Vol. 373: 295–302, 2008
doi: 10.3354/meps07823

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published December 23

Contribution to the Theme Section 'Effects of ocean acidification on marine ecosystems'

OPEN ACCESS

Fishes in high-CO_2, acidified oceans

Atsushi Ishimatsu[1,*], Masahiro Hayashi[1], Takashi Kikkawa[2]

[1]Institute for East China Sea Research, Nagasaki University, 1551-7 Tairamachi, Nagasaki 851–2213, Japan
[2]Central Laboratory, Marine Ecology Research Institute, Onjuku, Chiba 299-5105, Japan

ABSTRACT: Research interest in CO_2-driven ocean acidification has been centered on certain groups of calcifying marine organisms, but knowledge on the possible impacts of ocean acidification on fish is limited. Our survey of the existing literature on the effects of increased pCO_2 on fish (total of 116 papers) revealed that few studies were conducted under pCO_2 conditions relevant to the future scenarios of ocean acidification. Information is nearly absent on reproduction, early development, and behaviour of marine fish. The short experimental durations of these studies preclude forecasting of how mortality and growth of marine fish would be affected by future increases in seawater CO_2. Fish have been shown to maintain their oxygen consumption under elevated pCO_2 conditions, in contrast to declines seen in several marine invertebrates, in spite of possible additional energetic costs incurred by higher pCO_2. Impacts of prolonged CO_2 exposure on reproduction, early development, growth, and behaviour of marine fish are important areas that need urgent investigation. There is also a need to rapidly advance research into possible acclimation of marine fish to high pCO_2 environments, endocrine responses to prolonged CO_2 exposure, and indirect influences through food availability and quality on fish growth, survival and reproduction. Useful guidance could be gained from the rich literature on the effects of freshwater acidification.

KEY WORDS: Fish · Otolith · Ocean acidification · Mortality · Growth · Oxygen consumption

INTRODUCTION

Research interest pertaining to CO_2-driven ocean acidification has been centered on certain groups of calcifying marine organisms (Kleypas et al. 2006). In contrast, knowledge is limited on the possible impacts of ocean acidification on fish. We surveyed 116 papers (published 1969 through 2008) on the effects of high pCO_2 on fishes and summarize the results in Table 1. The survey revealed that the data from these studies are of limited value to predict the fate of fishes in the future acidified oceans for the following reasons: (1) the pCO_2 levels used were much higher (above 50 000 µatm in 92% of the papers: 1 µatm = 0.76 × 10^{-3} mmHg = 0.1013 Pa) than projected for the oceans in the next centuries (max. 1900 µatm at around the year 2300, Caldeira & Wickett 2003; see also Caldeira & Wickett 2005 for other projections), with only 2 studies covering the pCO_2 range below 2000 µatm (Jones et al. 1985, Ross et al. 2001); (2) CO_2 exposure periods were less than 4 d in 79% of the *in vivo* studies with only 8 experiments longer than 60 d; (3) marine species were used only in 25% of the studies; (4) research has focused largely on acid–base regulation and cardiorespiratory control (58% of the papers), and other aspects were little investigated; (5) effects on early development have been studied in only 2 papers (Kikkawa et al. 2003, this paper was counted under 'sequestration,' Sawada et al. 2008); and (6) all are laboratory experiments.

Another source of information that might give clues for considering CO_2 impacts on fish is the rich literature on freshwater acidification (Morris et al. 1989). However, extrapolations from freshwater acidification research must be made with caution: (1) the physicochemical nature of the milieu, and the taxonomy and physiology of the fish are vastly different between freshwater and seawater ecosystems; (2) the pH reductions envisaged in the future scenarios of ocean acidification (max. 0.77 pH units at around the year 2300,

*Email: a-ishima@nagasaki-u.ac.jp

Table 1. Summary of the literature survey on the effects of CO_2 on fishes. Numbers in parentheses indicate number of papers classified according to fish habitat, pCO_2 level used, exposure duration, and purpose of study. Total number of papers surveyed = 116. 'Miscellaneous' includes CO_2 anesthesia (6 studies), *in vitro* myocardium physiology (6), CO_2 sequestration (6), palatine CO_2 receptors (5), sperm motility (4), metabolism (2), behaviour (2), swimbladder gas (1), fillet attributes (1), Ca metabolism (1), ammonia (1), cataract (1), blood sugar (1), feed intake (1), and early development (1)

Habitat[a]	pCO_2 (µatm)[b]	Duration (d)[c]	Purpose of study[d]
Freshwater (88)	<5000 (9)	<1 (52)	Acid-base (38)
Seawater (30: teleosts 22,	5000 to 10 000 (52)	1 to 4 (27)	Cardiorespiratory (29)
elasmobranchs 8)	10 000 to 50 000 (44)	4 to 10 (3)	Growth (10)
	>50 000 (8)	>30 (18)	Miscellaneous (39)

