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Review

Biological Impact of Elevated Ocean CO_2 Concentrations: Lessons from Animal Physiology and Earth History

HANS O. PÖRTNER*, MARTINA LANGENBUCH and ANKE REIPSCHLÄGER

Alfred-Wegener-Institut für Polar- und Meeresforschung, Biologie I/Ökophysiologie, Columbusstraße, D-27568 Bremerhaven, F.R.G.

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CO_2 currently accumulating in the atmosphere permeates into ocean surface layers, where it may impact on marine animals in addition to effects caused by global warming. At the same time, several countries are developing scenarios for the disposal of anthropogenic CO_2 in the worlds' oceans, especially the deep sea. Elevated CO_2 partial pressures (hypercapnia) will affect the physiology of water breathing animals, a phenomenon also considered in recent discussions of a role for CO_2 in mass extinction events in earth history. Our current knowledge of CO_2 effects ranges from effects of hypercapnia on acid-base regulation, calcification and growth to influences on respiration, energy turnover and mode of metabolism. The present paper attempts to evaluate critical processes and the thresholds beyond which these effects may become detrimental. CO_2 elicits acidosis not only in the water, but also in tissues and body fluids. Despite compensatory accumulation of bicarbonate, acid-base parameters (pH, bicarbonate and CO_2 levels) and ion levels reach new steady-state values, with specific, long-term effects on metabolic functions. Even though such processes may not be detrimental, they are expected to affect long-term growth and reproduction and may thus be harmful at population and species levels. Sensitivity is maximal in ommastrephid squid, which are characterized by a high metabolic rate and extremely pH-sensitive blood oxygen transport. Acute sensitivity is interpreted to be less in fish with intracellular blood pigments and higher capacities to compensate for CO_2 induced acid-base disturbances than invertebrates. Virtually nothing is known about the degree to which deep-sea fishes are affected by short or long term hypercapnia. Sensitivity to CO_2 is hypothesized to be related to the organizational level of an animal, its energy requirements and mode of life. Long-term effects expected at population and species levels are in line with recent considerations of a detrimental role of CO_2 during mass extinctions in the earth's history. Future research is needed in this area to evaluate critical effects of the various CO_2 disposal scenarios.

Keywords:
- Rising tropospheric CO_2 concentrations,
- ocean disposal of CO_2,
- critical CO_2 thresholds in marine animals,
- physiological effects of hypercapnia,
- acid-base disturbances,
- CO_2 in marine ecosystems,
- mass extinction events.

1. Introduction

Anthropogenic CO_2 production and the direct and indirect effects of accumulating CO_2 on marine ecosystems have come increasingly into focus in recent years. The current trend of accumulating CO_2 goes hand in hand with regional trends in other climate factors, especially increasing temperatures and increasing temperature variability (IPCC 2001). Global warming in itself threatens to change the geographical distribution of marine and terrestrial animals with the potential consequence of local extinction of previously common species (Parmesan and Yohe, 2003; Thomas *et al.*, 2004). The CO_2 emitted by human activities may also play a role in this context as it already permeates into ocean surface layers, where it may impact on marine organisms combined with effects exerted by current trends of warming and eutrophication. Whereas the rise in atmospheric CO_2 and its equilibration with the surface waters of the world's oceans is expected to fertilize marine phytoplankton depending on the

* Corresponding author. E-mail: hpoertner@awi-bremerhaven.de

availability of other nutrients (e.g. Riebesell *et al*., 1993; Wolf-Gladrow *et al*., 1999) and to affect calcification processes in phytoplankton, other effects of CO_2 on the physiology of marine organisms, especially animals, are not very well understood. In 1996, the observed accumulation of CO_2 in surface water had already caused a decrease in water pH by 0.1 pH units (Haugan and Drange, 1996) and a detectable impact on planktonic algae was observed (Riebesell *et al*., 2000).

Additional interest in such effects has arisen from recent plans to dispose of anthropogenic CO_2 by introducing it into the ocean, especially the deep sea (an idea originally proposed by Marchetti, 1977, 1979) where it would affect particularly animals living in the aphotic zone. The basic idea is to accelerate the equilibration of rising atmospheric CO_2 with the deep sea, which would otherwise be delayed for centuries, and to reduce the transient atmospheric CO_2 peak otherwise expected to rise from 370 ppm (PCO_2 = 370 μatm) up to more than 1500 ppm (PCO_2 = 1,500 μatm) between the years 2100 and 2200, unless CO_2 emissions can be reduced (e.g. Wigley *et al*., 1996). Under all the different disposal scenarios, organisms will have to tolerate high local CO_2 concentrations at the site of disposal (Shirayama, 1995), the actual magnitude depending upon the strategy applied. Liquid and gaseous CO_2 would be brought into the deep sea by pipeline from land or even a travelling ship. It may be stored in the form of CO_2 lakes filling local basins on the sea floor, or may be more evenly distributed in deep water layers (e.g. Ohsumi, 1995).

The relevance of potential CO_2 effects on animals is emphasized by the present discussion of aquatic CO_2 oscillations, which played a key role in the Perm Trias mass extinction events (Knoll *et al*., 1996; Bambach *et al*., 2002; Berner, 2002). Moreover, the evolution of high performance life forms in the sea may have depended on the reduction of atmospheric CO_2 levels during early metazoan evolution. Cambrian CO_2 levels reached as high as 0.5% (PCO_2 around 0.5 kPa or 5,000 μatm) and mean values decreased progressively thereafter (cf. Dudley, 1998). Recently, Cornette *et al*. (2002) suggested a relationship between atmospheric CO_2 levels and the rate of species diversification in the marine realm, while the mechanisms and time scales involved are presently unclear.

Under present conditions, CO_2 represents an abiotic factor that remains more or less constant in most of the pelagic zones of the sea. CO_2 levels will fluctuate where volcanic emissions occur in the sea and where excessive respiration occurs in confined areas filled with plant and animal life, e.g. in rockpools of the intertidal zone at night. It also fluctuates in marine sediments or hypoxic bottom waters as it depends on the oxidation of organic matter, rates of oxygen consumption and anaerobic metabolism of bacteria, meio- and macrofauna in an environment where mixing with the surface water is poor. PCO_2 values as high as 1.60 kPa (16,000 μatm) are conceivable in anoxic environments (Knoll *et al*., 1996) and levels of up to 8.00 kPa (80,000 μatm) have been recorded close to deep sea hydrothermal vents where some hydrothermal vent fauna, like the vestimentifera, may make use of the high CO_2 levels for CO_2 fixation by symbiontic bacteria (Childress *et al*., 1993).

