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Review

The CO_2/pH ventilatory drive in fish ☆

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Abstract

That ventilation in fish is driven by O_2 has long been accepted. The O_2 ventilatory drive reflects the much lower capacitance of water for O_2 than for CO_2, and is mediated by O_2 receptors that are distributed throughout the gill arches and that monitor both internal and external O_2 levels. In recent years, however, evidence has amassed in support of the existence of a ventilatory drive in fish that is keyed to CO_2 and/or pH. While ventilatory responses to CO_2/pH may be mediated in part by the O_2 drive through CO_2/pH-induced changes in blood O_2 status, CO_2/pH also appear to stimulate ventilation directly. The receptors involved in this pathway are as yet unknown, but the experimental evidence available to date supports the involvement of branchial CO_2-sensitive chemoreceptors with an external orientation. Internally-oriented CO_2-sensitive chemoreceptors may also be involved, although evidence on this point remains equivocal. In the present paper, the evidence for a CO_2/pH-keyed ventilatory drive in fish will be reviewed.

Keywords: CO_2; pH; Ventilation; Gills; Fish; Chemoreceptors; O_2; Gas transfer; Hypercapnia; Hypoxia

1. Introduction

The essential function of the gas exchange system is to meet the metabolic requirements of the cells for oxygen and to remove the carbon dioxide produced by cellular metabolism. In fish, the gills are usually the primary interface for gas exchange between the animal and the aquatic environment. Given that the metabolic demands of the tissues for O_2 supply and CO_2 removal are highly variable and that large variations may occur in the environmental O_2 and CO_2 tensions, it is essential that the animal be able to sense and respond to changes in both environmental gas levels and metabolic O_2 demands. Ventilatory adjustments are one means through which gas exchange can be matched to the demands of oxidative metabolism.

Owing to the low capacitance of water for O_2 relative to that for CO_2, fish exhibit high convection requirements for O_2 uptake (ventilation volume per unit O_2 uptake), and correspondingly low arterial CO_2 tensions and high arterial pH (relative to an equivalent air-breather). It is widely accepted that, following also from the difference

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in water O_2 and CO_2 capacitance values, ventilation in fish is primarily keyed to O_2 rather than to CO_2 and/or pH, as is the case in air-breathing animals. Indeed, that CO_2 and/or pH played any role in modulating ventilation in fish was disputed for many years (see Perry and Wood, 1989; Milsom, 1995a,b). The O_2-linked ventilatory drive in fish appears to be mediated by branchial O_2-sensitive chemoreceptors that monitor both the internal (blood) and external (water) environment, allowing modulation of ventilation in response to both internal O_2 demands and external O_2 supply. While there is no doubt that ventilation in fish is driven by requirements for O_2 uptake, increasingly, experimental evidence suggests that a significant CO_2 and/or pH-keyed ventilatory drive also exists in fish. However, the relative importance of a CO_2/pH drive vs. the O_2-linked ventilatory drive remains to be ascertained. The identity and location of the receptors that mediate the CO_2/pH-ventilatory drive in fish also remain unknown, and in addition, the advantage of a hyperventilatory response to elevated water CO_2 (termed hypercarbia or external hypercapnia; the latter will be used here) in fish is not immediately obvious.

The objective of the present review is to summarise and examine the evidence for a CO_2/pH-keyed ventilatory drive in fish. As the regulation of ventilation in fish has been the subject of a number of detailed reviews (Shelton et al., 1986; Smatresk, 1988, 1990, 1994; Perry and Wood, 1989; Burleson et al., 1992; Fritsche and Nilsson, 1993; Burleson, 1995; Milsom, 1995a,b; Taylor et al., 1999), emphasis in the present review will be placed on recent experimental data that support a role for CO_2/pH in controlling ventilation in fish and also provide information on the identity and location of the receptors involved. For simplicity, the discussion will focus primarily on water-breathing fish and unless otherwise stated, ventilation amplitude (or stroke volume) and frequency refer to ventilation of the gills. To provide a context within which data on a CO_2/pH-mediated ventilatory drive may be evaluated, the O_2-keyed ventilatory drive in fish will also be reviewed. Finally, the functional significance of a CO_2/pH-mediated ventilatory drive in fish will be discussed in an attempt to address the question of why fish should hyperventilate in response to environmental hypercapnia.

2. Ventilation in fish is driven by O_2

There is abundant evidence that ventilation in fish is driven by O_2. Ventilatory responses to both hypoxaemia and aquatic hypoxia or hyperoxia have been well documented and indicate that fish sense and respond to both internal and external O_2 levels. Exposure to environmental hypoxia typically elicits significant increases in stroke volume (or ventilation amplitude) and/or ventilation frequency (Table 1), or gape in obligate ram ventilators (Bushnell et al., 1990; Bushnell and Brill, 1991), resulting in an elevation of ventilation volume (e.g. Forgue et al., 1989; Aota et al., 1990; Kinkead and Perry, 1990, 1991; Bushnell et al., 1990; Glass et al., 1990; Kinkead et al., 1991; Bushnell and Brill, 1991; Rantin et al., 1992; Soncini and Glass, 2000). Additionally, environmental hypoxia promotes continuous ventilation in species with intermittent ventilation under resting normoxic conditions (e.g. sturgeon, Nonnotte et al., 1993; Maxime et al., 1995; eel, Smith et al., 1983), air-breathing in species with bimodal respiration (e.g. gar, Smatresk et al., 1986; Smatresk, 1986; Burleson et al., 1998; Australian lungfish, Fritsche et al., 1993a; bowfin, McKenzie et al., 1991; Hedrick et al., 1991; Hedrick and Jones, 1999; armoured catfish, Brauner et al., 1995) and aquatic surface respiration in tambaqui (Sundin et al., 2000). The hyperventilation induced by aquatic hypoxia typically consists of large changes in stroke volume coupled to more modest increases of ventilation frequency (Table 1), a strategy that would be expected to elevate ventilation volume in an energetically efficient manner (Perry and Wood, 1989). Ventilatory responses opposite to those elicited by aquatic hypoxia are observed when fish are exposed to elevated water O_2 tensions (hyperoxia). Typically, ventilation volume is greatly reduced during hyperoxic exposure (e.g. Wood and Jackson, 1980; Wilkes et al., 1981; Thomas et al., 1983; Heisler et al., 1988; Takeda, 1990; Kinkead and Perry, 1991; Soncini and Glass, 2000), owing to significant decreases in ventilation frequency and/or stroke volume (ventilation amplitude) (Table 1). In addition, hyperoxia may elicit intermittent ventilation in species that normally breathe continuously (e.g. Heisler et al., 1988; Powell and Perry, 1997; S.G. Reid, L. Sundin, T. Rantin and W.K. Milsom, unpublished observations on tambaqui).

Table 1
Data from selected experimental studies over the past 10 years[a] on changes in ventilation frequency, ventilation amplitude or stroke volume, and arterial P_{CO_2} in response to acute environmental hypoxia or hyperoxia in various fish species

Species	P_{O_2} (torr) Time	ΔV_{amp} (%)	ΔV_f (%)	ΔP_{CO_2} (%)	Reference
Hypoxia					
Siberian sturgeon,	30, 10 min	+260	+124	−37	(Maxime et al., 1995)
Acipenser baeri	60, 1 h	+90	+121	−17	(Nonnotte et al., 1993)
	40, 1 h	+110	+127	−21	(Nonnotte et al., 1993)
	20, 1h	+165	+103	−47	(Nonnotte et al., 1993)
Adriatic sturgeon,					
A. naccarii	19, 20 min	+130	+39		(McKenzie et al., 1995)
Tambaqui,					
Colossoma macropomum	10, 10 min	+88	+63		(Sundin et al., 2000)
Traira,					
Hoplias malabaricus	10, 10 min	+88	+33		(Sundin et al., 1999a)
	35, unknown	+375†	+40		(Rantin et al., 1992)
Trairão,					
H. lacerdae	35, unknown	+163†	+160		(Rantin et al., 1992)
Spiny dogfish,					
Squalus acanthias	35, 30 min	+93	+16	−33	(Perry and Gilmour, 1996)
Bowfin,					
Amia calva	35, >1 h	+66	+67	−53	(Hedrick et al., 1991)
Carp,					
Cyprinus carpio	110, 1h	+9†*	+41	−26	(Glass et al., 1990)
	75, 1 h	+5†*	+205	−47	(Glass et al., 1990)
	97, 30 min	−59†	+179	−15*	(Soncini and Glass, 2000)
	89, 30 min	−21†*	+150		(Soncini and Glass, 2000)
Atlantic cod,					
Gadus morhua	46, 30 min	+37†	+19*		(Kinkead et al., 1991)
Rainbow trout,					
Oncorhynchus mykiss	40, 20 min	+44	+14*	−35	(Bindon et al., 1994)
	60, >30 min	+21	+18	−18	(Gilmour and Perry, 1994)
	40 ,20 min	+85	+25	−28	(Greco et al., 1995)
	72, 30 min	+179†	+8*	−16*	(Kinkead and Perry, 1990)
	45, 30 min	+240	+4*	−33	(Perry and Gilmour, 1996)
	55, 20 min	+190	+35	−49	(Perry and Thomas, 1991)
	60, 24 h		+21	−29	(Borch et al., 1993)
Hyperoxia					
Carp, *C. carpio*	285, 6 h	−2†*	−55	+141	(Takeda, 1990)
	460, 6 h	−13†*	−57	+190	(Takeda, 1990)
	245, 30 min	+62†	−63	+131	(Soncini and Glass, 2000)
Channel catfish,					
Ictalurus punctatus	250, 24 h	−13*	−8*	+31	(Burleson and Smatresk, 2000)
Spotted gar,					
Lepisosteus oculatus	500, unknown		−29	+145	(Smatresk and Cameron, 1982b)
White sucker,					
Catostomus commersoni	450, 4 h	−44†	−17*	+125	(Wilkes et al., 1981)
Rainbow trout,					
O. mykiss	500, 1h	−51†	−12*	+73	(Wood and Jackson, 1980)
	425, 1h	−59†	−26	+91	(Thomas et al., 1983)
	384, 1h	+5*	−15		(Aota and Randall, 1993)
	548, >30 min	+3*	−37	+36	(Gilmour and Perry, 1994)

Changes in stroke volume or ventilation amplitude (ΔV_{amp}; measurements of stroke volume are denoted by the superscript '†'), ventilation frequency (ΔV_f) and arterial P_{CO_2} (ΔP_{CO_2}) are expressed as a percentage of the control (normoxic) value; thus a negative value indicates a decrease while a positive value indicates an increase from the normoxic control value. Percent changes have been calculated from mean data reported in the original studies. * indicates changes that were not significant.