[a]2 studies used both freshwater and seawater fish

[b]Sum of the listed studies is 113 since several studies did not report pCO_2 values. For those studies in which several levels of pCO_2 were used, the lowest pCO_2 values were counted. Original papers reported CO_2 levels as concentration (mg l^{-1}) or pCO_2 in mmHg, torr or kPa. We calculated pCO_2 in µatm using reported experimental temperature and CO_2 solubility values (Dejours 1981)

[c]Sum of the listed studies is 100 since we excluded *in vitro* studies. For those studies in which several exposure durations were tested, the longest duration was counted

[d]For those studies in which more than 1 purpose was stated, we selected the major purpose

Caldeira & Wickett 2003) are of smaller magnitude and will develop on a longer timescale than those caused by freshwater acidification. Freshwater acidification in susceptible areas (several countries in Europe and North America) has occurred more rapidly with larger pH reductions than ocean acidification (e.g. a pH reduction of ~2.0 within 30 yr in a forest lake, Andersson & Olsson 1985), often accompanying episodic further pH declines of 1.0 to 2.5 due to heavy rainfall or snowmelt (Reader & Dempsey 1989); (3) CO_2 often has greater negative impacts on exposed animals than mineral acids at identical pH levels (Crocker & Cech 1996, Hayashi et al. 2004a, Kikkawa et al. 2004).

This review attempts to summarize currently available information about selected aspects of CO_2 impacts on fish to provide a basis for understanding consequences of ocean acidification on the biology of marine fish. We propose research areas that need urgent attention.

MORTALITY

High concentrations of CO_2 kill fish (Lee et al. 2003, Hayashi et al. 2004b, Ishimatsu et al. 2004). The results of Lee et al. (2003) suggested cardiac failure is an important factor in acute death of the yellowtail *Seriola quinqueradiata* when it is exposed to 50 000 µatm of CO_2. Hayashi et al. (2004b) demonstrated that fish death occurred after arterial blood pH was restored to the pre-exposure level. However, the acutely lethal pCO_2 levels (in less than 3 d, >30 000 to 50 000 µatm) used in these studies far exceed those pertaining to ocean acidification and, therefore, will not be considered further.

The information on the prolonged impact of somewhat lower pCO_2 on fish mortality can be found in the aquaculture literature, even though the pCO_2 levels used in these experiments are still higher than those projected for the future oceans (Table 2). We were able to find only 2 aquaculture papers reporting mortality of seawater fish in hypercapnic environments. Although fish mortality appears to be positively dependent on imposed pCO_2 levels and exposure duration, the data are somewhat variable between studies even for the same species, possibly due to differences in experimental temperature and fish size. Furthermore, the interpretation of the 3 freshwater aquaculture studies is complicated by possible involvement of aluminium in fish mortality, which is thought to be a main factor in acid-water toxicity to freshwater fish (Heath 1995). Aluminium is mobilized from the soil by reductions of surface water pH and can reach 100 µmol l^{-1} (total aluminium) during low pH episodes (Reader & Dempsey 1989). However, aluminium concentration in seawater is usually much lower (<20 nmol l^{-1} in open oceans but up to 150 nmol l^{-1} in semi-enclosed seas, Tria et al. 2007). In addition, calcium, which counteracts the toxic effects of aluminium, is higher in seawater (10 mmol l^{-1} in 35 ppt seawater, Thurman & Trujillo 1999) than in freshwater (0.05 to 5.0 mmol l^{-1}, Appelo & Postma 2006), which makes it unlikely for aluminium to be involved in CO_2 toxicity to seawater fish.

None of the aquaculture studies examined mortality during early developmental stages (see initial body weight in Table 2). Acute (up to 72 h) mortality under pCO_2 of 3000 to 148 000 µatm was studied for embryos and larvae of marine teleosts (*Pagrus major* and *Sillago japonica*), which demonstrated that the most susceptible stages were cleavage and juvenile, whereas the

Table 2. Mortality of fish under elevated CO_2 conditions reported in aquaculture papers. FW: freshwater; SW: seawater. (1) Fivelstad et al. (2007), (2) Fivelstad et al. (1999), (3) Fivelstad et al. (2003), (4) Hosfeld et al. (2008), (5) Fivelstad et al. (1998), (6) Foss et al. (2003)

Species	Medium	pCO_2 (µatm)[a]	Temp (°C)	Period (d)	Initial body weight (g)	Mortality (%)[b]	Source
Salmo salar							
Parr	FW	380	5	47	10 to 13	0	1
		15 800				0	
		660	15			0	
		15 800				0	
Smolt	FW[c]	2600	3–7	62	53	1.5	2
		6600				4.6	
		11 800				7.7	
	FW[c]	2600	7–9	60	66	3	3
		6600				2.4	
		9200				4.5	
	FW[c]	920	6.4 to 9	42	50	0	4
		7100				0	
Postsmolt	SW	790	15 to 16	43	170 to 260	0	5
		6400				0	
		15 800				1.1	
		26 300				4.3	
Anarhichas minor							
Juvenile	SW	480	6	70	16	0	6
		8000				0	
		14 700				0	
		26 100				0	

