with artificial cerebrospinal fluid with elevated levels of CO₂/[H⁺] had no effect on fictive air-breathing (Hoffman et al., 2009).

Interestingly, two other studies using similar isolated brainstem-spinal cord preparations in longnose gar (*Lepisosteus osseus*) (Wilson et al., 2000) and Siamese fighting fish (*Betta splendens*) (Corcoran et al., 2007) did report increases in fictive air breathing (but not fictive gill breathing) in response to increases in superfusate CO₂/[H⁺].

3.2.5. AIR-BREATHING LOBE-FINNED FISHES

This group includes all of the extant lungfishes (*Protopterus*, *Lepidosiren* and *Neoceratodus* species). Some species use air breathing to supplement O₂

uptake while the gills (and/or skin) remain the primary site for CO_2 excretion, other species rely completely on air breathing for gas exchange (Graham, 1997). As the reliance on air-breathing increases, the functional surface area of the gills is reduced with a consequent increase in arterial PCO_2 (Perry et al., 2009a).

While it has been shown that the African lungfish *Protopterus annectens* responds to aerial hypercapnia with pronounced pulmonary hyperventilation (Babiker, 1979), similar treatment had no effect on ventilation in other species (the slender lungfish *P. dolloi*, and the marbled lungfish *P. aethiopicus* as well as the South American lungfish, *Lepidosiren paradoxa*) (Burggren, 1979; Jesse et al., 1967; Johansen et al., 1967; Johansen and Lenfant, 1968; Lomholt and Johansen, 1974; Perry et al., 2008; Sanchez and Glass, 2001) even though it produced a respiratory acidosis (Perry et al., 2005). While these results suggest that this group of fishes also lacks any internal CO_2/H^+ sensitive respiratory chemoreceptors, the story may not be this straightforward. It has also been shown that *L. paradoxa* possesses CO_2 sensitive airway receptors (DeLaney et al., 1974). These receptors when stimulated by elevated CO_2 inhibit ventilation. Thus, giving lungfish CO_2 to breathe, to simulate elevated levels of arterial CO_2 arising either from uptake from hypercapnic water or from metabolically produced CO_2 , would mask the effects of any central CO_2/H^+ stimulation. In support of this, it has been shown that these fish exhibit a “post-hypercapnic hyperpnea” (Sanchez and Glass, 2001). That is, when animals subsequently return to breathing normocapnic air, inspired CO_2 levels fall immediately while arterial levels of CO_2 fall slowly as whole body CO_2 stores are eliminated. Thus, the inhibitory effect of elevated airway CO_2 is removed, while systemic CO_2 levels remain elevated, as does air-breathing frequency (see Milsom et al., 2004 for a review of this phenomenon) suggesting that this species, at least, does possess internal CO_2/H^+ receptors. Consistent with these data, it has been shown that pulmonary ventilation increases in *L. paradoxa* in response to independent changes in both CO_2 and pH of cerebrospinal fluid indicating that the internal receptors reside in the central nervous system (Amin-Naves et al., 2007a,b; Sanchez and Glass, 2001). These are the only unequivocal data in support of the presence of central CO_2/pH chemoreceptors. While similar studies have yet to be performed on other lungfish species, the African lungfish *P. annectens* also can adjust branchial and/or pulmonary ventilation appropriately to correct blood acid-base disturbances arising from arterial infusions of NaHCO_3 or NH_4Cl (reviewed in Perry and Gilmour, 2006). These findings support the suggestion that central CO_2/H^+ sensitive chemoreceptors are common to all lungfish species.

3.2.6. CELLULAR MECHANISMS OF CENTRAL CO_2 SENSING

Given the equivocal nature of the evidence in support of central CO_2/H^+ chemoreceptors in fish, it should not be surprising that there has been no work

done to date analyzing the possible mechanisms of central CO₂ sensing. The only group in which this has been studied in any detail is the mammals. In mammals, chemosensitive neurons are spread among numerous brain stem regions, and neurons from different regions have different levels of chemosensitivity. Recent evidence indicates that the retrotrapezoid nucleus (RTN) may be of particular importance. Two non-mutually exclusive mechanisms have been suggested to explain the sensitive response of RTN neurons to CO₂. Changes in pHi could excite RTN neurons directly mediated by the intrinsic acid sensitivity of subsets of potassium channels, or indirectly via specialized proton receptors and intracellular messengers. Alternately, CO₂ may activate RTN neurons by causing the surrounding glia to release ATP (reviewed in [Guyenet, 2012](#)). The signaling mechanisms for chemosensitivity at other sites may also involve changes of pHe, [Ca²⁺]_i, gap junctions, oxidative stress, [HCO₃⁻], or PCO₂. The normal target for these signals is generally believed to be a variety of K⁺ channels as well as Ca²⁺ channels (reviewed in [Putnam et al., 2004](#)).

3.3. Cellular Acid-Base Sensing

The peripheral and central CO₂/H⁺ chemosensing mechanisms described in the previous sections mediate cardiorespiratory processes through neural pathways. This section describes acid-base sensing mechanisms that modulate cellular physiology in response to local fluctuations in CO₂, pH and [HCO₃⁻], without the need for neuronal or hormonal inputs. Some of these acid-base sensing mechanisms can contribute to general cellular homeostasis, for example, by sensing metabolic CO₂ and H⁺ production and regulating pHi or gene expression. In addition, acid-base sensing mechanisms in specialized cells can modulate organ and whole animal physiology. In fact, peripheral and central chemosensing must ultimately rely on similar cellular A/B sensing mechanisms to trigger action potentials in response to CO₂/H⁺.

In fish, specialized ion-transporting cells (ionocytes) located in the gill are responsible for regulating blood plasma A/B status. Broadly speaking, gill ionocytes excrete excess H⁺ in exchange for environmental Na⁺ to compensate systemic acidosis, and excrete excess HCO₃⁻ in exchange for Cl⁻ to compensate systemic alkalosis. These processes also involve accumulation of HCO₃⁻ and H⁺, respectively. As explained in detail below, A/B sensing of blood plasma of elasmobranch fishes is mediated by the HCO₃⁻-sensing enzyme soluble adenylyl cyclase (sAC; *adcy10*) inside gill ionocytes ([Roa and Tresguerres, 2016](#); [Tresguerres et al., 2010a,b,c](#)). sAC is also present in gill ionocytes of bony fish where it likely contributes to blood A/B and ionic regulation ([Salmerón and Tresguerres, unpublished](#)); however, experimental confirmation still awaits.

The end result of branchial H^+ and HCO_3^- excretion and absorption is the maintenance of a relatively stable acid-base status in blood plasma that lessens the amount of energy necessary for regulating pH_i in the rest of the cells. However, those cells must still regulate (and therefore, be able to sense), the acid-base status in the cytosol. These mechanisms have not been characterized in fish, but they are most likely similar to those described in coral (Barott et al., 2017) and mammals (Tresguerres et al., 2010a).

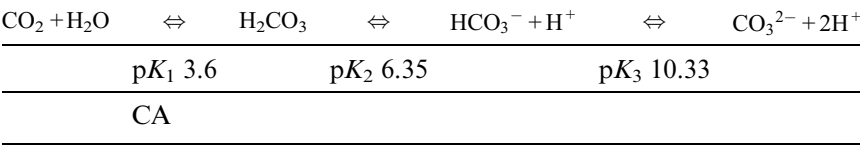
Additional sites that require acid-base sensing in fish include extracellular compartments such as the cerebrospinal fluid, otolith endolymph, and intestinal fluid. With the exception of the latter where sAC has again been implicated in sensing elevated HCO_3^- (Tresguerres et al., 2010a), the acid-base sensing mechanisms remain unknown. Importantly, the acid-base conditions in those external, extracellular, and intracellular sites can vary widely, implying the presence of acid-base sensing mechanisms specifically tuned for each site.

4. MOLECULAR A/B SENSORS

While the structure of all proteins is affected by pH to a certain extent, a molecular acid-base sensor must also be able to regulate and coordinate the activity of downstream effector proteins in a manner conducive to a homeostatic response (Fig. 2).

In animals, molecular acid-base sensors fall into two broad categories: (1) enzymes that induce the production of messenger molecules [e.g., cyclic adenosine monophosphate (cAMP), cyclic guanylyl monophosphate (cGMP), inositol 1,4,5-triphosphate (IP_3), diacylglycerol (DAG), Ca^{2+}] and whose activity is modulated by one or more acid-base parameter, and (2) pH-sensitive ion channels that modulate the membrane potential of chemosensing neurons, and thus action potentials and nerve impulse conduction. Multiple types of acid-base molecular sensors might cooperate together as part of a cellular acid-base sensing mechanism to allow the discrimination between the different types of acid-base stress, and regulate acid-base status around specific set points in different organs, cells, and subcellular compartments.