Accordingly, some animals appear to be characterized by the development of specialized metabolic features to tolerate CO_2 or may be exposed to hypercapnia only periodically. This includes those pre-adapted to the accumulation of internal CO_2 (during exercise). Such animal models can be used to study hypercapnic effects and the underlying mechanisms.

All of these observations lead us to ask about both the general and the specific effects of CO_2 on various animal groups, from molecular and cellular to whole animal levels. The results presented in this paper originate from a field (physiological ecology) which aims at a deeper understanding of the effects of environmental variables on the physiology and biochemistry of organisms. Investigations also address how marine organisms adapt to a changing environment and what the tradeoffs and constraints in this adaptability are. Species living in the pelagic zone are adapted to constantly low CO_2 levels and may be more sensitive, whereas species dwelling in sediments may tolerate CO_2 fluctuations to some extent, especially in the intertidal zone. It is the latter species that permit the general and long-term effects of CO_2 and the underlying mechanisms to be studied in more detail. It should be emphasized that the investigation of sublethal effects appears crucial in terms of the evaluation of long-term effects of CO_2 in those species which survive acute CO_2 exposure. These long-term effects, for intervals greater than the duration of the reproduction cycle or the life span of an individual, may be overlooked but may nevertheless drastically change an ecosystem.

In general, the present paper is intended to assess criteria for an evaluation of maximum CO_2 concentrations tolerated by different marine animals from a physiological point of view. It draws on research that addresses the physiological and biochemical processes affected by CO_2 in shallow water animals, since few comparable data are currently available for deep-sea species. However, most mechanisms affected by CO_2 should be similar in all animals and, wherever adequate, extrapolations to the deep-sea situation will be included, with adequate precaution. For a more unifying picture, we are also drawing on information available for some freshwater animals as well as for CO_2 effects on such long-term processes as growth and reproduction and on various life stages, such as eggs, sperm, larvae and juveniles.

2. Physiology of CO_2 Effects: Molecular to Organismic Levels

When atmospheric CO_2 partial pressures rise, the quantities of CO_2 dissolved in water increase in accordance with Henry's law, leading to levels similar to those in air due to the great physical solubility of the gas in water. CO_2 enters the organism by diffusion, equilibrates between all body compartments and acts predominantly through its acidifying effect on acid-base balance. With an elevation in PCO_2, pH drops and bicarbonate levels rise, depending on the effect of buffers other than bicarbonate (non-bicarbonate buffers), which are titrated by the rise in carbonic acid concentration. The drop in pH is higher and the rise in bicarbonate is less in those fluids with lower buffer capacity. Accordingly, the passive rise in bicarbonate caused by increasing water PCO_2 is much less in sea water than in extracellular fluids, and here again, much less than in intracellular compartments. In contrast, the largest pH drop can be observed in sea water due to the small amounts of non-bicarbonate buffers, and the smallest pH drop occurs intracellularly owing to high levels of non-bicarbonate buffering, which is five times higher than in extracellular compartments. In most animals, invertebrates and vertebrates, extracellular pH is set to values 0.5–0.8 pH-units above intracellular pH. In consequence, bicarbonate levels are higher in extracellular than in intracellular fluids.

The analysis of effects of external CO_2 on acid-base regulation in water breathing animals (investigated in numerous species) follows the same lines as the analysis of respiratory influences. Respiratory changes titrate non-bicarbonate buffer values with changing PCO_2 and lead to pH decreases and an accumulation of bicarbonate. In only a few species has this approach been combined with an analysis of CO_2 effects on metabolism, not only when hypoxia and anaerobic metabolism occur (Reipschläger *et al.*, 1997) but also in the sense that CO_2 may affect overall metabolic rate and the partitioning of energy between individual metabolic processes by changes in either one or a combination of the different acid-base parameters in the water or in body compartments (Pörtner and Reipschläger, 1996; Pörtner *et al.*, 2000; Langenbuch and Pörtner, 2002).

The present paper focuses on ectothermic, water breathing animals whose body fluid CO_2 tension is much lower than in air breathers. Ventilation is driven largely by the limiting factor of water oxygenation (e.g. Jouve-Duhamel and Truchot, 1983) but there is growing evidence for a direct ventilatory sensitivity to CO_2, e.g. in teleosts and elasmobranchs (Burleson and Smatresk, 2000; McKendry *et al.*, 2001). Thus, an acute stimulatory effect of hypercapnia on ventilation was demonstrated in European eel (McKenzie *et al.*, 2002). However, the capacity for ventilatory compensation of the effect of hypercapnia on acid-base status is very limited in water breathers due to the small diffusion gradient of CO_2 between the organism and the water (Scheid *et al.*, 1989). Water breathers therefore rely almost exclusively on ion exchange mechanisms for a compensation of hypercapnic disturbances in acid-base status.

The processes of membrane-bound ion regulation will respond to respiratory or metabolic acid loads as well as to changes in water acid-base parameters and will strive to re-establish original or new acid-base equilibria in the body fluids. The carriers involved transport H^+ and/or bicarbonate, leading to an accumulation of bicarbonate and to a partial or complete compensation of the pH drop. Exchange processes will occur in epithelial membranes between animal and water and in membranes separating intra- and extracellular compartments. Ion transport depends on the sodium gradient built by ATP consuming Na^+/K^+-ATPase or consumes energy (ATP) directly in the case of the H^+-ATPase (Heisler, 1993). Research has as yet given us a far from complete understanding of the regulatory integration of these proton equivalent ion transport mechanisms, but phenomenological descriptions exist for fish and marine invertebrates and some carriers have been identified (cf. Heisler, 1986b, 1993; Pörtner and Reipschläger, 1996; Ishimatsu and Kita, 1999; Pörtner *et al.*, 2000; Claiborne *et al.*, 2002).

Following Heisler (1986b), the principle response to hypercapnia in fish is similar in freshwater and marine teleosts and elasmobranchs. Environmental hypercapnia causes an almost immediate reduction of plasma pH induced by elevated plasma PCO_2. In fish, more than 90% of all acid-base equivalent ion transport processes occur across the branchial epithelium (Heisler, 1986a), nevertheless intestine (Grosell *et al.*, 2001) and kidney (Wood *et al.*, 1999) are involved to a minor degree. One has to bear in mind that acid-base relevant transfer is always coupled with the problem of osmoregulation due to the required uptake of appropriate counter ions, which lead to an additional NaCl load of up to 10% in marine fish (Evans, 1984). Therefore, freshwater adapted fish species mainly rely on apical H^+-ATPase for the electrogenic transfer of H^+ to the ambient water; a process that also drives the uptake of external Na^+ into gill cells via Na^+ channels (Lin *et al.*, 1994). In contrast, different isoforms of Na^+/H^+ exchangers (NHE) are thought to be the main component responsible for acid-base transfer in seawater adapted fishes. At high external [Na^+], thermodynamic considerations favour Na^+/H^+ exchange for acid-base regulation (Potts, 1994) and functional studies confirmed the role of apical NHE for H^+ extrusion from gill cells (Edwards *et al.*, 2001).