[a]Only studies that examined at least two of ventilation frequency, ventilation amplitude or stroke volume and arterial P_{CO_2} have been included. As data on the ventilatory responses of fish to hyperoxia are relatively sparse, studies from the past 20 years have been included.

Ventilatory responses to a decrease in the environmental O_2 tension are initiated very rapidly, within seconds of the hypoxic water contacting the gills (e.g. Smatresk et al., 1986; Burleson and Smatresk, 1990a,b; Fritsche and Nilsson, 1993; Sundin et al., 1999a, 2000). Such rapid responses are indicative of the involvement of externally-oriented branchial O_2-sensitive chemoreceptors, a supposition that has been confirmed by the demonstration that cyanide, a potent O_2-chemoreceptor stimulant, also elicits a hyperventilatory response when added to the inspired water (Burleson and Smatresk, 1990a,b; McKenzie et al., 1991, 1995; Burleson and Milsom, 1995a; Sundin et al., 1999a, 2000). Nerve sectioning and/or nerve recording techniques have been used to localise the O_2-sensitive chemoreceptors that mediate ventilatory responses to external hypoxia and have demonstrated that, in the teleost fish so far studied, these receptors are distributed throughout all gill arches and are innervated by branches of the ninth (glossopharyngeal) and/or tenth (vagus) cranial nerves (e.g. Milsom and Brill, 1986; Burleson and Smatresk, 1990a; McKenzie et al., 1991; Burleson and Milsom, 1993; Sundin et al., 1999a, 2000; reviewed by Burleson et al., 1992; Fritsche and Nilsson, 1993; Burleson, 1995). These externally-oriented O_2-chemoreceptors probably allow ventilatory adjustments to changes in environmental O_2 tension to be initiated before O_2 delivery to the tissues is affected (Burleson, 1995).

A second group of branchial O_2-sensitive chemoreceptors, functionally indistinguishable from the first group except in terms of their orientation, responds to changes in blood O_2 levels and to cyanide injected into the vasculature (Smatresk et al., 1986; Burleson and Smatresk, 1990b; McKenzie et al., 1991, 1995; Burleson and Milsom, 1993, 1995a,b; Sundin et al., 1999a, 2000; reviewed by Burleson et al., 1992; Fritsche and Nilsson, 1993; Burleson, 1995). These internally-oriented O_2-chemoreceptors may reinforce ventilatory stimuli initiated by externally-oriented receptors during environmental hypoxia that is severe enough to impact on blood O_2 levels. In addition, however, the internally-oriented O_2-chemoreceptors respond to hypoxaemia in the absence of environmental hypoxia, and may therefore be important in matching ventilation to metabolic O_2 demands under conditions of external normoxia (e.g. during exercise) (Burleson, 1995).

While relatively few studies have reported ventilatory responses to hypoxaemia in the absence of environmental hypoxia, some experimental data are available from studies in which the blood O_2 carrying capacity was manipulated or vasoactive chemical agents were investigated. With respect to blood O_2 carrying capacity, hyperventilation has been documented in rainbow trout and carp exposed to carbon monoxide (Holeton, 1971; Soncini and Glass, 2000), anecdotally in carp exposed to nitrite resulting in methaemoglobin formation (Jensen et al., 1987), and during the early stages (1–24 h) of anaemia in rainbow trout (Smith and Jones, 1982) and starry flounder (Wood et al., 1979). In most of these cases, the reduction in blood O_2 content was accompanied by a significant increase in the partial pressure of O_2 in the arterial blood (Holeton, 1971; Wood et al., 1979; Smith and Jones, 1982; Jensen et al., 1987), implying an O_2-chemoreceptor response to blood O_2 content rather than P_{O_2}. The question of whether branchial O_2-sensitive chemoreceptors respond to P_{O_2} or O_2 content (or both) has not yet been answered, although it is likely that the response at the actual O_2 receptor site is to partial pressure rather than content. Nerve recording studies carried out to date have used gills perfused with saline, where it is difficult to change P_{O_2} and O_2 content independently (Milsom and Brill, 1986; Burleson and Milsom, 1993, 1995b; see review by Burleson, 1995). Direct investigations of the sensitivity of the O_2-chemoreceptors to P_{O_2} vs. O_2 content are needed. The strong correlation between ventilation volume and blood total O_2 content under a variety of conditions reported by Randall (1982) using the data of Smith and Jones (1982) provides further support for the role of internally-oriented O_2 chemoreceptors in controlling ventilation in fish. Note, however, that the reduction in blood O_2 carrying capacity during anaemia is accompanied by an increase in P_{CO_2} and a lowering of pH (Wood et al., 1982), and therefore caution should be used in attributing the anaemic hyperventilation solely to the reduction in blood O_2 content.

Recent investigations into the effects of various vasoactive substances on cardiorespiratory parameters in fish have also yielded information on the control of ventilation. For example, injec-

tion of serotonin into rainbow trout (Fritsche et al., 1992; Burleson and Milsom, 1995a) and eel (Janvier et al., 1996a) caused significant increases in both ventilation frequency and amplitude. Marked, significant increases in ventilation frequency and amplitude were also observed in rainbow trout and spiny dogfish following administration of endothelin-1 (Perry et al., 2000). Both serotonin (Sundin et al., 1995, 1998; Forster et al., 1998) and endothelin-1 (Olson et al., 1991; Stenslokken et al., 1999; Hoagland et al., 2000) are potent constrictors of the gill vasculature that have been demonstrated to elicit significant reductions in arterial O_2 tension (endothelin-1, Perry et al., 2000; serotonin, Fritsche et al., 1992; Sundin et al., 1998; but note that an increase in arterial P_{O_2} was reported in the eel following serotonin injection by Janvier et al., 1996b). The hyperventilatory responses to these agents may be mediated indirectly through the decreases in internal O_2 status, although indirect mediation through changes in internal CO_2 levels and/or pH must also be taken into consideration (see below), as at least in trout, significant increases in arterial P_{CO_2} and a corresponding acidosis are observed following serotonin (Fritsche et al., 1992) or endothelin-1 (Perry et al., 2000) treatment. In addition, direct effects of the agents themselves on branchial (or extra-branchial) chemoreceptors cannot be excluded at this time (Burleson and Milsom, 1995b), nor can indirect effects mediated through the elevation of circulating catecholamine levels (see below) elicited by these compounds (Fritsche et al., 1993b; Perry et al., 2000). Further investigation of the effects on ventilation and blood gases of these and other vasoactive substances (e.g. adenosine, Sundin et al., 1999b; acetylcholine, Burleson and Milsom, 1995a; Forster et al., 1998) is clearly warranted. These substances may prove to be useful tools for distinguishing among potential mechanisms in the control of ventilation in fish.

Although great interspecies variability has been reported, there is evidence to suggest that extra-branchial O_2-chemoreceptors may also exist and play a role in mediating the O_2-keyed ventilatory drive in at least some species of fish (see reviews by Burleson et al., 1992; Fritsche and Nilsson, 1993; Burleson, 1995). For example, significant hyperventilatory responses to aquatic hypoxia and external cyanide injections were still observed in traira (Sundin et al., 1999a) and tambaqui (Sundin et al., 2000) even after complete branchial denervation, while tambaqui also responded to internal cyanide injection after complete branchial denervation (Sundin et al., 2000). These results point to the existence of both externally- and internally-oriented extra-branchial O_2-chemoreceptors. Several potential locations have been suggested as sites for extra-branchial O_2 receptors, including the orobuccal cavity (Butler et al., 1977), the arterial and venous vasculature, and the brain (Burleson et al., 1992; Burleson, 1995). Of these potential sites, only the orobuccal cavity would appear to be promising with respect to the existence of externally-oriented extra-branchial O_2-chemoreceptors. The available experimental support for central O_2-sensitive chemoreceptors in fish, and indeed in all vertebrates, is weak. While few studies have directly investigated the possibility of central chemoreception in fish, neither Hedrick et al. (1991), using manipulation of the extradural fluid surrounding the brain in bowfin, nor Rovainen (1977), using an isolated lamprey brain preparation, obtained convincing evidence for the presence of central O_2-sensitive chemoreceptors.