At 25 °C in pure water, the reversible equilibrium between H_2O , CO_2 , H^+ , HCO_3^- and CO_3^{2-} is pH-dependent and follows the equation:



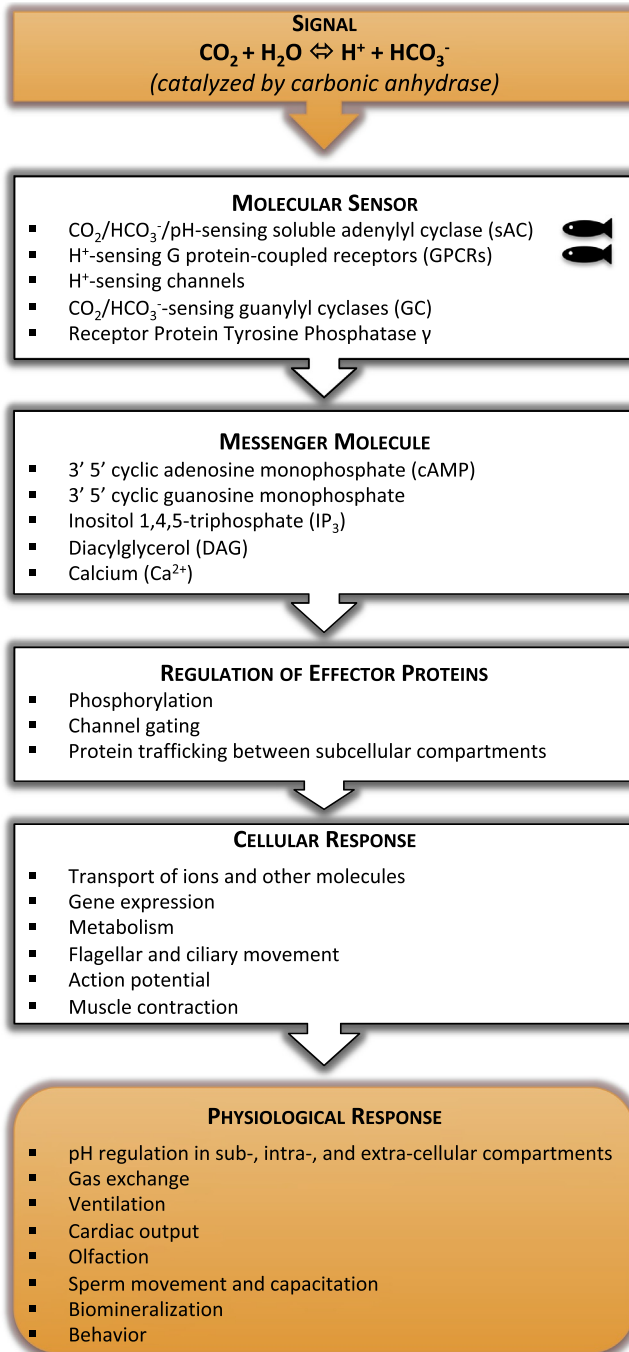


Fig. 2. Generalized acid-base sensing mechanisms. The fish icons indicate Molecular Sensors that have been characterized in fish. The other elements in this figure (Messenger Molecules, Regulation of Effector Proteins, Cellular and Physiological Responses) are conserved throughout the Animal kingdom.

As a result, at the physiological pH of most internal fluids the dominant carbon species is by far HCO_3^- . Furthermore, although the interconversion between CO_2 and H_2O and H_2CO_3 is relatively slow, catalysis by CA ensures the almost instantaneous equilibration of CO_2 with HCO_3^- and H^+ . An important implication for the purposes of acid-base sensing is that HCO_3^- and H^+ can be used as proxies for CO_2 levels. Indeed, the vast majority of acid-base molecular sensors identified to date sense HCO_3^- or H^+ . A variety of molecular acid-base sensing mechanisms have been identified in mammals, insects, plants, yeast and bacteria (Linder and Schultz, 2003; Steegborn, 2014; Tresguerras et al., 2010a, 2011). However in fish, the only molecular acid-base sensors identified to date are the $\text{CO}_2/\text{pH}/\text{HCO}_3^-$ sensor sAC (Fig. 3A) and the H^+ -sensing G-protein-coupled receptors (GPCRs) ovarian G-protein coupled receptor 1 (OGR1), G-protein coupled receptor 4 (GPR4), and G2A (Ichijo et al., 2016; Mochimaru et al., 2015) (Fig. 3B).

4.1. $\text{CO}_2/\text{pH}/\text{HCO}_3^-$ Sensing sAC

sAC is a cAMP-producing enzyme that is directly activated by HCO_3^- (Buck et al., 1999; Chen et al., 2000). However, due to the interrelationship with CO_2 and H^+ explained above, sAC can also act as a sensor for both internal and external CO_2 and pH (reviewed in Tresguerras et al., 2011) (Fig. 3A). Originally identified and characterized from rat testis, sAC is related to HCO_3^- -sensing adenylyl cyclases from cyano- (Buck et al., 1999; Chen et al., 2014; Steegborn, 2014) and chloroflexi bacteria (Kobayashi et al., 2004), and was later molecularly and biochemically characterized in phylogenetic diverse organisms including coral (Barott et al., 2017), sea urchin (Nomura et al., 2005), shark (Tresguerras et al., 2010c), and bony fish (Salmerón and Tresguerras, unpublished). Accordingly, sAC is now accepted as an evolutionarily conserved $\text{pH}/\text{CO}_2/\text{HCO}_3^-$ sensor.

4.1.1. sAC IN ELASMOBRANCH GILLS SENSES BLOOD ACID-BASE STATUS

In elasmobranch fishes, sAC is abundantly expressed in the cytoplasm of acid- and base-secreting cells where it acts as a sensor of blood acid-base status (Roa and Tresguerras, 2016, 2017; Tresguerras et al., 2010c). The mechanism is shown in Fig. 4, and is as follows: during a post-feeding blood alkalosis, plasma HCO_3^- is dehydrated into CO_2 by CAs in red blood cells and at the basolateral membrane of gill pillar cells. CO_2 then diffuses into the base-secreting cells, where it is rehydrated into HCO_3^- and H^+ by cytosolic CAs. The elevated HCO_3^- is sensed by sAC, resulting in increased cAMP production that triggers the translocation of vesicles containing V-type H^+ -ATPase (VHA) from the cell's cytoplasm to the basolateral membrane, and of vesicles containing the anion exchanger pendrin to the apical membrane

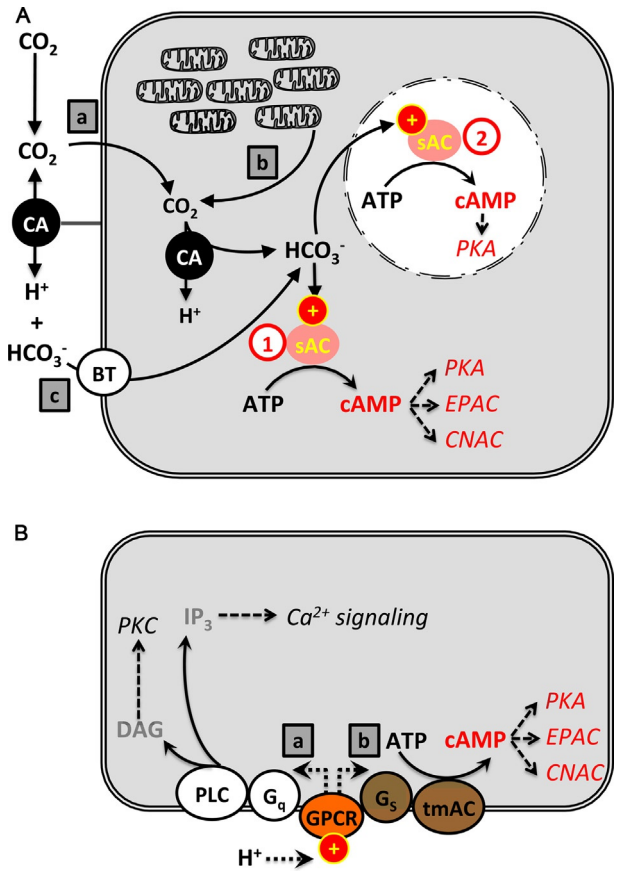


Fig. 3. Cellular and molecular mechanisms characterized in fish. (A) Soluble adenylyl cyclase (sAC) in the cytoplasm (1) and nucleus (2). sAC may be stimulated by intracellular HCO₃⁻ derived from CO₂ from [a] external and [b] metabolic origin after being hydrated in a reaction catalyzed by carbonic anhydrase (CA). [c] sAC may also be stimulated by HCO₃⁻ that enters the cell through HCO₃⁻ transporting proteins (BT). sAC-produced cAMP mediates physiological functions in the nucleus and cytosol (and possibly other subcellular compartments) through protein kinase A (PKA), exchange protein activated by cAMP (EPAC), and cyclic nucleotide-activated channels (CNAC). (B) H⁺-sensing G-protein-coupled receptors (GPCRs). Extracellular H⁺ (blood plasma, interstitial fluids) activates H⁺-sensing GPCRs. [a] If linked to protein G_q, it leads to phospholipase C (PLC) activation, production of diacylglycerol (DAG) and inositol triphosphate (IP₃), and modulation of downstream proteins by protein kinase C (PKC) and Ca²⁺ release from intracellular stores, respectively. [b] If linked to protein G_s, it stimulates transmembrane adenylyl cyclase (tmAC) to produce cAMP leading to subsequent PKA, EPAC, and/or CNAC modulation.

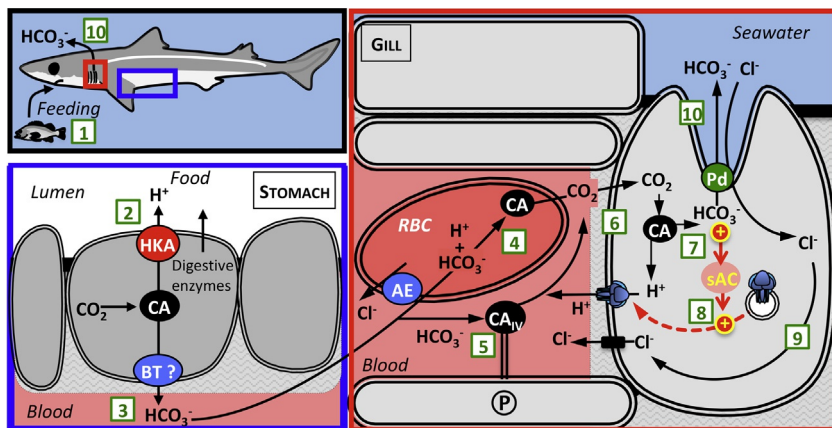


Fig. 4. Sensing of blood alkalosis by soluble adenylyl cyclase in elasmobranch gill cells. *Black Box:* (1) Sharks feed opportunistically on a variety of fish and invertebrate prey. *Blue Box:* (2) Gastric H^+/K^+ -ATPase (HKA), not to be confused with VHA, helps secrete HCl into the stomach lumen and, together with digestive enzymes, digest the food. (3) At the same time, HCO_3^- is absorbed into the blood through unidentified HCO_3^- transporters (BT), which induces a blood alkalosis. *Red Box:* HCO_3^- travels in blood plasma and also enters red blood cells (RBC) via anion exchangers (AE). (4) Inside RBCs, intracellular carbonic anhydrase (CA) hydrates HCO_3^- into CO_2 . (5) In addition, extracellular CA IV located in the cell membrane of pillar cells (P) hydrates plasma HCO_3^- into CO_2 . (6) CO_2 from both sources diffuses into VHA-rich base-secreting cells, where intracellular CA rehydrates it into HCO_3^- and H^+ . (7) Intracellular HCO_3^- stimulates soluble adenylyl cyclase (sAC), which (8) triggers the translocation of cytoplasmic vesicles containing VHA (blue icon) to the cell basolateral membrane. VHA then secretes H^+ into the blood. (9) A putative basolateral channel brings Cl^- from VHA-rich cells into the blood. (10) Intracellular HCO_3^- is secreted to seawater in exchange for Cl^- via apical pendrin (Pd)-like anion exchangers. The combined action of H^+ reabsorption by VHA and HCO_3^- secretion by pendrin corrects blood alkalosis. Water molecules have been omitted for simplicity. The shaded areas surrounding epithelial gastric and branchial cells signify connective tissue and other cell types that might separate them from the blood space. Modified from Tresguerres (2016). Based on Gilmour et al. (2007), Roa et al. (2014), Roa and Tresguerres (2016), Tresguerres et al. (2005, 2006, 2007), Tresguerres (2010a, 2010b, 210c), Wood et al. (2005, 2009).