[a]Original papers reported CO_2 levels as concentration (mg l^{-1}) or pCO_2 in mmHg, torr or kPa. We calculated pCO_2 in µatm using reported experimental temperature and CO_2 solubility values (Dejours 1981)
[b]Percent mortality recorded at the end of the experiments
[c]Fish were transferred to normocapnic seawater subsequent to the freshwater periods

preflexion and flexion stages were more tolerant (Kikkawa et al. 2003). Recently, Sawada et al. (2008) reported that 150 min exposure to pCO_2 of 92 000 µatm resulted in significantly higher mortality in the embryos of the striped jack *Pseudocaranx dentex*. Studies of CO_2 impacts on early developmental stages of marine fish are particularly important since freshwater acidification studies have revealed that embryonic and larval stages are often the most sensitive stages to acute acid stress (Morris et al. 1989, Sayer et al. 1993, Heath 1995). Kurihara (2008, this Theme Section) discusses effects of high pCO_2 on early development of marine invertebrates.

The cause for fish mortality in long-term high pCO_2 exposure remains unknown. Aquaculture studies often reported occurrence of calcareous precipitates in the kidney (nephrocalcinosis), which may obstruct the lumen of kidney tubules (Fivelstad et al. 1999, 2003). Among the 2 studies on seawater fish, Foss et al. (2003) found increased percentage of fish with nephrocalcinosis, whereas Fivelstad et al. (1998) did not. Long-lasting reductions of plasma Cl^-, possible reductions of hepatic metabolism, and a shift to anaerobic metabolism (see 'Energetic costs of living in high CO_2 oceans') deserve attention in elucidating mechanism(s) of fish mortality during long-term exposure to environmental hypercapnia. Recently, Kikkawa et al. (2008) indicated an inverse relationship between acute CO_2 mortality and oxygen consumption among marine animals.

ENERGETIC COSTS OF LIVING IN HIGH CO_2 OCEANS

Elevations of ambient pCO_2 may require fishes to spend more energy for physiological adaptations, in particular, acid–base regulation and cardiorespiratory control. Many excellent reviews have been already published on these topics (Milsom 2002, Perry & Gilmour 2002, Evans et al. 2005, Marshall & Grosell 2006, Perry & Gilmour 2006); therefore we limit our discussion to the energetic aspects of these physiological processes.

Cost of osmoregulation in seawater fish has been estimated to be 6 to 15% of resting oxygen consumption (Kirschner 1993, Kidder et al. 2006). On top of this baseline cost for osmoregulation, elevation in seawater pCO_2 would require additional energy expenditure for

acid–base regulation. When the body fluid becomes acidic, fish excrete excess H^+ ions into the ambient water across different epithelia (gills, kidney and intestine) to restore body fluid pH near to its normal level (Heisler 1986). Fish are usually more efficient in extracellular acid–base regulation than invertebrates (Widdicombe & Spicer 2008). One consistent finding for teleosts, but not elasmobranchs, is that plasma Cl^- concentration decreases at a nearly 1:1 ratio with increasing plasma bicarbonate in both freshwater and seawater species (Ishimatsu et al. 2005). Such reductions of plasma Cl^- persisted even after 70 d when a seawater spotted wolffish *Anarhichas minor* was exposed to 8000 to 26 000 µatm pCO_2 (Foss et al. 2003). Because Cl^- is actively extruded in marine fish (Marshall & Grosell 2006), the observed further reductions of plasma Cl^- during exposure to high CO_2 would require the fish to expend additional energy. Similar long-lasting reductions of plasma Cl^- were observed also in freshwater species exposed to high pCO_2 (Fivelstad et al. 1999, Danley et al. 2005).

Ventilation of water-breathing animals is energetically more costly than in air-breathing animals. This is due to the relative scarcity of oxygen in water compared to air, the higher density and viscosity of water than of air (Dejours 1981), and is reflected in much higher energetic cost of ventilation in water breathers (around 10% at rest and up to 70% during exercise in fish) than in air breathers (1 to 2%, Gilmour 1998). It appears that fish would show little respiratory acclimation during long-term exposure to a high pCO_2 environment. Fivelstad et al. (1999) found that ventilatory frequencies remained significantly higher (ca. 125% of the control) in Atlantic salmon smolt exposed to 12 000 µatm pCO_2 than in the control fish throughout a 62 d exposure period. Similarly, Hosfeld et al. (2008) reported significant increases in ventilatory frequency for the same species throughout a 36 d exposure to 7900 µatm pCO_2. These observations indicate that the fish needed to expend more energy in ventilation throughout the hypercapnic period.

