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Commentary

Biological impacts of deep-sea carbon dioxide injection inferred from indices of physiological performance

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Summary

A recent proposal to store anthropogenic carbon dioxide in the deep ocean is assessed here with regard to the impacts on deep-living fauna. The stability of the deep-sea has allowed the evolution of species ill-equipped to withstand rapid environmental changes. Low metabolic rates of most deep-sea species are correlated with low capacities for pH buffering and low concentrations of ion-transport proteins. Changes in seawater carbon dioxide partial pressure (P_{CO_2}) may thus lead to large cellular P_{CO_2} and pH changes. Oxygen transport proteins of deep-sea animals are also highly sensitive to changes in pH. Acidosis leads to metabolic suppression, reduced protein synthesis, respiratory stress, reduced metabolic scope and, ultimately, death. Deep-sea CO_2 injection as a means of controlling atmospheric CO_2 levels should be assessed with careful consideration of potential biological impacts. In order to properly evaluate the risks within a relevant timeframe, a much more aggressive approach to research is warranted.

Key words: carbon dioxide, global warming, deep sea, hypercapnia, acid–base balance, sequestration, cephalopoda, metabolism.

Introduction

Atmospheric carbon dioxide (CO_2) has increased by 31% from pre-industrial levels (Houghton et al., 2001). A growing consensus among environmental scientists that increased atmospheric CO_2 is causing global warming has spurred the development of various strategies to control CO_2 levels in the atmosphere. United States President George W. Bush has stated his belief that "technology offers great promise to significantly reduce emissions – especially carbon capture, storage and sequestration technologies" (Kerr, 2001). The ocean is an attractive site for possible storage of CO_2 because of its enormous volume. Disposal of CO_2 in the deep-ocean, first proposed by Marchetti (1977) nearly 25 years ago, is now actively being explored (Halmann and Steinberg, 1999). The first direct experiments on the behavior of liquid CO_2 in the deep ocean (Brewer et al., 1999), and on the reaction of deep-sea organisms to CO_2 *in situ* (Tamburri et al., 2000; Barry et al., 2002), have recently been conducted.

Addition of CO_2 to seawater will result in a decrease in pH due to the bicarbonate buffer system in the ocean (equation 1):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^- + H^+ . \quad (1)$$

Reductions in pH resulting from direct ocean storage of CO_2 are estimated to range anywhere from 1.0 to 4.0 (Adams et al., 1997) and 0.01 to 0.1 (Drange et al., 2001; Haugan, 1997) pH units for acute and long-term exposure, respectively, near the injection site. These pH reductions may occur in numerous regions spanning hundreds of kilometers each, depending on the density of inputs and the method of CO_2 disposal (Caulfield et al., 1997). Disposal of sufficient CO_2 to stabilize atmospheric levels at twice the pre-industrial level by the end of this century would lower the pH of the entire ocean on average by more than 0.1 unit (Seibel and Walsh, 2001). This is a large fraction of the normal variation of pH in open seawater (7.6–8.2), especially considering that pH is a log scale of proton concentration.

A variety of disposal schemes are being discussed (Adams et al., 1997). Shallow gas injection to form a dense sinking fluid, injection of ultra-cold liquid CO_2 to form a dense ice skin-hydrate phase, formation of a lake of CO_2 on the seafloor, and dissolution of a rising plume of liquid CO_2 are among the possible scenarios. The environmental 'friendliness' of CO_2 sequestration is thought to depend primarily on the method of CO_2 injection, general circulation models at the depth and location of CO_2 injection, and the general tolerance of deep-living organisms to reductions in pH and increased CO_2 (hypercapnia). This latter point is the focus of this paper.

Environmental stability and the evolution of physiological strategies

Control of intra- and extracellular pH is a ubiquitous feature of cellular physiology, reflecting the diversity of cellular processes dependent on pH or proton gradients (Hochachka and Somero, 2002). Tight control of pH, even in the face of large environmental pH perturbations, is required for proper physiological functioning. Organisms living in variably acidic and hypercapnic environments, such as deep-sea hydrothermal vents (Goffredi et al., 1997) or estuaries (Burnett, 1997), typically have a large capacity for acid–base regulation.

However, the projected perturbations in pH and CO_2 partial pressure (P_{CO_2}) due to CO_2 disposal are large relative to the pH variation experienced by most organisms in the deep-sea. While CO_2 and pH vary diurnally, or even hourly, within some shallow-water habitats (Truchot and Duhamel-Jouve, 1980; Burnett, 1997), P_{CO_2} (pH) in most of the deep-sea, like oxygen, is dependent on regional productivity and the age of bottom water (Miyake and Saruhashi, 1956; Park, 1968) and is, thus, stable over thousands of years (Kennett and Ingram, 1995). Deep-sea animals have evolved in the absence of substantial environmental variability and, as a result, lack the capacity adaptations that facilitate regulation of their internal milieu in the face of changing environmental characteristics (Childress and Seibel, 1998; Seibel et al., 1999). Angel (1992) stated that, as a result of environmental stability, deep-sea communities "*can be expected to contain the most highly tuned species with possibly the least tolerance of environmental change of all on earth*". Haedrich (1996) affirmed this sentiment stating "*any disturbance that takes place too quickly to allow for a compensating adaptive change within the genetic potential of finely adapted deep-water organisms is likely to be harmful. Deep-water faunas are sure to be sensitive to any change that occurs over a few generations and is significantly outside the probably rather narrow range of environmental conditions under which the fauna evolved*". Therefore, even small perturbations in CO_2 or pH could have important consequences for deep-sea organismal physiology and, by extrapolation, the ecology of the entire deep sea.