[although the involvement of sAC in latter has not been directly established (Roa et al., 2014)]. Cells with apical pendrin and basolateral VHA are thus activated to secrete HCO_3^- and absorb H^+ and Cl^- , which effectively counteracts the blood alkalosis. This acid-base sensing mechanism also takes place in isolated gill fragments (Tresguerres et al., 2010c) and isolated gill cells (Roa and Tresguerres, 2016), indicating acid-base sensing takes place locally in each gill base-secreting cell and is therefore independent of the peripheral and central chemosensors described in previous sections.

Some unknown aspects of this acid-base sensing mechanism include the processes that connect sAC-produced cAMP to VHA translocation (i.e., PKA or EPAC, regulation of vesicle movement along microtubules), and how gill acid- and base-secreting cells discriminate between the different types of acid-base stress. In this regard, it has been hypothesized that the coordinated action of sAC and H⁺ sensing GPCRs (described in the next section) may stimulate base-secreting cells during metabolic alkalosis and inhibit them during acidosis, while simultaneously having the opposite modulatory effect on acid-secreting cells (Roa and Tresguerres, 2016). However, this model requires experimental confirmation.

4.1.2. sAC IN OTHER ELASMOBRANCH TISSUES

In elasmobranchs, sAC has also been reported in rectal gland, cornea, intestine, skeletal and cardiac muscle (Roa and Tresguerres, 2017), and red blood cells (Tresguerres et al., 2014). However, the physiological roles of acid-base sensing by sAC in those organs are still unknown. Immunohistochemical analysis has found sAC can be present in the nucleus of cells from diverse organs. Furthermore, cell nuclei isolated from gill and rectal gland demonstrated HCO₃⁻-stimulated cAMP production that is inhibited by pharmacological sAC inhibition (Roa and Tresguerres, 2017), suggesting that sAC regulates gene expression in response to acid-base stress by phosphorylation of gene transcription factors as reported for mammals (Zippin et al., 2004).

4.1.3. sAC IN BONY FISH

Genes coding for sAC are also present in bony fish (Tresguerres, 2014; Tresguerres et al., 2014). Recent research has found that rainbow trout possesses multiple sAC splice variants and protein isoforms (Salmerón and Tresguerres, unpublished). Interestingly, some of the sAC isoforms are preferentially located in the cytoplasm while others are found in the nucleus or associated with the Golgi apparatus. This suggests different sAC isoforms sense acid-base and regulate specific physiological functions in each subcellular compartment.

sAC has also been detected in the head, midpiece and flagella of Atlantic salmon sperm (von Schalburg et al., 2018), and most likely regulates sperm flagellar movement and capacitation as described in mammals (Hess et al., 2005) and sea urchin (Beltrán et al., 2007; Nomura et al., 2005).

4.1.4. sAC IN FISH INTESTINE MODULATES NaCl-DRIVEN WATER ABSORPTION

In marine bony fishes, sAC is able to sense CO₂/HCO₃⁻ levels inside intestinal ionocytes and modulate transepithelial NaCl-driven water absorption (Fig. 5). Intestinal water absorption is essential for osmoregulation in marine fishes, and depends on massive HCO₃⁻ secretion into the intestinal lumen where

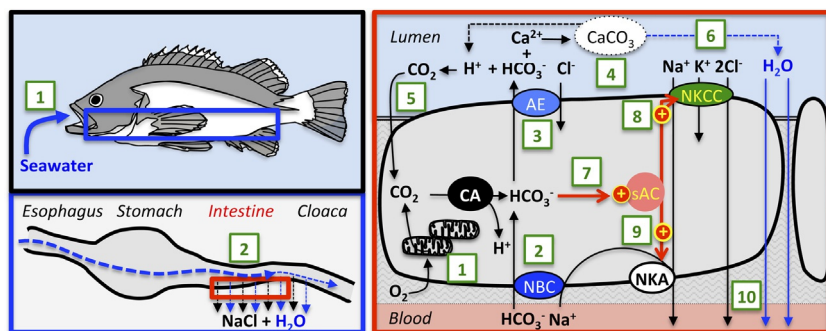


Fig. 5. Acid/Base sensing by soluble adenylyl cyclase in the intestine of marine teleost fish. (1) Marine teleosts drink large amounts of seawater to counteract dehydration. (2) NaCl-mediated water absorption takes place in the intestine. In intestinal cells, the mechanism is as follows: (1) HCO_3^- is generated from carbonic anhydrase (CA)-catalyzed hydration of metabolic CO_2 , and (2) imported into the cell via $\text{Na}^+/\text{HCO}_3^-$ exchangers (NBC). (3) An apical Anion Exchanger (AE) excretes HCO_3^- into the intestinal lumen. (4) The high luminal $[\text{HCO}_3^-]$ precipitates with Ca^{2+} (and Mg^{2+}) from the ingested seawater. (5) Precipitation of carbonates generates H^+ , resulting in formation of CO_2 that diffuses into the intestinal cells and is hydrated to HCO_3^- and (6) reduces the osmolality of the fluid within the intestinal lumen. Intracellular HCO_3^- derived from those three sources stimulates soluble adenylyl cyclase (sAC). The exact downstream mechanisms are unknown, but it has been hypothesized that cAMP produced by sAC stimulates protein kinase A, which in turns activates apical $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporters (NKCC) and/or Na^+/K^+ -ATPase (NKA). Overall, this results in transepithelial NaCl and H_2O absorption. Modified from Tresguerres et al. (2010b). Based on Carvalho et al. (2012), Taylor et al. (2010), Tresguerres et al. (2010b), Wilson et al. (2002).

it can reach concentrations in excess of 100mM (Wilson et al., 2002). This unique acid-base physiology prompted studies about the potential regulatory roles of HCO_3^- and sAC on intestinal NaCl and water transport. The evidence supporting a role for sAC includes immunohistochemical detection using heterologous antibodies against shark and rat sAC (Carvalho et al., 2012; Tresguerres et al., 2010b), and a reduction in transepithelial NaCl and water absorption upon sAC inhibition (Carvalho et al., 2012; Tresguerres et al., 2010b). sAC in intestinal ionocytes is stimulated by HCO_3^- that enters from blood plasma via $\text{Na}^+/\text{HCO}_3^-$ cotransporters (NBCs) and by HCO_3^- derived from CA-catalyzed hydration of metabolic CO_2 . Additionally, the HCO_3^- that stimulates sAC might be derived from CO_2 buildup in the intestinal lumen. Thus, sAC in intestinal ionocytes might integrate sensory inputs for $\text{CO}_2/\text{HCO}_3^-$ from three compartments (plasma, cells and lumen). The downstream pathway is not completely understood, but it has been proposed that sAC-produced cAMP activates PKA to modulate the activities of apical $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporters (NKCCs) and basolateral Na^+/K^+ -

ATPases by phosphorylation (Carvalho et al., 2012; Tresguerres et al., 2010b) (Fig. 5).

4.1.5. sAC IN HAGFISH HEART MODULATES CARDIAC FREQUENCY

Immunohistochemical detection and pharmacological experiments have also established a role for sAC in regulating Pacific hagfish cardiac frequency in response to HCO₃⁻ fluctuations (Wilson et al., 2016). In isolated hagfish systemic hearts, this mechanism contributes to bradycardia during anoxia and to tachycardia during the early phase of normoxia following anoxia. In vivo, this mechanism may induce similar responses when hagfish are feeding in anoxic environments.

sAC was immunohistochemically detected throughout the hagfish systemic heart, although not in all cells (Wilson et al., 2016). In cardiomyocytes, sAC signal was more intense in defined regions (presumably sacomeric bands). Based on the effect of pharmacological sAC inhibition on heart beat rate, sAC is likely to be present in pacemaker cells (although these have not been identified in hagfish yet). The mechanisms downstream of sAC are also unknown, but may include hyperpolarization-activated cyclic nucleotide-modulated ion channels (HCNs) in addition to PKA phosphorylation of multiple targets (Fig. 6). Similar to bony fish intestine, sAC in hagfish heart likely senses HCO₃⁻ derived from plasma that enters cardiac cells through NBCs, and from metabolic CO₂ production. The levels of both HCO₃⁻ sources decrease as a result of anaerobic metabolism during anoxia, and experience a sharp peak at the onset of aerobic metabolism when normoxic conditions return (Cox et al., 2011). Because the hagfish heart lacks innervation (Greene, 1902), sAC-mediated control of cardiac rate might reflect an ancestral characteristic potentially present in basal animals and vertebrate larval stages.

4.1.6. EMERGING PATTERNS OF ACID-BASE SENSING BY sAC

The few studies described above have revealed some interesting characteristics about acid-base sensing by sAC in fish and animals in general, as well as raising novel questions. One of them is that sAC proteins are tuned to the typical physiological HCO₃⁻ levels present in each species. This is evident in their EC₅₀^{HCO₃⁻} (the concentration of HCO₃⁻ that results in half-maximal cAMP production), which is ~5mM in shark, ~10mM in coral and trout, and ~20mM in hagfish and mammals (reviewed in Tresguerres et al., 2014). Because the EC₅₀ is at the midpoint of the steepest part of the HCO₃⁻/cAMP dose response curve, this implies that minor HCO₃⁻ fluctuations around a set point will result in relatively large changes in cAMP production, which is exactly what one would predict (and desire) for a physiological acid-base sensor.