Although the sensory pathways that mediate ventilatory responses to changes in internal or external O_2 levels remain to be fully elucidated, it is clear that O_2 is of great importance in controlling ventilation in fish. The importance of O_2 status as a ventilatory drive in fish has often been illustrated by the observation that ventilatory adjustments to optimise O_2 delivery have an impact on blood CO_2 tension and/or pH (Perry and Wood, 1989). The hypoventilation elicited by exposure to environmental hyperoxia typically results in a significant elevation of arterial P_{CO_2} accompanied by a corresponding lowering of arterial pH, the classical respiratory acidosis (Table 1). A respiratory alkalosis characterised by depressed arterial P_{CO_2} and elevated arterial pH occurs when ventilation volume is increased in response to aquatic hypoxia (Table 1). It would appear that, under these conditions, ventilation is being matched to O_2 demand, even at the expense of disturbances of acid–base balance. A similar situation occurs, however, in terrestrial vertebrates (in which it is widely accepted that ventilation is keyed primarily to internal CO_2 and/or pH status) during acute exposure to lowered environmental O_2 tensions, e.g. at high altitude — O_2 delivery to the tissues is maintained

by hyperventilation despite a concomitant respiratory alkalosis (West, 1989; Faraci, 1991). Thus, the argument that O_2 delivery is regulated at the expense of CO_2 excretion in fish should not be used to exclude the possibility that a CO_2/pH ventilatory drive exists.

3. Does CO_2 and/or pH drive ventilation in fish?

Compared to the research effort that has been directed towards hypoxia, data on the ventilatory responses of fish to changes in water CO_2 levels are sparse (Table 2). Nevertheless, the available data strongly support environmental hypercapnia, i.e. the elevation of water CO_2 tension, as a ventilatory stimulus. Exposure to aquatic hypercapnia typically elicits significant increases in stroke volume (or ventilation amplitude) and/or ventilation frequency (Table 2), resulting in an elevation of ventilation volume (e.g. Janssen and Randall, 1975; Smith and Jones, 1982; Graham et al., 1990; Kinkead and Perry, 1991; Kinkead et al., 1993; Soncini and Glass, 2000). Air-breathing may also be promoted by exposure to aquatic hypercapnia in those species that exhibit bimodal respiration (Smatresk and Cameron, 1982a; see reviews by Milsom, 1995a,b). Although some studies report only transient increases in ventilation in response to prolonged hypercapnia (> 2 h) (Janssen and Randall, 1975; Randall et al., 1976), more typically ventilation remains elevated throughout the hypercapnic exposure (e.g. Smatresk and Cameron, 1982a; Thomas and Le Ruz, 1982; Thomas et al., 1983; Graham et al., 1990; Crocker and Cech, 1998).

A first glance at Table 2 suggests that ventilatory responses to environmental hypercapnia may be more variable than those to aquatic hypoxia. For example, whereas fish almost universally appear to hyperventilate when subjected to hypoxia (e.g. Table 1), exposure to environmental CO_2 tensions of up to 6 torr did not elicit hyperventilation in eel (McKendry, 2000), carp (Soncini and Glass, 2000), tambaqui (Sundin et al., 2000) or traira (Reid et al., 2000). In addition, Dejours (1973) reported anecdotally that no obvious hyperventilation occurred in goldfish even at a water CO_2 tension of 24 torr. Tambaqui and traira did, however, hyperventilate when exposed to water of $P_{CO_2} > 10$ torr, a level well within the physiological range for these neotropical species (Sundin et al., 2000; Reid et al., 2000). Similarly, increased ventilatory effort was observed in carp at water P_{CO_2} values of 14 torr (Soncini and Glass, 2000) (the response of eels to higher CO_2 tensions has not been investigated; S.F. Perry, personal communication). These observations suggest that fish species vary in their tolerance of environmental CO_2 tensions and that CO_2 levels appropriate to the species under consideration must be selected for experimental investigation of ventilatory responses, a statement that also holds true for O_2 (see, for example, Thomas and Perry, 1992). Our knowledge of the CO_2 tolerances of different species of fish, however, is limited in comparison to the data available for O_2 tolerances.

Hyperventilatory responses to hypercapnia also occur under conditions of altered water O_2 levels. Despite the pronounced hyperventilation invoked by exposure to hypoxia, the ventilation frequency in rainbow trout exposed to a combination of hypercapnia and hypoxia ($P_{CO_2} = 5$ torr, $P_{O_2} = 60$ torr) was significantly higher than that in fish exposed to hypoxia alone; the combined treatment elevated ventilation frequency by 29% in comparison to 21% for hypoxia (Borch et al., 1993). Although ventilation is generally depressed in fish breathing hyperoxic water (Table 1), an increase in the P_{CO_2} of the inspired (hyperoxic) water can eliminate this hypoventilatory response (Kinkead and Perry, 1991) or elicit a hyperventilation similar to that observed under normoxic hypercapnic conditions (Thomas et al., 1983; Burleson and Smatresk, 2000). Furthermore, the hyperventilation induced in rainbow trout by exposure to water of $P_{CO_2} = 8–11$ torr can only be attenuated, not eliminated, by raising the inspired water P_{O_2}, although at a lower environmental CO_2 tension (~ 5 torr), ventilation returned to a level that was not significantly different from the normoxic normocapnic value when water P_{O_2} was increased (Smith and Jones, 1982). These observations demonstrate that fish are responsive to environmental CO_2 and/or pH levels even when O_2 ventilatory chemoreflexes are stimulated.

There appear to be very few reports of the responses to a lowering of water CO_2 levels, and not surprisingly, the few studies available have focused exclusively on recovery from chronic (> 24 h) hypercapnic exposure. In fact, it appears that ventilatory parameters have been measured

Table 2
Data from selected experimental studies over the past 30 years[a] on changes in ventilation frequency and ventilation amplitude or stroke volume in response to acute environmental hypercapnia under normoxic conditions in various fish species

Species	P_{CO_2} (torr), Time	ΔP_{aCO_2} (%)	ΔpHa	ΔP_{aO_2} (%)	ΔCaO_2 (%)	ΔV_{amp} (%)	ΔV_f (%)	Reference
Spiny dogfish, *S. acanthias*	20 min	+525	−0.43	NS	NS	+72	+38	(Perry and Gilmour, 1996)
	6, ~30 min					+93	+18	(McKendry, 2000)
Spotted dogfish, *Scyliorhinus stellaris*	5, 1 h	+333	−0.30	+62		+67†	NS	(Randall et al., 1976)
Atlantic big skate, *Raja ocellata*	7.5, 1 h	+925	−0.50	NS	NS	+100†	+23	(Graham et al., 1990)
Rainbow trout, *O. mykiss*	20 min	+150	−0.40	+87	NS	+317	NS	(Perry and Gilmour, 1996)
	5, 1 h	+208	−0.32	+18			+34	(Thomas and Le Ruz, 1982)
	5, 1 h	+200	−0.27	+23		+237†	+43	(Thomas et al., 1983)
	5, >30 min	+126	−0.28			+12	+17	(Gilmour and Perry, 1994)
	8, 20 min	+200	−0.34	NS		+133	NS	(Perry et al., 1999)
	6, 20 min		−0.20	NS	−25	+63†	NS	(Smith and Jones, 1982)
Pacific sanddab, *Citharichthys sordidus*	8, ~30 min					+181	+28	(McKendry, 2000)
Atlantic salmon, *Salmo salar*	6, ~30 min					+115	+36	(McKendry, 2000)
American eel, *Anguilla anguilla*	6, ~30 min					NS	NS	(McKendry, 2000)
Carp,	7, 30 min	NS	NS	NS	NS	NS	NS	(Soncini and Glass, 2000)
C. carpio	14, 30 min	+110	−0.15	+71	−13	NS	+140	(Soncini and Glass, 2000)
Brown bullhead, *I. nebulosus*	6, ~30 min					NS	+17	(McKendry, 2000)
Spotted gar, *L. oculatus*	6, 4 h	+129	−0.21	NS			+23	(Smatresk and Cameron, 1982a)
White sturgeon, *A. transmontanus*	30, 2 h	+1000	−0.55	NS	NS[b]		+70	(Crocker and Cech, 1998)
Tambaqui, *C. macropomum*	~38, 10 min					NS	+45	(Sundin et al., 2000)
Traira, *H. malabaricus*	~38, 15 min					+110	+21	(Reid et al., 2000)

Changes in arterial P_{CO_2} (ΔP_{aCO_2}), arterial P_{O_2} (ΔP_{aO_2}), arterial blood total O_2 content (ΔCaO_2), ventilation amplitude or stroke volume (ΔV_{amp}; measurements of stroke volume are denoted by the superscript '†') and ventilation frequency (ΔV_f) are expressed as a percentage of the control (normocapnic) value together with changes in arterial pH (ΔpHa); thus a negative value indicates a decrease while a positive value indicates an increase from the normocapnic control value. Percent changes have been calculated from mean data reported in the original studies. NS indicates that no significant change was reported.

[a] The table includes studies published in the last 30 years that reported ventilation frequency and/or ventilation amplitude (or stroke volume) data for fish acutely (<24 h) exposed to normoxic hypercapnic conditions.

[b] Based on in vitro O_2 equilibrium curves reported in the same study.

in only one such study. Return to normocapnia after 72 h at a CO_2 tension of approximately 30 torr elicited a lowering of ventilation frequency from a value of ~105 min^{-1} back to the control (pre-hypercapnic) value of ~61 min^{-1} by 24 h in white sturgeon (Crocker and Cech, 1998). The paucity of data on changes in ventilation when water CO_2 levels are lowered suggests that investigation of ventilatory responses to hypocapnia of fish species that are exposed to elevated CO_2 tensions in their natural environment is warranted as a means of examining CO_2 and/or pH ventilatory drives in fish.

