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

Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view

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ABSTRACT: Ocean warming and acidification occur at global scales and, in the case of temperature, have already caused shifts in marine ecosystem composition and function. In the case of CO_2-induced ocean hypercapnia and acidification, however, effects may still be so small that evidence for changes in the field is largely lacking. Future scenarios indicate that marine life forms are threatened by the specific or synergistic effects of factors involved in these processes. The present paper builds on the view that development of a cause and effect understanding is required beyond empirical observations, for a more accurate projection of ecosystem effects and for quantitative scenarios. Identification of the mechanisms through which temperature- and CO_2-related ocean physicochemistry affect organism fitness, survival and success, is crucial with this research strategy. I suggest operation of unifying physiological principles, not only of temperature but also CO_2 effects, across animal groups and phyla. Thermal windows of optimized performance emerge as a basic character defining species fitness and survival, including their capacity to interact with other species. Through effects on performance at the level of reproduction, behaviour and growth, ocean acidification acts especially on lower marine invertebrates, which are characterized by a low capacity to compensate for disturbances in extracellular ion and acid–base status and sensitivity of metabolism to such disturbances. Available data suggest that one key consequence of these features is a narrowing of thermal tolerance windows, as well as a reduced scope for performance at ecosystem level. These changes in bioenvelopes may have major implications for the ranges of geographical distribution of these organisms and in species interactions.

KEY WORDS: Ocean acidification · Global change · Temperature effects · Calcification · Metabolic performance · Acclimation · Ecosystems · Hypoxia

TEMPERATURE AND CO_2 SHAPING MARINE ECOSYSTEMS

The oceans cover 70% of the earth's surface. Due to their large volume and the ability of seawater to buffer CO_2, oceans have absorbed approximately half of all anthropogenic CO_2 emissions to the atmosphere, which amounts to more than 120 Gt C in total or 440 Gt CO_2 (Sabine et al. 2004) within the last 200 yr. CO_2 produced by human activities penetrates into the surface layers of the ocean and is transported by ocean currents to deeper waters. At present, the oceans take up about 2 of the 6 Gt C per annum from human activity. In this context, the contribution of ocean biology to CO_2 up-take is similarly large as that of the terrestrial biosphere. However, the ability of the ocean to take up CO_2 decreases with increasing atmospheric CO_2 concentrations due to the reduced buffering ability of seawater as CO_2 accumulates. The present increase in CO_2 levels in the atmosphere is approximately 100-fold faster than at the end of the last ice ages when CO_2 levels rose by about 80 ppm over 6000 yr (IPCC 2001, 2007). Now exceeding 380 ppm, the present CO_2 content is the highest in the atmosphere for the last 420 000 and possibly more than 10 million yr (IPCC 2001, 2007).

Ecosystem effects of CO_2 accumulation and their interaction with effects of warming, eutrophication, and hypoxia are attracting increasing international at-

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tention (Cicerone et al. 2004a,b, Orr et al. 2005, based on the UNESCO symposium 'Oceans in a High CO$_2$ World', http://ioc.unesco.org/iocweb/co2panel/HighOceanCO2.htm, or a corresponding discussion in the context of OSPAR, www.ospar.org/documents/dbase/publications/p00285_Ocean acidification.pdf, see also www.ocean-acidification.net). Once atmospheric CO$_2$ levels increase, the amount of CO$_2$ physically dissolved in the water follows in accordance with Henry's Law. Distribution kinetics and equilibria are modified by biological processes such as respiration and photosynthesis. In physical equilibrium CO$_2$ reaches concentrations which are similar in the 2 media due to the similar 'solubilities' in water and air. Increments in aquatic CO$_2$ levels cause associated changes in water physicochemistry or acid–base status, which have been detectable in upper ocean layers for some decades (Chen & Millero 1979, Brewer et al. 1997, IPCC 2007). The CO$_2$ budget of the ocean comprises about 1% physically dissolved CO$_2$, including H$_2$CO$_3$, as well as about 91% bicarbonate (HCO$_3^-$) and about 8% carbonate (CO$_3^{2-}$). Model calculations revealed that in comparison with pre-industrial times, the accumulation of CO$_2$ in 1996 had already caused a pH decrease beyond 0.1 units equivalent to an increase of H$^+$ ion activity by 30% in the surface ocean (Haugan & Drange 1996). With the continued use of fossil fuels, atmospheric CO$_2$ concentrations are expected to rise from current 380 ppm (pCO$_2$ = 380 µatm) to more than 750 ppm (IPCC scenario IS92a; Houghton et al. 2001) or even more than 1000 ppm (Royal Society 2005) in 2100 and will climb to more than 1500 ppm (pCO$_2$ = 1500 µatm) between 2100 and 2200 (e.g. Wigley et al. 1996). This will lead to a pH reduction in the upper ocean layers by 0.3 to 0.5 units up to 2100 (Zeebe & Wolf-Gladrow 2001, Caldeira & Wickett 2005). Acidification of the surface water by up to 0.77 pH units is finally expected if values of atmospheric CO$_2$ achieve levels of 1900 ppm by 2300 (Caldeira & Wickett 2003).