OXYGEN CONSUMPTION

Notwithstanding the possible higher energetic costs during hypercapnic exposure, oxygen consumption did not change significantly when resting fish were exposed to sublethal levels of CO_2 (Table 3). A transient increase in oxygen consumption was observed in 2 elasmobranchs, and a significant rise reported for *Leiostomus xanthurus* might be due to the short duration of the experiment. The constant oxygen uptake of fish during hypercapnic exposure is at variance with the data for invertebrates, where oxygen consumption decreased significantly (Table 3). Fabry et al. (2008) also reported unpublished data showing 20 to 50% reductions in oxygen consumption for marine invertebrates during hypercapnia. In spite of the insignificant changes in oxygen consumption of fish during hypercapnia, an *in vitro* study by Langenbuch & Pörtner (2003) demonstrated a reduction of oxygen consumption by hepatocytes of 2 Antarctic fish when incubated at a pCO_2 of 10 000 µatm. They estimated that 60% of the observed reduction in oxygen consumption was accounted for by a decline in protein synthesis in both species. A recent study on a seawater fish, *Sparus auratus*, subjected to a pCO_2 of 5000 µatm suggested a shift from aerobic to anaerobic metabolism on the basis of changes in metabolic enzyme activities (Michaelidis et al. 2007). Because published data on oxygen consumption in fish during hypercapnic exposure are all of short duration (<24 h) and under pCO_2 higher than levels projected for future oceans, long-term measurements of oxygen consumption are needed under pCO_2 conditions relevant to the ocean acidification scenarios.

GROWTH

It may be inferred that fish growth is reduced due to the possible additional energetic costs imposed by elevated pCO_2, when overall oxygen consumption re-

Table 3. Effect of hypercapnia on oxygen consumption of selected marine animals. The 5 upper fish species are teleosts; the bottom 2 fishes are elasmobranchs. FW: freshwater. SW: seawater. Source: (1) Kinkead et al. (1993), (2) Takeda (1991), (3) Cochran & Burnett (1996), (4) Graham et al. (1990), (5) Randall et al. (1976), (6) Michaelidis et al. (2005), (7) Pörtner et al. (1998)

Species	Medium	pCO_2 (µatm)	Temp (°C)	Duration (h)	Control %	Source
Fishes						
Oncorhynchus mykiss	FW	7500	9 to 11	0.5	No change	1
Cyprinus carpio	FW	13 200	25	6	No change	2
Fundulus heteroclitus	25 ppt	92 000	30	Not stated	No change	3
Palaemonetes pugio	25 ppt	92 000	30	Not stated	No change	3
Leiostomus xanthurus	25 ppt	92 000	30	Not stated	147	3
Raja ocellata	SW	9900	12	24	No change[a]	4
Scyliorhinus stellaris	SW	6600	16 to 19	4	No change[a]	5
Invertebrates						
Mytilus galloprovincialis	SW	5000	18	20 90 d	35 (adults) 65 (juveniles)	6
Sipunculus nudus	SW	10 300	15	2 to 3	80	7

[a]Transient significant increases at the onset of hypercapnia

mains unchanged. Again, information is only available from aquaculture investigations that employed relatively high pCO_2. Increments of body weight were in general unaffected by exposure to pCO_2 of up to 15 000 µatm irrespective of salinity (Fivelstad et al. 1998, 1999, 2003, Foss et al. 2003, Hosfeld et al. 2008). The condition factor ([100 × body weight] / [body length]^3) tended to decrease at high pCO_2, but the threshold for this effect appears to depend on species, fish size and salinity. Growth was invariably reduced at pCO_2 >26 000 µatm. Fivelstad et al. (2007) recently demonstrated that negative CO_2 effect on fish growth was more pronounced at a low temperature when exposed to the same pCO_2 (16 000 µatm). Feeding may be suppressed at a very high pCO_2 (55 000 µatm, Cecchini et al. 2001; 26 500 µatm, Foss et al. 2003).

Inspection of these growth studies revealed that the smallest initial fish size was 4 g (juvenile *Acipenser transmostanus*, Crocker & Cech 1996). To our knowledge, no paper has been published on growth from fish eggs or larvae under pCO_2 of < 2000 µatm. There is an urgent need to conduct CO_2 exposure experiments from fish eggs and larvae to compare subsequent growth and survival at pCO_2 of <2000 µatm.

SKELETONS AND OTOLITH FORMATION

Gil-Martens et al. (2006) is probably the only study that investigated effects of high pCO_2 on fish bones, minerals of which are composed of calcium phosphate in the form of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. After rearing Atlantic salmon for 135 d under control (pCO_2 3300 µatm) and gradually increasing pCO_2 conditions (4700 to 16 600 µatm), they found higher Ca and P contents in vertebral bones of the experimental fish than in control fish. Histological examinations suggested higher bone remodeling activities in the high CO_2 group, while no morphological difference was detected by X-ray radiography.