Physiological responses of animals to acid–base disturbance

The physiological responses of shallow-living organisms to intra- and extracellular acidosis are well characterized (for reviews, see Roos and Boron, 1981; Somero, 1985; Cameron, 1986, 1989; Truchot, 1987; Heisler, 1989; Walsh and Milligan, 1989; Pörtner and Reipschläger, 1996). The general mechanisms used by organisms to combat acid–base imbalance are similar regardless of whether the perturbation is exogenous (environmental) or endogenous (metabolic or respiratory) in origin. Primary mechanisms for regulating acid–base balance include (1) metabolic production and consumption of protons, (2) buffering of intra- and extracellular compartments and (3) active proton-equivalent ion transport (Fig. 1; Walsh and Milligan, 1989). These

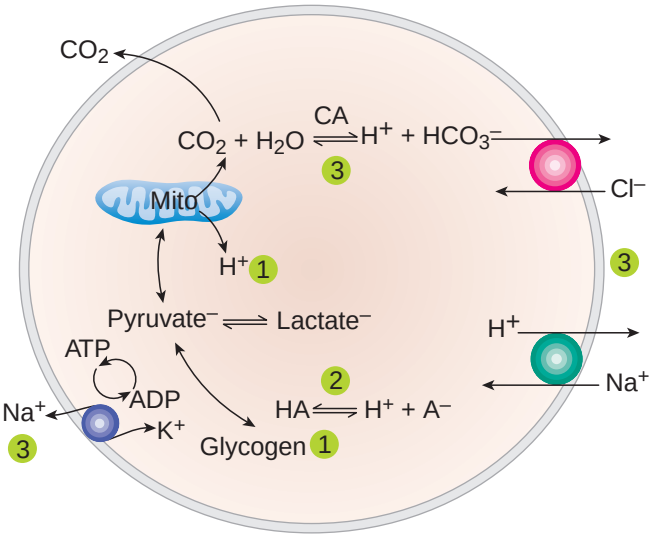

Fig. 1. Schematic representation of an animal cell with the potential means of regulating intracellular pH. (1), metabolic interconversion of acids and bases. (2), buffering; HA represents a weak acid or base with a dissociation constant in the physiological pH range. (3), transport of acids and bases across cell membranes; carbonic anhydrase (CA) catalyzes the hydration of CO_2 to yield H_2CO_3, which then dissociates to H^+, HCO_3^-, and CO_3^{2-} (an abbreviated reaction is shown).

mechanisms are highly conserved across animal phyla. In some animal groups, CO_2 and proton transport by respiratory proteins in the blood is also important (Bridges and Morris, 1989).

In the shallow-living species for which acid–base balance has been studied, elevated environmental P_{CO_2} leads directly to elevated internal P_{CO_2} until a new steady-state gradient sufficient to restore CO_2 excretion is established. When this occurs, the extracellular pH (pHe=blood pH) is depressed, and the bicarbonate concentration rises according to the bicarbonate buffer characteristics of the animal's extracellular fluid (see equation 1; Cameron, 1986). Extracellular pH and bicarbonate values directly impact intracellular pH (pHi; Pörtner et al., 1998). A secondary rise in bicarbonate ions due to active transport, ion exchange (Cameron, 1986; Cameron and Iwama, 1987; Pörtner et al., 1998) or dissolution of $CaCO_3$ exoskeletons (Lindinger et al., 1984) results in an increase in pHe towards control values over a time course of hours to days (Fig. 2). Intracellular compensation is typically completed within 48–72 h in the animals tested to date, and some return towards control values is usually observed within 24 h (Pörtner et al., 1998), often at the expense of pHe. An inability to control acid–base imbalances in the intra- and extracellular spaces may, as discussed in more detail below, lead directly to metabolic suppression, reduced scope for activity or loss of consciousness due to disruption of oxygen transport mechanisms and, ultimately, death.

Seibel and Walsh (2001) briefly reviewed the literature available for deep-sea organisms with relevance to acid–base