Among marine invertebrates, the system of membrane proteins responsible for intracellular pH homeostasis comprises v-type H^+-ATPase as well as Na^+/

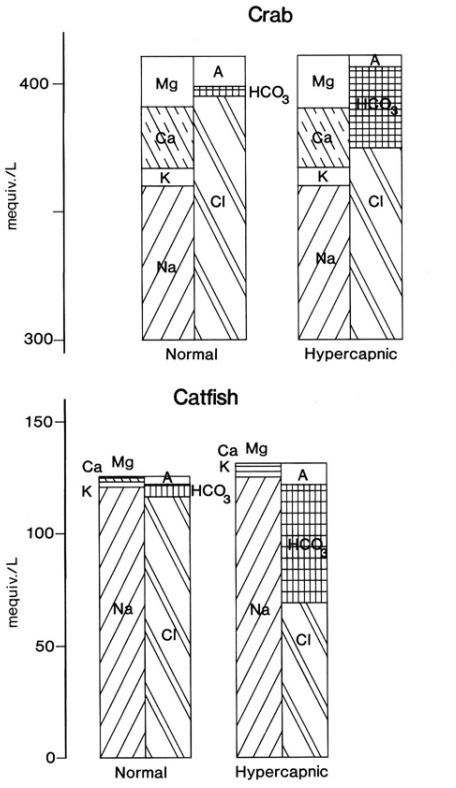


Fig. 1. Gamblegrams depicting the ionic composition of extracellular fluids in blue crabs and catfish (plasma) under control conditions and during environmental hypercapnia at a PCO_2 of 45 mm Hg (crab) and 56 mm Hg (catfish). A = unaccounted-for anion, probably mostly protein (adopted from Cameron and Iwama, 1989). Note the accumulation of bicarbonate under hypercapnia.

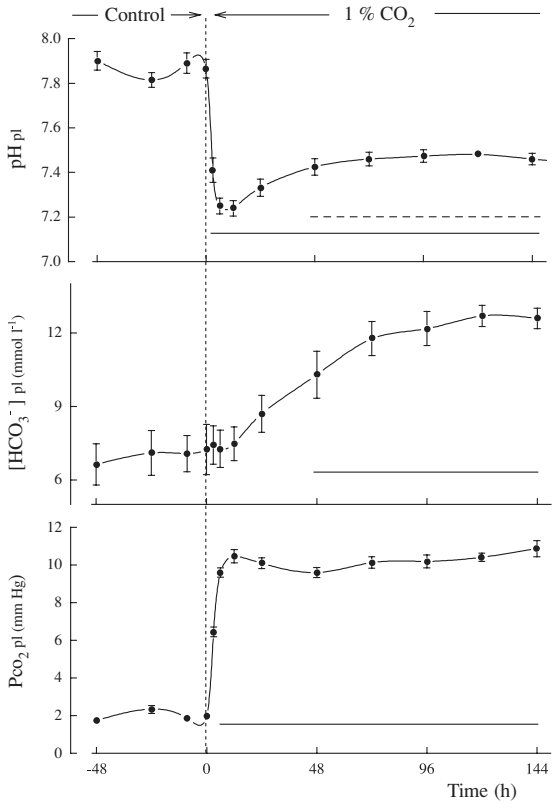


Fig. 2. Acid-base variables (pH, "$[HCO_3^-]$", PCO_2) in the coelomic plasma (extracellular fluid) of *Sipunculus nudus* under control conditions and subsequent hypercapnia (modified after Pörtner *et al.*, 1998). Note the incomplete compensation of the acidosis in the extracellular space.

H^+- and Na^+-dependent Cl^-/HCO_3^- exchange, as seen in the benthic sipunculid worm *Sipunculus nudus* (Pörtner *et al.*, 2000). The mechanisms of acid-base regulation operative at the organism water interface remain unidentified in this species.

In decapod crustaceans, HCO_3^- anion exchange plays an important role in the restoration of acid-base status after acid-base imbalance. The strongly euryhaline crab *Callinectes sapidus*, for example, uses the uptake of HCO_3^-, associated with an efflux of Cl^-, to compensate for hypercapnic acidosis (Truchot, 1979). Similar to fishes, a strong relationship between acid-base status and ion regulation has been found in decapod crustaceans. Compensation of the acidosis causes large and perhaps unfavorable changes in the ionic composition of plasma and other body fluids (see Fig. 1; Cameron and Iwama, 1989). Interestingly, changes in haemolymph acid-base status were only secondary to ion regulation and cell volume control in *Eriocheir sinensis* (Whiteley *et al.*, 2001).

In all organisms studied so far, acid-base disturbances elicited by hypercapnia are compensated for by an accumulation of bicarbonate anions (Heisler, 1986b; Wheatly, 1989; Pörtner *et al.*, 1998), a process that is most effective in the intracellular but not the extracellular compartment. In Atlantic cod, for instance, Larsen *et al.* (1997) observed a complete compensation of hypercapnia-induced acidosis in both extra- and intracellular compartments. However, while this process was rapid in the intracellular space of muscle tissue, extracellular pH reached control values only after 24 h via an increase in extracellular bicarbonate concentration paralleled by a reduction in chloride levels. Nevertheless, extracellular pH may not always return to the original value and the degree of compensation is lower, especially in marine invertebrates (e.g. 30% in a marine worm, see Fig. 2; Pörtner *et al.*, 1998), than in freshwater as well as marine teleost and elasmobranch fish under the same conditions. In the fish, extracellular pH also rarely reaches the original value (Heisler, 1986a). One limiting factor for the degree of compensation may be the extent to which bi-

carbonate is available from the ambient water for extracellular compensation, or from the extracellular fluid (blood, haemolymph) for intracellular compensation (Heisler, 1993; Pörtner *et al.*, 1998). However, in fish these relationships are also influenced by the level of sodium, which is used to extrude H^+ from the organism (Iwama and Heisler, 1991).