Clearly, then, ventilatory responses to aquatic hypercapnia (or a relative hypocapnia) occur in fish (see also Perry and Wood, 1989; Milsom, 1995a,b). The sensory pathway(s) through which fish detect and respond to changes in the environmental CO_2 tension are as yet unknown. Water P_{CO_2} changes are accompanied by corresponding alterations of water pH (the magnitude of water pH changes will depend upon the buffering ca-

pacity of the water), and exposure to aquatic hypercapnia results in a rapid and significant elevation of arterial P_{CO_2} together with a depression of arterial pH (Table 2). In addition, in those species with haemoglobin that exhibits a significant Root effect, the elevated blood P_{CO_2} and acid–base disturbance resulting from hypercapnic exposure may result in a depression of blood O_2 status that could trigger a hyperventilatory response through the internally-oriented O_2-sensitive chemoreceptors. Thus, any one (or a combination) of water CO_2 status, water pH, blood CO_2 status, blood pH or blood O_2 status could act as the proximate stimulus for ventilation (see also Wood et al., 1990).

Hypoxaemia linked to elevated blood CO_2 and/or reduced pH was long regarded as the prime force driving the hyperventilatory response to hypercapnia. The case for CO_2/pH-induced hypoxaemia as the stimulus for hypercapnic hyperventilation was argued most persuasively by Smith and Jones (1982), who reported that the hypercapnic hyperventilation to water of $P_{CO_2} = 5$ torr was eliminated by the addition of O_2 to the inspired water to achieve $P_{O_2} = 350$ torr, and thus ventilation volume could be correlated with arterial blood O_2 content, independent of the method used to manipulate content. Note, however, that at slightly higher P_{CO_2} values, the hypercapnic hyperventilation was not eliminated by hyperoxia, but as blood O_2 content measurements were not carried out in those experiments, the data could not be included in the analysis (Smith and Jones, 1982). Oxygen-chemoreceptor mediated reflexes likely play a role in stimulating the hypercapnic hyperventilation in some cases. However, the hyperventilatory responses to elevated water CO_2 tensions that have been observed in teleost fish in the absence of changes in blood O_2 content (Table 2), the marked hypercapnic hyperventilation reported for three species of elasmobranchs, which lack Root effect haemoglobins (Table 2), and the persistence of hyperventilatory responses to hypercapnia during hyperoxia (Smith and Jones, 1982; Thomas et al., 1983; Burleson and Smatresk, 2000), all argue strongly in favour of a direct effect of CO_2 and/or pH on ventilation (see also Perry and Wood, 1989; Milsom, 1995a,b).

Recent research effort has been directed towards localising and characterising the CO_2- and/or pH-sensitive chemoreceptors that presumably mediate the CO_2/pH-linked ventilatory drive. It is likely that more than one population of such receptors exists; for example, different receptors may be involved in controlling ventilation frequency and ventilation amplitude. Nerve sectioning studies have been carried out on four different fish species and have yielded similar results — denervation of the branchial branches of cranial nerves IX (glossopharyngeal) and X (vagus) to the gill arches was found to eliminate the hyperventilatory response to hypercapnia in traira (Reid et al., 2000), tambaqui (Sundin et al., 2000), channel catfish (Burleson and Smatresk, 2000) and spiny dogfish (Fig. 1; McKendry et al., 2001). In the studies on traira (Reid et al., 2000) and tambaqui (Sundin et al., 2000), selective denervation of only the first gill arch was also investigated; fish subjected to this treatment still responded to hypercapnia. Taken together, the results of these studies indicate that the CO_2/pH-sensitive chemoreceptors involved in producing ventilatory responses are distributed throughout all gill arches and are innervated by branches of cranial nerves IX and/or X.

While the data from nerve sectioning studies also tend to suggest an exclusively branchial localisation for the CO_2/pH-chemoreceptors that mediate ventilatory responses, there are a few indications that extra-branchial CO_2/pH-receptors may also exist. First, Reid et al. (2000) found that the increase in ventilation amplitude in traira elicited by aquatic hypercapnia was not completely abolished by total gill denervation in all fish. As with extra-branchial O_2-chemoreceptors, potential sites for extra-branchial CO_2/pH-sensitive chemoreceptors include the orobuccal cavity and the central nervous system (Burleson, 1995; Reid et al., 2000). Indeed, palatine chemoreceptors that are sensitive to CO_2 and pH or CO_2 alone have been found in rainbow trout (Yamashita et al., 1989), eel (Yoshii et al., 1980) and carp (Konishi et al., 1969); such chemoreceptors play a role in the gustatory system but whether they can also mediate ventilatory effects is not known.

Using selective denervation on a decerebrate, spinalised, spontaneously-breathing preparation, Reid and co-workers (S.G. Reid, L. Sundin, T. Rantin and W.K. Milsom, unpublished) have obtained data suggestive of central and olfactory CO_2/pH chemoreceptors in tambaqui. Tambaqui subjected to spinalisation (at the level of spinal

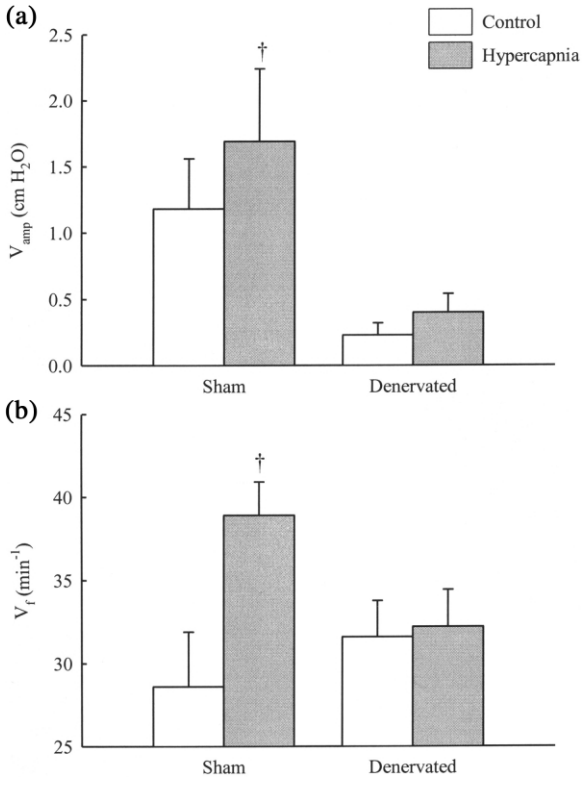

Fig. 1. Mean values of (a) ventilation amplitude (V_{amp}) and (b) ventilation frequency (V_f) for denervated ($N = 4–5$) or sham-operated ($N = 6$) spiny dogfish, *Squalus acanthias*, under normocapnic conditions (control; open bars) and during exposure to an inspired water P_{CO_2} of 6 torr (hypercapnia; filled bars). In denervated dogfish, the branchial branches of cranial nerves IX and X were sectioned; the nerves were exposed by means of a lateral incision dorsal to the gill slits. In sham-operated dogfish, the nerves were exposed but not sectioned. Pressure changes resulting from ventilation were recorded by means of a water-filled cannula (Clay Adams PE160) that was placed in the spiracle and connected to a pressure transducer (Bell and Howell) and computerised data acquisition system; ventilation amplitude and frequency were then calculated from the pressure changes using built-in functions of the data acquisition software (Perry et al., 2000 describe a similar experimental setup). Values are means ± S.E.M.; † indicates a significant difference between the hypercapnic value and its associated control (paired Student's *t*-test, $P < 0.05$). The data demonstrate that denervation of the gills in dogfish eliminated the hyperventilation induced by exposure to hypercapnia. (Data redrawn from McKendry et al., 2001).

nerve II) and decerebration (removal of the forebrain) responded to aquatic hypercapnia (5% CO_2), as did intact animals, with increases in ventilation frequency and amplitude (Fig. 2), indicating that neither the forebrain nor the spinal cord is required to stimulate ventilation in response to hypercapnia. This result is consistent with a branchial location for the CO_2/pH-sensitive chemoreceptors. However, a hypercapnic hyperventilation was still observed following central vagotomy (Fig. 2), a procedure that completely denervated the gills by sectioning cranial nerves IX and X at the point of exit from the brain and hence would be expected to abolish the hyperventilatory response to increases of P_{CO_2} in the inspired water (Sundin et al., 2000; Reid et al., 2000). Additional denervation of sensory branches of cranial nerves V (buccal cavity) and VII (palatine and opercular) also failed to eliminate the hyperventilatory response to increases of P_{CO_2} in the inspired water in the decerebrate, spinalised preparation. To account for these results, Reid and colleagues (S.G. Reid, L. Sundin, T. Rantin and W.K. Milsom, unpublished) have suggested that inhibitory CO_2/pH receptors may exist in the olfactory system/forebrain of tambaqui in addition to the already identified stimulatory branchial CO_2/pH chemoreceptors (Sundin et al., 2000). Interestingly, such an arrangement would be analogous to that observed in an amphibian as inhibitory olfactory CO_2-sensitive chemoreceptors have been shown to be present in bullfrog (Kinkead and Milsom, 1996). Other evidence for inhibitory olfactory chemoreceptors in fish exists; for example, branchial apnea can be induced in gar by placement of cyanide or strong acid or salt solutions on the anterior nares (Smatresk, 1988).

The presence of central CO_2/pH-sensitive chemoreceptors in fish is an attractive concept given their existence in and important role in controlling ventilation in terrestrial vertebrates (Milsom, 1995a,b). However, the experimental evidence for central CO_2/pH-chemoreceptors in fish is equivocal. Manipulation of the P_{CO_2} and/or pH of the extradural fluid surrounding the brain of bowfin was found to be without effect on ventilation (Hedrick et al., 1991), nor was hyperventilation during environmental hypercapnia in the skate correlated with cerebrospinal fluid pH or P_{CO_2} (Graham et al., 1990; Wood et al., 1990). On the other hand, Hughes and Shelton (1962) did report having observed changes in ventilation following the injection of bicarbonate solutions into the brain of tench.