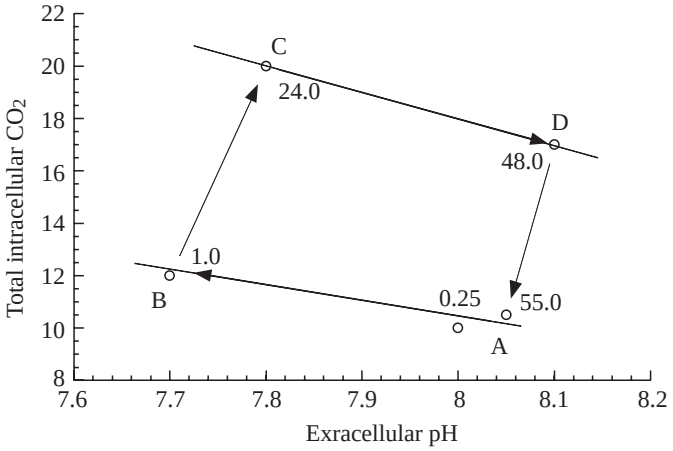

Fig. 2. A Davenport diagram, a graphical representation of the Henderson Hasselbalch equation ($pH=pK+\log[HCO_3^-]/[CO_2]$), demonstrating a typical time course for compensation of extracellular (blood) acidosis. Numbers between points represent time (h). Within 1 h of acidotic stress (A–B), extracellular pH generally drops according to the buffering capacity of the plasma. Over the next 12–24 h (B–C), bicarbonate (*y*-axis; mmol l^{-1}) is transported into the cell (or protons out) in order to shift the equilibrium towards higher pH values. Upon return to normal seawater CO_2 tensions, there is a rapid increase in pH (C–D), due again to passive reactions, followed by a slower decompensation phase (D–A) leading to restoration of the original acid–base status. Intracellular pH and bicarbonate concentrations generally follow those in the extracellular fluid. See Cameron (1989) for additional details on acid–base balance.

physiology. We concluded that, as a result of the relative environmental stability and low rates of metabolism in the deep-sea, organisms living there have little need, and thus little capacity, for robust acid–base regulation. Here, we reiterate these arguments and detail the extreme sensitivity of deep-sea organisms to even small changes in seawater chemistry. While great variation in CO_2 tolerance presumably exists within diverse animal assemblages at any given depth, enough data exist to illustrate differences in the magnitude of acid–base imbalance between generalized deep- and shallow-living species predicted to result from deep-sea injection of CO_2. However, precise thresholds for individual species cannot yet be predicted. Furthermore, it should be pointed out that regulation of acid–base balance typically occurs at the expense of ionic homeostasis and cell volume regulation (Cameron and Iwama, 1987; Whiteley et al., 2001) such that mechanisms for compensation of short-term hypercapnia may not be possible during longer exposures.

Metabolism

Over the past 30 years, a number of *in situ* studies of the oxygen consumption rates of deep-sea animals have been conducted (e.g. Smith, 1983; Smith and Hessler, 1974). Others have successfully recovered deep-sea animals and measured respiratory rates in the laboratory (for a review, see Childress, 1995; Childress and Seibel, 1998). From these studies, a general conclusion has been established that deep-living animals, both fishes and invertebrates, have low metabolic rates. In some cases, deep-living species have metabolic rates more than two orders of magnitude lower than their shallow-living relatives, even after correction for temperature

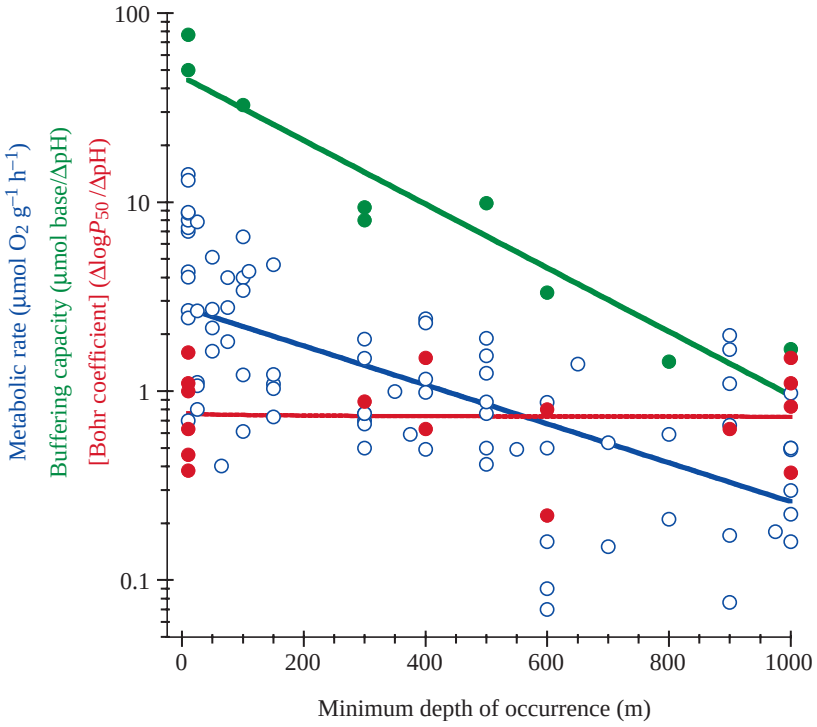

Fig. 3. Metabolic rates (open blue circles) of fishes, cephalopods and crustaceans as a function of minimum depth of occurrence (the depth below which 90% of the individuals in a population are captured). Also shown is the capacity for buffering of intracellular fluids in cephalopods (green circles) and the pH sensitivity of respiratory proteins (red circles) in crustaceans, fishes and cephalopods. Buffering capacity is measured in 'slykes', here equal to the quantity of base that must be added to a homogenate made from a 1 g sample of muscle to titrate the pH from approximately 6 to 7. The Bohr coefficient is the change in the log of respiratory oxygen affinity (P_{50}; defined as the oxygen partial pressure at which the respiratory protein is half-saturated) over the change in pH. Bohr coefficients in these animal groups are negative but are presented here as absolute values. The metabolic rates are normalized to a common body mass of 10 g and measurement temperature of 5°C using measured scaling coefficients and Q_{10} values where available or assuming a scaling coefficient of –0.25 and a Q_{10} of 2. Data are from Childress and Seibel (1998) and references therein. Note that the *y*-axis is a log scale.