An assessment of the adaptive and ecophysiological importance of these patterns gives rise to the questions of what are the benefits of acid-base regulation for the organism? And what is the effect of changes in acid-base parameters under conditions of environmental stress, like elevated concentrations of ambient CO_2? To start with, work on two invertebrate species of completely different mode of life may illustrate the levels at which CO_2 exerts its specific effects. The level of organization of the organism, its activity level, mobility and reactivity are likely to be correlated with the sensitivity level. The organisms chosen are at the two extreme ends of the spectrum, one relatively tolerant to CO_2 and the other one of the most sensitive species known so far. One of them is *Sipunculus nudus*, a protostome invertebrate (body weight up to 80 g and body length up to 30 cm) living in marine sediments where fluctuations of environmental parameters like oxygen and CO_2 are regular. The species range extends from the intertidal zone up to water depths of 2300 m, making it a suitable model organism for studying principal CO_2 effects. The overall physiological characters of this animal comprise a low metabolic rate, slow body movements, oxyconformity of oxygen consumption, biochemical characteristics of a facultative anaerobe, and the abilities to reduce metabolic rate under extreme conditions and to undergo long-term starvation (Pörtner and Grieshaber, 1993).

The second model organism is the squid *Illex illecebrosus*, which lives in the pelagic zone of the open ocean and displays a level of activity which is comparable to, or even exceeds that of similar sized fish (O'Dor and Webber, 1986). Squids are invertebrates with high metabolic power compared to fish and must therefore be considered even more sensitive to fluctuations in environmental parameters than highly active fish (cf. Pörtner, 1994). Finally, the literature available on fishes indicates that their sensitivity to CO_2 is intermediate (see below).

2.1 CO_2 effects on apparently tolerant species

In many animal groups tolerant to CO_2 oscillations, CO_2 has long been known to cause a drop in metabolic rate and even anaesthesia, especially in insects. A narcotic effect of high, non-determined CO_2 levels was also observed in deep-sea hagfish after CO_2 disposal in situ, possibly as the result of respiratory distress (reduced O_2 carrying capacity of the blood, Tamburri *et al.*, 2000). *Sipunculus nudus* experiences a long-term depressing effect of CO_2 on aerobic energy metabolism which is related to the level of hypercapnia (depression by 35% at 20000 μatm PCO_2, water pH ≈ 6.6). Further study revealed that under both normo- and hypercapnia, acidosis elicits a drop in metabolic rate which can only be explained by the decrease in extracellular pH (see Fig. 3; Reipschläger and Pörtner, 1996). Neither intracellular pH nor intra- or extracellular bicarbonate levels or PCO_2 were involved in eliciting metabolic depression under hypercapnia. These relationships have not yet been investigated in fish.

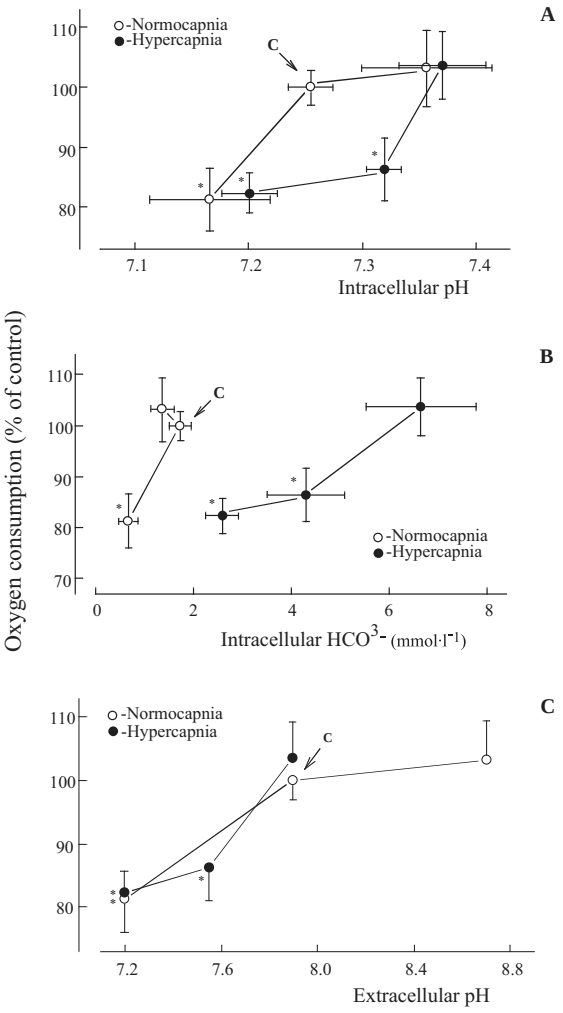


Fig. 3. Oxygen consumption rates of isolated body wall musculature of *Sipunculus nudus* during normocapnia and during hypercapnia depicted as a function of intracellular pH values (A), intracellular bicarbonate concentrations (B), and values of extracellular pH (C). Only plot C is consistent for both normo- and hypercapnic data and demonstrates that oxygen consumption is significantly depressed below a threshold value of extracellular pH (modified after Reipschläger and Pörtner, 1996).

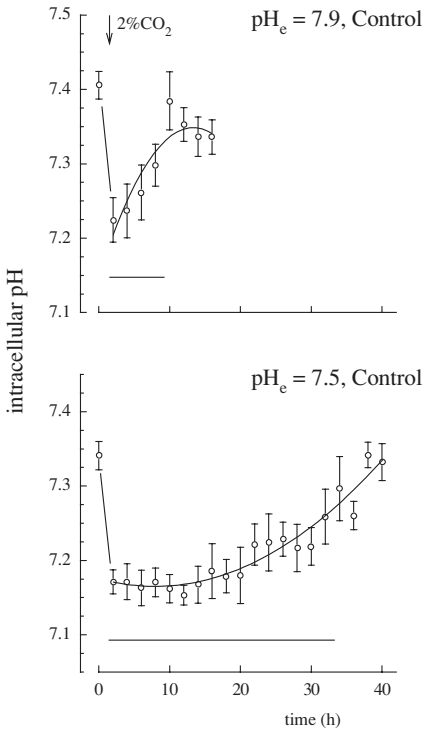


Fig. 4. Time course of pHi recovery in isolated body wall musculature of *S. nudus* during acute hypercapnia under 2% CO_2 at high and low extracellular pH. Recovery of intracellular pH takes longer during acidosis due to the specific effect of low extracellular pH on transmemembrane ion exchange (see text and Pörtner *et al*., 2000).