Due to this high storage capacity, the ocean at first appeared to be a suitable place for the disposal of CO$_2$, either directly, via diffusive entry or industrial scale deep-sea release, or indirectly, via iron fertilization, consecutive net particle export and CO$_2$ release during deep-sea respiration. However, CO$_2$ develops specific effects on marine life which exclude or at least limit the ocean's use as a solution to rising atmospheric CO$_2$ concentrations. This impact is exacerbated when combined with temperature extremes, potential problems of oxygen deficiency that arise from global warming, eutrophication, or potential CO$_2$ disposal strategies through iron fertilization (Pörtner et al. 2005). Effects go beyond the potential changes in the fluxes of carbon or nutrients which still require investigation (Riebesell et al. 2007).

The current trend of increasing atmospheric CO$_2$ is accompanied by regional changes in other climatic factors, primarily temperature and its variability (IPCC 2001, 2007). Global warming alone has already affected the geographical distribution of aquatic and terrestrial animals with enhanced risk of local extinction of species or even ecosystems, in the case of coral reefs (Parmesan & Yohe 2003, Thomas et al. 2004, Perry et al. 2005, Hoegh-Guldberg 2005). Within conditions set by geomorphology, ocean currents, water depth and stratification or salinity, large scale geographical distribution of marine animals is shaped decisively by temperature. Depending on the level of mobility and tolerance windows for physical factors, organisms can achieve particular geographical ranges. Mode of life (e.g. passive versus active) in relation to living conditions, food supply or competition for food, are additional factors shaping the final biogeography of individual species and the functional structure of communities in open water (pelagic) and on the bottom (benthic). These considerations also apply for reproductive stages (eggs or sperm) as well as adult phases of the life cycle. It is clear, however, that the tolerances to climate-related factors might be very different between larvae and adult organisms (e.g. pelagic larvae versus benthic adults) as well as between species, thereby influencing species interactions within ecosystems. It is also important to point out that the future distribution of organisms also depends on how fast required habitats are being changed by climate change and how fast a species can spread and follow a changing climate. In some cases organisms may migrate, or be dispersed through reproductive stages. At this point geographical barriers such as deep-sea trenches or currents (e.g. the circum-Antarctic current) may become important (Thatje et al. 2005). Overall, the physiological principles setting performance, on the one hand, and climate dependent ecological patterns, on the other hand, may be more intertwined than traditionally thought (Pörtner & Farrell 2008).

The importance of combined temperature and CO$_2$ effects, and the limited capacities of marine organisms (from microbes to phytoplankton to animals) to acclimatize or adapt to elevated CO$_2$ concentrations, is illustrated through current discussions of a pivotal role played by CO$_2$ and temperature oscillations in mass extinction events, e.g. during the Permian–Triassic (Knoll et al. 1996, 2007, Bambach et al. 2002, Berner 2002, Pörtner 2004, Pörtner et al. 2005). The course of evolutionary history might thus have been decisively influenced by atmospheric and aquatic CO$_2$ concentrations. It is conceivable that the evolution of very mobile marine life-forms became possible in geological history only with the decrease in atmospheric CO$_2$ levels. CO$_2$ levels in the Cambrian atmosphere ranged up to

about 0.5 % (i.e. a pCO_2 of 0.5 kPa or 5000 µatm). Average atmospheric levels fell more or less continuously in the following phases of earth history (cf. Dudley 1998, Berner 2002). Cornette et al. (2002) suggested that the level of atmospheric CO_2 concentrations influenced the rate of speciation in the sea, however, mechanisms and time scales involved are unclear.

Currently, CO_2 is an abiotic factor which can vary strongly in some marine habitats. It remains constant in large stretches of the open ocean but will oscillate considerably where excessive metabolic or photosynthetic activities occur and where gas exchange with the atmosphere or open sea is at least periodically constrained. CO_2 absorption is increased by increasing solubility at low water temperatures, whereas warming favours CO_2 release. Variable values of pH and CO_2 partial pressure in the seawater are therefore linked with water temperatures, ocean currents, CO_2 consumption due to photosynthetic activity at the sea surface or by oxygen demand arising from high contents of organic materials in deeper layers. The latter is also causal in the formation of hypoxic layers in the oceans. Correspondingly, CO_2 partial pressure rises and water pH falls progressively in seawater in the course of large-scale deep-ocean currents ('conveyor belt') from the North Atlantic to the North Pacific. In the oxygen minimum zones of the North Pacific, CO_2 partial pressures of 1200 µatm result and contrast with corresponding values of 500 µatm in the North Atlantic (Millero 1996). CO_2 partial pressures are increased and pH values reduced at the surface of upwelling zones (e.g. Feely et al. 2008). This trend is exacerbated when the water is warming. Starting out from a slightly alkaline pH of 8.2 at the surface, a pH variability of more than ±0.3 pH units can result depending on region, season and phytoplankton activity (Hinga 2002).

The classic example of short term CO_2 oscillations is seen in the rock pools of the intertidal zone where respiration dominates by night and the consumed oxygen is replaced by accumulating CO_2 (Truchot & Duhamel-Jouve 1980, Morris & Taylor 1983). In the same pools, low tide in the middle of the day is characterised by excessive photosynthetic activity relative to respiration, and the precipitous drop in CO_2 concentrations and increase in pH.