differences (Fig. 3; Childress, 1995; Seibel et al., 1997). Reduced metabolism in the deep sea appears, in part, to reflect relaxed selection for locomotory capacity due to light limitation on predator–prey interactions. Some animal groups, such as infaunal invertebrates and gelatinous zooplankton, tend towards low metabolic rates regardless of depth. However, metabolism is further reduced by cold temperature and is also suppressed by limitations on food supply in some cases (Gage and Tyler, 1991). These metabolic patterns hold true to varying degrees for all phyla (Childress, 1995) and regions studied (Ikeda, 1988; Torres et al., 1994) and extend to the deepest depths of the ocean. As outlined below, a correlation is found between metabolic rate and capacity indices for acid–base balance such that we expect animals with low metabolic rates (and low capacities for burst locomotion) to have low tolerances for metabolic end products such as CO_2 and protons.

Buffering capacity

During stress conditions, such as production of protons and CO_2 during exercise, or during exogenous hypercapnia, protons may accumulate due to rate limitations of the proton-elimination pathways. Buffering is then the primary mechanism immediately available that can hold intra- and extracellular pH to values compatible with life functions (Heisler, 1989). In biological fluids, non-bicarbonate buffers are primarily protein residues characterized by pK′ values close to physiological pH (i.e. histidine). Maintenance of pHi *via* buffering of metabolic end products is imperative for conservation of protein structure and function (Somero, 1985).

The ability to buffer metabolic end products correlates with metabolic capacity. Thus, non-bicarbonate buffering capacities are as much as 100 times higher in muscles of shallow-living species than in comparable deep-living species (Fig. 3; Castellini and Somero, 1981; Morris and Baldwin, 1984; Pörtner, 1990; Seibel et al., 1997). The muscles of deep-sea organisms have low amounts of both dialyzable buffers and proteins (Somero, 1985). The consequences of reduced intracellular buffering capacity are illustrated clearly in Fig. 4. A doubling of P_{CO_2} causes only a 0.02 pH change in the intracellular space of the shallow-living squid *Stenoteuthis oualaniensis*. However, a similar increase in P_{CO_2} leads to a 0.2 pH change in the deep-living pelagic octopod *Japetella heathi*. The ability to buffer extracellular fluids against pH change is similarly dependent on the concentration of proteins in the blood (Wells et al., 1988). The majority of deep-living animals measured have extremely low extracellular protein contents compared with closely related shallow-living relatives (Douglas et al., 1976; Brix, 1983; Childress and Seibel, 1998; Seibel et al., 1999). Respiratory protein concentrations generally decrease with metabolic rate and, therefore, depth.

Ion-exchange capacity

Over the longer-term (hours to days), acid–base relevant ion transfer processes are required for elimination of H^+-equivalent ions, whether produced as metabolic end products or accumulated during environmental hypercapnia (Cameron, 1986). In crustaceans, for example, the coupled transfer of acid/base equivalents to ion exchange is the principal mechanism of acid–base regulation (Wheatly and Henry, 1992). This is why external salinity is known to influence acid–base status in some organisms (Whiteley et al., 2001). Ion transport is, in most cases, carrier mediated and thus limited by the capacity or concentration of carrier mechanisms, particularly in gas-exchange tissue (Cameron, 1986). Carriers may include Na^+/H^+ or Cl^-/HCO_3^- exchangers and pumps (i.e. ATPases). Partial correction of acidosis may also be accomplished by accumulation of HCO_3^- in the tissues and blood (Fig. 2), a process that also requires substantial ion-exchange capacity or, in some cases, modifications of gill morphology (Perry and Laurent, 1993).

Gas-exchange (e.g. gill) tissue has been identified as the primary site responsible for acid–base regulation in both fishes and invertebrates (McDonald, 1983; McDonald et al., 1991; Whiteley et al., 2001), although significant gas and ion exchange may take place across all epithelial surfaces in some organisms (e.g. cephalopods; Pörtner, 1994). Reduced gill surface area among deep-sea species may, therefore, limit ion-exchange capacity. Aside from those adapted for residence in oxygen minimum layers (Childress and Seibel, 1998), the few deep-sea species studied have much lower gill surface areas than their more-active, shallower-living counterparts (Henry et al., 1990; Marshall, 1971; Voss, 1988).

