

Synergistic effects of temperature extremes, hypoxia, and increases in CO₂ on marine animals: From Earth history to global change

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[1] Currently rising CO₂ levels in atmosphere and marine surface waters as well as projected scenarios of CO₂ disposal in the ocean emphasize that CO₂ sensitivities need to be investigated in aquatic organisms, especially in animals which may well be the most sensitive. Moreover, to understand causes and effects, we need to identify the physiological processes that are sensitive to CO₂ beyond the current emphasis on calcification. Few animals may be acutely sensitive to moderate CO₂ increases, but subtle changes due to long-term exposure may already have started to be felt in a wide range of species. CO₂ effects identified in invertebrate fauna from habitats characterized by oscillating CO₂ levels include depressed metabolic rates and reduced ion exchange and protein synthesis rates. These result in shifts in metabolic equilibria and slowed growth. Long-term moderate hypercapnia has been observed to produce enhanced mortality with as yet unidentified cause and effect relationships. During future climate change, simultaneous shifts in temperature, CO₂, and hypoxia levels will enhance sensitivity to environmental extremes relative to a change in just one of these variables. Some interactions between these variables result from joint effects on the same physiological mechanisms. Such interactions need to be considered in terms of future increases in atmospheric CO₂ and its uptake by the ocean as well as in terms of currently proposed mitigation scenarios. These include purposeful injection of CO₂ in the deep ocean or Fe fertilization of the surface ocean, which reduces subsurface O₂ levels. The resulting ecosystem shifts could develop progressively, rather than beyond specific thresholds, such that effects parallel CO₂ oscillations. It is unsure to what extent and how quickly species may adapt to permanently elevated CO₂ levels by microevolutionary compensatory processes.

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1. Introduction

[2] Global climate change includes the ongoing accumulation of CO₂ in the atmosphere as well as regional trends in other climate factors, due to increases in average temperatures and temperature variability [Intergovernmental Panel on Climate Change (IPCC), 2001]. Global warming threatens to change species specific geographical distributions, including local extinction of previously common species [Parmesan and Yohe, 2003; Thomas *et al.*, 2004]. In this context, the CO₂ emitted by human activities (anthropogenic CO₂) will also play a role as it permeates into ocean surface layers. There it will affect marine organisms in combination with current trends of warming and eutrophication, which also involve a reduction of oxygen levels.

[3] Various strategies of reducing greenhouse gas emissions or of carbon sequestration have been discussed during recent years to mitigate effects of climate change [Lackner, 2003]. Such strategies would reduce the degree of CO₂ accumulation in surface waters; however, they would also transfer CO₂ effects expected from accumulation in the upper ocean to the deeper ocean. Direct ocean disposal scenarios include injecting CO₂ into the deep ocean, with some dispersal over part of the water column or in the form of concentrated lakes of liquid CO₂ [Ohsumi, 1993]. It has also been proposed to neutralize added CO₂ by adding limestone [Kheshgi, 1995; Caldeira and Rau, 2000; Lackner, 2003] as a means to extend storage time and minimize pH changes. Alternatively, iron fertilization of surface waters has been proposed as an indirect means of ocean disposal. Thus atmospheric CO₂ is bound up by organic matter production, which is exported to the deep ocean and released as dissolved inorganic

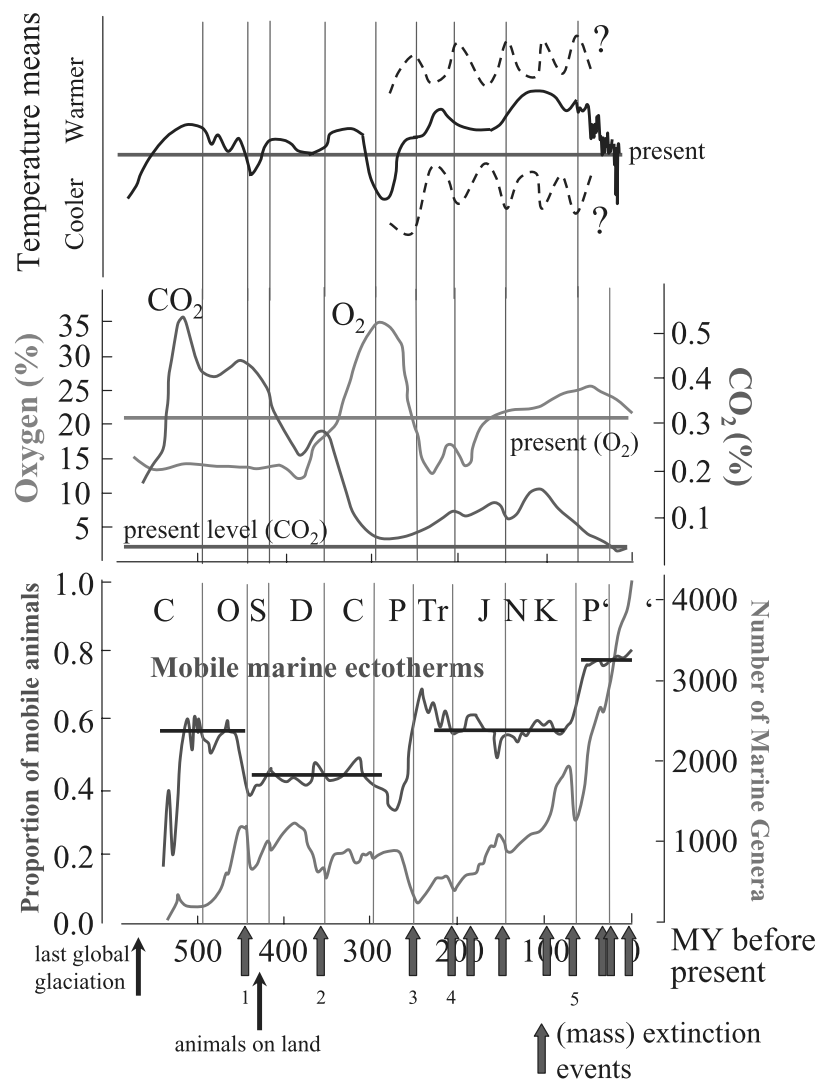


Figure 1. Changes in mean global temperatures, atmospheric oxygen, and CO₂ levels in Earth history compared to present atmospheric levels and the evolution of marine fauna (figure modified after Dudley [1998], Berner and Kothalova [2001], Huch et al. [2001], and Bambach et al. [2002]). High CO₂ and low oxygen levels are interpreted to favor hypometabolic life forms. Once ambient O₂ levels were high and CO₂ levels low, a stepwise evolutionary shift to more mobile animal forms starting with the Permian Triassic mass extinction events appear as a consequence of (climate-induced) evolutionary crises. Such shifts are consistent with the “cost of eurythermy” hypothesis [Pörtner, 2004]. Survival of high-energy turnover lifestyles and elevated capacities of circulatory and ventilatory structures was favored by cold exposures during excessive climate oscillations. During more stable climate periods the upward shift of performance levels and energy turnover likely supported the exponential rise in the number of marine genera over the last 55–65 million years, when atmospheric CO₂ levels were low (see text). See color version of this figure in the HTML.

carbon (DIC) through bacterial activity [Gribbin, 1988]. In this case, the detour used to bind CO₂ first into organic matter by biological activity causes an increase in the complexity of environmental impacts, ranging from a large-scale loss of surface macronutrients to enhanced rates of oxygen consumption in the deep sea. The latter could reduce oxygen availability for higher life to the point that deep sea fauna could suffer from combined effects of hypercapnia and hypoxia. Such proposals need to be

examined not only in terms of feasibility or cost, but also with respect to potential environmental impacts. Benefits and risks must be weighed carefully before such proposals are implemented.