In contrast to bones, fish otoliths usually deposit aragonite, the orthorhombic polymorph of calcium carbonate ($CaCO_3$) (Carlström 1963). Aragonite is more soluble than calcite, the other most common marine $CaCO_3$ (Zeebe & Wolf-Gladrow 2001, Morse et al. 2007). Thus, elevated environmental pCO_2 could reduce $CaCO_3$ saturation of the endolymph, in which the fish otolith is formed, and thereby affect otolith growth. There is limited information available on aragonite saturation and on the acid–base status of the endolymph of the inner ear sacs, and on the mechanisms of otolith formation in fish. Takagi (2002) and Takagi et al. (2005) reported that endolymph is supersaturated with respect to aragonite in rainbow trout under normocapnic conditions. However, the reported pCO_2 of the saccular endolymph (11 000 to 16 500 µatm) is high compared with values commonly reported for arterial plasma of chronically cannulated fish (2600 to 5300 µatm, Heisler 1986); therefore, this value needs confirmation. Payan et al. (1997, 1998) demonstrated that endolymph in rainbow trout is characterized by higher pH and total CO_2 than in plasma, although the reported values of low arterial pH (7.2 to 7.3, as opposed to the typical 7.8 to 8.0 at the experimental temperature of Payan et al. 1997, 1998, see Heisler 1986) and high pCO_2 (8000 to 12 000 µatm) might be due to some sampling and/or analytical problem. We are not aware of any study that addressed the impacts of high CO_2 on otolith formation in fish.

Fish otolith is involved in both sound perception and the maintenance of postural equilibrium. The data by Gagliano et al. (2008) suggest that asymmetry of the saccular otolith affects the ability of larvae to distinguish between different sound frequencies in a coral reef fish (*Pomatocentrus ambioinensis*), which possibly lead to higher mortality by impairing navigation in coral reefs. As a more extreme case, Riley & Moorman (2000) demonstrated that bilateral loss of utricular otoliths disrupts vestibular functions and is invariably lethal for zebrafish larvae. Gagliano et al. (2008) also found that otolith asymmetries arising early in the embryonic stage were not corrected during the subsequent larval stage. Otoliths, as well as labyrinth, are formed before hatching (Noakes & Godin 1988) when the capacity for acid–base regulation may not be fully developed (Alderdice 1988). Thus, there is a need to investigate effects of CO_2 on otolith growth, including asymmetry.

SUMMARY AND RESEARCH NEEDS

Here we summarize some of the research areas of high priority to understand effects of ocean acidification on fish. Several recent reviews have discussed future research needs in broader contexts (Fabry et al. 2008, Guinotte & Fabry 2008, Doney et al. 2009).

(1) Effect of CO_2 acidified seawater on reproduction of fish needs urgent attention. To our knowledge, no information is available on fecundity, egg viability and hatching, and progeny survival of marine fish under high pCO_2 conditions, for which ample evidence for negative impacts is available in the freshwater acidification literature (Heath 1995). Kitamura & Ikuta (2000) reported that nest-digging behaviour of female hime salmon (land-locked *Oncorhynchus nerka*) was significantly inhibited by a pH reduction of 6.8 (control) to only 6.4.

(2) Long-term exposure experiments covering entire life stages need to be conducted under realistic future

ocean CO_2 conditions and sublethal impacts must be carefully investigated on developmental and homeostatic processes from molecular, biochemical and physiological viewpoints, with particular attention to early developmental stages.

(3) Behaviour (e.g. feeding, prey capture, escape from predators) must be quantitatively analyzed using fish reared under hypercapnic conditions. Behavioural responses can be a sensitive indicator of environmental stress and have significant ecological implications (Roast et al. 2001).

(4) Possible acclimation of marine fish to hypercapnic marine environments needs to be studied. One example is the finding that a strain of Japanese dace, inhabiting an acid lake (pH 3.5), exhibits a marked acid tolerance, while individuals of the same species inhabiting circumneutral lakes died rapidly when exposed to pH 3.5 conditions (Kaneko et al. 1999, Hirata et al. 2003). Interspecific differences in acclimation capacity could alter species composition of fish communities.

(5) Endocrine responses to prolonged exposure to high pCO_2 are not known. Acute CO_2 exposure did not affect blood concentrations of catecholamines and somatolactin in rainbow trout (Kakizawa et al. 1997, Julio et al. 1998). Endocrine responses to acidic freshwater stress were reviewed by Wendelaar Bonga & Balm (1989).

(6) Indirect impacts through changes in food availability and quality are another important issue in considering the fate of fish in high CO_2 oceans (Guinotte & Fabry 2008). Effect of high pCO_2 on the appetite of fish is not well understood. Yoshii & Yoshii (1997) reported suppression of taste nerve responses by CO_2.