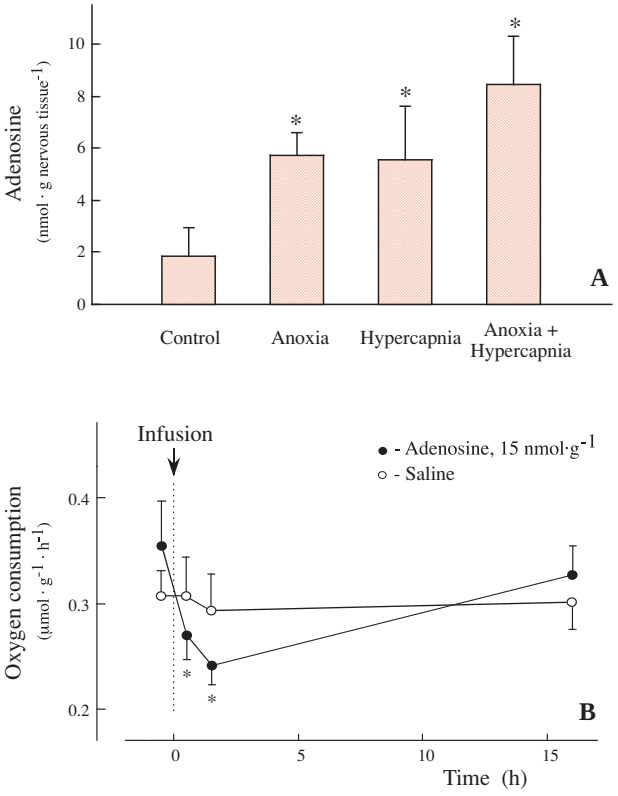


Fig. 5. (A) Adenosine levels in the nervous tissue of *Sipunculus nudus* after 24h of exposure to hypercapnia, anoxia and anoxic hypercapnia. (B) Effect of adenosine infusion on the oxygen consumption rate of *Sipunculus nudus*. Adenosine solution or saline were infused into the coelomic fluid via an indwelling catheter (after Reipschläger *et al*., 1997).

Decreasing extracellular pH slows down the rate of H^+ equivalent ion exchange by both Na^+/H^+- and Na^+-dependent Cl^-/HCO_3^--transporters which are responsible for the regulation of the intracellular acid-base status. Therefore, less sodium needs to be pumped by the Na^+/K^+-ATPase, thus diminishing the energy requirements of acid-base regulation (see Fig. 4; Pörtner *et al*., 2000). The general relevance of these relationships among water breathing animals is confirmed by data collected on tilapia from alkaline Lake Magadi, which indicate that the cost of acid-base regulation can even range above 50% of baseline metabolism under extreme environmental conditions (Wood *et al*., 2002).

Further investigation at the whole animal (systemic) level revealed that a modulation in the cost of acid-base regulation cannot fully explain metabolic depression since ventilatory activity was reduced under hypercapnia, suggesting a contribution from a central nervous mechanism (Pörtner *et al*., 1998). Among neurotransmitters, adenosine accumulated in the nervous tissue of *S. nudus* during hypercapnia, hypoxia, and even more so during combined hypoxia and hypercapnia (Fig. 5). Infusion of adenosine caused metabolic depression (Reipschläger *et al*., 1997). A similar role for adenosine is also known for some vertebrates (e.g. freshwater fish and turtles) subjected to anoxic conditions (Hylland *et al*., 1997; for review see Lutz and Nilsson, 1997). No studies addressing the specific role of adenosine or other neurotransmitters in marine fish during hypercapnia are available as yet.

Most importantly, not only metabolic depression but also metabolic imbalance may arise under hypercapnia, depending on the degree of compensation in acid-base status. Under conditions of extreme acidosis (metabolic depression by up to 45% of control values), Langenbuch and Pörtner (2002) found lowered atomic ratios of oxygen consumed (O) to nitrogen produced (N) in muscle tissue of *S. nudus*, despite a concomitant drop in oxygen consumption and ammonia excretion rates (Fig. 6). On the one hand, these results indicate a shift in N metabolism with a preferred degradation of amino acids like asparagine, glutamine or their dicarboxylic acids, which yields low O/N ratios. This results in an increased pro-

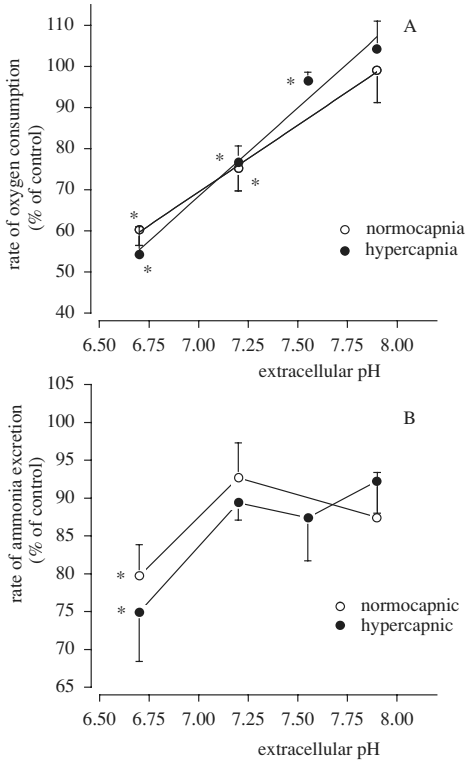


Fig. 6. Rates of oxygen consumption (A) and ammonia excretion (B) at different values of extracellular pH under normocapnic and hypercapnic conditions in *Sipunculus nudus* isolated muscle tissue. Note the progressive drop in oxygen consumption with falling extracellular pH and the delayed decrease in the rate of ammonia excretion under conditions of severe acidosis (after Langenbuch and Pörtner, 2002).

duction of bicarbonate, which supports the regulation of intracellular pH. On the other hand, the decrease in O/N ratios suggests that hypercapnia may cause a long-term drop in protein synthesis, possibly induced by the drop in intracellular pH (Langenbuch and Pörtner, 2002). This context remains largely uninvestigated for other organisms but it can be postulated to affect the long-term physiology of the animals: a reduction in growth and reproduction is expected, processes which largely depend on protein synthesis and which support the maintenance of a population. Reduced growth rates under hypercapnia were found in marine penaeid prawns (Wickins, 1984) and in juvenile white sturgeon, with reductions in foraging activity being involved in the latter (Crocker and Cech, 1996).

Much work has focused on pH as a stress factor affecting reproduction and associated processes in fresh water and marine species. Low water pH decreases the size of the eggs and delays hatching in perch, *Perca fluviatilis* (Vinogradov and Komov, 1985). In clams (*Tivela stultorum*) fertilization was most successful at slightly alkaline sea water pH (Alvarado-Alvarez *et al.*, 1996), suggesting that even small reductions of pH may reduce reproductive success in some species (see also Desrosiers *et al.*, 1996). Thus, in white sturgeon (*Acipenser transmontanus*), sperm motility was shown to be affected by low pH or increased PCO_2 (Ingermann *et al.*, 2002).