[4] Clearly, future scenarios not only involve changes in oceanic CO₂ inventories and associated effects on marine organisms. Business-as-usual scenarios lead to continued CO₂ enrichment along with warming and eutrophication trends which cause a decrease in dissolved oxygen in

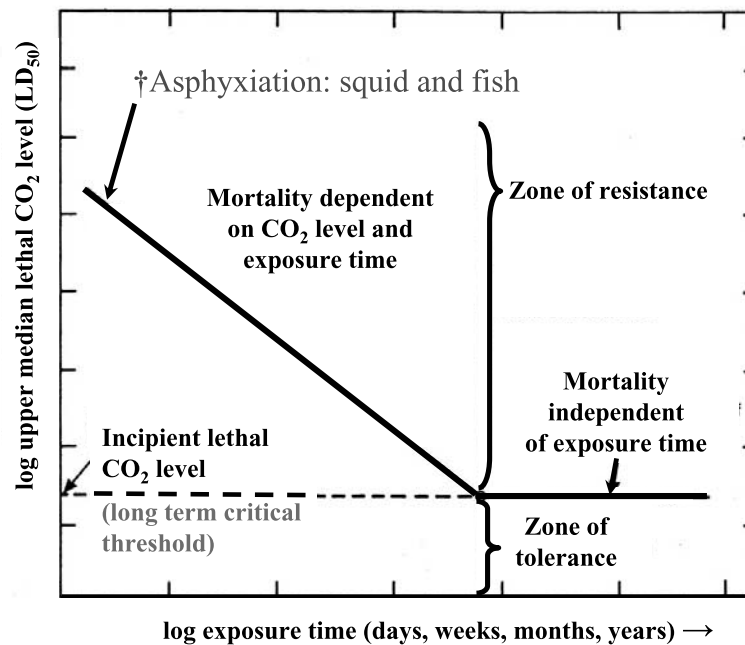


Figure 2. Roles of timescale and CO₂ concentration in causing time- and concentration-dependent mortality in animals (conceptual considerations). Long-term critical thresholds (= incipient lethal CO₂ levels) may exist at the species level. However, the onset of long-term effects in individual species at low levels of CO₂ accumulation (see text) and the likelihood of differences between sensitivities of species suggest that ecosystem shifts may develop progressively rather than suddenly beyond thresholds. In consequence, effects may parallel environmental CO₂ oscillations. Acute effects involve asphyxiation as seen (for different reasons and at different concentrations) in squid and fish (see text). Note that the depiction is hypothetical as such data and graphs have not been generated for any organism under CO₂. Long-term critical levels and the underlying physiological mechanisms are unknown (figure developed based on studies of thermal stress in fish by Doudoroff [1945], after Cossins and Bowler [1987]).

surface waters. Indirect disposal during iron fertilization would be associated with eutrophication and oxygen depletion in the deep sea. Temperature and oxygen will thus change in parallel with CO₂ levels. Increased CO₂ could affect the sensitivity of organisms to other environmental factors and vice versa. Thus the combined effects of these variables must also be considered. Here we aim to provide a conceptual analysis of how effects from these factors may be intertwined and to identify gaps in our knowledge. Our goal is to develop a mechanistic cause-and-effect understanding and to explore possibly relevant tolerance windows or thresholds which are set by the integrated changes in all of these factors. Sensitivities of different life stages also require consideration. Here we rely on Shelford's law of tolerance, which has been used in thermal physiology to identify ecologically relevant thermal limitation thresholds in marine ectothermal animals [Frederich and Pörtner, 2000; Pörtner, 2001, 2002a, 2002b].

2. CO₂ Effects: Timescales and Tolerance Limits

[5] Extant animal life, especially that with high levels of performance, evolved when atmospheric CO₂ had fallen far below the Cambrian levels of 5000 ppm (see Figure 1; in this paper the partial pressure of CO₂, pCO₂, is expressed as a ppm fraction of atmospheric pressure). Oscillating CO₂ levels may have been relevant in animal evolutionary

history. For example, a detrimental role of elevated aquatic CO₂ levels has been suggested to contribute to the Permian/Triassic mass extinction events in Earth history [Knoll *et al.*, 1996]. In present day marine ecosystems, CO₂ partial pressures may be elevated (hypercapnia) periodically or permanently due to natural phenomena in sediments of the intertidal zone, in estuaries [Diaz and Rosenberg, 1995] or in hypoxic bottom waters [Knoll *et al.*, 1996]. In most of the pelagic zones of the sea, however, CO₂ levels remain relatively constant. Physiological and ecological studies of CO₂ effects should support an evaluation of the relevance of CO₂ as an evolutionary factor and as a potential stress factor in extant marine environments.

[6] Effects of elevated CO₂ levels on marine animals have recently been reviewed [Pörtner *et al.*, 2004a]. Here, after summarizing and updating this work, we emphasize the need for further study from a conceptual point of view. Studies of CO₂ effects have distinguished acute from chronic, and lethal from sublethal effects. However, the continuum between time- and concentration-dependent effects has not been clearly elaborated for any species studied (Figure 2), especially with respect to the existence of critical thresholds limiting long-term survival. The physiological mechanisms underlying long-term critical levels are also unknown although individual physiological mechanisms that respond to CO₂ have been identified. The integration of individual effects into a whole animal or

even whole ecosystem scenario is still in its infancy. The early onset of long-term effects in individual species and the likelihood of different sensitivity levels in various species would suggest that ecosystem shifts develop progressively rather than simply at given thresholds, and thus they may follow environmental CO₂ oscillations.

2.1. Mechanisms of Short-Term Sensitivity to CO₂

[7] Available studies indicate that physiological CO₂ effects are mediated through low pH in acidified water as well as through diffusive CO₂ entry into the organism. Elevated CO₂ elicits an acidosis in tissues and body fluids. Acute effects may occur because plasma pH is lowered rapidly and oxygen transport by pH sensitive blood pigments is disrupted. For example, in cephalopods, a limited concentration of pigment (haemocyanin) is dissolved extracellularly in the plasma. Efficient oxygen transport by haemocyanin strongly depends on pH. This dependence may be what defines critical CO₂ levels for short-term exposure (min to hours). Critical CO₂ levels can be as low as about 3 kPa (30,000 ppm) in the coastal squid *Loligo pealei* [Redfield and Goodkind, 1929] or less than 1 kPa (10,000 ppm) in the more active open ocean squid *Illex illecebrosus* [Pörtner, 1990].

[8] In fish, Ishimatsu et al. [2004] consider cardiac failure as the main physiological perturbation responsible for acutely limited tolerance to high CO₂ levels. In yellowtail tuna (*Seriola quinqueradiata*), they found a dramatic drop in cardiac output at 5% CO₂. As a result, there was severely limited oxygen delivery to tissues even though oxygen concentrations in arterial blood remained nearly constant [see also Lee et al., 2003]. Thus under hypercapnia in fish, circulation rather than ventilation is limiting, similar to recent findings for fish under thermal stress (reviewed by Pörtner et al. [2004b]).

[9] Susceptibility to elevated CO₂ levels is higher for early developmental stages (eggs, sperm, larvae, juveniles) than for adults [Crocker and Cech, 1996; Ingermann et al., 2002; Kikkawa et al., 2003; Ishimatsu et al., 2004]. In adult fish, mortality due to elevated pCO₂/and thus lower pH sets in when pCO₂ goes beyond 30,000 to 50,000 ppm (for incubation periods up to 72 hours) [Takeda and Itazawa, 1983; Grottum and Sigholt, 1996; Ishimatsu et al., 2004], whereas the equivalent limit is 13,000 to 28,000 ppm in fish larvae.

[10] These studies generally indicate, that the sensitivity to acutely elevated CO₂ levels is lower in fish than in most invertebrates, especially those having high performance levels, e.g., the coleoid cephalopods (see above). Fish may be less sensitive to acute effects because they use intracellular haemoglobin in oxygen transport as opposed to the extracellular haemocyanin used by cephalopods. Furthermore, vertebrates have a higher regulatory capacity of ion exchange and they have epithelia, which limit diffusive ion losses. Such may promote higher short-term as well as long-term CO₂ tolerance in vertebrates relative to invertebrates.

[11] The trend to accumulate CO₂ and to lose the capacity to transport oxygen in the blood because of lowered pH might be counteracted by enhanced ventilation, as observed repeatedly in fish [e.g., McKenzie et al., 2002]. This would cause enhanced CO₂ release and an alleviation of the pH disturbance. Yet, ventilatory capacity to enhance CO₂ re-

lease is extremely limited in water breathers because of the close to equilibrium distribution of CO₂ between the organism and the water [Scheid et al., 1989]. Water breathers must therefore rely nearly exclusively on transepithelial ion exchange mechanisms to compensate for the disturbance of pH by high CO₂. With elevated PCO₂, pH drops and bicarbonate levels rise depending on the amount of non-bicarbonate buffers [Heisler, 1986a]. Bicarbonate is observed to increase in all animals studied so far, e.g., fish [Heisler, 1986b; Larsen et al., 1997; Ishimatsu et al., 2004], crustaceans [Wheatly, 1989] and other invertebrates (*Sipunculus nudus* [Pörtner et al., 1998]). Hence acid-base and ion equilibria reach new steady state values, associated with specific, long-term shifts in metabolic equilibria. In most cases such processes may not be acutely life threatening for the individual; nevertheless, they are still expected to hamper slow processes like growth and reproduction. Thus they are potentially harmful on longer timescales and at the population, species, and ecosystem levels.