Few research efforts have been directed to marine fishes to test possible impacts of ocean acidification. The present review has demonstrated that the existing knowledge of CO_2 impacts on fish could provide no more than useful starting points to understand possible alterations of marine fish populations in future oceans. We hope this review will provide momentum in research into fish biology in high-CO_2, acidified oceans.

LITERATURE CITED

Alderdice DF (1988) Osmotic and ionic regulation in teleost eggs and larvae. In: Hoar WS, Randall DJ (eds) Fish physiology, Vol XIA. Academic Press, San Diego, CA, p 163–251

Andersson F, Olsson B (1985) Lake Gårdsjön — an acid forest lake and its catchment. AiO Press, Odense

Appelo CAJ, Postma D (2006) Geochemistry, groundwater and pollution, 2nd edn. A. A. Balkema, Leiden

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

Caldeira K, Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. J Geophys Res 110, C09S04, doi:10.1029/2004JC002671

Carlström D (1963) A crystallographic study of vertebrate otoliths. Biol Bull 125:441–463

Cecchini S, Saroglia M, Caricato G, Terova G, Sileo L (2001) Effects of graded environmental hypercapnia on sea bass (*Dicentrarchus labrax* L.) feed intake and acid-base balance. Aquacult Res 32:499–502

Cochran RE, Burnett LE (1996) Respiratory responses of the salt marsh animals, *Fundulus heteroclitus, Leiostomus xanthurus*, and *Pelaemonetes pugio* to environmental hypoxia and hypercapnia and to the organophosphate pesticide, azinphosmethyl. J Exp Mar Biol Ecol 195: 125–144

Crocker CE, Cech JJ Jr (1996) The effects of hypercapnia on the growth of juvenile white sturgeon, *Acipenser transmontanus*. Aquaculture 147:293–299

Danley M, Kenney P, Mazik P, Kiser R, Hankins J (2005) Effects of carbon dioxide exposure on intensively cultured rainbow trout *Oncorhynchus mykiss*: physiological responses and fillet attributes. J World Aquacult Soc 36: 249–261

Dejours P (1981) Principles of comparative respiratory physiology, 2nd edn. Elsevier, Amsterdam

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO_2 problem. Annu Rev Mar Sci 1:169–192

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

Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J Mar Sci 65:414–432

Fivelstad S, Haavik H, Løvik G, Olsen AB (1998) Sublethal effects and safe levels of carbon dioxide in seawater for Atlantic salmon postsmolts (*Salmo salar* L.): ion regulation and growth. Aquaculture 160:305–316

Fivelstad S, Olsen AB, Kløften H, Ski H, Stefansson S (1999) Effects of carbon dioxide on Atlantic salmon (*Salmo salar* L.) smolts at constant pH in bicarbonate rich freshwater. Aquaculture 178:171–187

Fivelstad S, Olsen AB, Åsgård T, Baeverfjord G, Rasmussen T, Vindheim T, Stefansson S (2003) Long-term sublethal effects of carbon dioxide on Atlantic salmon smolts (*Salmo salar* L.): ion regulation, haematology, element composition, nephrocalcinosis and growth parameters. Aquaculture 215:301–319

Fivelstad S, Waagbø R, Stefansson S, Olsen AB (2007) Impacts of elevated water carbon dioxide partial pressure at two temperatures on Atlantic salmon (*Salmo salar* L.) parr growth and haematology. Aquaculture 269:241–249

Foss A, Rønes BA, Øiestad V (2003) Graded environmental hypercapnia in juvenile spotted wolffish (*Anarhichas minor* Olafsen): effects on growth, food conversion efficiency and nephrocalcinosis. Aquaculture 220: 607–617

Gagliano M, Depczynski M, Simpson SD, Moore JAY (2008) Dispersal without errors: symmetrical ears tune into the right frequency for survival. Proc R Soc Lond B Biol Sci 275:527–534

Gilmour KM (1998) Gas exchange. In: Evans DH (ed) The physiology of fishes, 2nd ed. CRC Press, Boca Raton, FL, p 101–127

Graham MS, Turner JD, Wood CM (1990) Control of ventilation in the hypercapnic skate *Raja ocellata*: I. Blood and extradural fluid. Respir Physiol 80:259–277

Guinotte JM, Fabry VJ (2008) Ocean acidification and its potential effects on marine ecosystems. Ann NY Acad Sci 1134:320–342

Hayashi M, Kita J, Ishimatsu A (2004a) Comparison of the acid-base responses to CO_2 and acidification in Japanese flounder (*Paralichthys olivaceus*). Mar Pollut Bull 49: 1062–1065

Hayashi M, Kita J, Ishimatsu A (2004b) Acid-base responses to lethal aquatic hypercapnia in three marine fishes. Mar Biol 144:153–160

Heath A (1995) Water pollution and fish physiology, 2nd edn. CRC Press, Boca Raton, FL

Heisler N (1986) Acid-base regulation in fishes. In: Heisler N (ed) Acid-base regulation in animals. Elsevier, Amsterdam, p 309–356