One additional aspect not yet considered is that elevated CO_2 levels cause a disturbance of calcification processes in organisms relying on shells or other calcified structures (Wickins, 1984). Barker and Elderfield (2002) demonstrated that glacial-interglacial changes in the shell weights of several species of planktonic foraminifera are, in a negative feedback mechanism, related to changes in ambient carbonate ion concentrations over time in response to changing atmospheric PCO_2. Thus, marine calcification seems to be directly affected by elevated atmospheric carbon dioxide. There will even be a dissolution of calcified structures that are already present (e.g. Bamber, 1987; Shirayama, 1995). These disturbances will contribute to the predicted reduction in growth and reproduction as seen at low pH in molluscs (Bamber, 1987, 1990). There may also be differences between animal groups in this respect: if CO_2 was a key factor in late Permian mass extinctions it affected corals, articulate brachiopods, bryozoans and echinoderms to a greater extent than molluscs, arthropods and chordates, which may partly be due to the greater reliance of the former groups on heavily calcified skeletons (Knoll *et al.*, 1996).

In general, the tolerance limits to CO_2 have been seen as related to the acidifying action (cf. Shirayama, 1995; Auerbach *et al.*, 1997); however, specific effects exerted by CO_2 and bicarbonate have to be included in a complete consideration of CO_2 effects (Pörtner and Reipschläger, 1996). Recent studies confirmed this hypothesis, providing evidence for different sensitivities to CO_2- versus HCl (fixed acid)-induced water acidification. Higher mortalities resulted in all CO_2 exposed groups of tested fish larvae compared to groups exposed to the same water pH set by fixed acid (Ishimatsu *et al.*, 2004). Observed differences may partly result from the more rapid entry of highly diffusive CO_2 into body fluids, whereas disturbances may take longer to develop in HCl^-acidified seawater. Overall, CO_2-related water pH values as low as 7 and 6.5 may be tolerated, at least temporarily, by individual animals, but it is reasonable to predict that CO_2 will cause a change in the mode of metabolism. Reduced growth and reproduction may result, with long term effects at the population level. These effects may set in rather early. According to recent data reported by Shirayama (2002), even very moderate increases in CO_2 (200 μatm above control levels) are able to cause a re-

duction in growth rate and even survival of shelled animals like echinoderms and gastropods. Accordingly, many species would tolerate transient CO_2 fluctuations but may not be able to settle and thrive in areas where CO_2 remains permanently elevated (with the potential exception of hydrothermal vent fauna). For an estimation of true critical thresholds in tolerant species, long-term exposures are required as well as studies of growth and reproduction. No such comprehensive studies are available as yet.

2.2 CO_2 effects on acutely "intolerant" species

The number of species acutely sensitive to even small changes in CO_2 levels may be rather small but among them are the most fascinating and powerful invertebrate species, squid. A look at the special metabolic and respiratory design of squid explains why these creatures are so sensitive to ambient CO_2 fluctuations (Pörtner and Zielinski, 1998). In contrast to the low metabolic rate associated with undulatory swimming in fish, the squid's oxygen demand is much higher due to their less efficient mode of swimming by jet propulsion (O'Dor and Webber, 1986). The high metabolic rate of these animals needs to be supported by blood oxygen transport which occurs by use of an extracellular pigment, the haemocyanin (Fig. 7). The pigment concentration determines the capacity of the blood to bind and transport oxygen. However, there is a limit to the concentration of pigment, since soluble protein binds water (colloidal osmotic pressure) and can remove it from the tissues. High protein concentrations also increase the viscosity of the blood. Nonetheless, oxygen transport by the haemocyanin needs fine tuning to allow efficient oxygen transport. This occurs by pH, seen as a large Bohr effect, which causes a reduced binding affinity for oxygen with falling pH. Therefore, control of arterial pH as well as pH changes between arterial and venous blood are very important for oxygen transport. This conclusion is supported by the existence of large, pH-dependent cooperativity (i.e. interaction between haemocyanin subunits) which enhances the level of coordination between pH and oxygen binding. The high metabolic rate of the mantle causes haemocyanin to fully release its load in the tissue even under resting conditions, and not just during active periods. In contrast to the situation in fish, there is no venous oxygen reserve (Pörtner, 1990).

The relationship between O_2 binding, oxygen partial pressure (PO_2) and pH is clearly illustrated by pH saturation analysis (see Fig. 7). The oxygen isobars (lines of constant PO_2) show which pH changes in the blood enable the pigment to buffer dissolved oxygen or PO_2, as it unloads and saturation drops from 100% in the artery to the venous level. In accordance with a maximum cooperativity, the slope of oxygen isobars is steepest where pH is close to that of *in vivo* blood (about 7.4).

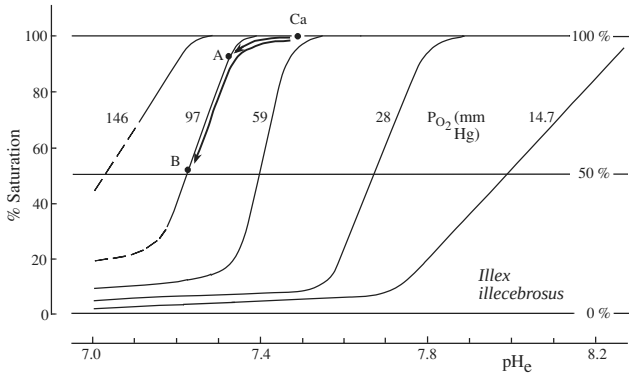


Fig. 7. Oxygen saturation of the blood of *Illex illecebrosus* depicted in a pH/saturation diagram (modified after Pörtner, 1990, 1994). The oxygen isobars show the change in hemocyanin bound oxygen levels with extracellular pH at constant PO_2. The acid-base and gas status of arterial blood under control resting conditions is depicted by C_a, changing into A during moderate (1.5 mm Hg) and into B during slightly more severe hypercapnia (5 mm Hg), thus reflecting the drop in arterial oxygenation with pH.

This shows that extracellular pH must be closely controlled and protected against fluctuations. While a moderate decrease in pH (by 0.15 pH-units) caused by a rise in water PCO_2 above 2,000 μatm would decrease the aerobic scope for activity, a larger rise in PCO_2 (up to 6,700 μatm) would cause blood pH to fall by about 0.25 pH units, reduce oxygen capacity by about 50%, and most likely induce lethal effects in the ommastrephid, open ocean squid *Illex illecebrosus* (Pörtner and Reipschläger, 1996). Squid of lower activity levels appear to be less sensitive. For example, Redfield and Goodkind, as early as 1929, demonstrated in the coastal squid *Loligo pealei* that CO_2 partial pressures of about 26,500 μatm would be acutely lethal, associated with a similar drop in blood oxygenation (Redfield and Goodkind, 1929). Acute critical CO_2 thresholds may be alleviated to some extent by long-term adjustments to rising ambient CO_2, allowing for some compensatory acid-base regulation.