[12] Many studies have identified detrimental effects of low ambient pH on reproductive success, growth and survival of marine animals [e.g., Wickins, 1984; Vinogradov and Komov, 1985; Crocker and Cech, 1996; Desrosiers et al., 1996; Alvarado-Alvarez et al., 1996; Kurihara et al., 2004]. In adult organisms, tolerance to pH excursions varies greatly, ranging from 50% mortality observed in mesopelagic copepods after 6 days of exposure to waters with a pH level 0.2 units below the control [Yamada and Ikeda, 1999] to the same level of mortality reached after a 60 day exposure to a pH of 6.90 in *Ostrea edulis* [Bamber, 1990]. Adverse effects increase with the intensity of the pH disturbance and the duration of exposure [Bamber, 1987, 1990; Grottum and Sigholt, 1996; Adams et al., 1997] (see Figure 2). In addition to the effects due to changes in pH, we also need to reconsider those due to increased CO₂ and bicarbonate [Pörtner and Reipschläger, 1996]. Ishimatsu et al. [2004] found that at the same pH, seawater with CO₂ had higher acute toxicity than that acidified with HCl. Hence CO₂ acts more quickly than fixed acid, probably because of its rapid diffusive entry into the organism.

2.2. Mechanisms of Long-Term Sensitivity to CO₂

[13] Overall, the critical thresholds for the onset of detrimental effects during long-term CO₂ exposures (see Figure 2) are not yet available, with most studies only analyzing LC₅₀ values for short to medium exposure periods (hours to a couple of weeks). Long-term critical thresholds (Figure 2) are likely to be found at unexpectedly low levels of CO₂ for many animals. Shirayama [2002] and Shirayama and Thornton [2005] demonstrated that a reduction in growth rate and survival of echinoderms and gastropods sets in long term at CO₂ concentrations only 200 ppm (0.02 kPa PCO₂) above extant levels. High sensitivity to long-term exposure is emphasized by recent data obtained in the marine mussel *Mytilus galloprovincialis* grown during a 3 month hypercapnic incubation in water at pH 7.3. This pH is close to that expected in marine surface waters around 2300 [Caldeira and Wickett, 2003]. Under these conditions, the mussels displayed a 55% reduction in shell growth rate and soft body growth (Figure 3), combined with a 65% reduction in metabolic rate [Michaelidis et al., 2005]. Thus we suspect substantial effects in marine surface waters

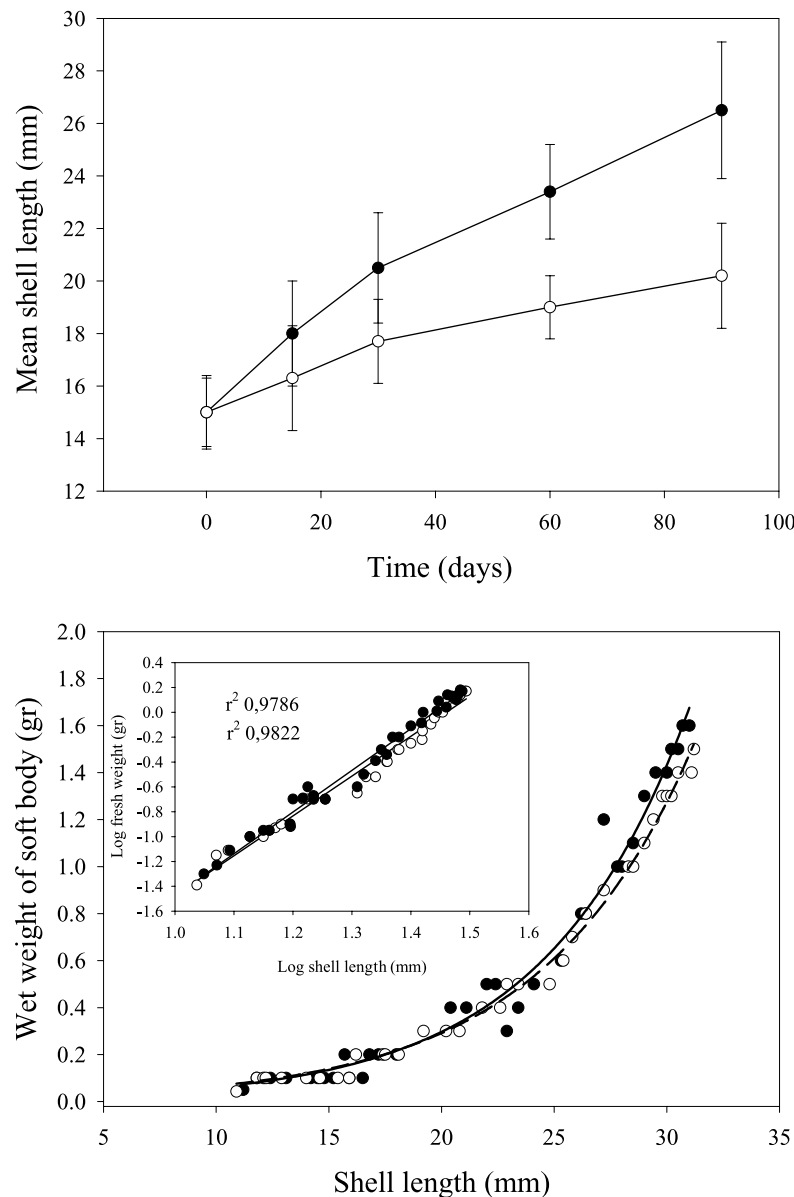


Figure 3. (top) Mean rate of shell growth of normocapnic (closed cycles) and hypercapnic (open cycles) mussels *Mytilus galloprovincialis* at 18°C. (bottom) Fresh weight was closely related to shell length in growing mussels under both normocapnia (solid line, closed cycles) and hypercapnia (dashed line, open circles) (data by Michaelidis *et al.* [2005]). Hypercapnic mussels kept at sea water pH 7.3 as set by controlled additions of gaseous CO₂ displayed a 55% reduction in growth rate. The chosen water pH value is close to that expected from business-as-usual scenarios of anthropogenic CO₂ production and release into the atmosphere by year 2300 [Caldeira and Wickett, 2003].

from CO₂ levels reached during business-as-usual scenarios of anthropogenic CO₂ production and release.

[14] Considering the spectrum of species-specific findings and sensitivities, we will be able to assess the patterns of sensitivity and the capacities for adaptation only if we can identify the unifying principles of the underlying physiological mechanisms that limit long-term performance and fitness under hypercapnia. Within an organism, changes in CO₂, pH and bicarbonate affect molecular, cellular, tissue, and whole organism functions. Similar to effects of temperature [Pörtner, 2002a], effects of CO₂ at lower levels of organization (e.g., on various molecular functions) likely integrate into functional changes at higher levels of biolog-

ical organization. These higher-level functions may result more sensitive than each individual molecular function. The first sign of sensitivity would be a decrease in functional capacity [Pörtner *et al.*, 2004a] (see Figure 5).

[15] Existing information on physiological effects in marine animals originates mostly from studies of invertebrates adapted to frequent CO₂ oscillations within their natural habitat. The most prominent adaptive strategy of these invertebrates is their ability to suppress aerobic energy turnover rates (“metabolic depression”) in response to environmental stress such as hypercapnia [Barnhart and McMahon, 1988; Barnhart, 1989; Rees and Hand, 1990; Pörtner *et al.*, 1998] (for a review, see Guppy and Withers

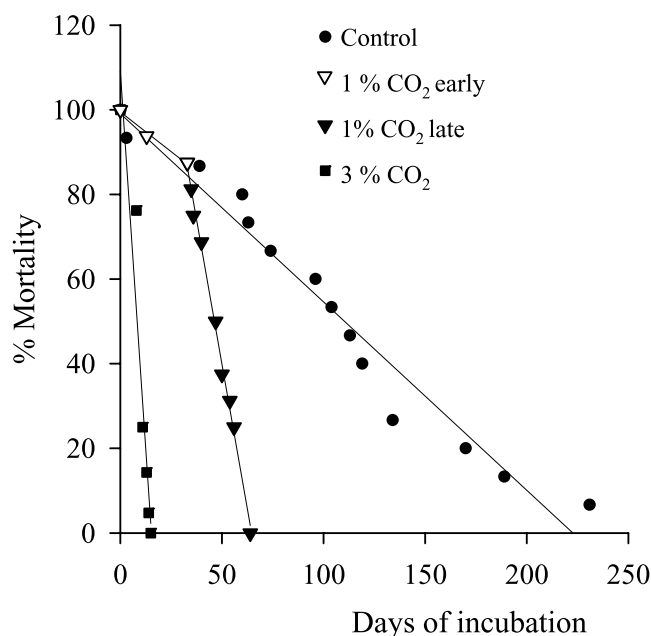


Figure 4. Limited tolerance of the eurybathic noncalcifying sipunculid *S. nudus* (L.) to long-term “disturbed” maintenance under control conditions and at elevated CO₂. Animals were stimulated to repeatedly rebury into sediment. Note the delayed onset of enhanced mortality under 1% CO₂. Under these conditions, no decrease in body energy stores occurred; however, animals were unable to continue repeated reburying (data by Langenbuch and Pörtner [2004]).