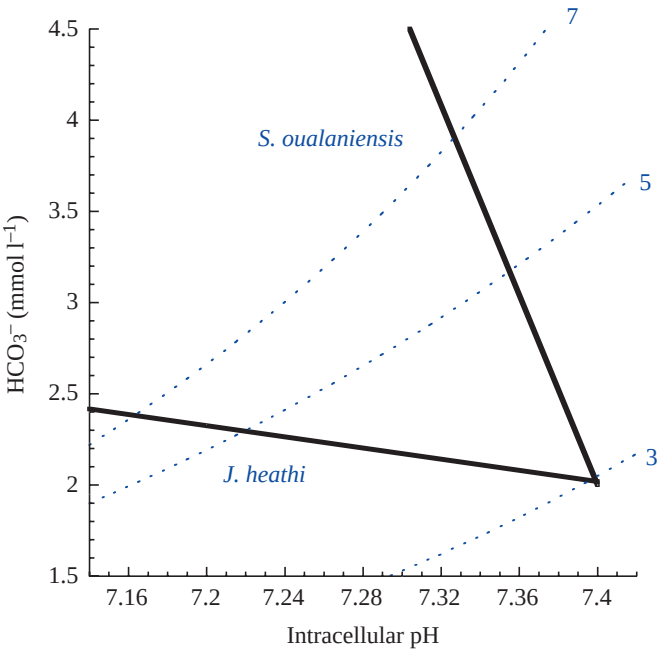

Fig. 4. Davenport diagram depicting passive buffering of intracellular pH (black lines) in two cephalopod species. Similar increases in CO_2 partial pressure (in mmHg; 1 mmHg=133.3 Pa; represented by the blue isopleths and numbers) will result in dramatically different changes in intracellular pH in shallow- (*Stenoteuthis oualaniensis*) and deep-living (*Japetella heathi*) cephalopods. Buffering data are from Seibel et al. (1997).

Gibbs and Somero (1990) reported greatly reduced capacities for active ion regulation *via* ATPases in gills of deep-sea fishes relative to shallower species. Although their study focused on Na^+/K^+-ATPases, their data show that activities of total ATPases declined with increasing depth as well. Similarly, deep-sea animals, other than those inhabiting hydrothermal vents, appear from limited data to have activities of carbonic anhydrase (CA) in gas-exchange tissue that are lower than those of shallower-living species (Henry, 1984; Kochevar and Childress, 1996). CA catalyzes the reversible hydration/dehydration reaction of CO_2 and water (equation 1) and, thus, plays an important role in CO_2 excretion and acid–base balance in marine animals by maintaining availability of H^+ and HCO_3^- for transporters (Burnett, 1997; Henry, 1984). Deep-sea species presumably have a much lower requirement for branchial ion transport due to the extreme ionic stability of seawater at depth and their low rates of metabolic and locomotory activity (Henry et al., 1990).

Oxygen transport, pH sensitivity and metabolism

Respiratory proteins in many animals are responsible for the transport of oxygen to the tissues and of CO_2 and protons to the gills for removal. The oxygen transport function of respiratory proteins (e.g. hemoglobin, Hb) is typically dependent on the pH of the blood (Bohr coefficient=$\Delta\log P_{50}\Delta pH^{-1}$, where P_{50} is the oxygen concentration at which respiratory proteins are half saturated; equation 2):

$$Hb-O_2+H^+ \leftrightarrow Hb-H^+ + O_2\,. \quad (2)$$

Protons produced in equation 1 upon addition of CO_2 reduce the affinity of respiratory proteins for O_2 according to equation 2. In some cases, the direct interaction of CO_2 with Hb can influence oxygen binding. Dependency of respiratory protein oxygen binding on pH facilitates release of oxygen at the tissues where CO_2 and protons are produced and oxygen uptake at the gills, where CO_2 is excreted (Bridges and Morris, 1989). Decreased seawater pH will diminish the effectiveness of oxygen uptake at the gills.

These interactions are well understood in a variety of organisms (see Bridges and Morris, 1989; Toulmond, 1992 for a review). However, the specific effects of CO_2 and pH on respiratory protein-mediated gas exchange vary widely between taxa. For example, limited data suggest that CO_2 increases the affinity of hemocyanin (molluscs and crustaceans) but decreases the affinity of Hb (vertebrates and annelids) for oxygen (Bridges and Morris, 1989; Toulmond, 1992). Only a few measurements of the specific effect of CO_2 on oxygen binding have been made in deep-sea species other than those inhabiting hydrothermal vents. No specific effect of CO_2 was found for vertically migrating mesopelagic shrimps (Sanders and Childress, 1990b) or for the deep-sea benthic shrimp *Glyphocrangon vicaria* (Arp and Childress, 1985). The specific CO_2 effect observed for the midwater shrimp *Notostomus gibbosus* was slight and masked by physiological concentrations of ammonium used for buoyancy in this species (Sanders et al., 1992). Regardless, increased CO_2 will result in decreased pH and a subsequent reduction of oxygen-binding affinity in most species. In addition to a large Bohr effect, many fish Hbs possess a Root effect, where a respiratory acidosis, as incurred during exposure to hypercapnia, can result in a dramatic reduction in hemoglobin-oxygen carrying capacity (up to 50%). The Root effect is thought, in some cases, to facilitate excretion of oxygen into a gas-filled swim-bladder following acid loading in the blood at the Rete Mirable. Most fishes that possess a Root effect also possess the ability to regulate red blood cell pH (pHi) during an acidosis through the release of catecholamines (adrenaline and noradrenaline) that indirectly activate Na^+/H^+ exchange (Tufts and Randall, 1989). Several deep-sea fishes investigated appear to possess a Root effect (Noble et al., 1986; Pelster, 1997). However, there are no data on whether deep-sea fishes have the ability to regulate pHi in the face of an acidosis that might be incurred during exposure to hypercapnia.