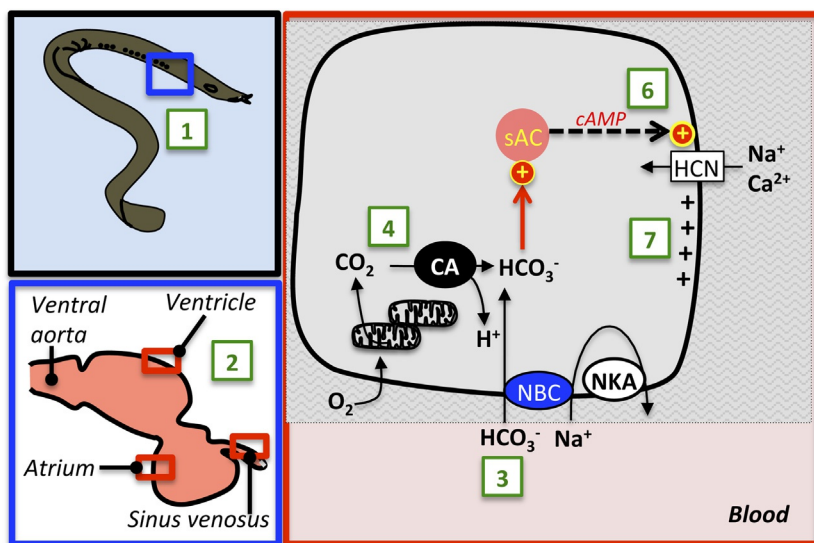


Fig. 6. Acid/Base sensing by soluble adenylyl cyclase in hagfish heart. (1) The hagfish systemic heart lacks innervation so it relies on local mechanisms to regulate heart beat frequency. (2) Soluble adenylyl cyclase is present in many cells (but not all) in the atrium, ventricle, and sinus venosus. The proposed mechanisms of sAC activation in atrial and ventricular cardiomyocytes involves (3) HCO_3^- entry from blood plasma via $\text{Na}^+/\text{HCO}_3^-$ exchangers (NBC) and (4) HCO_3^- that is generated from carbonic anhydrase (CA)-catalyzed hydration of metabolic CO_2 . (6) sAC-produced cAMP is proposed to activate hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels (a type of cyclic nucleotide-activated channel). (7) This leads to cell membrane depolarization and Ca^{2+} influx which control pacemaker activity. Based on Greene (1902), Wilson et al. (2013, 2016).

Another emerging pattern is that sAC can act as a sensor of metabolic CO_2 production as reported in fish intestine and hagfish heart, but also in coral cells (Barott et al., 2017). Intriguingly, sAC is also essential for maintaining pH homeostasis in coral. Considering that corals belong to the ancestral phylum Cnidaria, a universal role of sAC in this fundamental physiological function certainly is a possibility.

A third consideration concerns the interaction between sAC and the other sources of cAMP, the traditional, hormone-activated transmembrane adenylyl cyclases (tmACs). This relates to the model of intracellular cAMP-signaling microdomains whereby cAMP from different pools of sACs and tmACs are present in discrete subcellular regions and specifically regulate effector proteins within each domain (reviewed in Tresguerras and Salmeron, 2018). Fish provide several examples in support of the cAMP-signaling microdomain model. Indeed, sAC and tmACs have opposite effects on the VHA translocation in

elasmobranch gill cells and on NaCl and H₂O absorption and secretion across the intestine of marine bony fish, and induce different responses on hagfish heart rate despite both producing the same messenger molecule, cAMP. The cAMP-signaling microdomain model is also relevant for acid-base sensing because some of the H⁺-sensing GPCRs described in the next section also signal through cAMP.

Finally, the mechanism of acid-base sensing by sAC and its downstream responses in fish gill, intestine and heart provide hints about how acid-base sensing might take place in other cell types, physiological functions, and acid-base disturbances, including those associated with ocean acidification.

4.2. H⁺ Sensing GPCRs

A subset of GPCRs act as H⁺ sensors in mammals (reviewed in [Tresguerres et al., 2010a](#)). These GPCRs are stimulated by a drop in pHe ([Ludwig et al., 2003](#)); i.e., pH in blood plasma and interstitial fluids. Some of those H⁺-sensing GPCRs have been described in zebrafish, specifically OGR1, GPR4 ([Mochimaru et al., 2015](#)), and G2A ([Ichijo et al., 2016](#)).

The molecular mechanism that confers mammalian and zebrafish GPCRs their H⁺-sensing properties relies on conserved histidine residues that, upon protonation in the physiological pH range, induce a conformational change that initiates a signaling cascade ([Liu et al., 2010](#); [Ludwig et al., 2003](#)). If linked to Gs protein, this leads to tmACs stimulation, cAMP production, and modulation of target effector proteins by PKA phosphorylation, EPAC, and CNACs. However, if linked to Gq protein, H⁺-sensing GPCRs activate phospholipase C leading to diacylglycerol-PKC phosphorylation and to IP₃-mediated Ca²⁺ release from intracellular stores and subsequent modulation of effector proteins ([Fig. 2B](#)). Based on heterologous expression in mammalian cell lines, zebrafish H⁺-sensing GPCRs can act through all of those pathways and differentially regulate gene transcription ([Ichijo et al., 2016](#); [Mochimaru et al., 2015](#)). Those in vitro studies suggest zebrafish OGR1, GPR4, and G2A can regulate the expression of different genes; however, these functions have not been studied in native zebrafish cells.

Zebrafish have two G2A homologs (G2A-a and G2A-b). Their mRNAs are widely present throughout tissues including brain, pituitary, eye, gill, heart, gas bladder, gut, gallbladder, spleen, kidney, testis, ovary, muscle and scales ([Ichijo et al., 2016](#)). GPR4 and OGR1 mRNAs are present in zebrafish embryos ([Mochimaru et al., 2015](#)). However, the expression of OGR1 and GPR4 in adult zebrafish tissues has not been reported.

In mammals, H⁺-sensing GPCRs regulate multiple physiological process in response to systemic acidosis including (but not limited to) renal H⁺

secretion, angiogenesis, and tumor growth (Codina et al., 2011; Sun et al., 2010; Wyder et al., 2011; Yang et al., 2007). In zebrafish, H^+ -sensing GPCRs have been studied from a biomedical perspective. However, H^+ -sensing GPCRs orthologs are present in genomic and transcriptomic databases of multiple fish species, and their H^+ sensing functions likely play important physiological roles during acid-base disturbances.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Decades of research have identified peripheral and to a lesser extent, central sites of CO_2 sensing in diverse fish, and established evolutionary patterns and downstream whole animal cardiorespiratory responses. More recent research has identified molecular and cellular aspects of CO_2 sensing in peripheral NECs, as well as two molecular chemosensing enzymes that can regulate the activity of effector proteins via posttranslational modifications: $CO_2/pH/HCO_3^-$ -sensing sAC, and H^+ -sensing GPCRs. Because these molecular sensors are responsive to physiologically relevant CO_2 , HCO_3^- and pH levels and are widely expressed throughout fish tissues, they are poised to sense metabolic and environmental acid-base disturbances and modulate multiple homeostatic responses. However, there is still much to learn including other organs and processes under regulatory control by those sensors, as well as additional acid-base sensors. In this regard, promising candidates include the pH_i sensor Pyk2, extracellular CO_2 sensors that rely on CA in combination with HCO_3^- -sensing guanylyl cyclases or H^+ gated channels (reviewed in Tresguerras et al., 2010a), and the recently identified extracellular CO_2/HCO_3^- sensor Protein Tyrosine Phosphatase γ (Zhou et al., 2016). However, these candidate CO_2 -sensing mechanisms are based on mammalian systems, so their potential fish counterparts must be tuned to the different acid-base physiology of fishes.

Another interesting area of future investigation is the potential interaction between molecular chemosensors. As described throughout this chapter, some of them can use the same signaling pathways and therefore precise regulatory mechanisms must exist to ensure adaptive signal specificity. Moreover, the ability to differentiate between different types of acid-base stress and conditions (i.e., metabolic and respiratory acidosis and alkalosis, compensated respiratory acidosis, etc.) likely requires at least two different chemosensors (see Roa and Tresguerras, 2016).

A final intriguing question is the potential for acid-base sensing mechanisms to adjust their set points or mediate homeostatic responses to chronic changes in environmental CO_2 levels such as those experienced in fish

aquaculture facilities and expected ocean acidification. The answer to this question could determine fish universal or species-specific vulnerability or resilience.

ACKNOWLEDGMENTS

Supported by grants from the National Science Foundation (NSF IOS #1754994 to M.T.), and by the National Sciences and Engineering Research Council of Canada (NSERC Discovery and R.T.I. grants to W.K.M. and S.F.P.).