[1999]). Under hypercapnia, this suppression is mediated by the incomplete compensation of disturbances in intra- and/or extracellular pH levels (pHi/pHe). In brine shrimp embryos, this suppression is mainly attributed to an acidotic shift in pHi, whereas in *Sipunculus nudus*, a reduction in pHe contributes to a depression of aerobic energy turnover [Reipschläger and Pörtner, 1996].

[16] Mechanisms contributing to hypercapnia-induced metabolic depression have been extensively investigated in the sipunculid *S. nudus*, a marine eurybathic worm from intertidal and subtidal sediments. Under conditions of severe respiratory acidosis (PCO₂ = 10,000 ppm), the oxygen consumption of whole animals is reduced [Pörtner et al., 1998]. This reduction involves a 45% drop in the metabolic rate of (isolated) muscle tissue [Langenbuch and Pörtner, 2002]. During this metabolic depression less ATP is consumed because of the lower cost of net H⁺ excretion from the muscle tissue at a decreased rate of intracellular acid-base regulation [Pörtner et al., 2000]. In addition to metabolic depression, a metabolic imbalance may arise from acidosis. Langenbuch and Pörtner [2002] found lowered ratios of oxygen consumed (O) to nitrogen produced (N) in muscle tissue of *S. nudus* despite simultaneous declines in ammonia excretion and oxygen consumption rates. Probably this implies a change in the use of amino acid substrates in catabolism, namely a preferred degradation of low O/N amino acids like asparagine or glutamine, or of their dicarboxylic acids. As a result, there is increased production of bicarbonate, which

may partially compensate for the intracellular acidosis [Langenbuch and Pörtner, 2002]. Moreover, the decrease in O/N ratios as well as a drop in phenylalanine incorporation rates into muscle protein that has been determined under severe acidosis suggests that hypercapnia-induced acidosis leads to long-term reduction in protein biosynthesis rates [Langenbuch and Pörtner, 2002; M. Langenbuch et al., manuscript in preparation, 2005]. Eventually this could enhance mortality (Figure 4) [Langenbuch and Pörtner, 2004]. These findings may explain the permanent decrease in growth found during long-term uncompensated extracellular acidosis induced by hypercapnia in the mussel, *Mytilus galloprovincialis* [Michaelidis et al., 2005] (see Figure 3). This growth reduction is likely to be due to the pH-dependent reduction in the rates of both calcification and protein synthesis. In extreme cases, hypercapnia and anoxia-induced hypometabolism is associated with a global arrest of cellular transcription and translation, as found with *Artemia franciscana* embryos [Hofmann and Hand, 1994; van Breukelen et al., 2000]. All these data suggest a progressive limitation of essential cellular functions under elevated CO₂ concentrations. Dose-response analyses of expected anthropogenic CO₂ accumulation and pH reduction scenarios are urgently needed to provide a quantitative understanding of CO₂ effects. They are also needed to determine whether shifts develop progressively rather than beyond thresholds (Figure 2).

[17] Further whole animal investigations of *S. nudus* revealed that metabolic depression under hypercapnia goes beyond just modulating the cost of cellular acid-base regulation. Oxygen consumption and associated ventilatory activity were also reduced in response to an accumulation of the neuromodulator adenosine, which suggests that a central nervous mechanism also contributes [Reipschläger et al., 1997; Pörtner et al., 1998]. In vertebrates, adenosine has been shown to depress neuronal excitability (e.g., in the brains of mammals) [Rudolphi et al., 1992] as well as the excitability at neuromuscular endplates [Robitaille et al., 1999; Thomas and Robitaille, 2001; Schwartz et al., 2003]. Infusion of adenosine causes metabolic depression in *S. nudus*, suggesting a beneficial metabolic response to temporary CO₂ accumulation [Reipschläger et al., 1997]. This short-term benefit may switch to being harmful under long-term exposure due to depression of whole organism functions which would reduce fitness for survival. Yet, we understand neither the CO₂-dependent mechanism of adenosine production and release nor the extent to which the substance remains accumulated under chronic CO₂ stress. In conclusion, available data for *S. nudus* shows that CO₂ fluctuations are felt at all levels of organization: from specific cellular metabolic pathways to, ultimately, the functional integration of different tissue types into whole organism functioning and its control by the central nervous system (see Figure 5). Studies addressing how hypercapnia affects the role of adenosine or other neurotransmitters in marine fish are not yet available. It remains to be seen if the key physiological process ultimately limiting CO₂ tolerance may be located at a higher, i.e., systemic or central nervous, level of the organism.

[18] At the ambient CO₂ levels that elicit metabolic depression in invertebrates, such depression is not visible at the whole animal level in most fish. Probably there is

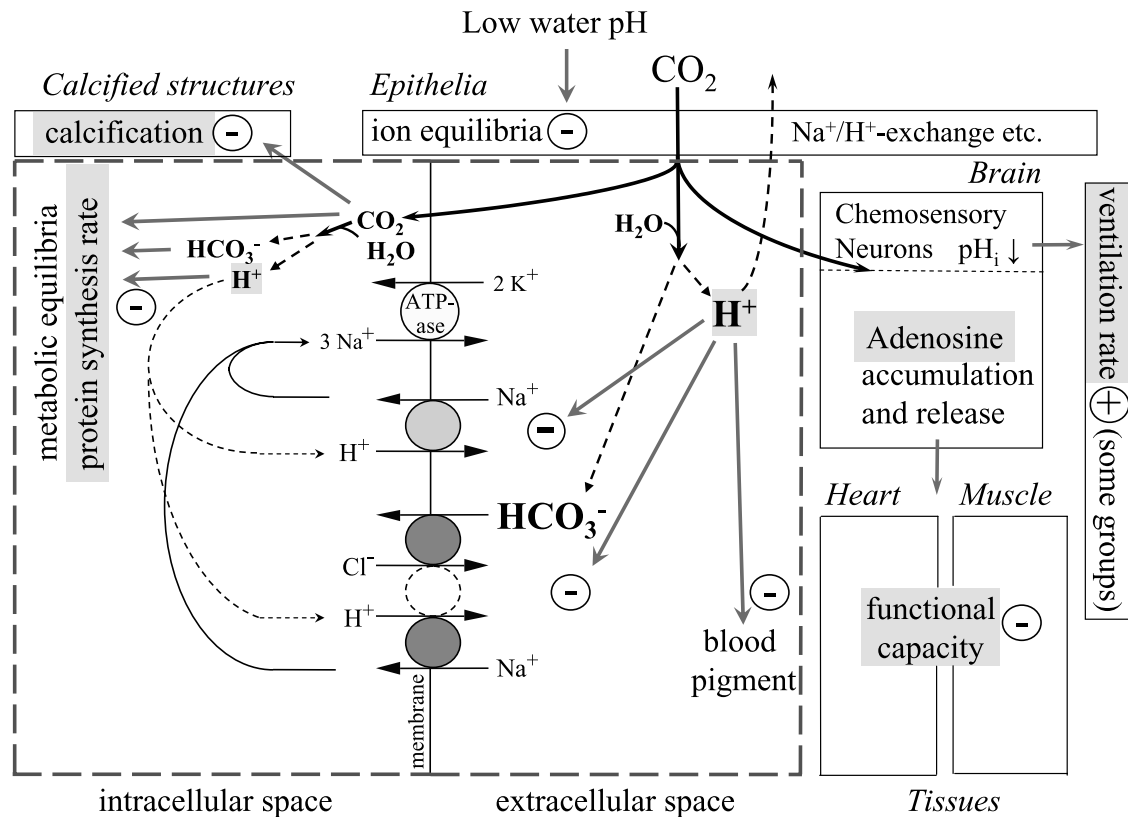


Figure 5. Processes relevant for growth and energy budget (shaded areas) in a concert of molecular to organismic CO₂ effects depicted for a generalized marine water breathing animal (updated from Pörtner *et al.* [2004a] (for further explanations see text)). Sublethal effects on short to medium timescales include the modulation of cellular processes, functional capacity of tissues, shifts in energy budget due to changing energy demand of individual processes, and of behavioral aspects, including the functional capacity for food uptake and consumption. An increase in ventilation rate under elevated CO₂ levels was observed in fish (see text) and in cephalopods (*Sepia officinalis* (S. Schmidt *et al.*, unpublished manuscript, 2005)). Several of these processes are also affected by temperature (due to temperature-induced hypoxia) and by ambient hypoxia itself (e.g., acid-base regulation, functional capacity and aerobic scope, protein synthesis rate, and adenosine formation and release (see text and Figure 6)). See color version of this figure in the HTML.