Water CO_2 content also fluctuates in marine sediments (e.g. at low tide) or in hypoxic bottom waters if high levels of organic material elicit increased oxygen consumption and finally anaerobic metabolism of bacteria, meio- and macrofauna in surroundings where the exchange with surface waters is low. CO_2 partial pressures of 1.60 kPa (16 000 µatm) are conceivable in anoxic environments (Knoll et al. 1996.). Deep-sea areas are anoxic in the Black Sea because no lateral oxygen import by ocean currents takes place. In other oceans where the deep sea is oxygenated and supports animal life, special habitats have developed at hydrothermal vents where the water is enriched with CO_2 due to volcanic activity. High CO_2 partial pressures of 8.00 kPa have been measured (80 000 µatm) and are exploited by hydrothermal fauna like the Vestimentifera (giant tube worms) during CO_2 fixation by their symbiotic bacteria (Childress et al. 1993).

Overall, marine animal life has adapted and possibly specialized in a range of ambient CO_2 conditions, from the high concentrations found at deep sea vents to the widely fluctuating levels typical of the intertidal zone. Certain life forms have also specialised to live in the permanently low CO_2 levels in the open ocean. These adaptive responses likely partially define the extent to which a species reacts sensitively to the progressively higher CO_2 levels of the future.

There are few field observations of specific CO_2 effects associated with climate dependent phenomena in marine ecosystems. Such phenomena have frequently been related to temperature effects. Even the decreasing calcification rates over the last decades in coral reefs have not been clearly explained and may be caused by combined temperature and CO_2 effects (Cooper et al. 2008). Oscillating calcification rates in phytoplankton during the anthropocene (Iglesias-Rodriguez et al. 2008), palaeo-records during glacial to interglacial periods (Barker & Elderfield 2002) or mass extinction events, such as during the Permian–Triassic period (Knoll et al. 1996, 2007) are being discussed as related to specific CO_2 effects. In all of these phenomena temperature is again a crucial factor. Current statements concerning the effects of CO_2 on marine organisms and ecosystems are therefore largely based on experimental studies in the laboratory or in mesocosms. Moreover, experiments at volcanic sites or after experimental release of CO_2 into the deep sea have investigated specific CO_2 effects. Experimental studies that explore the effect of CO_2 at ecosystem level are also few, except for recent studies in mesocosms which focus on primary production and the export of organic material (Riebesell et al. 2007) or on nutrient flux in sediments (Widdicombe & Needham 2007) and on calcification as well as community changes in coral reefs (Jokiel et al. 2008).

The current situation is also characterized by a large uncertainty in assessing the role of ocean hypercapnia and acidification in the context of climate change effects on marine ecosystems. This uncertainty mirrors the insufficient consideration of a mechanistic cause and effect understanding which has also been emphasized in the context of interpreting climate-induced ecosystem change in general (cf. Jensen 2003). The present paper is intended to provide a perspective on the physiological mechanisms involved in effects of

ocean acidification, in the context of rising temperatures and higher frequencies of hypoxia events. Such research may benefit from recent progress in the field of thermal biology, where organismal limitations in response to temperature could recently be identified as being responsible for warming-induced ecosystem level changes in the abundance and well-being of a species (Pörtner & Knust 2007).

PHYSIOLOGICAL PRINCIPLES OF CO_2 VS. TEMPERATURE EFFECTS ON MARINE ANIMALS

Similar to thermal effects (Pörtner 2002), CO_2 effects may extend from the highest level of sensitivity seen in whole organism functioning, down to cellular and molecular levels, reflecting a systemic to molecular hierarchy of tolerance limits. This emphasizes that complex macro-organisms specialize more on environmental parameters and thus respond more sensitively to environmental extremes than unicellular eukaryotes and much more so than prokaryotes (Pörtner 2002).

The integration of molecular and biochemical mechanisms into whole organism functional networks and their performance capacity is thus a crucial element in understanding cause and effect visible at an ecosystem level. This requires knowledge of the molecular and cellular mechanisms of CO_2 effects and their whole organism consequences, and in this context, knowledge of the mechanistic links between CO_2-dependent functional levels from molecule to ecosystem.

As for other environmental factors, unifying principles of CO_2 effects across groups of organisms (e.g. animal phyla, phytoplankton species) need to be distinguished from those possibly specific and typical for certain groups. This applies particularly to the different physiological strategies (e.g. extracellular versus intracellular blood pigments, open versus closed circulatory systems) displayed by various animal phyla. Such physiological studies of CO_2 effects, via development of a cause-and-effect understanding, will support the development and assessment of predictive scenarios of ecosystem changes (Cicerone et al. 2004a,b, Orr et al. 2005, Royal Society 2005, Pörtner & Farrell 2008).

Realistic scenarios also require integrated analyses of effects of CO_2, temperature and oxygen deficiency since all of these factors change concomitantly in the real world and their effects influence each other (Reynaud et al. 2003, Hoegh-Guldberg 2005, Pörtner et al. 2005, Hoegh-Guldberg et al. 2007, Pörtner & Farrell 2008). According to the postulated central role of physiology, the principles of CO_2 effects thus have to be evaluated in the light of interacting temperature (and hypoxia) effects.