Pörtner and Reipschläger (1996) predicted that extremely active animals, in particular, squids, would be disproportionately impacted by anthropogenic decreases in seawater pH. Epipelagic squids such as *Illex illecebrosus* have extremely high rates of oxygen consumption and low blood-oxygen carrying capacity relative to fishes with intracellular respiratory proteins. Therefore, squids have little venous oxygen reserve and are highly dependent on a large Bohr shift to ensure complete release of oxygen at the tissues. Not surprisingly, pHe is tightly controlled in squids (Pörtner, 1994). A blood pH change of as little as 0.15 units is predicted to reduce the scope for activity in species such as *I. illecebrosus*, while a change of 0.25 units is lethal (Pörtner and Reipschläger, 1996).

Conversely, they argued that the specific effect of pH on oxygen binding is small in animals with low metabolic rates, such as those in the deep-sea, and that such species will be less affected by CO_2 disposal (Pörtner and Reipschläger, 1996). Although some deep-living species have respiratory proteins with low pH sensitivities (e.g. the vampire squid *Vampyroteuthis infernalis*; Seibel et al., 1999), no relationship exists between metabolic rate and the Bohr coefficient or between the Bohr coefficient and depth for a variety of deep- and shallow-living marine species (Fig. 3). The shrimp *Glyphocrangon vicaria*, for example, living on the sea floor at depths near 3000 m (oxygen=30% air saturation) has a Bohr coefficient similar to those of its shallow-living relatives despite having a low metabolic rate (Arp and Childress, 1985). Similarly, all octopodids (Cephalopoda) measured, like squids, have hemocyanins that are extremely sensitive to pH ($\Delta\log P_{50}/\Delta pH > -1.0$) regardless of depth, oxygen or metabolic rate (Bridges, 1994). For example, in the deep-sea octopod *Benthoctopus* sp., a drop in arterial pH by just 0.3 units would reduce oxygen saturation of the blood by 40% at ambient oxygen levels (Fig. 5B; A. Seibel unpublished data).

Mickel and Childress (1978) examined the effects of reduced pH on oxygen consumption of *Gnathophausia ingens*,

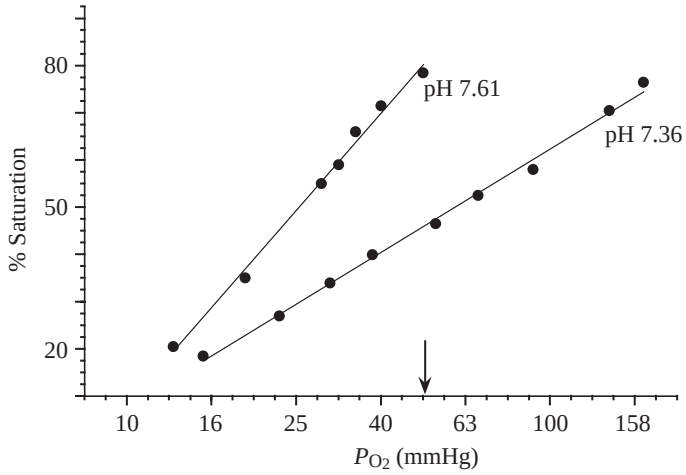

Fig. 5. Percentage hemocyanin-oxygen saturation as a function of oxygen partial pressure P_{O_2} (mmHg; 1 mmHg=133.3 Pa) at pH 7.61 and 7.36 for *Benthoctopus* sp. (B. A. Seibel, unpublished data). Ambient P_{O_2} at capture depth (51 mmHg) is indicated by an arrow. At ambient P_{O_2}, a drop in blood pH of 0.3 units results in a 40% decrease in hemocyanin saturation. All measurements were on dialyzed hemolymph at 5°C. Changes in pH were achieved by varying CO_2 concentrations, thus we cannot distinguish between pH and CO_2 effects on oxygen binding.

a crustacean living in extreme hypoxia at mid-depths off California where seawater pH values are as low as 7.6. They found no specific effect of pH on the rates of oxygen consumption or the abilities of this species to regulate its oxygen consumption rate. However, they did note a large increase in the percentage of oxygen extracted from the respiratory stream at pH 7.1 as opposed to pH 7.9. They reasoned that, as the increase in oxygen extraction does not improve the ability of *G. ingens* to regulate its oxygen uptake, there must be a loss in effectiveness of oxygen uptake at some other point along the oxygen-transport pathway at low pH. If, as is suggested by the large negative Bohr coefficient for this species (Sanders and Childress, 1990a), hemocyanin-oxygen affinity is reduced by low extracellular pH, then perhaps *G. ingens* is slowing either the respiratory or circulatory stream at low pH in order to increase the amount of oxygen extracted. Such a strategy would greatly reduce the scope for activity at low pH for this species.