Experiments on isolated brain preparations have also yielded results that are suggestive of central CO_2/pH-chemoreceptor activity. For example, the frequency of respiratory discharges

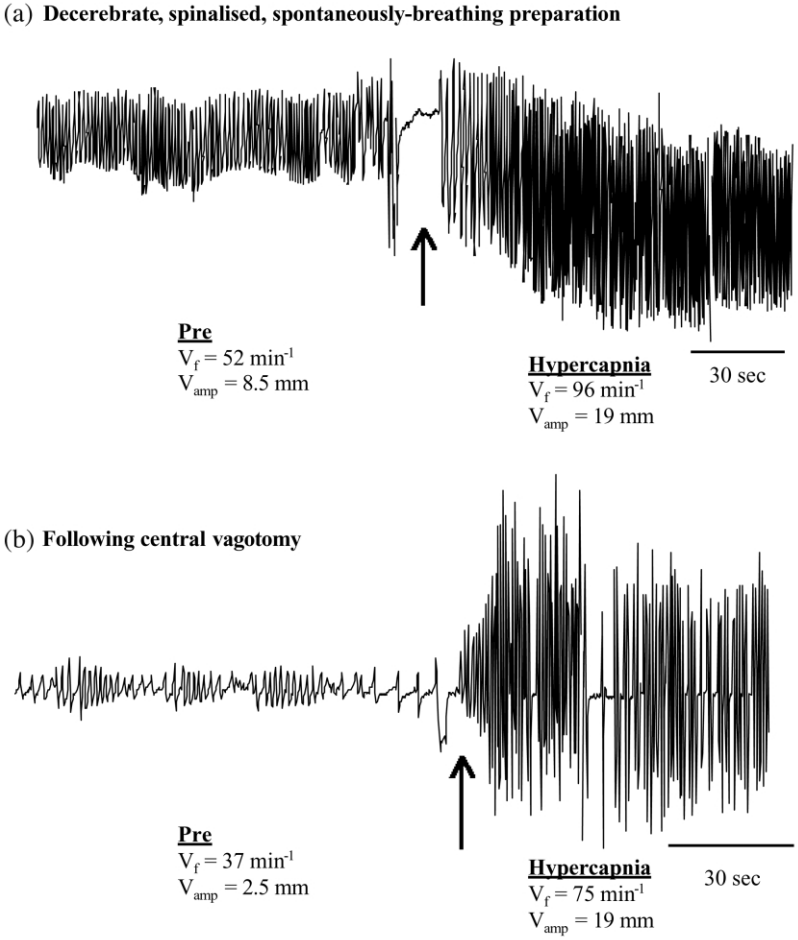

Fig. 2. Original, representative data recordings for ventilation in a tambaqui (*Colossoma macropomum*) decerebrate, spinalised, spontaneously-breathing preparation exposed to aquatic hypercapnia (P_{CO_2} = 38 torr). Ventilation was recorded as displacement of the opercula by means of small electrodes sutured to the opercular covers and connected to an impedance converter and computerised data acquisition system as described by Sundin et al. (2000). In the decerebrate, spinalised, spontaneously-breathing preparation, the brain was exposed to allow transection of the spinal cord at the level of spinal nerve II (spinalisation) and removal of the forebrain (decerebration). Fish were held in a stereotaxic device that kept the top of the head above water and ventilation was assisted by directing a flow of water into the mouth. The gill innervation was intact in this preparation and the response to hypercapnia involved increases in both ventilation frequency and amplitude (a). The recording presented in (b) illustrates the response to aquatic hypercapnia in the decerebrate, spinalised, spontaneously-breathing preparation following branchial denervation by central vagotomy, a procedure in which cranial nerves IX and X were sectioned at the point of exit from the brain. The persistence of the hypercapnic hyperventilation in this preparation suggests the existence of extra-branchial inhibitory CO_2/pH chemoreceptors (see text for further explanation of the results). (Unpublished data of S.G. Reid, L. Sundin, T. Rantin and W.K. Milsom.)

from the lamprey brain was increased, albeit moderately, by superfusion with an acidic solution and decreased by superfusion with a bicarbonate solution (Rovainen, 1977). More recently, a rigorous assessment of central chemosensitivity in fish has been carried out using isolated brain preparations developed for gar (Wilson et al., 2000) and Siamese fighting fish (R.J.A. Wilson, M.B. Harris, S.F. Perry and J.E. Remmers, personal communication), both species that exhibit bimodal respiration. These preparations show two patterns of burst activity that have been characterised as the neural equivalents of air-breathing and gill ventilation. When the preparations were superfused with solutions containing elevated CO_2/lowered pH, the frequency of fictive air-breathing increased, indicating that central chemosensitivity to CO_2/pH is present in both species. Fictive gill ventilation, on the other hand, was unaffected by changes in solution CO_2/pH. Many questions

remain unanswered, including the importance of central chemosensitivity vs. that of peripheral receptors, the responsiveness of central receptors to CO_2 vs. H^+ ions, and the importance (if any) of central chemosensitivity to gill ventilation. However, the results that have been obtained to date coupled with the general lack of information on central chemosensitivity indicate that the possibility of central chemosensitivity in fish cannot be dismissed and should be investigated further.

Although there are now convincing data for the existence of branchial CO_2/pH-sensitive chemoreceptors in fish (Sundin et al., 2000; Reid et al., 2000; Burleson and Smatresk, 2000; McKendry et al., 2001; see also Fig. 1), the orientation of these receptors, i.e. external (water) and/or internal (blood), as well as their sensitivity to CO_2 vs. H^+ ions, have yet to be determined. As with ventilatory responses to hypoxia, the rapid onset of ventilatory responses to increases in the inspired water CO_2 tension (e.g. Reid et al., 2000; see also Fig. 2) suggests that at least one population of branchial CO_2/pH receptors must be oriented externally. This hypothesis is supported by recent experiments on spiny dogfish (J.E. McKendry and S.F. Perry, personal communication) and Atlantic salmon (McKendry, 2000), in which ventilation frequency and amplitude were monitored as bolus injections of CO_2-equilibrated seawater were delivered to the gills via a cannula placed in the spiracle (dogfish) or buccal cavity (salmon). As illustrated by the representative traces presented in Figs. 3 and 4, injection of normocapnic seawater had, at most, a small, very transient effect on ventilation frequency in dogfish and ventilation amplitude in salmon. By contrast, injection of seawater equilibrated with 2% CO_2 (dogfish; Fig. 3) or 4% CO_2 (salmon; Fig. 4) caused marked increases in both ventilation frequency and amplitude that persisted for several minutes. In an attempt to distinguish between the effects of CO_2 and H^+ ions, aliquots of seawater were first thoroughly aerated to drive off CO_2 and were then titrated with HCl to pH 7.0 (the pH of the 2% CO_2-equilibrated seawater) or pH 6.3 (the pH of the 4% CO_2-equilibrated seawater). The effects of injecting the pH-adjusted seawater were modest at best in dogfish (Fig. 3), and while slightly more pronounced in salmon (Fig. 4), still considerably smaller than the effects induced by injecting CO_2-equilibrated seawater. Similarly, external acid injections into the inspired water via a cannula in the snout were found to be without significant effect on ventilation in tambaqui (Sundin et al., 2000) and traira (Reid et al., 2000). In addition, it has been known for some time that environmental acidification in the absence of elevated water P_{CO_2} has little effect on ventilation (Janssen and Randall, 1975; Neville, 1979; Thomas and Le Ruz, 1982). Taken together, these data suggest that externally-oriented chemoreceptors that are located in the gills and that respond specifically to CO_2 are involved in mediating ventilatory responses to hypercapnia. Although the receptors appear to respond to CO_2 specifically, a role for H^+ ions in triggering receptor activation at the intracellular level cannot be excluded at present and indeed is likely — in mammalian CO_2 chemoreceptors, H^+ ions rather than CO_2 molecules appear in most cases to be the stimulus modality at the level of the receptor cell (González et al., 1992; Nattie, 1999). Confirmation of this hypothesis will likely require investigations of afferent neural activity in isolated, perfused gill arch preparations, an approach that has previously been used successfully to confirm the existence, and examine the pharmacology, of branchial O_2-sensitive chemoreceptors (e.g. Milsom and Brill, 1986; Burleson and Milsom, 1993, 1995b) as well as gustatory CO_2/pH chemoreceptors (e.g. Yamashita et al., 1989).