Future scenarios of CO_2 effects require consideration that on macro-ecological scales, the distribution of marine fish and invertebrates is strongly defined by temperature gradients (Murawski 1993, Jacob et al. 1998). These observations reflect that complex macro-organisms are specialized for a certain window of bio-climate. They also emphasize the fact that the thermal windows of species in an ecosystem differ despite the fact that they overlap at those temperatures where species coexist. The loss or replacement of a species in a community may therefore relate to the climate-driven change in its geographical distribution since species would follow their preferred thermal niches. Changes in occurrence then become predictable from the temperature regime (Pearson & Dawson 2003). The respective 'climate envelope models' were successfully applied in the terrestrial realm and are currently considered to be the best approach in determining the effects of climate change on biodiversity (Huntley et al. 2004).

In this context, mechanistic knowledge is needed to explain the specialization of organisms on limited and specific thermal windows. Considerable progress has been made in the field of thermal biology, where relevant physiological mechanisms defining thermal windows and linking climate to ecosystem change have been identified (Pörtner 2001, 2002, Pörtner & Knust 2007). The principles involved even lead to explanations of regime shifts, changes in species interaction and food web structure (Pörtner & Farrell 2008). Although it is currently unclear whether windows of CO_2 tolerance exist in similar ways to thermal windows, conventional physiological knowledge has many examples of such specialization. Defence mechanisms against hypo- or hypercapnia effects on acid–base status exist within groups from different CO_2 environments (see previous section). Circumstantial observations indicate higher sensitivity to hypocapnia of fauna living in marine sediments as compared to epibenthic or pelagic fauna. This line of thought is also supported by shifting CO_2 windows during evolution of air breathing ectotherms from water breathers (Ultsch 1987) and furthermore of endotherms from ectotherms.

STRATEGIES FOR PHYSIOLOGICAL RESEARCH

How should one go about studying specific CO_2 effects and then integrate these findings with studies of temperature and hypoxia effects? In physiology, laboratory studies apply defined scenarios of environmental parameters and are used to identify the mechanisms causing changes at molecular to organismic levels of biological organization. For a clear elabora-

tion of effects and mechanisms involved, extreme conditions are applied first, before intermediate values of environmental parameters are tested. For example, this strategy was used to characterize the effects of anoxia and hypoxia effects on marine animals, such as invertebrates dwelling in the intertidal zone (for review see Grieshaber et al. 1994). Although full, long-term anoxia is experienced by few of these facultative anaerobes, anoxia exposure was used to identify the biochemical mechanisms, their capacities and the ATP yield of anaerobic energy production. Consecutive studies then explored the relevance and use of these mechanisms in more moderate and more realistic levels of hypoxia under field conditions.

In the case of CO_2, earlier physiological work used levels of 10 000 ppm and higher in aquatic (including marine) animals as a tool to challenge and investigate the mechanisms of acid–base regulation, as well as their capacity to compensate for acid–base disturbances (e.g. Heisler 1986a,b). In this context, the question arose as to what extent CO_2 is effective as a variable natural factor in various aquatic environments (see above) and whether it has ecologically relevant effects, such as in metabolic depression (e.g. during low tide) (Reipschläger & Pörtner 1996, Burnett 1997, Pörtner et al. 1998). A perspective emerged of how CO_2 oscillations on longer time scales might have been involved in mass extinction events in earth history (Pörtner et al. 2004, 2005, Knoll et al. 2007). These studies also became relevant from an applied point of view, namely as a guideline for assessment of environmental impact of projected ocean storage scenarios, as compiled in the IPCC special report on carbon capture and storage (Caldeira et al. 2006). Such scenarios of ocean disposal involve local effects of CO_2 on marine organisms and ecosystems at levels similar to those used in earlier physiological work. Present knowledge of such effects contributed to the recent banning by OSPAR (Oslo-Paris Commission, www.ospar.org) in 2007 of CO_2 placement strategies in the water column or on the sea bed.

In contrast, scenarios of anthropogenic ocean acidification from atmospheric CO_2 release involve much lower CO_2 levels and, therefore, long term rather than acute effects (cf. Pörtner et al. 2005) (Fig. 1). Nonetheless, for a clear and comprehensive identification of the mechanisms and of the detailed regulatory pathways involved in responding to CO_2, the use of high concentrations is still required, especially given the limited time frame of experimental studies. Consecutively, various CO_2 levels need to be applied including, but also beyond, those expected from CO_2 accumulation scenarios, in order to find out when effects set in and why and to what extent such mechanisms respond to the relatively low concentrations involved. It is also
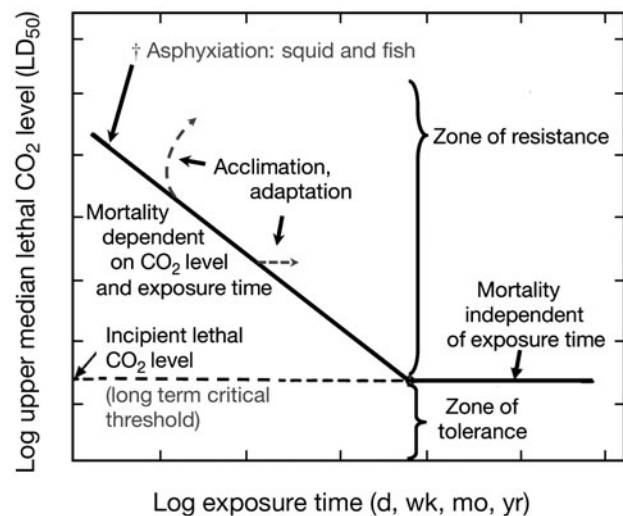