2.3 CO_2 sensitivity in fish

In comparison to squid, fish are much better protected from CO_2 effects since they have a lower metabolic rate and some venous oxygen reserve. Their hemoglobin is located in erythryocytes, where it is being protected from extracellular pH disturbances by the great capacity for intracellular acid-base regulation. This will reduce acute sensitivity to elevated CO_2 as opposed to squid, and critical levels may not be reached even at CO_2 partial pressures of 4 kPa or more (40,000 μatm, e.g. Crocker and Cech, 1996). Nevertheless, the question of pH sensitivity

of the oxygen-transport system in fishes, especially concerning deep sea animals, remains controversial, because available data are very sparse. As some deep sea animals display relatively large pH sensitivities of respiratory pigments at high oxygen affinities, similar to deep water Antarctic species (e.g. among octopods; Zielinski *et al.*, 2001), Seibel and Walsh (2001) postulated that deep sea animals would experience serious problems in oxygen supply under conditions of increased environmental CO_2 concentrations. They refer to midwater organisms which possess pigments with large Bohr effects and high O_2 affinities, like the mysid crustacean *Gnathophausia ingens* and some fish species (Childress and Seibel, 1998; Sanders and Childress, 1990). However, it remains unclear whether these animals are representative of the deep sea fauna because they are residents of so called "oxygen minimum layers" at intermediate depths with special adaptations for the efficient extraction of oxygen from hypoxic waters. For a definite evaluation of CO_2 sensitivity under these conditions, combined hypoxia and CO_2 exposure scenarios would have to be considered. In contrast, Graham *et al.* (1985) found very low hemoglobin levels in the blood of three deep sea fishes, comparable to those of a number of Antarctic fishes (Wells *et al.*, 1980). The hemoglobins of these fish species displayed hyperbolic oxygen dissociation curves, low Hill constants and small Bohr effects, associated with their low activity habits and a reduced importance of blood oxygen transport.

Moreover, fish (and squid) lead a sluggish life at depths below 300 to 400 m. A decline in metabolic activity of pelagic animals, including fishes and cephalopods, with increasing minimum depth of occurrence has been reported in several studies (Childress, 1995; Seibel *et al.*, 1997). A comparative study of Antarctic stenothermal and eurythermal temperate cephalopods showed that the magnitude of the Bohr effect also depends upon the level of activity. Furthermore, the Bohr effect was decreased and the pH-insensitive oxygen reserve increased during cooling, suggesting that the relevance of pH sensitivity is reduced in the cold (Zielinski *et al.*, 2001). In addition, the hemoglobins of Antarctic notothenioids like *Trematomus newnesi*, *Artedidraco orianae* and *Pogonophryne scotti* are characterized by a modest Bohr effect, very weak or no Root effect, and very low cooperativity of oxygen binding, features most likely related to their low resting metabolic rate (Tamburrini *et al.*, 1998; D'Avino and DeLuca, 2000). Similar trends may be found in deep sea fishes below the oxygen minimum layer.

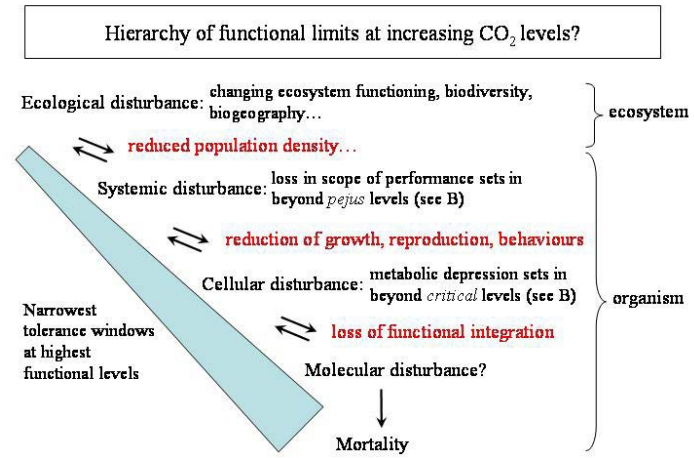
Once again, it may not be the acute toxic effects of hypercapnia but the long-term sublethal effects on deep sea fauna which are more detrimental to population structure and species distribution. Due to their low metabolic activity, the efficiency of cellular and tissue acid-base regulation is likely to be greatly diminished in deep sea fishes compared to their shallow-living counterparts (Seibel and Walsh, 2001). A lower intracellular non-bicarbonate buffering capacity (Seibel *et al.*, 1997) and reduced rates of active ion exchange in the gills (Goffredi and Childress, 2001) may be typical of deep sea fauna. In consequence, the capacity to fully compensate systemic acid-base disturbances may be limited, suggesting shifting acid-base and ion equilibria and associated metabolic consequences (see above). This may in fact lead to constantly depressed energy turnover rates and subsequent constraints in growth and reproduction processes as outlined above.

3. Conclusions and Perspectives

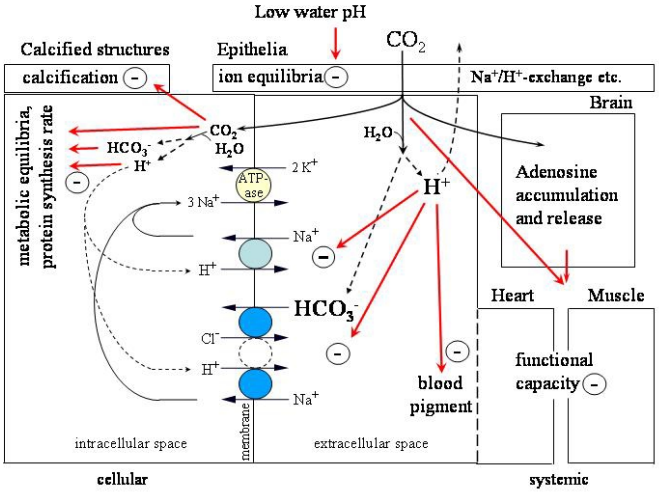
The number of species suffering from acute CO_2 toxicity will be limited. In general, long-term steady-state elevations in CO_2 levels may be tolerated by animals with a low activity mode of life or pre-adapted to large fluctuations in environmental parameters. Tolerance also depends upon the capacity to compensate for associated acid-base disturbances. However, this refers only to short to medium term survival of the individuals of a population. At present it is difficult, if not impossible, to give numbers for a critical threshold and to really qualify animal species as being permanently tolerant to elevations in ambient CO_2 since long-term effects have not yet been sufficiently investigated. The data reported by Shirayama (2002) indicate that shallow water calcifying species may respond rather early with reduced growth and survival (see above).