complete compensation of extracellular and intracellular acidosis (in inner organs and musculature) (e.g., in eelpout, *Pachycara brachycephalum*, or Atlantic cod, *Gadus morhua*) [Heisler, 1986a, 1986b; Larsen *et al.*, 1997; Ishimatsu *et al.*, 2004; C. Burghard *et al.*, unpublished manuscript, 2005]. Metabolism may even be stimulated by hypercapnia. However, at the cellular level, mechanisms leading to a marked reduction in energy turnover seem to be similar in fish and marine invertebrates (see above). Similar to *S. nudus* muscle tissue, hepatocytes of two species of Antarctic fish also showed a decrease of oxygen consumption rates under high CO₂ stress; simultaneously there was an almost complete shut down of cellular protein biosynthesis [Langenbuch and Pörtner, 2003].

[19] In contrast to observations in *S. nudus*, ventilatory frequency and effort increase under hypercapnia in teleosts and elasmobranchs [Burlinson and Smatresk, 2000; McKendry *et al.*, 2001; McKenzie *et al.*, 2002] and also in cephalopods (S. Schmidt *et al.*, unpublished manuscript, 2005). Such increase may require enhanced metabolic costs. Unfortunately no comprehensive data are available

to quantify the contribution of elevated ventilatory activity to CO₂-induced increases in energy turnover. We also do not know the consequences of elevated ventilation for energy reallocation in an animal's overall energy budget (e.g., at the expense of growth, under long-term hypercapnic exposure *in vivo*).

[20] Such enhanced ventilation under high CO₂ in fish is similar to that observed in mammals. For mammals, enhanced respiratory drive under hypercapnia (observed at 5–10% CO₂ in air) has been attributed to the uncompensated intracellular acidosis in chemosensitive neurons causing an increased firing rate [Filosa *et al.*, 2002; Wang *et al.*, 2002]. Simultaneously, elevated ambient CO₂ causes bradycardia, i.e., a slowing of heart rate [e.g., Brevard *et al.*, 2003]. Elevated CO₂ also causes vasodilation of cerebral blood vessels due to combined effects of adenosine, NO, and low plasma pH, which affect ATP-sensitive K⁺ channels and cause enhanced potassium ion conductance across vascular smooth muscle [Nakahata *et al.*, 2003; Brevard *et al.*, 2003]. Finally, very high CO₂ levels result in respiratory depression and anaesthesia in humans [Dean *et al.*, 2003].

[21] Given these findings for mammals, the responses of ventilation (enhanced frequency) and circulation (bradycardia) to hypercapnia also need to be studied in terms of their CO₂-dependent regulation in fish, particularly regarding their influence on the central nervous system. *Söderström and Nilsson* [2000], for instance, reported that the hypercapnia-induced increase in cerebral blood flow velocity in rainbow trout is independent of NO production. Thus mediating mechanisms may be different in lower vertebrates and must be carefully analyzed. Neurotransmitter or hormonal activity may also change. For example, environmental CO₂ stress is known to induce secretion of catecholamines into the circulation of fish [Perry *et al.*, 1989; Aota *et al.*, 1990]. There is also a clear influence of catecholamines on cardiovascular function in trout [Bernier and Perry, 1996]. Catecholamine secretion from chromaffine kidney cells is controlled by cholinergic nerve fibers of the sympathetic nervous system [Bernier and Perry, 1996]. In contrast, in more “primitive” organisms like hagfish or lampreys, adenosine may play a role in the control of hormone release [Reid *et al.*, 1998].

[22] Comparing the patterns seen in mammals and fish with those found in invertebrates suggests once again that the effect of metabolic suppression due to CO₂ sets in with lower CO₂ increments in invertebrates. The uncompensated acidosis found in some invertebrates, e.g., *Sipunculus nudus*, *Mytilus galloprovincialis*, and *Sepia officinalis* [Pörtner *et al.*, 1998; Michaelidis *et al.*, 2005; S. Schmidt *et al.*, unpublished manuscript, 2005], may contribute to their lower resistance and enhanced mortality under long-term moderate hypercapnia, particularly if there is also metabolic depression. In contrast, acidosis under the same degree of hypercapnia in fish is nearly fully compensated. This reflects the enhanced capacity of the vertebrates to avoid early metabolic depression, and their comparatively high resistance to long-term elevated CO₂ levels. Available observations indicate a complex interaction of cellular, neural, and humoral factors in the control of essential systemic functions under hypercapnia (Figure 5). These observations also reflect changes in energy use and allocation at the cellular and organismic levels, with potential consequences for long-term survival.

3. Sensitivities to Temperature, Hypoxia, and CO₂

[23] The increasing importance of CO₂ as a general stress factor opens the question as to what extent CO₂ will interfere or combine with other factors shaping marine environments. Meridional temperature gradients and global climate patterns are seen as the most important factors governing large-scale biogeography of marine ectothermic animals [Angel, 1991]. Oscillations of ambient CO₂ and temperature may naturally parallel the concomitant development of ambient hypoxia, which occurs, for example in oxygen minimum layers at intermediate depths, in isolated bottom waters [Knoll *et al.*, 1996], in tide pools at night [Bridges, 1993], and in marine sediments (for a review, see Grieshaber *et al.* [1994]). These patterns may involve excessive respiration by macroalgae and metazoa (in tide pools) or by microbial life (in the sediment or bottom waters or oxygen minimum layers) which contribute to enhanced

CO₂ accumulation via the oxidation of organic matter. Climate fluctuations and corresponding changes in ocean circulation (slowing of the thermohaline circulation) are seen as key processes that affect the oxygen budget of the ocean [Matear *et al.*, 2000; Plattner *et al.*, 2002; Joos *et al.*, 2003]. Concurrent changes in temperature (due to global warming), oxygen (due to eutrophication and less oxygen export to the deep due to increased stratification), and anthropogenic CO₂ accumulation could interact in terms of their combined effect on marine ecosystems. Although studies have investigated the relevance of each of these factors, no experimental studies have focused on their combined effects. Thus we provide here a conceptual analysis of whether and how these factors may exert synergistic effects on marine fauna.

3.1. Temperature

[24] Compared with terrestrial fauna, marine organisms cover larger ranges of geographical distribution and exhibit latitudinal zonation which is more distinct than that of terrestrial animals [Pielou, 1979; Rapoport, 1994]. Temperature is a key factor in these patterns. Therefore the identification of mechanisms of thermal limitation and adaptation [e.g., Johnston and Bennett, 1996] is important, particularly in the light of future shifts in the geographical distribution and in the physiological performance of ectothermic animals due to global warming [e.g., Wood and MacDonald, 1997; Pörtner *et al.*, 2001].

[25] In various phyla of marine invertebrates and in fish, the transition to internal (systemic) hypoxia was found to characterize the borders of the thermal tolerance window, in fully oxygenated waters. Finally, anaerobic metabolism sets in at both cold and warm temperature extremes. These observations led to the development of the concept that animals have an oxygen-limited thermal tolerance [Pörtner *et al.*, 2000, 2004b; Pörtner, 2001, 2002a, 2002b] (see Figure 6). This concept suggests that the first level of thermal intolerance at low and high temperature extremes in animals is due to a loss in whole organism aerobic scope when near the low and high edges of the thermal envelope, i.e., the so-called pejus thresholds. This loss is not caused by reduced concentrations of ambient oxygen, but by the limited capacity of oxygen supply mechanisms (ventilation, circulation) to cover an animal's temperature-dependent oxygen demand. With continued cooling or warming, aerobic scope finally disappears at low or high critical threshold temperatures (T_c), where there occurs a transition to anaerobic mitochondrial metabolism and cellular energy levels become progressively insufficient. Anaerobic metabolism, combined with molecular protection mechanisms, thus enable the animal to survive temporary periods of exposures to temperature extremes.