REFERENCES

- Abdallah, S.J., Perry, S.F., Jonz, M.G., 2012. CO₂ signaling in chemosensory neuroepithelial cells of the zebrafish gill filaments: role of intracellular Ca²⁺ and pH. *Adv. Exp. Med. Biol.* 758, 143–148.
- Abdallah, S.J., Jonz, M.G., Perry, S.F., 2015. Extracellular H⁺ induce Ca²⁺ signals in respiratory chemoreceptors of zebrafish. *Pflugers Arch.* 467, 399–413.
- Amin-Naves, J., Giusti, H., Hoffmann, A., Glass, M.L., 2007a. Components to the acid–base related ventilatory drives in the South American lungfish *Lepidosiren paradoxa*. *Resp. Physiol. Neurobiol.* 155, 35–40.
- Amin-Naves, J., Giusti, H., Hoffmann, A., Glass, M.L., 2007b. Central ventilatory control in the South American lungfish, *Lepidosiren paradoxa*: contributions of pH and CO₂. *J. Comp. Physiol. B* 177, 529–534.
- Aota, S., Holmgren, K.D., Gallagher, P., Randall, D.J., 1990. A possible role for catecholamines in the ventilatory responses associated with internal acidosis or external hypoxia in rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* 151, 57–70.
- Babiker, M., 1979. Respiratory behaviour, oxygen consumption and relative dependence on aerial respiration in the African lungfish (*Protopterus annectens*, Owen) and an air-breathing teleost (*Clarias lazera*, C.). *Hydrobiologia* 65, 177–187.
- Bailly, Y., Dunelrb, S., Laurent, P., 1992. The neuroepithelial cells of the fish gill filament—indolamine-immunocytochemistry and innervation. *Anat. Rec.* 233, 143–161.
- Barott, K.L., Barron, M.E., Tresguerres, M., 2017. Identification of a molecular pH sensor in coral. *Proc. Biol. Sci.* 284, 20171769.
- Beltrán, C., Vacquier, V.D., Moy, G., Chen, Y., Buck, J., Levin, L.R., Darszon, A., 2007. Particulate and soluble adenylyl cyclases participate in the sperm acrosome reaction. *Biochem. Biophys. Res. Commun.* 358, 1128–1135.
- Boijink, C.L., Florindo, L.H., Leite, C.A.C., Kalinin, A.L., Milsom, W.K., Rantin, F.T., 2010. Hypercarbic cardiorespiratory reflexes in the facultative air-breathing fish jeju (*Hoplerthrinus unitaeniatus*): role of branchial CO₂ chemoreceptors. *J. Exp. Biol.* 213, 2792–2807.
- Buck, J., Sinclair, M.L., Schapal, L., Cann, M.J., Levin, L.R., 1999. Cytosolic adenylyl cyclase defines a unique signaling molecule in mammals. *Proc. Natl. Acad. Sci. U.S.A.* 96, 79–84.
- Burggren, W.W., 1979. Bimodal gas exchange during variation in environmental oxygen and carbon dioxide in the air-breathing fish *Trichogaster trichopterus*. *J. Exp. Biol.* 82, 197–213.

- Burleson, M.L., Smatresk, N.J., 2000. Branchial chemoreceptors mediate ventilatory responses to hypercapnic acidosis in channel catfish. *Comp. Biochem. Physiol. A* 125, 403–414.
- Burleson, M.L., Smatresk, N.J., Milsom, W.K., 1992. Afferent inputs associated with cardio-ventilatory control in fish. In: Hoar, W.S., Randall, D.J., Farrell, A.P. (Eds.), *The Cardio-vascular System*. Academic Press, San Diego, pp. 389–423.
- Burleson, M.L., Shipman, B.N., Smatresk, N.J., 1998. Ventilation and acid-base recovery following exhaustive activity in a fish. *J. Exp. Biol.* 201, 1359–1368.
- Burleson, M.L., Mercer, S.E., Wilk-Blaszczak, M.A., 2006. Isolation and characterization of putative O₂ chemoreceptor cells from the gills of channel catfish (*Ictalurus punctatus*). *Brain Res.* 1092, 100–107.
- Carvalho, E.S.M., Gregório, S.F., Power, D.M., Canário, A.V.M., Fuentes, J., 2012. Water absorption and bicarbonate secretion in the intestine of the sea bream are regulated by transmembrane and soluble adenylyl cyclase stimulation. *J. Comp. Physiol. B* 182, 1069–1080.
- Chen, Y., Cann, M.J., Litvin, T.N., Iourgenko, V., Sinclair, M.L., Levin, L.R., Buck, J., 2000. Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. *Science* 289, 625–628.
- Chen, X., Baumlin, N., Buck, J., Levin, L.R., Fregien, N., Salathe, M., 2014. A soluble adenylyl cyclase form targets to axonemes and rescues beat regulation in soluble adenylyl cyclase knockout mice. *Am. J. Respir. Cell Mol. Biol.* 51, 750–760.
- Coccimiglio, M.L., Jonz, M.G., 2012. Serotonergic neuroepithelial cells of the skin in developing zebrafish: morphology, innervation and oxygen-sensitive properties. *J. Exp. Biol.* 215, 3881–3894.
- Codina, J., Opyd, T.S., Powell, Z.B., Furdul, C.M., Petrovic, S., Penn, R.B., DuBose, T.D., 2011. pH-dependent regulation of the α -subunit of H⁺-K⁺-ATPase (HK α 2). *Am. J. Physiol. Renal Physiol.* 301, F536–F543.
- Coolidge, E.H., Ciuhandu, C.S., Milsom, W.K., 2008. A comparative analysis of putative oxygen-sensing cells in the fish gill. *J. Exp. Biol.* 211, 1231–1242.
- Corcoran, A., Wilson, R., Harris, M., 2007. Central CO₂/pH chemo-sensitivity in a modern air-breathing teleost; evidence in vitro and in vivo. *Soc. Neurosci. Abstr.* 297, 11.
- Cox, G.K., Sandblom, E., Richards, J.G., Farrell, A.P., 2011. Anoxic survival of the Pacific hagfish (*Eptatretus stoutii*). *J. Comp. Physiol. B* 181, 361–371.
- Crocker, C.E., Farrell, A.P., Gamperl, A.K., Cech Jr., J.J., 2000. Cardiorespiratory responses of white sturgeon to environmental hypercapnia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 279, R617–R628.
- Cutz, E., Jackson, A., 1999. Neuroepithelial bodies as airway oxygen sensors. *Respir. Physiol.* 115, 201–214.
- Dejours, P., 1973. Problems of control of breathing in fishes. In: Bolis, L., Schmidt-Nielsen, K., Maddrell, S.H.P. (Eds.), *Comparative Physiology: Locomotion, Respiration, Transport, and Blood*. North Holland/American Elsevier, Amsterdam New York, pp. 117–133.
- Delaney, R., Lahiri, S., Fishman, A., 1974. Aestivation of the African lungfish *Protopterus aethiopicus*: cardiovascular and respiratory functions. *J. Exp. Biol.* 61, 111–128.
- Duarte, C.M., Hendriks, I.E., Moore, T.S., Olsen, Y.S., Steckbauer, A., Ramajo, L., Carstensen, J., Trotter, J.A., McCulloch, M., 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries Coast* 36, 221–236.
- Dunel-Erb, S., Bailly, Y., Laurent, P., 1982. Neuroepithelial cells in fish gill primary lamellae. *J. Appl. Physiol.* 53, 1342–1353.
- Ellis, R.P., Urbina, M.A., Wilson, R.W., 2017. Lessons from two high CO₂ worlds—future oceans and intensive aquaculture. *Glob. Chang. Biol.* 23, 2141–2148.

- Esbaugh, A., Perry, S.F., Bayaa, M., Georgalis, T., Nickerson, J.G., Tufts, B.L., Gilmour, K.M., 2005. Cytoplasmic carbonic anhydrase isozymes in rainbow trout *Oncorhynchus mykiss*: comparative physiology and molecular evolution. *J. Exp. Biol.* 208, 1951–1961.
- Farrell, A.P., Sobin, S.S., Randall, D.J., Crosby, S., 1980. Intralemmellar blood flow patterns in fish gills. *Am. J. Physiol.* 239, R428–R436.
- Florindo, L.H., Reid, S.G., Kalinin, A.L., Milsom, W.K., Rantin, F.T., 2004. Cardiorespiratory reflexes and aquatic surface respiration in the neotropical fish tambaqui (*Colossoma macropomum*): acute responses to hypercarbia. *J. Comp. Physiol. B* 174, 319–328.
- Frieder, C.A., Nam, S.H., Martz, T.R., Levin, L.A., 2012. High temporal and spatial variability of dissolved oxygen and pH in a nearshore California kelp forest. *Biogeosciences* 9, 3917–3930.
- Gilmour, K.M., 2001. The CO₂/pH ventilatory drive in fish. *Comp. Biochem. Physiol. A* 130, 219–240.
- Gilmour, K.M., Perry, S.F., 1996. The effects of metabolic acid-base disturbances and elevated catecholamines on the acid-base disequilibrium in the arterial blood of rainbow trout. *J. Exp. Zool.* 274, 281–290.
- Gilmour, K.M., Perry, S.F., 2007. Branchial chemoreceptor regulation of cardiorespiratory function. In: Hara, T.J., Zielinski, B. (Eds.), *Sensory Systems Neuroscience*. Academic press, pp. 97–151.
- Gilmour, K.M., Milsom, W.K., Rantin, F.T., Reid, S.G., Perry, S.F., 2005. Cardiorespiratory responses to hypercarbia in tambaqui (*Colossoma macropomum*): chemoreceptor orientation and specificity. *J. Exp. Biol.* 208, 1095–1107.
- Gilmour, K.M., Bayaa, M., Kenney, L., McNeill, B., Perry, S.F., 2007. Type IV carbonic anhydrase is present in the gills of spiny dogfish (*Squalus acanthias*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R556–R567.
- Graham, J.B., 1997. *Air-Breathing Fishes: Evolution, Diversity, and Adaptation*. Academic Press, San Diego.
- Graham, M.S., Turner, J.D., Wood, C.M., 1990. Control of ventilation in the hypercapnic skate, *Raja ocellata*: I. Blood and extradural fluid chemistry. *Respir. Physiol.* 80, 259–277.
- Greene, C.W., 1902. Contributions to the physiology of the California hagfish, *Polistotrema stouti*—II. The absence of regulative nerves for the systemic heart. *Am. J. Physiol.* 6, 318–324.
- Guyenet, P., 2012. How does CO₂ activate the neurons of the retrotrapezoid nucleus? *J. Physiol.* 590, 2183–2184.
- Hedrick, M., Bursleson, M., Jones, D., Milsom, W., 1991. An examination of central chemosensitivity in an air-breathing fish (*Amia calva*). *J. Exp. Biol.* 155, 165–174.
- Heisler, N., Toews, D.P., Holetson, G.F., 1988. Regulation of ventilation and acid-base status in the elasmobranch *Scyliorhinus stellaris* during hyperoxia induced hypercapnia. *Respir. Physiol.* 71, 227–246.
- Hess, K.C., Jones, B.H., Marquez, B., Chen, Y., Ord, T.S., Kamenetsky, M., Miyamoto, C., Zippin, J.H., Kopf, G.S., Suarez, S.S., et al., 2005. The “soluble” adenylyl cyclase in sperm mediates multiple signaling events required for fertilization. *Dev. Cell* 9, 249–259.
- Hockman, D., Burns, A.J., Schlosser, G., Gates, K.P., Jevans, B., Mongera, A., Fisher, S., Unlu, G., Knapik, E.W., Kaufman, C.K., et al., 2017. Evolution of the hypoxia-sensitive cells involved in amniote respiratory reflexes. *Elife* 6, e21231. <https://doi.org/10.7554/eLife.21231>.
- Hoffman, M., Harris, M.B., Taylor, B.E., 2009. Characterization and validation of aerial respiration and central CO₂ chemosensitivity in the Alaska blackfish, *Dallia pectoralis*. *FASEB J.* 23 (Suppl. 1), 598.516–598.516.
- Hofmann, G.E., Smith, J.E., Johnson, K.S., Send, U., Levin, L.A., Micheli, F., Paytan, A., Price, N.N., Peterson, B., Takeshita, Y., et al., 2011. High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PLoS ONE* 6, e28983.