Several lines of evidence also point to the existence of chemoreceptors that monitor the internal CO_2/pH status, although the location of such receptors (branchial, central or some other location) cannot be inferred from the data available at present. The elevation of arterial P_{CO_2} and/or accompanying acidosis that occur following exhaustive exercise in fish have been implicated as stimuli for the maintenance of hyperventilation during the recovery from exhaustive exercise (Wood and Perry, 1985; Perry and Wood, 1989; Wood, 1991; Wood and Munger, 1994). Severe, exhaustive exercise in fish elicits a state characterised by elevated O_2 consumption, hyperventilation, greatly elevated arterial P_{CO_2} and correspondingly depressed arterial pH, but relatively normal arterial P_{O_2} and arterial O_2 content (reviewed by Wood and Perry, 1985; see also Perry and Wood, 1989; Wood, 1991). Given the relatively normal O_2 status in the post-exhaustive state and the fact that post-exhaustive fish are generally motionless, eliminating the proprioceptive stimuli that likely contribute to the ventila-

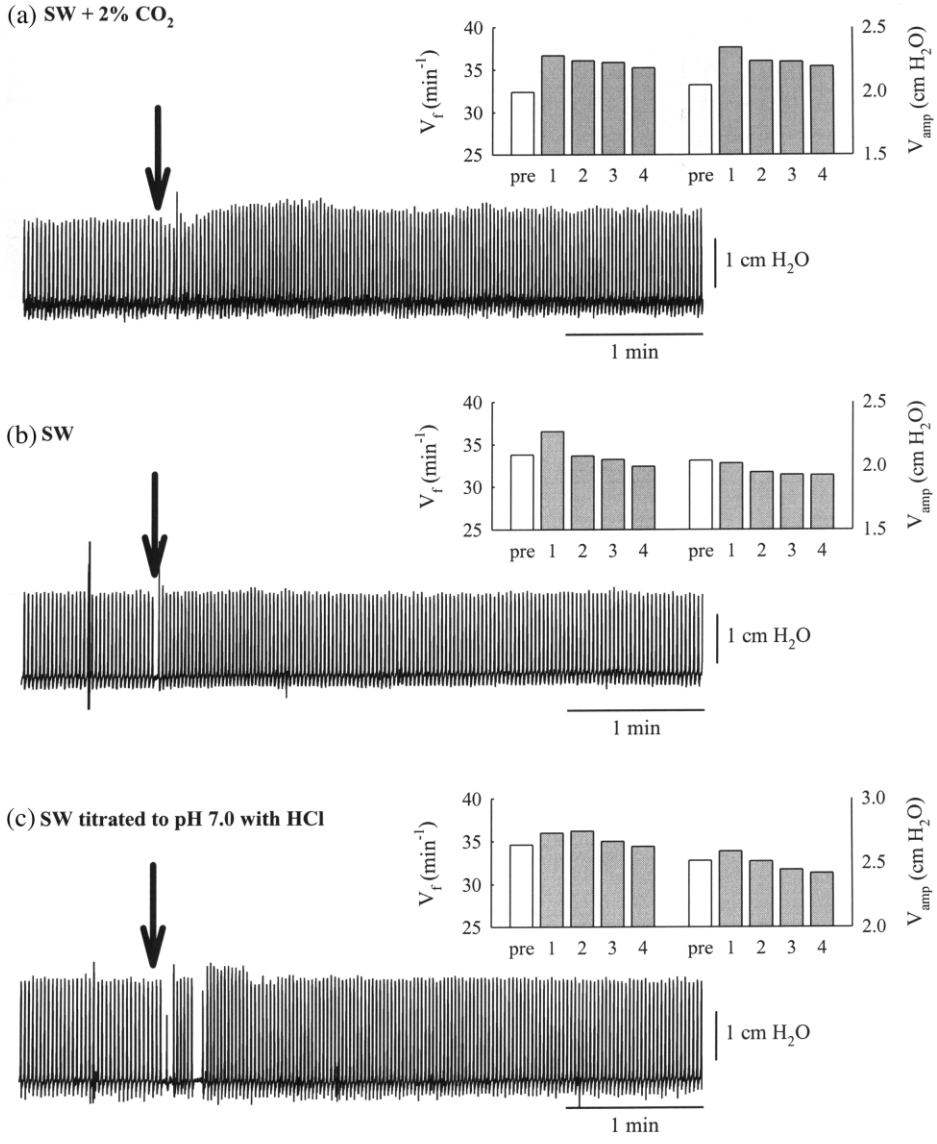

Fig. 3. Original, representative data recordings of changes in pressure due to ventilation in the spiracular cavity of spiny dogfish (*Squalus acanthias*) during bolus injections (60 ml) of seawater equilibrated with 15 torr CO_2 (a), seawater (b), or seawater titrated to pH 7.0 with HCl (c) into the inspired water via a cannula placed in the opposite spiracle. Pressure changes were recorded by means of a water-filled cannula (Clay Adams PE160) that was placed in the spiracle and connected to a pressure transducer (Bell and Howell) and computerised data acquisition system; bolus injections were made via an identical cannula placed in the other spiracle. The point of injection is noted on the recordings with an arrow. Ventilation amplitude (V_{amp}; the difference between the maximum and minimum pressures in a breathing cycle) and frequency (V_f) were calculated from the pressure changes using built-in functions of the data acquisition software (Perry et al., 2000 describe a similar experimental setup). The inset graphs present values of V_f and V_{amp} determined over 30-s intervals 1 min before the bolus injection (pre), and at 1-min intervals for 4 min following the injection. See text for discussion of the data. (Unpublished data of J.E. McKendry and S.F. Perry.)

tory drive during exercise, the profound respiratory acidosis is an obvious candidate for the driving force behind the post-exhaustion hyperventilation (Wood and Perry, 1985). A potential confounding factor, however, is that circulating catecholamines are elevated following exhaustive exercise (Wood and Perry, 1985) and may themselves play a role in controlling ventilation (see below). The case for the elevated P_{CO_2} and/or associated acidosis induced by exhaustive exercise as a stimulus for the post-exhaustion hyperventilation was argued most convincingly by Wood and

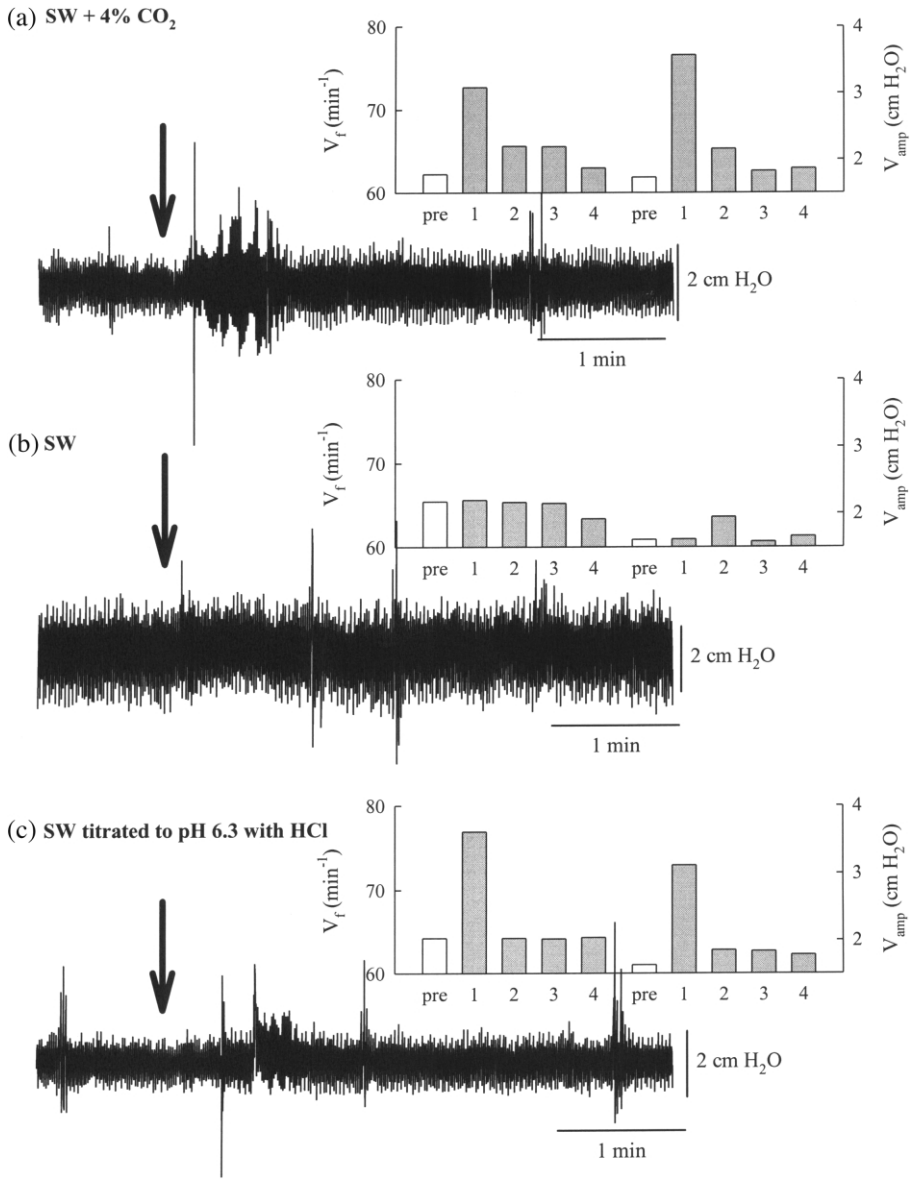

Fig. 4. Original, representative data recordings of changes in pressure due to ventilation in the opercular cavity of Atlantic salmon (*Salmo salar*) during bolus injections (60 ml) of seawater equilibrated with 30 torr CO_2 (a), seawater (b), or seawater titrated to pH 6.3 with HCl (c) into the flow of inspired water via a cannula placed in the buccal cavity. Pressure changes were recorded by means of a water-filled cannula (Clay Adams PE160) that was placed in the opercular cavity through the opercular cover and connected to a pressure transducer (Bell and Howell) and computerised data acquisition system; bolus injections were made via a similar cannula placed in the buccal cavity through the snout. The point of injection is noted on the recordings with an arrow. Ventilation amplitude (V_{amp}; the difference between the maximum and minimum pressures in a breathing cycle) and frequency (V_f) were calculated from the pressure changes using built-in functions of the data acquisition software (Perry et al., 2000 describe a similar experimental setup). The inset graphs present values of V_f and V_{amp} determined over 30-s intervals 1 min before the bolus injection (pre), and at 1-min intervals for 4 min following the injection. See text for discussion of the data. (Unpublished data of J.E. McKendry and S.F. Perry; see also McKendry, 2000.)