Figure 8 summarizes our knowledge of carbon dioxide effects at different organisational levels. Analogous to a recent treatment of thermal sensitivities (Pörtner, 2002), an ecosystem to molecular hierarchy of CO_2 tolerance is proposed on the basis of current physiological data. The data given above give rise to the general prediction that CO_2 will cause a change in the mode of metabolism. Reduced growth and reproduction may result in apparently tolerant species and populations, contributing to a long-term decrease of population density or even local extinction of species. However, such effects have yet to be demonstrated in long term field investigations.

Nevertheless, such effects are in accordance with CO_2 effects that have been supposed to contribute to the extinction of species during the Perm/Trias mass extinction event (cf. Knoll *et al.*, 1996). In the mass extinction scenario, PCO_2 levels were reached in surface waters that are in the low range of those expected in CO_2 disposal scenarios. The notion by Knoll *et al.*, that animals without gills, with a weak internal circulation and low metabolic rate are more sensitive to hypercapnia is not supported by the data compiled in this review. Ventilatory compensation of hypercapnia does not represent a possi-



A



B

Fig. 8. Overview of effects of elevated ocean CO_2 levels on marine animal organisms and ecosystems. (A) Application of Shelford's law of tolerance and of the concept of a systemic to molecular hierarchy of tolerance limits, as recently elaborated in thermal physiology, supports a categorization of CO_2 dependent phenomena. The integrated concert of different functional levels leads to narrowest tolerance windows at high hierarchical levels which shape ecological functions and their shift under elevated CO_2 concentrations. Note that tolerance thresholds (pejus and critical levels) likely vary between species and phyla, partly depending on the level of energy turnover (see text). Long-term tolerance limits (pejus levels), which define the onset of performance limitations, still await quantification. (B) Summary of physiological functions and their changes and interactions under the effect of CO_2 in a generalized marine water breathing animal. The processes depicted here are hypothesized to elicit the patterns depicted in (A). Note that this picture is incomplete and hypothetical with respect to some details and ignores the specific phylogenetic constraints characterizing individual phyla and species (see text). The generalized cellular processes depicted on the left probably have their specific functional consequences in tissues like brain, heart or muscle depicted on the right (experimental results and concepts adopted from Pörtner and Reipschläger, 1996; Reipschläger and Pörtner, 1996; Reipschläger *et al.*, 1997; Larsen *et al.*, 1997; Pörtner *et al.*, 2000; Langenbuch and Pörtner, 2002, 2003; Pörtner, 2002; Shirayama, 2002; Ishimatsu *et al.*, 2004; Kurihara *et al.*, 2004).

ble advantage for the more active water breathers since PCO_2 gradients between water and blood are too low. Rather, the much greater reliance on calcified structures may have defined the limits in those groups of articulates, echinoderms, bryozoans and cnidarians which were severely affected by mass extinction. Calcification is usually less expressed in the more active animal groups since this will hamper mobility.

However, although CO_2 may have been one of the effective parameters, the relevant analysis by Knoll *et al.* neglects the role of temperature oscillations, which most probably contributed to mass extinction events through the long term effects of repeated exposure to extreme climate oscillations and associated cooling events (Pörtner, 2001, 2004). These considerations indicate that current trends of warming and CO_2 accumulation in marine surface waters may exert synergistic effects on marine fauna. According to a recently proposed principle, thermal tolerance windows of animals are set by limitations of the integrated capacities of ventilation and cardio-circulation for oxygen uptake and distribution in the organism (Pörtner, 2001, 2002). Warming and anthropogenic eutrophication will cause falling oxygen levels in marine environments and thereby a reduction of tolerance to thermal extremes, finding reflection in a narrowing of thermal windows. This trend is likely to be exacerbated by accumulating CO_2 since it reduces animal performance, as outlined above and will probably act in the same way as hypoxia. Synergistic effects of warming and CO_2 accumulation will probably be harmful to animal populations, especially at the borders of their biogeographical range.

As a corollary, future research is required to study the long-term effects of elevated CO_2 levels and evaluate critical threshold levels for a detrimental effect of CO_2 in both apparently tolerant and acutely intolerant species. This research should consider the physiological importance of each individual CO_2 species in addition to pH effects. Furthermore, and on a long-term basis, combined effects of CO_2 accumulation and global warming should be studied to develop our understanding of potential synergistic effects.

It seems to be important to find a model organism to represent the large group of deep sea fishes which are barely accessible *in vivo* for laboratory work. Their metabolic requirements are on the low side among fishes, even lower than in polar fish (Torres and Somero, 1988). Those species uniquely adapted to little or no light, low temperatures (below 5°C) and huge hydrostatic pressures show a great diversity, are endemic to these extreme environments, and are characterized by slow growth and reproduction (Gage and Tyler, 1991). This picture already suggests that deep sea fauna is sensitive to any change that may occur suddenly and is well beyond the range of conditions under which this fauna has evolved (Hädrich, 1996). Due to the difficulties in the collection and handling of live deep sea animals, we have started to work on benthic Antarctic eelpout *Pachycara brachycephalum* (Zoarcidae). In particular, the physiological characteristics of benthic Antarctic fauna should be close to those of deep sea fish. These zoarcids are also highly adapted to constantly low temperatures (around 0°C) and their sluggish benthic lifestyle contributes to an exceptionally low metabolic rate (Anderson, 1990, 1994; van Dijk *et al.*, 1999). In the future, results obtained in studies on the physiological tolerance of *P. brachycephalum* to hypercapnia should yield valuable information which should be applicable to deep sea fish species.

Present data indicate that relatively moderate CO_2 increases of 200 μatm may have significant long term effects. This already casts doubt on whether CO_2 should be disposed by even distribution in the sea, although this would possibly keep levels below those which elicit acute lethal effects on the most active organisms of the pelagic. Due to the danger of long term detrimental effects, and if CO_2 disposal strategies to the ocean cannot be avoided, CO_2 should be pH neutralized and converted to bicarbonates or rather be trapped in confined stable areas like bottom depressions (canyons) where virtually no life will continue under those anoxic and hypercapnic conditions. These sites in the deep sea filled by CO_2 lakes could be looked at in the same way as landfills. If they were to use only a minor percentage of the total surface area of the deep-sea floor, such strategies should leave a large fraction of the marine environment unaffected so that the net damage to marine ecosystems should be less than with an even distribution of CO_2.

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