Fig. 1. Mortality in animals corresponding to exposure time and concentration of ambient CO_2 (conceptual considerations, after Pörtner et al. 2005). Priorities among effective mechanisms in causing mortality likely shift between short-term exposure to high concentrations (hampering oxygen supply) versus long-term exposure to low concentrations (hampering growth and reproduction). Acclimation and evolutionary adaptation cause a shift in steepness and position of the sensitivity curve (broken arrows). Sensitivity likely differs between species such that ecosystem shifts may develop progressively rather than suddenly beyond thresholds

important to consider whether such effects occur over short or long time scales and also, whether they can be compensated for during acclimation or adaptation processes.

These considerations put into perspective claims that previous investigations are invalid because they have used high CO_2 levels that are beyond expected scenarios of ocean acidification. This criticism would imply that a completely different picture might develop once effects of 'realistic' values are being studied. From an empirical point of view the exclusive study of expected CO_2 accumulation scenarios appears sufficient, however, the identification of some mechanisms above noise levels will rely on the use of higher concentrations. While some processes such as calcification may well begin to show clear early effects even under low levels, others such as protein synthesis may also be affected, but significant changes may not yet be detectable during limited experimental periods or for methodological reasons (cf. Langenbuch et al. 2006). Since protein synthesis is involved in growth, demonstration of this effect (e.g. Michaelidis et al. 2005) and identification of the mechanisms causing reduced protein synthesis are crucial for an understanding of CO_2 effects. For any mechanism, clear-cut and significant effects should develop on relatively short time scales under a high CO_2 regime.

Mechanisms responsive to low CO_2 levels will also respond to high levels, albeit to different degrees and on different time scales (Pörtner et al. 2005). At present there is no evidence of mechanisms which exclusively respond to low CO_2 levels and thus escape identification in experiments that use these elevated levels. However, mechanisms responding to high levels might not yet do so to low levels, such that fewer mechanisms might be affected by low than high CO_2 levels. Some mechanisms effective during long-term moderate exposures, like reductions in protein synthesis, will also be involved during short-term exposures but the period may be too short for them to become detrimental, even under extreme conditions. Other mechanisms, such as those involved in oxygen supply, respond strongly in this case and thereby take priority (Fig. 2). Apparently different patterns at various CO_2 concentrations may result from a change in the priorities of CO_2 effects. Studies at high levels are thus important for a comprehensive identification of affected

mechanisms and should not be dismissed based on premature paradigms. Conceptually, it is important to study the extreme and then 'titrate' responses and mechanisms at various intermediate levels of physicochemical parameters including the range of expected values.

The scale and magnitude of CO_2 effects depend on both concentration and time scale. Acute effects are usually only observed under very high CO_2 levels. In animals, oxygen supply is affected, e.g. via fast disturbance of blood oxygen transport through oxygen binding proteins as in squid (Pörtner et al. 2004) or via the onset of cardiocirculatory collapse as in fish (Ishimatsu et al. 2005). These processes may be only minimally affected under long-term moderate CO_2 exposures with no significant harm seen under laboratory conditions. Recent insight into thermal effects and their ecological consequences in the field indicates, however, that full performance capacity and aerobic scope is crucial for successful competition and survival in the
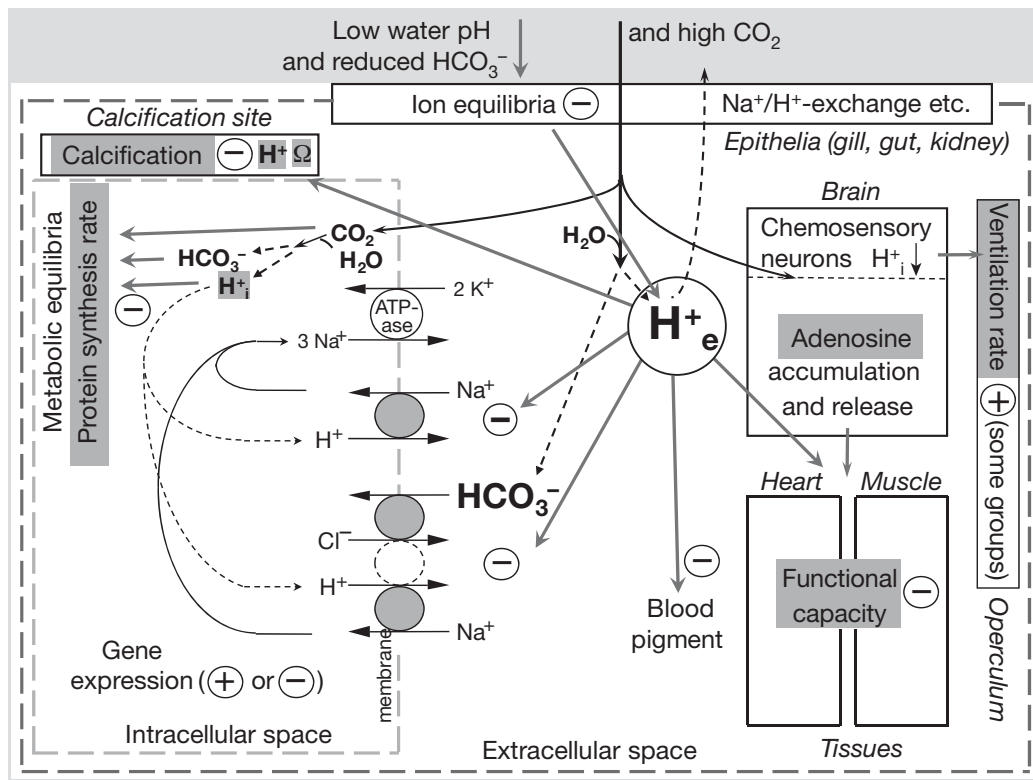