[26] The growing inability of oxygen supply to match demand thus represents the first line of sensitivity to both cold and warm temperature extremes. This mismatch will affect all higher organismic functions beyond those relevant for basic maintenance. Such higher functions include motor activity, behavior, feeding, digestion, growth, and reproduction. Thus inadequate O₂ supply will affect the long-term fate of organisms, populations, and species in various climates. Only when an organism is within the optimum range of the window, where maximum aerobic

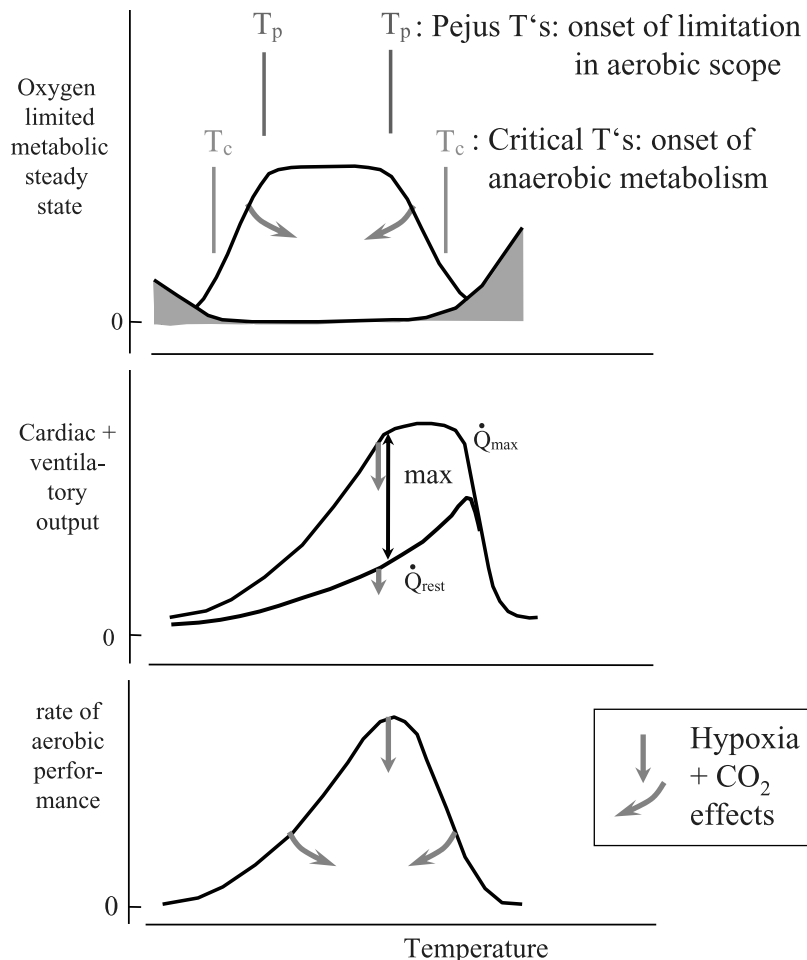


Figure 6. Schematic of oxygen-limited thermal tolerance and performance capacity in fish and other metazoa, set by the capacity of oxygen supply mechanisms (after Pörtner [2001, 2002a, 2002b] and Pörtner *et al.* [2004b]). (top) First limitation at thermal extremes is the onset of a loss in aerobic scope, i.e., the flexibility of the organism to respond to changing energy demand. This loss becomes severe at critical temperatures, when anaerobic metabolism sets in due to temperature-induced hypoxia. (center) Maximum scope (Δ_{max}) between resting and maximum rate of oxygen supply through ventilatory and cardiac activity results at the upper pejus temperature T_p , before aerobic scope becomes thermally limited. (bottom) This supports an asymmetric performance curve of the whole organism. Arrows indicate how the thermal tolerance window is narrowed under the effects of enhanced CO₂ and hypoxia levels because several physiological processes respond unidirectionally under the effects of CO₂ as well as temperature-induced and ambient hypoxia (Figure 5). While CO₂ will support passive but time-limited survival of hypoxia and thermal extremes, it will at the same time cause a decrease in aerobic scope, with prospected decrements in long-term aerobic performance and growth functions as a result as well as a narrowing of thermal windows. For further explanations, see text. See color version of this figure in the HTML.

scope is reached, is there adequate flexibility of aerobic metabolic rate.

[27] The high and low limits of thermal tolerance are interdependent. Such has been interpreted to result from tradeoffs between optimized tissue functional capacity, and baseline oxygen and energy demand. The biochemical factors that are involved and modified during thermal adaptation, are common properties of all eukaryotes. They relate to primary, energy producing metabolism and to energy consuming functions and have whole organism implications. These functions include the glycolytic pathway and mitochondrial metabolism such as respiratory chain and the Krebs cycle, as well as interactions of

membrane bound and catabolic processes. The thermal responses of such fundamental biochemical mechanisms contribute to defining performance levels including the overall capacity of oxygen supply and delivery (ventilation and circulation), which is optimal only within a limited window of thermal tolerance.

[28] Hence there are links between the different levels of functional hierarchy from molecules to the whole animal. For example, temperature-induced hypoxia from thermal extremes in an intact animal elicits limitations in tissue functional capacity due to insufficient aerobic energy. That is followed by a transition to anaerobic metabolism, associated metabolic depression, and ultimately disturbance of

tissue as well as cellular and molecular functions. Consequently, the earliest limits of thermal tolerance are set at the highest level of organizational complexity, i.e., the functional coordination of molecular, cellular, and tissue components works toward the larger unit and toward the resulting whole organism functional capacity. This functional capacity defines how animals exert their ecological functions through their mode of life and behavioral traits [e.g., Pörtner, 2002b]. It is likely that there is a systemic to molecular hierarchy of thermal limitations (for a review, see Pörtner [2002a]). A similar hierarchy may also exist with respect to the tolerance to elevated CO₂ [Pörtner et al., 2004a].

3.2. Temperature and Hypoxia Interactions

[29] Adaptations and limitations associated with hypoxia have been studied in animals from various marine environments. Studies have focused on the mechanisms that allow animals to extract oxygen from the environment down to the lowest possible level of ambient oxygen while they remain fully aerobic. At this low level, termed the critical PO₂ (for a review, see Pörtner and Grieshaber [1993]), anaerobic metabolism sets in, being associated with disturbances of acid-base status and metabolic equilibria (for a review, see Grieshaber et al. [1994]). An organism responds to oxygen deficiency by metabolic down-regulation that is elicited by a wide range of organismal and cellular mechanisms [Hand and Hardewig, 1996; Guppy and Withers, 1999]. These include intra- and extracellular acidosis as well as central nervous mechanisms, e.g., via adenosine accumulation. Such a role for adenosine is not only found among marine invertebrates [Reipschläger et al., 1997] but also in lower vertebrates (e.g., freshwater fish and turtles) that have been subjected to anoxic conditions [Hylland et al., 1997] (for a review, see Lutz and Nielsson [1997]).

[30] Heat- or cold-induced hypoxia is also likely to elicit such responses that appear beneficial. At least, heat-induced hypoxia could counteract the warm-induced acceleration of baseline metabolic costs. The interdependence of thermal tolerance and aerobic scope has only recently been introduced as a unifying principle in animals [Pörtner, 2001]. Therefore the effect of temperature-induced hypoxia on metabolic regulation has not been widely investigated. There is evidence from invertebrates in the intertidal zone that supports a role for hypoxic adaptations in the survival of thermal extremes. When these organisms are exposed to both low tide and midday heat they experience both, extreme temperatures and hypoxia from lack of respiratory water [Sokolova and Pörtner, 2003]. Under such conditions, they switch to an anaerobic metabolism and undergo metabolic depression which contributes to energy savings during low tide. Metabolic depression likely occurs throughout the period when temperatures go beyond the maximum (or minimum) critical or even pejus temperatures.

[31] The combined response to hypoxia and temperature is elucidated by the observation that ectotherms exposed to hypoxia also decrease their preferred body temperature. More specifically, hypoxia-induced behavioral hypothermia is provoked by the onset of oxygen limitation at the critical oxygen tension of amphibians [Wood and Malvin, 1991; Pörtner et al., 1994], reptiles [Branco et al., 1993], fish [Schurmann and Steffensen, 1992], and crustaceans

[Robertson et al., 2002]. The onset of anaerobic metabolism linked to succinate and lactate accumulation characterizes the critical PO₂ in similar ways as it typifies the critical temperature [Frederich and Pörtner, 2000]. Lactate may well be involved in eliciting the downward shift of preferred body temperature in ectotherms [Pörtner et al., 1994; de Wachter et al., 1997; Branco and Steiner, 1999]. These findings underscore the close relationships between aerobic scope, thermal preferences and the range of thermal tolerance. They also emphasize the relevance of hypoxia-temperature interactions in setting tolerance to environmental extremes.