Animals without well-developed circulatory systems, such as cnidarians and echinoderms, may also be sensitive to hypercapnia and reduced pH. As far as is known, they depend solely on a favorable tissue-environment gradient for CO_2 excretion. Elevated environmental P_{CO_2}, therefore, will lead directly to intracellular pH reductions. During brief bouts of environmental hypercapnia, echinoderms may use their large volume of coelomic fluid to buffer environmental changes (Spicer, 1995; Burnett et al., 2002); however, this mechanism will be ineffective during longer exposures (see below). Echinoderms and bivalves may also rely on dissolution of calcareous tests and shells to buffer pH changes (see below; Burnett et al., 2002; Lindinger et al., 1984; Spicer, 1995).

Metabolic suppression

Metabolic suppression is, in many cases, an adaptive strategy for survival of temporary energy limitation in aquatic organisms. As energy (oxygen or food) becomes limiting, ATP demand is reduced to a level that can be matched by the reduced rates of oxidative phosphorylation. Recent evidence suggests that low pH and hypercapnia are sufficient to trigger a reduction in total energy expenditure (i.e. metabolic suppression) in a variety of organisms (for a review, see Guppy and Withers, 1999), perhaps because oxygen and CO_2 are often inversely correlated in aquatic habitats (Truchot and Duhamel-Jouve, 1980). This reversible response ranges from dormancy periods lasting years in brine shrimp embryos to metabolic suppression over just minutes to hours in some intertidal organisms (Guppy and Withers, 1999).

Not all animals are able to suppress metabolism. Organisms unable to reduce oxygen demand sufficiently are subject to depletion of high-energy phosphate levels during energy limitation, resulting in death. Although not specifically investigated, metabolic suppression is suspected for midwater (mesopelagic) species migrating diurnally into low oxygen regions as well as for deep-sea benthic fauna living in burrows, crevices or shells that may become hypoxic periodically (Hunt and Seibel, 2000; Seibel and Childress, 2000). Furthermore, metabolism of some deep-living macrofauna is depressed nearly fivefold between periods of feeding (Smith and Baldwin, 1982), and many copepod species are known to overwinter in a dormant state in deep water (Alldredge et al., 1984; Hand, 1991).

The mechanisms that bring about reductions in metabolism are under active investigation. Metabolic suppression is typically associated with a decrease in pHi that may lead to rapid adjustments in pH-sensitive metabolic processes such as glycolysis in muscle tissue *via* alterations in the activities of glycolytic enzymes (reviewed by Somero, 1985). For example, phosphorylation of glycolytic enzymes is involved in the transition into dormancy in some marine molluscs (Brooks and Storey, 1997). In some cases, high CO_2 levels trigger metabolic suppression independently of pH (Hand, 1998; Pörtner et al., 1998), but reduced pH is also sometimes sufficient to trigger metabolic suppression (Hand, 1998; Kwast and Hand, 1996).

Suppression of metabolism is accomplished, at least in part, by shutting down expensive cellular processes such as protein synthesis (Guppy and Withers, 1999). For example, Reid et al. (1997) found that low pH inhibited protein synthesis in trout living in lakes rendered acidic through anthropogenic input. Mitochondrial protein synthesis in brine shrimp is also acutely sensitive to pH changes (Kwast and Hand, 1996). Metabolic suppression under conditions of environmental hypercapnia is accompanied by changes in nitrogen excretion in the marine worm *Sipunculus nudus*, which is attributable, in part, to a reduction in protein synthesis rates (Langenbuch and Pörtner, 2002). Reduced protein synthesis, by definition, restricts both growth and reproduction.

Takeuchi et al. (1997) investigated growth rate in one deep- and several shallow-living nematode species in relation to pH.

Growth rate in the deep-sea species was reduced by nearly 50% at pH 6.9, while the shallow-living species showed similar growth rates to the control at pH 6.2. This may reflect the suppression of metabolic rate due to an inability to compensate for an intracellular acidosis in the deeper-living species.

Mortality

Yamada and Ikeda (1999) recently investigated the effect of low seawater pH on zooplankton mortality, and reported that marine zooplankton are more sensitive than similar freshwater species. Furthermore, the single mesopelagic species in this study, a euchaetid copepod, was more sensitive than epipelagic species, perhaps as a result of its lower metabolic rate relative to the calanid and metridinid species with which it was compared (Thuesen et al., 1998). In the mesopelagic copepod *Paraeuchaeta elongata*, a reduction in pH of only 0.2 units from that typical at mid-depths off California is sufficient to cause 50% mortality after 6 days exposure. Mortality was directly proportional to exposure time and inversely proportional to pH (Yamada and Ikeda, 1999).

In situ investigations suggest that deep-sea echinoid (sea urchin) shells are extremely susceptible to fatal dissolution when caged near small pools of liquid CO_2 on the seafloor at 3600 m depth (Barry et al., 2002). Equation 3 demonstrates clearly how addition of CO_2 may enhance $CaCO_3$ dissolution:

$$CO_2 + H_2O + CaCO_3 \leftrightarrow 2HCO_3^- + Ca^{2+}. \quad (3)$$

Shell dissolution under modest hypercapnia can facilitate compensation of temporary acid–base imbalance. However, long-term exposure under CO_2 disposal scenarios will be fatal if CO_2 excursions are sufficient to initiate shell dissolution. Deep-sea holothuroids (sea cucumbers) were also killed by pH excursions associated with small-scale *in situ* CO_2 sequestration experiments (Barry et al., 2002). These studies suggest that echinoids and holothurians, both dominant components of the invertebrate epibenthic fauna in many areas of the deep sea (Gage and Tyler, 1991), are especially susceptible to small changes in pH (see above).