Hirata T, Kaneko T, Ono T, Nakazato T and others (2003) Mechanism of acid adaptation of a fish living in a pH 3.5 lake. Am J Physiol 284:R1199–R1212

Hosfeld CD, Engevik A, Mollan T, Lunde TM and others (2008) Long-term separate and combined effects of environmental hypercapnia and hyperoxia in Atlantic salmon (*Salmo salar* L.) smolts. Aquaculture 280:146–153

Ishimatsu A, Kikkawa T, Hayashi M, Lee KS, Kita J (2004) Effects of CO_2 on marine fish: larvae and adults. J Oceanogr 60:731–741

Ishimatsu A, Hayashi M, Lee KS, Kikkawa T, Kita J (2005) Physiological effects on fishes in a high-CO_2 world. J Geophys Res 110, C09S09, doi:10.1029/2004JC002564

Jones KA, Hara TJ, Scherer E (1985) Locomotor responses by Arctic char (*Salvelinus alpinus*) to gradients of H^+ and CO_2. Physiol Zool 58:413–420

Julio AE, Montpetit CJ, Perry SF (1998) Does blood acid-base status modulate catecholamine secretion in the rainbow trout (*Oncorhynchus mykiss*)? J Exp Biol 201: 3085–3095

Kakizawa S, Ishimatsu A, Takeda T, Kaneko T, Hirano T (1997) Possible involvement of somatolactin in the regulation of plasma bicarbonate for the compensation of acidosis in rainbow trout. J Exp Biol 200:2675–2683

Kaneko T, Hasegawa S, Uchida K, Ogasawara T, Oyagi A, Hirano T (1999) Acid tolerance of Japanese dace (a cyprinid teleost) in Lake Osorezan, a remarkable acid lake. Zoolog Sci 16:871–877

Kidder GI, Petersen C, Preston R (2006) Energetics of osmoregulation: I. Oxygen consumption by *Fundulus heteroclitus.* J Exp Zool 305A:309–317

Kikkawa T, Ishimatsu A, Kita J (2003) Acute CO_2 tolerance during the early developmental stages of four marine teleosts. Environ Toxicol 18:375–382

Kikkawa T, Kita J, Ishimatsu A (2004) Comparison of the lethal effect of CO_2 and acidification on red sea bream (*Pagrus major*) during the early developmental stages. Mar Pollut Bull 48:108–110

Kikkawa T, Watanabe Y, Katayama Y, Kita J, Ishimatsu A (2008) Acute CO_2 tolerance limits of juveniles of three marine invertebrates, *Sepia lycidas, Sepioteuthis lessoniana*, and *Marsupenaeus japonicas.* Plankton Benthos Res 3:184–187

Kinkead R, Aota S, Perry SF, Randall DJ (1993) Propranolol impairs the hyperventilatory response to acute hypercapnia in rainbow trout. J Exp Biol 175:115–126

Kirschner LB (1993) Energetics of osmotic regulation in ureotelic and hypoosmotic vertebrates. J Exp Zool 267: 19–26

Kitamura S, Ikuta K (2000) Acidification severely suppresses spawning of hime salmon (land-locked sockeye salmon, *Oncorhynchus nerka*). Aquat Toxicol 51:107–113

Kleypas JA, Feely RA, Fabry VJ, Langdon C, Sabine CL, Robbins LL (2006) Impacts of ocean acidification on coral reefs and other marine calcifiers: a guide for future research, report of a workshop held 18–20 April 2005, St. Petersburg, FL

Kurihara H (2008) Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar Ecol Prog Ser 373:275–284

Langenbuch M, Pörtner HO (2003) Energy budget of hepatocytes from Antarctic fish (*Pachycara brachycephalum* and *Lepidonotothen kempi*) as a function of ambient CO_2: pH-dependent limitations of cellular protein biosynthesis? J Exp Biol 206:3895–3903

Lee KS, Kita J, Ishimatsu A (2003) Effects of lethal levels of environmental hypercapnia on cardiovascular and blood-gas status in yellowtail, *Seriola quinqueradiata.* Zoolog Sci 20:417–422

Marshall WS, Grosell M (2006) Ion transport, osmoregulation, and acid-base balance. In: Evans DH, Claiborne JB (eds) The physiology of fishes, 3rd edn. CRC Press, Boca Raton, FL, p 177–230

Gil-Martens L, Witten P, Fivelstad S, Huysseune A, Sævareid B, Vikeså V, Obach A (2006) Impact of high water carbon dioxide levels on Atlantic salmon smolts (*Salmo salar* L.): effects on fish performance, vertebrae composition and structure. Aquaculture 261:80–88

Michaelidis B, Ouzounis C, Paleras A, Pörtner H (2005) Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis.* Mar Ecol Prog Ser 293:109–118