Fig. 2. Overview of processes and mechanisms affected by CO_2 in a generalized water-breathing animal, emphasizing a key role for extracellular pH in defining sensitivity to ocean hypercapnia and acidification (after Pörtner et al. 2005). As with thermal sensitivity, the first line of hypercapnia tolerance is set at the level of functional capacity of whole animals defined e.g. by tissues involved in oxygen supply (cf. Fig. 4). Dark shaded areas indicate processes involved in changing energy budget. Grey arrows indicate signalling through water or body fluid physicochemistry, with a key role for intra- and extracellular H^+ (H^+_i and H^+_e) or other factors like adenosine, K^+, Na^+, or Cl^-. Ω quantifies the saturation of carbonates, e.g. aragonite, where K^*_{sp} is the solubility product

$$\Omega_{aragonite} = \frac{[Ca^{2+}][CO_3^{2-}]}{K^*_{sp,\,aragonite}}$$

field (Pörtner & Knust 2007). Therefore, minor disturbances of oxygen transport pathways may significantly depress performance and affect the capacity of organisms to forage and compete for resources, to reproduce, display various behaviours or just avoid predators (Pörtner & Farrell 2008).

Similar concerns argue for a consideration of time scale in studies of CO_2 effects, especially during mild exposures (Fig. 1). A recent example of this is the study by Gazeau et al. (2007) which focussed on changes in calcification upon acute exposure (2 h) to various CO_2 levels in marine bivalves (mussels *Mytilus edulis* and oysters *Crassostrea edule*). Calcification was progressively reduced with rising CO_2 levels. Assuming the unlikely, namely that no acclimation occurs, the authors projected a decrease in calcification rates by 25 and 10% upon exposure to year 2100 CO_2 accumulation scenarios. A threshold value of 1800 ppm was elaborated for *M. edulis* where shell dissolution would exceed calcification. However, the data from Michaelidis et al. (2005) on *Mytilus galloprovincialis* and those from Berge et al. (2006) on *Mytilus edulis* rather suggest that acclimation sets in within days and supports net (including shell) growth and calcification even beyond that threshold. Studies of acute responses (e.g. Gazeau et al. 2007) thus do not yet provide a realistic picture of how animals respond over weeks or months to various CO_2 levels, and need to be complemented by long-term investigations that allow acclimation to occur.

As a corollary, acclimation is relevant and also affects calcification. If acclimation capabilities are to be evaluated properly, physiological mechanisms need to be identified which mediate the decrease in performance including calcification rates. These mechanisms need to be evaluated in how they vary between species, during acclimation and adaptation, and thereby contribute to the species-specific level of sensitivity on various time scales. In this context, calcification should not be treated as an isolated phenomenon. In other words, the drop in calcification rates is a crucial effect but, except for the different nature of the carbonates (predominantly aragonite in *Mytilus edulis* versus calcite in *Crassostrea edule*), a full mechanistic explanation needs to consider the physiological (within animal) mechanisms and processes setting calcification rates.

UNIFYING MECHANISMS OF CO_2 EFFECTS

Current literature emphasizes the sensitivity of calcifiers to ocean acidification (e.g. Royal Society 2005), but this view may not be sufficient for understanding ecosystem effects. Calcification plays a role in the stabilization of body form and function and in the protection against predators or, in the case of corals, in the

building of a reef as a specific habitat. Some forms such as corals and phytoplankton can exist (for extended periods) without their calcareous shell (Fine & Tchernov 2007), whereas others such as echinoderms cannot as their skeletons support organismal functioning. The question is whether effects on calcification are currently considered very crucial only because effects on calcified exoskeletons are so very obvious. Is calcification really a key bottleneck or simply one among several physiological processes concomitantly affected in sensitive organisms? This section builds on the view that such physiological processes are usually closely coordinated and that, in the case of a calcifier, the control of calcification is integrated into the control of other processes equally relevant for survival, such as growth, neural functioning, and regulation of body fluid pH and intracellular pH in various tissues. However, knowledge of the mechanisms regulating calcification is limited. Moreover, it is not clear whether the responses of calcifiers and non-calcifiers are shaped via similar mechanisms. Such knowledge is needed to answer this question and is critical for a comparative assessment of sensitivities. Previous studies using relatively high CO_2 levels in fact provide physiological background information which indicates that unifying principles define sensitivity to CO_2 in both calcifying and non-calcifying animals.