Mobility of deep-sea fauna

The most serious pH excursions (below pH 7) are expected to occur within a few hundred kilometers of the source CO_2 (Adams et al., 1997). Active avoidance is typically the first response of mobile organisms to low pH (Davies, 1991). As demonstrated above, deep-sea organisms in general have reduced locomotory capacity relative to shallower-living species. Although the majority of benthic fauna, as well as many planktonic forms, are not expected to be able to avoid a large plume of acidic seawater, some, particularly fishes, are sufficiently motile to avoid liquid CO_2 pools or plumes. However, the ability to swim does not guarantee that such species *will* avoid CO_2 plumes.

Tamburri et al. (2000) recently conducted the first *in situ* experiments attempting to directly assess the impacts of liquid CO_2 on deep-sea organisms. By mixing a fish slurry with liquid CO_2, they were able to attract fish (primarily hagfish, Myxinidae) to the liquid CO_2 itself. Hagfish appeared not to detect the CO_2 and swam directly to the beaker that contained the CO_2/fish slurry. Upon contact with the CO_2, the fish immediately lost consciousness and fell to the bottom. Rattails (*Macrouridae*), by far the dominant fishes near the deep-sea floor, rely on olfaction to find food. Studies with baited cameras on the deep-sea floor have shown that fish abundance increases dramatically, up to 1 per m^2, with the intensity of the current carrying the bait smell (Gage and Tyler, 1991). Rattails are known to root about in the ooze, sucking in the top layer of sediment and straining off small infaunal invertebrates (Gage and Tyler, 1991). Preliminary *in situ* observations suggest that rattails are also not able to detect liquid CO_2 or accompanying pH decreases. Individual rattails did, however, demonstrate dramatic reactions upon making direct contact with liquid CO_2 (B. A. Seibel, personal observation). These results are worrying because they suggest that the smell of decaying animals that is certain to accompany the initial impact from any sequestered CO_2 may attract additional scavengers.

Perspective

It is assumed that direct, prolonged, contact with liquid CO_2 will result in death and that any disposal scheme will cause mortality of at least some organisms within some distance of the injection point. The chore set forth for deep-sea biologists with respect to CO_2 sequestration is to determine what pH and CO_2 limits will be tolerated by deep-living organisms and what spatial and temporal scales are acceptable. Survival of short-term hypercapnia is dependent on the capacity to buffer, transport and eliminate acid–base equivalents, to tolerate compromised ionic balance during compensation of acidosis, and to suppress metabolism in order to wait out periods of intolerably high CO_2 or low pH. Deep-sea organisms are poorly equipped with respect to these abilities, with capacities 10–100 times lower than comparable shallow-living species. However, very few studies have directly investigated the survival of deep-sea organisms under hypercapnic conditions (Barry et al., 2002; Takeuchi et al., 1997; Yamada and Ikeda, 1999). Also important for survival of entire deep-sea ecosystems is the ability of organisms to tolerate, acclimate and adapt to subtle, prolonged elevations in CO_2. Currently, no data exist that address this issue. As stated above, deep-sea organisms are not expected to be highly plastic in their responses to environmental change.

Given the substantial research investment in deep-sea biology over the past few decades, and the relatively limited understanding of deep-sea processes that endures, a very aggressive research campaign must be initiated in order to provide the necessary information within a relevant time frame, unless a ‘no effect’ strategy is adopted.

Priorities should include:

(1) additional mechanistic studies to confirm the generality of the strategies employed to combat acid–base imbalance and

develop physiological indices useful for prediction of hypercapnic responses;

(2) studies to determine the pH and CO_2 levels below which survival is reduced and to assess the acclimatory responses of deep-living organisms over longer timescales;

(3) determination of long-term energetic (i.e. growth and reproduction) and, by extrapolation, ecological consequences of acid–base and ionic imbalance with CO_2 exposure and

(4) studies aimed at predicting potential impacts of localized CO_2 injection on ecosystem-wide processes.

Many deep-living species, if captured carefully, can be kept alive indefinitely at atmospheric pressure (e.g. Seibel and Childress, 2000). Methods are also available for successful maintenance of deep-sea animals under their respective habitat pressures in the laboratory (e.g. Girguis et al., 2002) and for laboratory culture (Omori et al., 1998; Young and George, 2000). Such methods allow long-term hypercapnia studies that are essential prior to instigating deep-sea CO_2 injection.

What can be stated with confidence, based on our present knowledge, is that shallow-living organisms are already generally intolerant of hypercapnia (Knutzen, 1981) and that deep-sea organisms will be even more so. Slow recolonization of deep-sea habitats and a tendency towards slow growth and longevity among deep-sea organisms (Gage and Tyler, 1991) suggests that recovery from any anthropogenic insult will be slow at best. Should deep-sea CO_2 injection be deemed necessary, great care and caution must be employed and further research initiated to ensure that it is done in the most environmentally benign manner possible.

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