Michaelidis B, Spring A, Pörtner H (2007) Effects of long-term acclimation to environmental hypercapnia on extracellular acid-base status and metabolic capacity in Mediterranean fish *Sparus aurata.* Mar Biol 150:1417–1429

Milsom WK (2002) Phylogeny of CO_2/H^+ chemoreception in vertebrates. Respir Physiol Neurobiol 131:29–41

Morris R, Taylor EW, Brown DJA, Brown JA (1989) Acid toxicity and aquatic animals. Cambridge University Press, Cambridge

Morse JW, Arvidson RS, Lüttge A (2007) Calcium carbonate formation and dissolution. Chem Rev 107:342–381

Noakes D, Godin JGJ (1988) Ontogeny of behavior and concurrent developmental changes in sensory systems in teleost fishes. In: Hoar WS, Randall DJ (eds) Fish physiology, Vol XIB. Academic Press, San Diego, CA, p 345–395

Payan P, Kossmann H, Watrin A, Mayer-Gostan N, Boeuf G (1997) Ionic composition of endolymph in teleosts: origin and importance of endolymph alkalinity. J Exp Biol 200: 1905–1912

Payan P, Borelli G, Boeuf G, Mayer-Gostan N (1998) Relationship between otolith and somatic growth: consequence of starvation on acid-base balance in plasma and endolymph in the rainbow trout *Oncorhynchus mykiss.* Fish Physiol Biochem 19:35–41

Perry SF, Gilmour KM (2002) Sensing and transfer of respiratory gases at the fish gill. J Exp Zool 293:249–263

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

Pörtner HO, Reipschläger A, Heisler N (1998) Acid-base regulation, metabolism and energetics in *Sipunculus nudus* as a function of ambient carbon dioxide level. J Exp Biol 201:43–55

Randall DJ, Heisler N, Drees F (1976) Ventilatory response to hypercapnia in the larger spotted dogfish *Scyliorhinus stellaris.* Am J Physiol 230:590–594

Reader JP, Dempsey CH (1989) Episodic changes in water quality and their effects on fish. In: Morris R, Taylor EW,

Brown DJA, Brown JA (eds) Acid toxicity and aquatic animals. Cambridge University Press, Cambridge, p 67–83

Riley BB, Moorman SJ (2000) Development of utricular otoliths, but not saccular otoliths, is necessary for vestibular function and survival in zebrafish. J Neurobiol 43: 329–337

Roast SD, Widdows J, Jones MB (2001) Impairment of mysid (*Neomysis integer*) swimming ability: an environmentally realistic assessment of the impact of cadmium exposure. Aquat Toxicol 52:217–227

Ross RM, Krise WF, Redell LA, Bennett RM (2001) Effects of dissolved carbon dioxide on the physiology and behavior of fish in artificial streams. Environ Toxicol 16:84–95

Sawada Y, Higuchi K, Haga Y, Ura K and others (2008) Effects of hypoxia and hypercapnia on the embryonic development of striped jack, *Pseudocaranx dentex.* Nippon Suisan Gakkai Shi 74:144–151

Sayer MDJ, Reader JP, Dalziel TRL (1993) Freshwater acidification: effects on the early life stages of fish. Rev Fish Biol Fish 3:95–132

Takagi Y (2002) Otolith formation and endolymph chemistry: a strong correlation between the aragonite saturation state and pH in the endolymph of the trout otolith organ. Mar Ecol Prog Ser 231:237–245

Takagi Y, Tohse H, Murayama E, Ohira T, Nagasawa H (2005) Diel changes in endolymph aragonite saturation rate and mRNA expression of otolith matrix proteins in the trout otolith organ. Mar Ecol Prog Ser 294:249–256

Takeda T (1991) Regulation of blood oxygenation during short-term hypercapnia in the carp, *Cyprinus carpio.* Comp Biochem Physiol 98A:517–521

Thurman HV, Trujillo AP (1999) Essentials of oceanography, 6th edn. Prentice Hall, Upper Saddle River, NJ

Tria J, Butler ECV, Haddad PR, Bowie AR (2007) Determination of aluminium in natural water samples. Anal Chim Acta 588:153–165

Wendelaar Bonga SE, Balm PHM (1989) Endocrine responses to acid stress in fish. In: Morris R, Taylor EW, Brown DJA, Brown JA (eds) Acid toxicity and aquatic animals. Cambridge University Press, Cambridge, p 243–263

Widdicombe S, Spicer JI (2008) Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? J Exp Mar Biol Ecol 366:187–197

Yoshii K, Yoshii C (1997) NH_3- and CO_2-induced suppression of taste nerve responses in clawed toads and eels. Brain Res 757:202–208

Zeebe RE, Wolf-Gladrow D (2001) CO_2 in seawater: equilibrium, kinetics, isotopes. Elsevier, Amsterdam

Submitted: December 4, 2007; Accepted: November 12, 2008

Proofs received from author(s): December 18, 2008