Munger (1994), who demonstrated that both the post-exercise hyperventilation and the post-exercise respiratory acidosis in rainbow trout were significantly reduced when bovine carbonic anhydrase was injected into the circulation prior to exercise to enhance CO_2 excretion. In addition, strong, significant correlations were found between the relative increase in ventilation and

the decrease in arterial pH or increase in arterial P_{CO_2}, with the stronger relationship being between ventilation and arterial pH (Wood and Munger, 1994).

Correlations between changes in ventilation and acid–base status have also been documented for elasmobranch fish exposed to environmental hyperoxia or hypercapnia (Heisler, 1988; Heisler et al., 1988; Graham et al., 1990; Wood et al., 1990). Elasmobranch fish are particularly advantageous for such studies because they lack a Root effect and hence their blood O_2 content should not be impaired by the respiratory acidosis that occurs during hyperoxia or hypercapnia. Changes in ventilation in skate during 24 h of aquatic hypercapnia occurred in the absence of alterations in blood O_2 status and were more closely related to changes in arterial pH than to changes in cerebrospinal fluid pH, brain tissue intracellular pH or arterial P_{CO_2} (Graham et al., 1990; Wood et al., 1990). Similarly, a relative hyperventilation observed in dogfish during profound hypoventilation induced by exposure to aquatic hyperoxia was explained on the basis of a requirement to regulate arterial acid–base status (Heisler et al., 1988). In addition, manipulation of arterial acid–base status in hyperoxic dogfish yielded a strong correlation between changes in ventilation and arterial pH but not arterial P_{CO_2} (unpublished data reported in Heisler, 1988).

Such correlations between changes in ventilation and acid–base status in elasmobranch fish, as well as the marked attenuation of the post-exhaustive exercise hyperventilation in trout treated with carbonic anhydrase, argue in favour of a specific CO_2 and/or pH effect on ventilation. However, other lines of evidence suggest that ventilation in fish is insensitive to internal CO_2 and/or pH. For example, a 30% reduction in gill surface area in rainbow trout (achieved by ligation of two gill arches) caused a 1 torr increase in P_{aCO_2} without affecting ventilation volume (Julio et al., 2000). Manipulation of the gill blood-to-water diffusion distance using hormone treatments similarly caused a significant elevation of P_{aCO_2} in the absence of an effect on ventilation (Bindon et al., 1994). Thomas and Le Ruz (1982) found that ventilation frequency in rainbow trout was unaffected by transfer of the fish to acidified water (at the same water P_{CO_2}), despite a significant increase in arterial P_{CO_2} (from 2.10 to 3.30 torr 15 min after transfer) and a corresponding depression of arterial pH (from 7.97 to 7.62). These authors interpreted their data as evidence that environmental acidification in the absence of increased water P_{CO_2} does not stimulate ventilation (Thomas and Le Ruz, 1982), but the data additionally imply that elevated blood P_{CO_2} and/or lowered pH are not, on their own, sufficient stimuli to trigger ventilation increases.

A similar inference must be drawn on the basis of ventilation measurements carried out following acetazolamide injection. Acetazolamide is a carbonic anhydrase inhibitor that readily permeates the red blood cell membrane. In fish treated with acetazolamide, arterial P_{CO_2} increases rapidly and arterial pH falls as acetazolamide penetrates the red blood cell and inhibits erythrocyte carbonic anhydrase activity, significantly impairing CO_2 excretion (e.g. Gilmour et al., 1994; Henry et al., 1995; Currie et al., 1995). However, in three of four species for which ventilation data following acetazolamide treatment were available in the literature, neither ventilation frequency nor ventilation amplitude was influenced by acetazolamide injection, despite significant increases in arterial P_{CO_2} of, on average, 120%, and corresponding decreases in arterial pH (Table 3). Although O_2 status was not monitored in these studies, any decrease in O_2 status resulting from the respiratory acidosis would be expected to stimulate ventilation, and hence the lack of an effect on ventilation is particularly telling. A significant increase in ventilation frequency was, however, observed in gar following acetazolamide treatment (Table 3). A notable difference between this study and the others reported in Table 3 is that ventilation measurements were made 4–7 h after acetazolamide injection rather than 30 min. If the signal transduction pathway of the putative internal CO_2/pH chemoreceptor requires carbonic anhydrase (e.g. if CO_2 is detected as H^+ ions following diffusion of molecular CO_2 across a cell membrane at either a branchial or central chemoreceptor), then acetazolamide treatment could conceivably impact on CO_2/pH detection, resulting in a delayed ventilatory response. In this context, it is noteworthy that carbonic anhydrase is present in brain tissue from dogfish and that acetazolamide appears to diffuse freely through the body fluids, including the cerebrospinal fluid, of dogfish (Maren, 1962). Furthermore, carbonic anhydrase activity is present in chemosensory (glomus) cells of the carotid body of mammals

Table 3
Selected data on the effect of intra-arterial injection of acetazolamide (1–30 mg kg^{-1} wet wt.) on ventilation parameters and arterial P_{CO_2}

Species	Control			Acetazolamide			Reference
	P_{aCO_2} (torr)	V_f (min^{-1})	V_{amp}	P_{aCO_2} (torr)	V_f (min^{-1})	V_{amp}	
Spotted gar (4)	8.1 ± 0.2	34 ± 2 (11)		14.2 ± 2.4*	46 ± 11*		(Smatresk and Cameron, 1982b)
Spiny dogfish (6)	1.4 ± 0.3	41 ± 2	2.5 ± 0.7[a]	3.0 ± 0.5*	39 ± 1	2.2 ± 0.5	(Gilmour et al., 2001)
(6)	1.0 ± 0.1	28 ± 2	3.1 ± 1.0[b]	2.5 ± 0.3*	28 ± 2	3.1 ± 1.0	(Gilmour et al., 1997)
Channel catfish (5)	3	70	7.5[b]	5.1*	70	6	(Henry et al., 1988)
Rainbow trout (6)	1.9 ± 0.1	96 ± 7	0.2 ± 0.03[a]	4.9 ± 0.4*	98 ± 8	0.2 ± 0.04	(Gilmour et al., 2001)

Values are means ± S.E.M. except where values have been estimated from figures, in which case only the mean value is reported. N numbers are reported in parentheses after each species number; note that the ventilation frequency for gar under control conditions is a mean value for $N = 11$ whereas all other measurements are for $N = 4$ gar. P_{aCO_2}, partial pressure of CO_2 in the arterial blood; V_f, ventilation frequency; V_{amp}, ventilation amplitude measured in cm[a] or cm H_2O[b]. * indicates a significant difference from the control value.

(Nurse, 1990) and appears to be essential for the speed and amplitude of the response to changes in CO_2 since inhibition of carbonic anhydrase activity in vivo or in vitro reduces the carotid body chemoreceptor response (Iturriaga et al., 1991, 1993; see also review by Iturriaga, 1993). Experiments involving longer term monitoring of ventilation parameters and blood acid–base status following acetazolamide treatment are certainly warranted.

A number of workers have attempted to determine whether ventilation in fish is sensitive to internal CO_2 and/or pH status by means of intra-arterial injections of acid, bicarbonate or CO_2-equilibrated saline. The results of such experiments have been very mixed. No significant changes in ventilation occurred in response to bolus injections of acid (0.125 mmol l^{-1} HCl in 0.2–0.3 ml saline) into the ventral aorta of tambaqui (Sundin et al., 2000) or traira (Reid et al., 2000), nor did bolus intra-arterial injections of saline (3 ml) equilibrated with 30 torr CO_2 have any consistent effect on ventilation in salmon or dogfish (Fig. 5). Intra-arterial infusion of bicarbonate (over 10 min) in rainbow trout was also without effect on ventilation (Gilmour and Perry, 1996). In contrast, 0.025 mmol l^{-1} HCl in 1 ml saline injected into the dorsal aorta of rainbow trout decreased arterial pH by approximately 0.2 units and elicited a significant three- to fourfold increase in ventilation volume that persisted for ~ 10 min (Janssen and Randall, 1975); increases in ventilation frequency (Gilmour and Perry, 1996) or ventilation amplitude (Aota et al., 1990) have also been reported in rainbow trout in response to acid injection. Janssen and Randall (1975) also observed a significant threefold increase in ventilation volume in rainbow trout injected intra-arterially with 1 mmol l^{-1} $NaHCO_3$ in 1 ml saline. The fact that acid and bicarbonate injections had opposite effects on arterial blood pH but would both be expected to increase the arterial CO_2 tension led these authors to propose that elevations of arterial P_{CO_2} act as a ventilatory stimulus (Janssen and Randall, 1975).

One difficulty with all of these studies in terms of demonstrating CO_2/pH-linked ventilatory effects, however, is that O_2 status was not monitored and hence O_2-linked effects on ventilation cannot be eliminated in those studies where changes in ventilation were observed. A second difficulty is that acid injection can stimulate catecholamine release (e.g. Aota et al., 1990). The role of circulating catecholamines in stimulating ventilation directly in fish has been greatly debated (see reviews by Perry et al., 1992 and Randall and Taylor, 1991 for the two opposing views). While the experimental evidence increasingly suggests that a direct effect of circulating catecholamines on ventilation is unlikely (unless exerted through a central nervous system mechanism), an indirect role through adrenergically-mediated changes in O_2 status remains a possibility (e.g. Perry and Gilmour, 1996; see also review by Burleson, 1995).