- Hughes, G.M., 1966. The dimensions of fish gills in relation to their function. *J. Exp. Biol.* 45, 177–195.
- Hughes, G.M., Morgan, M., 1973. The structure of fish gills in relation to their respiratory function. *Biol. Rev.* 48, 419–475.
- Hughes, G.M., Shelton, G., 1962. Respiratory mechanisms and their nervous control in fish. *Adv. Comp. Physiol. Biochem.* 1, 275–364.
- Ichijo, Y., Mochimaru, Y., Azuma, M., Satou, K., Negishi, J., Nakakura, T., Oshima, N., Mogi, C., Sato, K., Matsuda, K., et al., 2016. Two zebrafish G2A homologs activate multiple intracellular signaling pathways in acidic environment. *Biochem. Biophys. Res. Commun.* 469, 81–86.
- Iversen, N.K., McKenzie, D.J., Malte, H., Wang, T., 2010. Reflex bradycardia does not influence oxygen consumption during hypoxia in the European eel (*Anguilla anguilla*). *J. Comp. Physiol. B* 180, 495–502.
- Janssen, R.G., Randall, D.J., 1975. The effects of changes in pH and P_{CO_2} in blood and water on breathing in rainbow trout, *Salmo gairdneri*. *Respir. Physiol.* 25, 235–245.
- Jesse, M.J., Shub, C., Fishman, A.P., 1967. Lung and gill ventilation of the African lung fish. *Resp. Physiol.* 3, 267–287.
- Johansen, K., 1966. Air breathing in the teleost *Symbranchus marmoratus*. *Comp. Biochem. Physiol.* 18, 383–395.
- Johansen, K., 1970. Air breathing in fishes. In: Hoar, W.S., Randall, D.J. (Eds.), *Fish Physiology*, vol. 4. Academic Press, New York, pp. 361–411.
- Johansen, K., Lenfant, C., 1968. Respiration in the African lungfish, *Protopterus aethiopicus*. II. Control of breathing. *J. Exp. Biol.* 49, 453–468.
- Johansen, K., Lenfant, C., Grigg, G.C., 1967. Respiratory control in the lungfish, *Neoceratodus forsteri* (Kreffl). *Comp. Biochem. Physiol.* 20, 835–854.
- Jones, D.R., Schwarzfeld, T., 1974. The oxygen cost to the metabolism and efficiency of breathing in trout (*Salmo gairdneri*). *Respir. Physiol.* 21, 241–253.
- Jonz, M.G., 2018. Insights into the evolution of polymodal chemoreceptors. *Acta Histochem.* 120, 623–629.
- Jonz, M.G., Nurse, C.A., 2003. Neuroepithelial cells and associated innervation of the zebrafish gill: a confocal immunofluorescence study. *J. Comp. Neurol.* 461, 1–17.
- Jonz, M.G., Nurse, C.A., 2005. Development of oxygen sensing in the gills of zebrafish. *J. Exp. Biol.* 208, 1537–1549.
- Jonz, M.G., Nurse, C.A., 2008. New developments on gill innervation: insights from a model vertebrate. *J. Exp. Biol.* 211, 2371–2378.
- Jonz, M.G., Fearon, I.M., Nurse, C.A., 2004. Neuroepithelial oxygen chemoreceptors of the zebrafish gill. *J. Physiol.* 560, 737–752.
- Jonz, M.G., Zachar, P.C., Da Fonte, D.F., Mierzwa, A.S., 2015. Peripheral chemoreceptors in fish: a brief history and a look ahead. *Comp. Biochem. Physiol. A* 186, 27–38.
- Jonz, M.G., Buck, L.T., Perry, S.F., Schwerte, T., Zaccane, G., 2016. Sensing and surviving hypoxia in vertebrates. *Ann. N. Y. Acad. Sci.* 1365, 43–58.
- Kline, D.I., Teneva, L., Schneider, K., Miard, T., Chai, A., Marker, M., Headley, K., Opdyke, B., Nash, M., Valetich, M., et al., 2012. A short-term in situ CO_2 enrichment experiment on Heron Island (GBR). *Sci. Rep.* 2, 413.
- Kobayashi, M., Buck, J., Levin, L.R., 2004. Conservation of functional domain structure in bicarbonate-regulated “soluble” adenylyl cyclases in bacteria and eukaryotes. *Dev. Genes Evol.* 214, 503–509.
- Koudrina, N., 2017. The Role of TASK-2 Channels in CO_2 Sensing in Zebrafish (*Danio rerio*). Masters thesis. Department of Biology, Faculty of Science, University of Ottawa, Canada.
- Linder, J.U., Schultz, J.E., 2003. The class III adenylyl cyclases: multi-purpose signalling modules. *Cell. Signal.* 15, 1081–1089.

- Liu, J.-P., Nakakura, T., Tomura, H., Tobo, M., Mogi, C., Wang, J.-Q., He, X.-D., Takano, M., Damirin, A., Komachi, M., et al., 2010. Each one of certain histidine residues in G-protein-coupled receptor GPR4 is critical for extracellular proton-induced stimulation of multiple G-protein-signaling pathways. *Pharmacol. Res.* 61, 499–505.
- Lomholt, J.P., Johansen, K., 1974. Control of breathing in *Amphipnous cuchia*, an amphibious fish. *Respir. Physiol.* 21 (3), 325–340.
- Lopes, J.M., De Lima Boijink, C., Florindo, L.H., Leite, C.A.C., Kalinin, A.L., Milsom, W.K., Rantin, F.T., 2010. Hypoxic cardiorespiratory reflexes in the facultative air-breathing fish jeju (*Hoplerthrinus unitaeniatus*): role of branchial O₂ chemoreceptors. *J. Comp. Physiol. B* 180, 797–811.
- Ludwig, M.-G., Vanek, M., Guerini, D., Gasser, J.A., Jones, C.E., Junker, U., Hofstetter, H., Wolf, R.M., Seuwen, K., 2003. Proton-sensing G-protein-coupled receptors. *Nature* 425, 93–98.
- Maren, T.H., 1967. Carbonic anhydrase: chemistry, physiology and inhibition. *Physiol. Rev.* 47, 598–781.
- McKendry, J.E., Milsom, W.K., Perry, S.F., 2001. Branchial CO₂ receptors and cardiorespiratory adjustments during hypercapnia in Pacific spiny dogfish (*Squalus acanthias*). *J. Exp. Biol.* 204, 1519–1527.
- McKenzie, D.J., Aota, S., Randall, D.J., 1991. Ventilatory and cardiovascular responses to blood pH, plasma PCO₂, blood O₂ content, and catecholamines in an air-breathing fish, the bowfin (*Amia calva*). *Physiol. Zool.* 64, 432–450.
- McKenzie, D.J., Randall, D.J., Lin, H., Aota, S., 1993. Effects of changes in plasma pH, CO₂ and ammonia on ventilation in trout. *Fish Physiol. Biochem.* 10, 507–515.
- McKenzie, D.J., Skov, P.V., Taylor, E.W., Wang, T., Steffensen, J.F., 2009. Abolition of reflex bradycardia by cardiac vagotomy has no effect on the regulation of oxygen uptake by Atlantic cod in progressive hypoxia. *Comp. Biochem. Physiol. A* 153, 332–338.
- McMahon, B.R., Burggren, W.W., 1987. Respiratory physiology of intestinal air breathing in the teleost fish *Misgurnus anguillicaudatus*. *J. Exp. Biol.* 133 (1), 371393.
- Miller, S., Pollack, J., Bradshaw, J., Kumai, Y., Perry, S.F., 2014. Cardiac responses to hypercapnia in larval zebrafish (*Danio rerio*): the links between CO₂ chemoreception, catecholamines and carbonic anhydrase. *J. Exp. Biol.* 217, 3569–3578.
- Milsom, W.K., 2012. New insights into gill chemoreception: receptor distribution and roles in water and air breathing fish. *Respir. Physiol. Neurobiol.* 184, 326–339.
- Milsom, W.K., Burleson, M.L., 2007. Peripheral arterial chemoreceptors and the evolution of the carotid body. *Respir. Physiol. Neurobiol.* 157, 4–11.
- Milsom, W.K., Reid, S.G., Rantin, F.T., Sundin, L., 2002. Extrabranhial chemoreceptors involved in respiratory reflexes in the neotropical fish *Colossoma macropomum* (the tambaqui). *J. Exp. Biol.* 205, 1765–1774.
- Milsom, W.K., Abe, A.S., Tattersall, G.J., 2004. Evolutionary trends in airway CO₂/pH chemoreception. *Resp. Physiol. Neurobiol.* 144, 191–202.
- Mochimaru, Y., Azuma, M., Oshima, N., Ichijo, Y., Satou, K., Matsuda, K., Asaoka, Y., Nishina, H., Nakakura, T., Mogi, C., et al., 2015. Extracellular acidification activates ovarian cancer G-protein-coupled receptor 1 and GPR4 homologs of zebra fish. *Biochem. Biophys. Res. Commun.* 457, 493–499.
- Nomura, M., Beltrán, C., Darszon, A., Vacquier, V.D., 2005. A soluble adenylyl cyclase from sea urchin spermatozoa. *Gene* 353, 231–238.
- Peña-Munzenmayer, G., Niemeyer, M.I., Sepulveda, F.V., Cid, L.P., 2014. Zebrafish and mouse TASK-2 K⁺ channels are inhibited by increased CO₂ and intracellular acidification. *Pflugers Arch.* 466, 1317–1327.
- Perry, S.F., Abdallah, S.J., 2012. Mechanisms and consequences of carbon dioxide sensing in fish. *Resp. Physiol. Neurobiol.* 184, 309–315.