3.3. Integrated Effects of Temperature, Hypoxia, and CO₂

[32] We hypothesize that CO₂ affects several mechanisms that are also affected by oxygen deficiency and thermal extremes. Exposure to reduced ambient oxygen levels or to thermal extremes associated with increased ambient CO₂ would thus imply integrated effects of hypoxia and CO₂. Moreover, once animals respond to environmental extremes (e.g., hypoxia, thermal extremes) by metabolic depression, the rate of gas exchange across respiratory epithelia is reduced, internal oxygen stores are depleted, and respiratory CO₂ usually accumulates. Systemic hypoxia combined with hypercapnia will thus emphasize metabolic depression. This extends the period an organism is able to passively withstand environmental extremes, particularly in species pre-adapted to hypoxic environments.

[33] All individual mechanisms affected by CO₂, temperature, or hypoxic extremes (see Figure 5) result in reduced metabolism and functional flexibility or are otherwise protective during passive tolerance of adverse environmental conditions. This tolerance is time limited. Similar to hypoxia, high CO₂ affects the metabolic rate by shifting the steady state acid-base equilibria [e.g., Pörtner, 1993; Pörtner et al., 1998], and by reducing the rates of relevant transmembrane ion exchange [Pörtner et al., 2000]. Shifted steady state acid-base equilibria also limit the rate of protein synthesis with long-term consequences for growth and reproduction. Another similarity is that protective behavioral hypothermia is not only elicited by hypoxia (and the transition to anaerobic metabolism) but also by environmental hypercapnia [Riedel and Wood, 1988], possibly mediated by NO [Barros and Branco, 1998]. Combining high CO₂ and anoxia causes a synergistic metabolic depression via the effect of adenosine on central nervous functions [e.g., Reipschläger et al., 1997]. Adenosine which accumulates during both hypercapnia and anoxia acts as a central mediator of behavioral hypothermia [Branco et al., 2000]. Thus high CO₂ probably affects the metabolism of neurotransmitters and it may be involved in dampening the functional capacity of neuromuscular junctions, e.g., in response to hypercapnia-induced adenosine accumulation. On short to medium timescales, CO₂ accumulation may well be protective in terms of reducing metabolic energy turnover, and in doing so it may also minimize oxidative damage [Vesela and Wilhelm, 2002].

[34] However, all of these findings imply that high CO₂-induced shifts in cellular and organismic equilibria synergistically reduce the functional capacity of the whole organism with consequences on behavior, growth, repro-

duction, and thus long-term survival in a changing ecosystem. Several physiological processes (e.g., regulation of acid-base or ionic equilibria, aerobic energy turnover, and protein synthesis) are depressed due to the effects of CO₂ and temperature-induced or ambient hypoxia. While the consecutive depression of aerobic metabolic rate under CO₂ will support extended survival of hypoxia and thermal extremes, it will simultaneously cause a decrease in the capacity of an animal to increase its rate of aerobic energy turnover, even at temperatures that remain within the optimal range of thermal tolerance. Thus transgression to pejus (worse) conditions occurs more rapidly, resulting in limited performance and growth functions. Thus high CO₂ likely narrows the thermal window of an animal by enforcing such limitations in aerobic scope (see Figure 6).

[35] Such effects and interactions may well have been important during mass extinction events in Earth history. They may explain the role of CO₂ in the Permian-Triassic extinctions as previously suggested [Knoll *et al.*, 1996]. Temperature oscillations most probably contributed repeatedly to mass extinction events via the long-term effects of repeated exposure to extreme climate oscillations and associated cooling events [Pörtner, 2001, 2004]. The narrowing of thermal windows caused by both the transient CO₂ accumulation and hypoxia would have enhanced the detrimental effect of large-scale temperature fluctuations on marine ecosystems. Only the most extreme eurytherms, which were also the more active life forms, survived these events. This survival of active eurytherms could explain the stepwise increase in the fraction of more active marine species in evolution depicted in Figure 1 [Pörtner, 2004].

[36] These considerations suggest that current trends of warming, of CO₂ increase, and of oxygen reduction in marine waters may exert synergistic effects on marine fauna. These factors may be protective early on by extending the period of passive tolerance to environmental extremes, but they will become harmful as these conditions persist and worsen.

4. Comparison of Scenarios

[37] Such integrated effects of temperature, hypoxia, and CO₂ must be considered in the analysis of environmental impacts of climate change scenarios. Moreover, they will need to be considered in regard to various proposed scenarios of anthropogenic CO₂ accumulation and disposal. These include an uncompensated rise in the release of fossil fuel derived CO₂ to the atmosphere and upper ocean, or various strategies of direct sequestration of CO₂ into the deep ocean as well as enhanced CO₂ uptake through iron fertilization. All of these strategies involve specific tradeoffs where the degree of harm to the natural environment must be considered before any implementation.

4.1. Business as Usual

[38] The ongoing combustion of fossil fuels has led to a progressive rise in surface ocean CO₂ concentration and a drop in seawater pH that is already detectable today [Haugan and Drange, 1996; Brewer, 1997; Wolf-Gladrow *et al.*, 1999]. Atmospheric CO₂ levels could reach 970 ppm by the end of this century [IPCC, 2001]. During the next 300 years, there could be a further increase to 1900 ppm,

which is unprecedented probably for at least the last 300 million years; with that surface ocean pH would decrease by 0.77 units [Caldeira and Wickett, 2003]. Continued uptake of anthropogenic CO₂ by the ocean indicates that this abiotic factor will progressively become a general stress factor in aquatic environments. The first studies to focus on related biotic impacts have considered a potential CO₂ fertilization of marine phytoplankton growth [e.g., Riebesell *et al.*, 1993; Wolf-Gladrow *et al.*, 1999] as well as the disturbance of biogenic calcification [Done, 1999; Marubini and Atkinson, 1999; Riebesell *et al.*, 2000]. More recently, there is evidence (see above) that indicates that CO₂ effects will progressively compromise marine water breathing macrofauna, including calcifying invertebrates but also noncalcifying species.

[39] The progressive increase in marine surface water CO₂ content will most certainly not reach thresholds of acute effects among animals (e.g., via disturbance of pH-dependent oxygen transport in high performance invertebrates like squid). However, long-term moderate effects are likely. These will begin at relatively low CO₂ levels and will first result in decreased calcification rates in sessile animals, most visibly among corals. On a continuum between low CO₂ levels and the highest levels expected, the ratio of noncalcified over calcified organisms may increase progressively. Additionally and unless compensated for during microevolutionary adaptation, a progressively enhanced reduction in organismic energy turnover will likely affect marine animals more widely, especially invertebrates. These limitations may be less severe or may set in later in fish, if they occur at all, because of the greater capacity of fish to compensate acid-base disturbances. Metabolic depression involves a reduction in metabolism associated with a reduction in physical activity as well as in growth and reproduction. While transient exposure allows survival by metabolic depression, such depression probably becomes detrimental during long-term exposure. Both calcified and noncalcified organisms likely experience decreases in population densities once growth and reproduction rates begin to be affected. Furthermore, elements higher in the food chain, e.g., vertebrates, will also be affected because of reduced food availability.

[40] The principle interactions between hypoxia, temperature, and CO₂ along with the available evidence that indicates significant animal responses to CO₂ increases of just 200 ppm [Shirayama, 2002], suggest that effects of rising CO₂ levels on animal performance likely set in early on (see above). Thus they will aggravate current ecosystem responses to enhanced levels of environmental hypoxia and temperature extremes. The expected narrowing of the thermal window (see Figure 6) is likely to affect those animals living at the edge of their temperature-dependent (northern or southern) distribution, thereby also causing a narrowing of geographical distribution windows. Consequently, progressive shifts in marine and terrestrial ecosystems should be analyzed in terms of whether they are caused by the synergistic action of global warming and CO₂ accumulation. As the availability of ambient oxygen and the thermal sensitivity of animal organisms are closely intertwined, trends of global warming, associated decrements in oxygen availability despite increasing demand, as well as thermal and CO₂ sensitivities may closely interact to cause large-

and small-scale shifts in geographical distribution as well as ecosystem composition and functioning.