Thus, the question of whether internal CO_2 and/or pH status plays a role in controlling ventilation in fish remains open. To clearly demon-

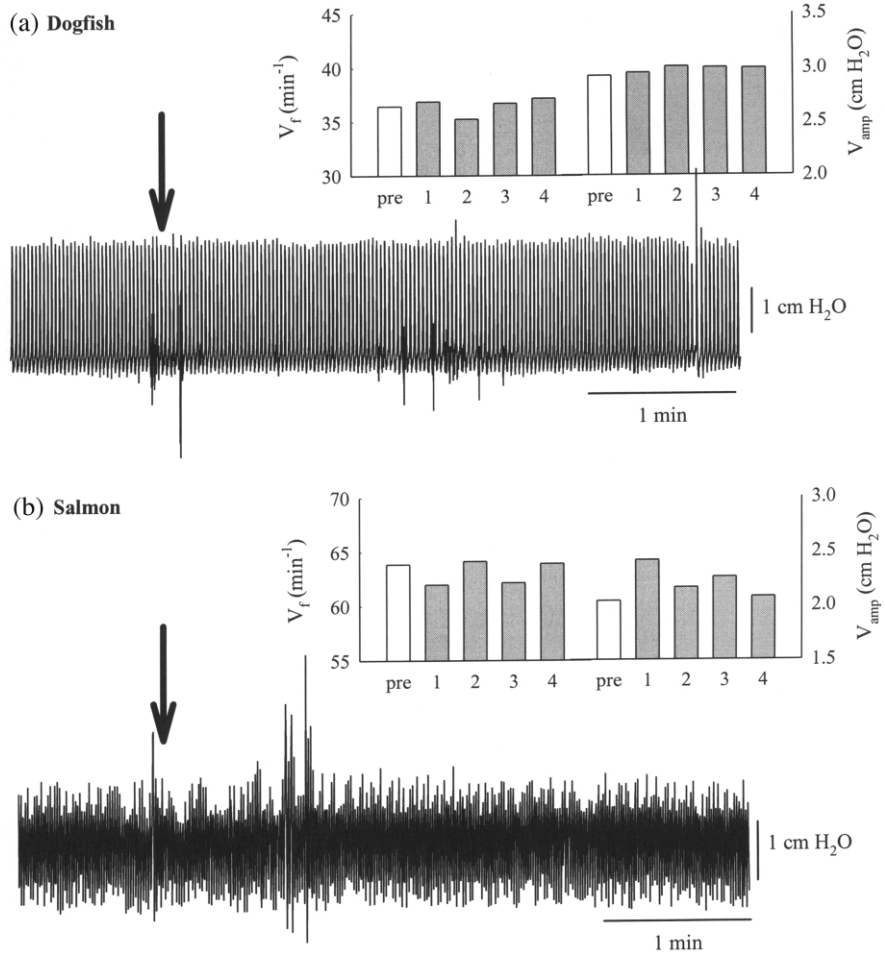

Fig. 5. Original, representative data recordings of changes in pressure due to ventilation in (a) the spiracular cavity of spiny dogfish or (b) the opercular cavity of Atlantic salmon during bolus intra-arterial injections (3 ml) of saline equilibrated with 30 torr CO_2. Pressure changes were recorded by means of a water-filled cannula (Clay Adams PE160) that was placed in either a spiracle or the opercular cavity and connected to a pressure transducer (Bell and Howell) and computerised data acquisition system. The point of injection is noted on the recordings with an arrow. Ventilation amplitude (V_{amp}; the difference between the maximum and minimum pressures in a breathing cycle) and frequency (V_f) were calculated from the pressure changes using built-in functions of the data acquisition software (Perry et al., 2000 describe a similar experimental setup). The inset graphs present values of V_f and V_{amp} determined over 30-s intervals 1 min before the bolus injection (pre), and at 1-min intervals for 4 min following the injection. See text for discussion of the data. (Unpublished data of J.E. McKendry and S.F. Perry.)

strate a specific effect of internal CO_2 and/or pH on ventilation will require that CO_2 and pH be manipulated independently, and in the absence of changes in O_2 status. The need for such experiments in distinguishing among different potential ventilatory stimuli in fish was pointed out by Perry and Wood (1989) over a decade ago. The current lack of conclusive data in this regard attests to the difficulty of such experiments. Significant progress has been made, however, in other respects since Perry and Wood (1989) first reviewed the case for a CO_2 and/or pH-mediated ventilatory drive in fish (see also Milsom, 1995a,b). It is now clear that fish respond to environmental hypercapnia with an increase in ventilation that is not due simply to a CO_2/pH-induced hypoxaemia. Indeed, hypercapnic hyperventilation can occur in the absence of CO_2/pH-induced hypoxaemia and appears to be mediated at least in part by branchial chemoreceptors that likely respond

specifically to CO_2 in the inspired water. The role, if any, of internally-oriented CO_2/pH-sensitive chemoreceptors remains equivocal.

4. Links between O_2- and CO_2/pH-ventilatory drives (or why hyperventilate during hypercapnia?)

Dejours (1973) first raised the issue of the functional significance of a hyperventilatory response to environmental hypercapnia in water-breathers, pointing out that 'hyperventilation cannot be a protection against hypercapnia, because the difference between inspired and expired water is always very small'. When coupled with the contention that the gill is 'hyperventilated with respect to CO_2 excretion' (because of the difference in water O_2 and CO_2 capacitances) and the concomitant implication that blood CO_2 status is insensitive to changes in ventilation, this argument appears to have led to the widely held view that ventilation in fish is driven primarily, if not solely, by O_2 status. Given our current state of knowledge on CO_2/pH-mediated ventilatory effects in fish, it is clearly time to reassess this view.

There are a number of reasons why hyperventilation could be of functional significance as a response to hypercapnia (environmental or internal) (see also Perry and Wood, 1989; Ultsch, 1996; Burleson and Smatresk, 2000). First, hypoxia typically occurs in combination with hypercapnia in vegetation-rich aquatic environments (see review by Ultsch, 1996). Such environments could well favour sensory systems that are attuned to both environmental O_2 and CO_2/pH levels (Dejours, 1973). It is relevant in this regard that the peripheral chemoreceptors of air-breathing vertebrates generally respond to alterations of CO_2/pH as well as P_{O_2} or O_2 content (Smatresk, 1990). Furthermore, in the two fish species in which respiratory reflexes to O_2 and to CO_2/pH have been examined simultaneously, traira (Sundin et al., 1999a; Reid et al., 2000) and tambaqui (Sundin et al., 2000), the receptors involved in producing the increases in ventilation frequency and amplitude during environmental hypercapnia appeared to have a similar although not necessarily identical distribution to those involved in mediating ventilatory responses to aquatic hypoxia. A similar conclusion was reached by Burleson and Smatresk (2000) following separate studies of ventilatory responses to hypoxia and hypercapnia in the channel catfish. Single-fibre recordings from branchial chemoreceptors during exposure to changes in O_2 and CO_2/pH status are essential to address this possibility by more completely characterising the sensitivity of branchial chemoreceptors to environmentally-relevant stimuli.

Secondly, hyperventilation is an appropriate and potentially relevant response for the correction of a respiratory acidosis of internal origin (e.g. exercise, hyperoxia-induced hypercapnia). Hyperventilation induced by exposure to hypoxia elicits a lowering of arterial P_{CO_2} — for example, arterial P_{CO_2} decreased by, on average, 32% in the studies reported in Table 1. Hypoventilation in response to environmental hyperoxia (Table 1) or under normoxic conditions (Iwama et al., 1987) causes P_{CO_2} to increase and arterial pH to fall. At the low CO_2 tensions characteristic of fish blood, only small changes in P_{CO_2} are required to elicit substantial changes in pH. Thus ventilatory adjustments to changes in internal CO_2/pH status could constitute a mechanism involved in the maintenance of acid–base homeostasis. As discussed above, however, the experimental evidence for ventilatory responses to internal CO_2/pH status are inconclusive at present. Confirmation of this hypothesis requires a careful analysis of ventilatory responses to changes in internal CO_2/pH status in the absence of changes in external CO_2/pH or internal O_2 status.

Finally, while hyperventilation may be ineffective as a means of protecting arterial P_{CO_2} and/or acid–base status in the face of environmental hypercapnia owing to the small blood-to-water P_{CO_2} gradients characteristic of the fish gill (e.g. Ultsch, 1996), it may still be an appropriate response for the maintenance of blood O_2 status. That is, the ultimate origin of hypercapnic hyperventilation may be the protection of O_2 status even though the proximate stimulus is the elevation of environmental CO_2 and/or fall in pH. The rapid hyperventilation invoked when hypercapnic water contacts the gill often results in an increase in arterial P_{O_2} that may serve to help maintain O_2 delivery under conditions in which O_2 delivery could otherwise be compromised. Investigation of this hypothesis, which would apply to teleost fish but not elasmobranchs, would involve assessing O_2 status during hypercapnia in

the presence and absence of a hyperventilatory response.

Clearly, both the proximate and ultimate causes of the ventilatory responses to CO_2 and/or pH in fish warrant further investigation. In addition, the relative roles of the O_2- and CO_2/pH-keyed ventilatory drives with respect to the control of breathing in fish need to be assessed. The primacy of the O_2-mediated ventilatory drive in fish has never been challenged, in part because the low O_2 capacitance of water places such a premium on obtaining O_2 from the environment and in part owing to the observation that ventilation is adjusted in response to environmental O_2 levels despite concomitant changes in internal CO_2 and/or pH status. The latter statement is also true, however, of air-breathers, in which it is widely accepted that ventilation is keyed primarily to internal CO_2 and/or pH status. Furthermore, in a number of cases where it has been assumed that ventilation in fish was being increased simply to maintain O_2 status (e.g. anaemia, treatment with branchial vasoconstrictors), potentially confounding changes in CO_2 and/or pH status would also have been expected to occur. It is quite conceivable that the CO_2/pH-mediated ventilatory drive in fish is of much greater significance than has previously been acknowledged.

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