The carbonate concentration and saturation levels of calcium carbonates in seawater are widely reported to set calcification rates. Calcification, however, rarely occurs at surfaces exposed to sea water. Rather, it occurs in relatively isolated compartments where ion transport across various epithelia establishes an environment suitable for calcification. Therefore, the perspective that water carbonate saturation directly sets calcification rates would be too simplistic physiologically. The influence of aquatic physicochemistry is important but often indirect, via effects on calcium and proton equivalent ion transport through the outermost barriers (e.g. gill or equivalent epithelia). These mechanisms do not usually transport carbonate, but rather bicarbonate; calcium channels and proton pumps may also be involved (Carre et al. 2006). Carbonate precipitated in calcified structures is therefore not directly originating from water carbonate, but generated or modulated via several reactions from imported bicarbonate and/or CO_2 trapped in the alkaline compartment at calcification sites. Water carbonate levels (CO_3^{2-}) and calcium carbonate saturation levels thus are useful proxies but usually not direct drivers of calcification. These proxies also mirror the effects on ion transport mechanisms of associated water parameters, such as pH, calcium or bicarbonate levels and thereby influence the setting of more direct effectors of calcification which comprise a range of physiological para-

meters inside the organism and compartments involved. Although not directly effective at the calcification site either (Fig. 2), extracellular body fluid including blood or haemolymph in animals is the first compartment affected by water physicochemistry. The extracellular acid–base status, as reflected in extracellular pH, responds in a species-specific way and acts as a mediator of the effects of water physicochemistry on calcification in most animals.

It is important to note that intracorporeal acid–base status not only comprises adjustments in compartmental pH values. pH compensation occurs through the accumulation of bicarbonate in mostly extracellular, but also intracellular compartments. Extracellular bicarbonate accumulation will support compensation of intracellular acidosis through transmembrane ion exchange (Pörtner et al. 1998). Bicarbonate accumulation will lead to higher saturation levels of the calcium carbonates, quantified by Ω (Fig. 2). At calcification sites, this may even lead to a counter-intuitive improvement of conditions for calcification under hypercapnia. Examples exist where such upregulation of calcification is visible in marine invertebrates (e.g. cephalopod *Sepia officinalis*, Gutowska et al. 2008, this Theme Section [TS]; infaunal ophiurids, Wood et al. 2008) and even in marine phytoplankton (Iglesias-Rodriguez et al. 2008). In the case of ophiurids, improved calcification came at the cost of muscle wastage, indicating a disturbance of energy budget not visible in the cuttlefish. We require quantification of the levels of intracorporeal physicochemistry to be maintained by ion and acid–base regulation for adequate calcification and for adequate coordination of calcification with whole body systemic functioning.

Extracellular acid–base status thus not only modulates calcification rates but also influences other physiological processes. The comparison of non-calcifying with calcifying marine invertebrates in fact supports the view that extracellular acid–base status and especially extracellular pH (pH_e) may be a unifying parameter which is operative in both calcifiers and non-calcifiers to set CO_2 sensitivity. Work on a non-calcifying worm, *Sipunculus nudus*, has provided the most comprehensive data set on physiological effects under hypercapnia to date. Key effects include metabolic depression and associated patterns of transepithelial acid–base regulation (Pörtner et al. 1998), reduced rates of tissue acid–base regulation (Pörtner et al. 2000), reduced rates of protein synthesis (Langenbuch et al. 2006) and enhanced levels of adenosine in nervous tissue and associated depression of behaviours (Reipschläger et al. 1997). These responses were associated with hypercapnia-induced acidosis which initially developed in both extra- and intracellular fluid compartments (of muscle tissue) but over time,

resulted in incompletely compensated extracellular but fully compensated intracellular acidosis (Pörtner et al. 1998). More detailed study has identified extracellular pH as a key variable mediating metabolic depression (Reipschläger & Pörtner 1996) through reduced rates of ion exchange (Pörtner et al. 2000), at maintained rates of ammonia excretion (Pörtner et al. 1998). Modified amino-acid metabolism or reduced rates of protein synthesis are mediated via modified intracellular acid–base variables, especially under conditions of severe extracellular acidosis (Langenbuch & Pörtner 2002, Langenbuch et al. 2006). Maintenance of extracellular pH thus appears as the first line of defence against hypercapnia induced disturbances of metabolic and tissue functioning as well as of behavioral performance. The key role of extracellular pH is emphasized by the fact that a lowering of pH_e is similarly effective in metabolic depression regardless of hypercapnic or normocapnic conditions (Reipschläger & Pörtner 1996).

In mussels *Mytilus galloprovincialis*, a study by Michaelidis et al. (2005) used elevated CO_2 levels to set water pH to 7.3, close to the maximum degree of acidification expected during realistic emission scenarios (Caldeira & Wickett 2003). Despite lower levels of ambient pCO_2, compensation of the extracellular acidosis occurred but was even less than in *Sipunculus nudus*. Under these conditions shell growth was largely reduced, in line with the finding of depressed calcification in *M. edulis* (Gazeau et al. 2007). Most importantly, the reductions of shell and soft body growth were found closely coordinated in *M. galloprovincialis*, indicating a common mechanism modulating the rate of both processes including the rate of calcification. Moreover, the metabolic effects of hypercapnia were the same in *S. nudus* and *M. galloprovincialis*. In line with phenomena seen in the sipunculid worm, Michaelidis et al. (2005) reported a decrease in metabolic rate, associated with a rise in ammonia excretion during partially compensated extracellular acidosis. These findings strongly suggest that as in *S. nudus*, the lowered extracellular pH in mussels is key to the observed metabolic depression. It is also very likely that the low capacity of sipunculids and bivalves to compensate for disturbances in extracellular pH explains the reduction in growth and calcification.