4.2. Ocean Disposal: Direct Injection

[41] The proposed dumping of anthropogenic CO₂ into the deep sea [Marchetti, 1977, 1979] will cause immediate increments in CO₂ levels with the highest CO₂ concentrations and largest pH excursions directly at the site of injection. Minimum levels of pH 4.0–5.0 are expected to occur depending on the sequestration technique [Auerbach *et al.*, 1996, 1997; Adams *et al.*, 1997]. Formation of CO₂ lakes would leave no benthic animal life in the chosen area. Large-scale mitigation options (e.g., towed pipe scenario) would reduce the degree of local impacts; however, they would still include significant increases in CO₂ partial pressure and lowering of pH in the entire affected ocean. These changes would probably affect deep sea biology. As a major benefit, direct CO₂ injection would introduce one individual stress factor only. That is, it would not substantially alter deep sea O₂ and temperature. Deep sea disposal would occur in a natural environment characterized by stable temperatures mostly between 1° and 4°C (with the exception of the Mediterranean, 11°–12°C, and the Red Sea, 21°C). This would exclude a role for a change in thermal sensitivity as a result of CO₂ effects unless CO₂ not only elicits a narrowing but also a shift of the thermal tolerance window by depressing aerobic scope.

[42] However, deep sea animals depend on oxygen supply by the thermohaline ocean circulation which is seen as the major process shaping the deep sea oxygen inventory. Deep water oxygen levels remaining somewhat below air saturation indicate that oxygen supply may be limited. Deep sea oxygen demand due to oxidation of organic matter is apparently in a sensitive equilibrium with lateral oxygen import from high latitudes, reflected by reduced steady state oxygen levels in the deep ocean. In line with a synergistic effect of hypoxia and CO₂ on metazoan physiology, CO₂ effects will be exacerbated if animals experience concomitant exposure to more severe hypoxia in response to global warming.

[43] Initial field experiments in the deep [Tamburri *et al.*, 2000] have not determined the tolerable limits for pH and CO₂ fluctuations over long timescales. Acute lethal effects were seen during experimental CO₂ exposure in some deep sea animals [see Barry *et al.*, 2005]. However, dose-response relationships remain again obscure. Generally it is thought that deep sea ecosystems may experience significant shifts in composition from even moderate pH changes of ~0.1–0.3 such as might be caused by long-term repeated large-scale CO₂ injection. These changes would drive shifts in population dynamics due to initially sublethal impacts that involve reduced rates of growth and reproduction. Effects would be more detrimental under long-term exposure.

[44] Onset of CO₂-related effects due to long-term exposure at low levels of CO₂ already casts doubt on whether large-scale strategies of CO₂ disposal should be implemented. Although this would possibly keep levels below those which elicit acute lethal effects on the most active pelagic organisms, there is considerable danger for long-term detrimental effects, and associated risks have not been

properly quantified. Nevertheless, if ocean disposal were to be implemented, strategies would have to be developed to leave a large fraction of the marine environment unaffected, i.e., so that the net long-term damage to marine ecosystems should be less than with a large-scale distribution of CO₂. The degree of pH reduction and of PCO₂ increase as well as harmful effects could be limited by carbonate neutralization strategies [Kheshgi, 1995; Caldeira and Rau, 2000] which would also extend the ocean's retention time of disposed CO₂ to several thousand years [Lackner, 2003]. Still such would need to be found practicable in terms of its environmental impact.

4.3. Ocean Disposal: Iron Fertilization

[45] Concerns about large-scale ecosystem effects have also arisen in response to proposals to fertilize the ocean with iron [Chisholm *et al.*, 2001]. The goal of iron fertilization is to stimulate phytoplankton growth by alleviating the limiting availability of iron in ocean waters, thereby increasing the export of organic carbon to the deep sea, which would increase some air-sea CO₂ flux [Gribbin, 1988]. Increased degradation of organic matter in the deep ocean would progressively enhance CO₂ levels, but probably much more slowly and moderately than with direct injection. However, the efficiency of this process, i.e., the degree of net export of organic material to the deep ocean is likely to be rather low [Buesseler and Boyd, 2003; see Baar, 2005]. Moreover, the export of carbon and its subsequent oxidation along the way and after sedimentation will lead to deep sea eutrophication with enhanced oxygen consumption in those areas used for iron fertilization. Thus depending on where iron fertilization is carried out, oxygen minimum layers found at 500–1500 m depths in low latitudes will be more intense.

[46] The deep sea below these layers is more oxygenated due to lateral import of cold oxygenated water from polar areas, but steady state oxygen levels still remain below air saturation. These observations emphasize that the deep sea represents an oxygen limited environment with a steady state equilibrium of limited supply vs. moderate demand. Most parts of the extant deep are permanently oxygenated because oxygen demand is low due to low nutrient content, scarcity of food, low density of oxygen demanding organisms and their low metabolic rates. Furthermore, oxygen gradients exist, especially close to the sediment, depending on the density of O₂ consuming organisms present. This present situation has not persisted throughout Earth history and may thus not be well stabilized. On evolutionary timescales, large-scale oscillations in oxygen content and associated increments in CO₂ levels have likely influenced evolutionary patterns (see Figure 1 and above). Repeated extinctions due to oxygen deficiency suggest a relatively recent recruitment of deep sea faunas [Jacobs and Lindberg, 1998].

[47] In conclusion, the oxygen inventory of the deep ocean could be most sensitive to human interference. In a deep ocean limited by oxygen supply, the equilibrium between oxygen supply and demand would be shifted to lower oxygen levels by the increased import and subsequent oxidation of organic matter [Sarmiento and Orr, 1991; Chisholm *et al.*, 2001]. The aerobic or anaerobic degradation of organic matter in bottom layers would

cause net accumulation of CO₂. Animals would be exposed to combined hypoxia and hypercapnia at rather stable temperatures. In response to iron fertilization, integrated effects of hypoxia and CO₂ on higher organisms would thus cause and exacerbate detrimental long-term ecosystem consequences.

5. Synopsis

[48] Available evidence suggests that the long-term consequences of anthropogenic CO₂ accumulation have already begun in surface waters. When combined with effects of global warming and enhanced hypoxia, this will cause and exacerbate shifts in ecosystem equilibria which affect higher marine organisms, i.e., the metazoa. Yet, ocean biota will also be affected by all mitigation scenarios which involve ocean CO₂ disposal. Direct storage in the deep ocean would reduce effects in surface waters from the passive invasion of anthropogenic CO₂ at the expense of similar overall effects in the deep ocean. Direct injection would also avoid the combined effects of eutrophication, oxygen depletion, and CO₂ accumulation that would come with an iron fertilization strategy. If ocean disposal were deemed necessary (e.g., due to limited capacities of geological storage), a scenario with little large-scale impact should be chosen. To avoid or minimize large-scale impacts, pH neutralization strategies combined with localized CO₂ disposal in stable deep sea dump sites (lake option) have been suggested [Pörtner et al., 2004a]. Thus higher priority should be given to evaluating those options with less severe overall impact.

[49] We conclude from the results of available physiological studies, that the mechanistic understanding of CO₂ effects on animals is still incomplete, as are potential synergistic effects with increasing temperature and hypoxia. No simple cause-and-effect relationship of CO₂ effects was found. Rather there may exist a complex hierarchy of CO₂ sensitivities, ranging from systemic functions and their central nervous control down to the tissue, cellular, and molecular levels [Pörtner et al., 2004a]. Whereas cellular processes display high flexibility in response to acute hypercapnia, in terms of acid-base regulation [Pörtner et al., 2000], and protein synthesis [Langenbuch and Pörtner, 2003; M. Langenbuch et al., manuscript in preparation, 2005], little is known about the limiting physiological processes effective on extended timescales for higher organizational levels. Such mechanisms, including neural or hormonal control, may partly determine the time-dependent onset of mortality that was observed under long-term exposure to moderate CO₂ levels. In the future, these mechanisms need to be identified.

[50] Furthermore, for a clear assessment and comparison of environmental impacts of any ocean CO₂ enrichment scenario CO₂-dependent effects in relation to those of temperature and hypoxia need to be understood and quantified at the whole organism and ecosystem levels. Knowledge of CO₂-dependent mechanisms, their role in ecosystem structure, and functioning and their "titration" by increasing CO₂ (lower pH), at various temperatures and oxygen levels, are crucial to understand cause-and-effect relationships. Field observations of ecosystem trends and fluctuations need to be carried out at the same time.

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