- Perry, S.F., Desforges, P.R., 2006. Does bradycardia or hypertension enhance gas transfer in rainbow trout (*Oncorhynchus mykiss*) exposed to hypoxia or hypercarbia? *Comp. Biochem. Physiol. A* 144, 163–172.
- Perry, S.F., Gilmour, K.M., 1996. Consequences of catecholamine release on ventilation and blood oxygen transport during hypoxia and hypercapnia in an elasmobranch (*Squalus acanthias*) and a teleost (*Oncorhynchus mykiss*). *J. Exp. Biol.* 199, 2105–2118.
- Perry, S.F., Gilmour, K.M., 2006. Acid-base balance and CO₂ excretion in fish: unanswered questions and emerging models. *Respir. Physiol. Neurobiol.* 154, 199–215.
- Perry, S.F., McKendry, J.E., 2001. The relative roles of external and internal CO₂ versus H⁺ in eliciting the cardiorespiratory responses of *Salmo salar* and *Squalus acanthias* to hypercarbia. *J. Exp. Biol.* 204, 3963–3971.
- Perry, S.F., Reid, S.G., 2002. Cardiorespiratory adjustments during hypercarbia in rainbow trout (*Oncorhynchus mykiss*) are initiated by external CO₂ receptors on the first gill arch. *J. Exp. Biol.* 205, 3357–3365.
- Perry, S.F., Tzaneva, V., 2016. The sensing of respiratory gases in fish: mechanisms and signalling pathways. *Respir. Physiol. Neurobiol.* 224, 71–79.
- Perry, S.F., Wood, C.M., 1989. Control and coordination of gas transfer in fishes. *Can. J. Zool.* 67, 2961–2970.
- Perry, S.F., Malone, S., Ewing, D., 1987. Hypercapnic acidosis in the rainbow trout (*Salmo gairdneri*). I. Branchial ionic fluxes and blood acid-base status. *Can. J. Zool.* 65, 888–895.
- Perry, S.F., Fritzsche, R., Hoagland, T., Duff, D.W., Olson, K.R., 1999. The control of blood pressure during external hypercapnia in the rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* 202, 2177–2190.
- Perry, S.F., Gilmour, K.M., Swenson, E.R., Vulesevic, B., Chew, S.F., Ip, Y.K., 2005. An investigation of the role of carbonic anhydrase in aquatic and aerial gas transfer in the African lungfish (*Protopterus dolloi*). *J. Exp. Biol.* 208, 3805–3815.
- Perry, S.F., Euverman, R., Wang, T., Loong, A.M., Chew, S.F., Ip, Y.K., Gilmour, K.M., 2008. Control of breathing in African lungfish (*Protopterus dolloi*): a comparison of aquatic and cocooned (terrestrialized) animals. *Resp. Physiol. Neurobiol.* 160, 8–17.
- Perry, S.F., Jonz, M.G., Gilmour, K.M., 2009a. Oxygen sensing and the hypoxic ventilatory response. In: Richards, J.G., Brauner, C.J., Farrell, A.P. (Eds.), *Hypoxia*. Academic Press, Amsterdam, pp. 193–251.
- Perry, S.F., Vulesevic, B., Braun, M., Gilmour, K.M., 2009b. Ventilation in Pacific hagfish (*Eptatretus stoutii*) during exposure to acute hypoxia or hypercapnia. *Respir. Physiol. Neurobiol.* 167, 227–234.
- Porteus, C.S., Brink, D.L., Milsom, W.K., 2012. Neurotransmitter profiles in fish gills: putative gill oxygen chemoreceptors. *Respir. Physiol. Neurobiol.* 184, 316–325.
- Porteus, C.S., Pollack, J., Tzaneva, V., Kwong, R.W., Kumai, Y., Abdallah, S.J., Zacccone, G., Lauriano, E.R., Milsom, W.K., Perry, S.F., 2015. A role for nitric oxide in the control of breathing in zebrafish (*Danio rerio*). *J. Exp. Biol.* 218, 3746–3753.
- Porteus, C.S., Wright, P.A., Milsom, W.K., 2014. Characterisation of putative oxygen chemoreceptors in bowfin (*Amia calva*). *J. Exp. Biol.* 217, 1269–1277.
- Putnam, R.W., Filosa, J.A., Ritucci, N.A., 2004. Cellular mechanisms involved in CO₂ and acid signaling in chemosensitive neurons. *Am. J. Physiol. Cell Physiol.* 287, C1493–C1526.
- Qin, Z., Lewis, J., Perry, S.F., 2010. Zebrafish (*Danio rerio*) gill neuroepithelial cells are sensitive chemoreceptors for environmental CO₂. *J. Physiol. (Lond.)* 588, 861–872.
- Randall, D.J., 1982. The control of respiration and circulation in fish during exercise and hypoxia. *J. Exp. Biol.* 100, 275–288.
- Randall, D.J., Daxboeck, C., 1984. Oxygen and carbon dioxide transfer across fish gills. In: Hoar, W.S., Randall, D.J. (Eds.), *Fish Physiology*. Academic Press, New York, pp. 263–314.

- Randall, D.J., Heisler, N., Drees, F., 1976. Ventilatory response to hypercapnia in the larger spotted dogfish *Scyliorhinus stellaris*. *Am. J. Physiol.* 230, 590–594.
- Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., 2005. Ocean Acidification Due to Increasing Atmospheric Carbon Dioxide. The Royal Society Policy Document 12.05 Cardiff. Clyvedon Press, UK.
- Regan, K.S., Jonz, M.G., Wright, P.A., 2011. Neuroepithelial cells and the hypoxia emersion response in the amphibious fish *Kryptolebias marmoratus*. *J. Exp. Biol.* 214, 2560–2568.
- Reid, S.G., Sundin, L., Kalinin, A.L., Rantin, F.T., Milsom, W.K., 2000. Cardiovascular and respiratory reflexes in the tropical fish, traíra (*Hoplias malabaricus*): CO₂/pH chemoresponses. *Respir. Physiol.* 120, 47–59.
- Roa, J.N., Tresguerres, M., 2016. Soluble adenylyl cyclase is an acid-base sensor in epithelial base-secreting cells. *Am. J. Physiol. Cell Physiol.* 311, C340–C349.
- Roa, J.N., Tresguerres, M., 2017. Bicarbonate-sensing soluble adenylyl cyclase is present in the cell cytoplasm and nucleus of multiple shark tissues. *Physiol. Rep.* 24, e13090.
- Roa, J.N., Munévar, C.L., Tresguerres, M., 2014. Feeding induces translocation of vacuolar proton ATPase and pendrin to the membrane of leopard shark (*Triakis semifasciata*) mitochondrion-rich gill cells. *Comp. Biochem. Physiol. A* 174, 29–37.
- Rovainen, C.M., 1977. Neural control of ventilation in the lamprey. *Fed. Proc.* 36, 2386–2389.
- Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., Wanninkhof, R., Wong, C.S., Wallace, D.W.R., Tilbrook, B., et al., 2004. The oceanic sink for anthropogenic CO₂. *Science* 305, 367–371.
- Salmerón, C. and Tresguerres, M., Molecular and biochemical characterization of soluble adenylyl cyclase from a bony fish, the rainbow trout *Oncorhynchus mykiss* (unpublished).
- Saltys, H.A., Jonz, M.G., Nurse, C.A., 2006. Comparative study of gill neuroepithelial cells and their innervation in teleosts and *Xenopus* tadpoles. *Cell Tissue Res.* 323, 1–10.
- Sanchez, A., Glass, M., 2001. Effects of environmental hypercapnia on pulmonary ventilation of the South American lungfish. *J. Fish Biol.* 58, 1181–1189.
- Shelton, G., Jones, D.R., Milsom, W.K., 1986. Control of breathing in ectothermic vertebrates. In: Cherniack, N.S., Widdicombe, J.G. (Eds.), *Handbook of Physiology, Section 3: The Respiratory System, Vol. II: Control of Breathing, part 2*. American Physiological Society, Bethesda, MD, pp. 857–909.
- Short, S., Taylor, E.W., Butler, P.J., 1979. The effectiveness of oxygen transfer during normoxia and hypoxia in the dogfish (*Scyliorhinus canicula* L.) before and after cardiac vagotomy. *J. Comp. Physiol. B* 132, 289–295.
- Smatresk, N.J., 1988. Control of the respiratory mode in air-breathing fishes. *Can. J. Zool.* 66, 144–151.
- Smatresk, N.J., Cameron, J.N., 1982. Respiration and acid-base physiology of the spotted gar, a bimodal breather. II. Responses to temperature change and hypercapnia. *J. Exp. Biol.* 96, 281–293.
- Smith, F.M., Jones, D.R., 1982. The effect of changes in blood oxygen carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* 97, 325–334.
- Somero, G.N., 1986. Proteins, osmolytes, and fitness of internal milieu for protein function. *Am. J. Physiol.* 251, R197–R213.
- Soncini, R., Glass, M.L., 2000. Oxygen and acid-base status related drives to gill ventilation in carp. *J. Fish Biol.* 56, 528–541.
- Steebhorn, C., 2014. Structure, mechanism, and regulation of soluble adenylyl cyclases—similarities and differences to transmembrane adenylyl cyclases. *Biochim. Biophys. Acta* 1842, 2535–2547.
- Sun, X., Yang, L.V., Tieg, B.C., Arend, L.J., McGraw, D.W., Penn, R.B., Petrovic, S., 2010. Deletion of the pH sensor GPR4 decreases renal acid excretion. *J. Am. Soc. Nephrol.* 21, 1745–1755.

- Sundin, L., Reid, S.G., Rantin, F.T., Milsom, W.K., 2000. Branchial receptors and cardiorespiratory reflexes in the neotropical fish, Tambaqui (*Colossoma macropomum*). J. Exp. Biol. 203, 1225–1239.
- Taylor, E.W., Barrett, D.J., 1985. Evidence of a respiratory role for the hypoxic bradycardia in the dogfish *Scyliorhinus canicula* L. Comp. Biochem. Physiol. A 80, 99–102.
- Taylor, J.R., Mager, E.M., Grosell, M., 2010. Basolateral NBCe1 plays a rate-limiting role in transepithelial intestinal HCO₃⁻ secretion, contributing to marine fish osmoregulation. J. Exp. Biol. 213, 459–468.
- Thomsen, M.T., Wang, T., Milsom, W.K., Bayley, M., 2017. Lactate provides a strong pH-independent ventilatory signal in the facultative air-breathing teleost *Pangasianodon hypophthalmus*. Sci. Rep. 7 (1), 6378.
- Tresguerres, M., 2014. sAC from aquatic organisms as a model to study the evolution of acid/base sensing. Biochim. Biophys. Acta 1842 (12 pt. B), 2629–2635.
- Tresguerres, M., 2016. Novel and potential physiological roles of vacuolar-type H⁺-ATPase in marine organisms. J. Exp. Biol. 219, 2088–2097.
- Tresguerres, M., Salmeron, C., 2018. Molecular, enzymatic and cellular characterization of soluble adenylyl cyclase from aquatic animals. Methods Enzymol. 605, 525–549.
- Tresguerres, M., Katoh, F., Fenton, H., Jasinska, E., Goss, G.G., 2005. Regulation of branchial NHE2, Na⁺/K⁺-ATPase and H⁺-ATPase in response to acid and base infusions in the dogfish (*Squalus acanthias*). J. Exp. Biol. 208, 345–354.
- Tresguerres, M., Parks, S.K., Katoh, F., Goss, G.G., 2006. Microtubule-dependent relocation of branchial V-H⁺-ATPase to the basolateral membrane in the Pacific spiny dogfish (*Squalus acanthias*): a role in base secretion. J. Exp. Biol. 209, 599–609.
- Tresguerres, M., Parks, S.K., Wood, C.M., Goss, G.G., 2007. V-H⁺-ATPase translocation during blood alkalosis in dogfish gills: interaction with carbonic anhydrase and involvement in the post-feeding alkaline tide. Am. J. Physiol. Reg. Int. Comp. Physiol. 292, 2012–2019.
- Tresguerres, M., Buck, J., Levin, L.R., 2010a. Physiological carbon dioxide, bicarbonate, and pH sensing. Pflugers Arch. 460, 953–964.
- Tresguerres, M., Levin, L.R., Buck, J., Grosell, M., 2010b. Modulation of NaCl absorption by [HCO₃⁻] in the marine teleost intestine is mediated by soluble adenylyl cyclase. Am. J. Physiol. Regul. Integr. Comp. Physiol. 299, R62–R71.
- Tresguerres, M., Parks, S.K., Salazar, E., Levin, L.R., Goss, G.G., Buck, J., 2010c. Bicarbonate-sensing soluble adenylyl cyclase is an essential sensor for acid/base homeostasis. Proc. Natl. Acad. Sci. U.S.A. 107, 442–447.
- Tresguerres, M., Levin, L.R., Buck, J., 2011. Intracellular cAMP signaling by soluble adenylyl cyclase. Kidney Int. 79, 1277–1288.
- Tresguerres, M., Barott, K.L., Barron, M.E., Roa, J.N., 2014. Established and potential physiological roles of bicarbonate-sensing soluble adenylyl cyclase (sAC) in aquatic animals. J. Exp. Biol. 217, 663–672.
- Truchot, J.P., Duhamel-Jouve, A., 1980. Oxygen and carbon dioxide in the marine intertidal environment: diurnal and tidal changes in rockpools. Respir. Physiol. 39, 241–254.
- Tufts, B.L., Perry, S.F., 1998. Carbon dioxide transport and excretion. In: Perry, S.F., Tufts, B.L. (Eds.), Fish Respiration, vol. 17. Academic Press, New York, pp. 229–281.
- Tuong, D.D., Borowiec, B., Clifford, A.M., Filogonio, R., Somo, D., Huong, D.T.T., Phuong, N.T., Wang, T., Bayley, M., Milsom, W.K., 2018. Ventilatory responses of the clown knifefish, *Chitala ornata*, to hypercarbia and hypercapnia. J. Comp. Physiol. B 1–9.
- Tuong, D.D., Huong, D.T.T., Phuong, N.T., Bayley, M., Milsom, W.K., 2019. Ventilatory responses of the clown knifefish *Chitala ornata* to arterial hypercapnia remain after gill denervation. J. Exp. Biol. under review.

- Tzaneva, V., Perry, S.F., 2010. Control of breathing in goldfish (*Carassius auratus*) experiencing thermally induced gill remodelling. *J. Exp. Biol.* 213, 3666–3675.
- von Schalburg, K.R., Gowen, B.E., Leong, J.S., Rondeau, E.B., Davidson, W.S., Koop, B.F., 2018. Subcellular localization and characterization of estrogenic pathway regulators and mediators in Atlantic salmon spermatozoal cells. *Histochem. Cell Biol.* 149, 75–96.
- Vulesevic, B., Perry, S.F., 2006. Developmental plasticity of ventilatory control in zebrafish, *Danio rerio*. *Respir. Physiol. Neurobiol.* 154, 396–405.
- Vulesevic, B., McNeill, B., Perry, S.F., 2006. Chemoreceptor plasticity and respiratory acclimation in the zebrafish, *Danio rerio*. *J. Exp. Biol.* 209, 1261–1273.
- Wilson, R., Harris, M., Remmers, J., Perry, S., 2000. Evolution of air-breathing and central CO₂/H⁺ respiratory chemosensitivity: new insights from an old fish? *J. Exp. Biol.* 203, 3505–3512.
- Wilson, R.W., Wilson, J.M., Grosell, M., 2002. Intestinal bicarbonate secretion by marine fish: why and how? *Biochim. Biophys. Acta* 1566, 182–193.
- Wilson, C.M., Stecyk, J.A.W., Couturier, C.S., Nilsson, G.E., Farrell, A.P., 2013. Phylogeny and effects of anoxia on hyperpolarization-activated cyclic nucleotide-gated channel gene expression in the heart of a primitive chordate, the Pacific hagfish (*Eptatretus stoutii*). *J. Exp. Biol.* 216, 4462–447.
- Wilson, C.M., Roa, J.N., Cox, G.K., Tresguerres, M., Farrell, A.P., 2016. Introducing a novel mechanism to control heart rate in the ancestral pacific hagfish. *J. Exp. Biol.* 219, 3227–3236.
- Wood, C.M., 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. *J. Exp. Biol.* 160, 285–308.
- Wood, C.M., Munger, R.S., 1994. Carbonic anhydrase injection provides evidence for the role of blood acid-base status in stimulating ventilation after exhaustive exercise in rainbow trout. *J. Exp. Biol.* 194, 225–253.
- Wood, C.M., Perry, S.F., 1985. Respiratory, circulatory, and metabolic adjustments to exercise in fish. In: Gilles, R. (Ed.), *Circulation, Respiration, Metabolism*. Springer-Verlag, Berlin, pp. 2–22.
- Wood, C.M., Turner, J.D., Munger, S., Graham, M.S., 1990. Control of ventilation in the hypercapnic skate *Raja ocellata*: II. Cerebrospinal fluid and intracellular pH in the brain and other tissues. *Respir. Physiol.* 80, 279–297.
- Wood, C.M., Kajimura, M., Mommsen, T.P., Walsh, P.J., 2005. Alkaline tide and nitrogen conservation after feeding in an elasmobranch (*Squalus acanthias*). *J. Exp. Biol.* 208, 2693–2705.
- Wood, C.M., Schultz, A.G., Munger, R.S., Walsh, P.J., 2009. Using omeprazole to link the components of the post-prandial alkaline tide in the spiny dogfish, *Squalus acanthias*. *J. Exp. Biol.* 212, 684–692.
- Wyder, L., Suply, T., Ricoux, B., Billy, E., Schnell, C., Baumgarten, B.U., Maira, S.M., Koelbing, C., Ferretti, M., Kinzel, B., et al., 2011. Reduced pathological angiogenesis and tumor growth in mice lacking GPR4, a proton sensing receptor. *Angiogenesis* 14, 533–544.
- Yang, L.V., Radu, C.G., Roy, M., Lee, S., McLaughlin, J., Teitell, M.A., Iruela-Arispe, M.L., Witte, O.N., 2007. Vascular abnormalities in mice deficient for the G protein-coupled receptor GPR4 that functions as a pH sensor. *Mol. Cell. Biol.* 27, 1334–1347.
- Zaccone, G., Lauweryns, J.M., Fasulo, S., Tagliaferro, G., Ainis, L., Licata, A., 1992. Immunocytochemical localization of serotonin and neuropeptides in the neuroendocrine paraneurons of teleost and lungfish gills. *Acta Zool.* 73, 177–183.
- Zaccone, G., Mauceri, A., Fasulo, S., 2006. Neuropeptides and nitric oxide synthase in the gill and the air-breathing organs of fishes. *J. Exp. Zool. A Comp. Exp. Biol.* 305, 428–439.

- Zacccone, G., Lauriano, E.R., Kuciel, M., Capillo, G., Pergolizzi, S., Alesci, A., Ishimatsu, A., Ip, Y.K., Icardo, J.M., 2017. Identification and distribution of neuronal nitric oxide synthase and neurochemical markers in the neuroepithelial cells of the gill and the skin in the giant mudskipper, *Periophthalmodon schlosseri*. *Zoology (Jena)* 125, 41–52.
- Zachar, P.C., Jonz, M.G., 2012. Neuroepithelial cells of the gill and their role in oxygen sensing. *Respir. Physiol. Neurobiol.* 184, 301–308.
- Zhang, L., Nurse, C.A., Jonz, M.G., Wood, C.M., 2011. Ammonia sensing by neuroepithelial cells and ventilatory responses to ammonia in rainbow trout. *J. Exp. Biol.* 214, 2678–2689.
- Zhou, Y., Skelton, L.A., Xu, L., Chandler, M.P., Berthiaume, J.M., Boron, W.F., 2016. Role of receptor protein tyrosine phosphatase γ in sensing extracellular CO_2 and HCO_3^- . *J. Am. Soc. Nephrol.* 27, 2616–2621.
- Zipin, J.H., Farrell, J., Huron, D., Kamenetsky, M., Hess, K.C., Fischman, D.A., Levin, L.R., Buck, J., 2004. Bicarbonate-responsive “soluble” adenylyl cyclase defines a nuclear cAMP microdomain. *J. Cell Biol.* 164, 527–534.