Low capacity of acid–base regulation through proton equivalent ion exchange may be a general pattern explaining the elevated sensitivity of lower marine invertebrates and their life stages to CO_2 (Pörtner et al. 2004, 2005, Shirayama & Thornton 2005, Dupont et al. 2008, this TS). The reduced capacity of lower marine invertebrates to regulate extracellular acid–base status becomes explainable in the light of their hypometabolic mode of life. Acid–base regulation bears a signif-

icant cost (Pörtner et al. 2000) which can be reduced at the expense of capacity and of the baseline idling of ion-exchange mechanisms. At the same time these organisms need to modulate the acid–base status of large volumes of extracellular fluid in open circulatory systems (more than 50% in the sipunculid). A larger degree of acidification upon acute CO_2 exposure is facilitated by much lower non-bicarbonate buffer values than found in vertebrate blood. As a consequence, sensitivity is enhanced as reduced capacity meets the requirement to adjust pH in large fluid compartments. Low capacity also means that the setpoint of extracellular pH even fluctuates passively depending on water physicochemistry as seen in *Sipunculus nudus* in response to fluctuating water bicarbonate levels (Fig. 3). Comparative work emphasizes that acid–base regulation capacity in relation to the rate of energy turnover is not only dependent on phylogeny but is also influenced by mode of life and habitat. For example, reduced capacity to regulate extracellular pH was recently found in deep-sea versus shallow-water crustaceans (Pane & Barry 2007, see also Spicer et al. 2007) where the slow and hypometabolic mode of life in deep-sea species is reflected in a reduced rate (and thus cost) for acid–base regulation.

Contrasting these data with findings in teleost fish supports the existence of a common mechanism of CO_2 sensitivity in marine water-breathing animals. Teleost fish *in vivo* do not display similar patterns of acid–base compensation as the invertebrates (Heisler 1986b, Larsen et al. 1997, Ishimatsu et al. 2004). The extracellular acidosis is rapidly and more or less fully compensated, and there is no metabolic depression at moderate CO_2 levels around 1%. Transient metabolic stimulation may even occur instead, as seen in Antarctic eelpout

Fig. 3. Body fluid extracellular pH (pH_e) in a lower marine invertebrate, the sipunculid *Sipunculus nudus*, depending on water bicarbonate levels ($[HCO_3^-]_w$) at constant levels of gaseous CO_2 (author's unpubl. data). The data emphasize a rapid response to changing water physicochemistry and its relevance for extracellular acid–base equilibria and associated physiological processes (see Fig. 2)

(G. Lannig pers. comm.). However, similarities between fish and marine invertebrate responses do exist. Metabolic depression can occur in fish and has been observed in European eels at CO_2 levels above 2% (Cruz-Neto & Steffensen 1997). Moreover, when isolated hepatocytes of Antarctic eelpout were investigated during exposure to respiratory and non-respiratory extracellular acidosis (Langenbuch & Pörtner 2003), they displayed metabolic phenomena strikingly similar to those observed in invertebrate tissues and whole animals. In fish, these cellular responses are alleviated at the whole-animal level due to the large capacity of the intact organism to more or less fully compensate for the acid–base disturbance in relatively high levels of hypercapnia. This line of evidence supports the conclusion that while cellular responses may be similar, whole-animal responses, and thus, resulting sensitivities, are largely different in the (lower) marine invertebrates and in fish due to different capacities to compensate for an extracellular acidosis. Nonetheless, the sensitivity of tissues to extracellular acid–base disturbances may also be modulated and vary among species.

These considerations confirm that the capacity of these organisms to maintain extracellular pH under various CO_2 conditions is crucial in mediating or alleviating hypercapnia effects (Fig. 2). Both acute and long term CO_2 sensitivity are likely highest in those lower marine invertebrates with a poor capacity to compensate for deviations from control extracellular pH which then affects systemic processes such as calcification as well as cellular processes like those involved in growth. According to mode of life and energy turnover, the most heavily calcified groups such as articulates, echinoderms (cf. Miles et al. 2007), bryozoans and cnidarians may be among those with the poorest capacity to regulate acid–base status. These were also those most severely affected during the Permian–Triassic mass extinction events (Knoll et al. 1996, 2007, Pörtner et al. 2004, 2005). In contrast, sensitivity is lowest in fish with a high capacity for extracellular pH compensation. Further study of these various groups is needed to further support this hypothesis. Such a hypothesis also needs testing in the light of possibly differential capacities of various groups to acclimate long term to ocean hypercapnia. While current data emphasize steady state in acid–base status reached within hours to days after an initial CO_2 disturbance, this steady-state value may well shift progressively during a long term acclimation process. Such long term analyses are not yet available and should help to elucidate the capacity to acclimate or adapt to ocean acidification scenarios. Long-term adjustments (within weeks) occur in the gene expression of ion exchangers contributing to acid–base regulation in teleost gills
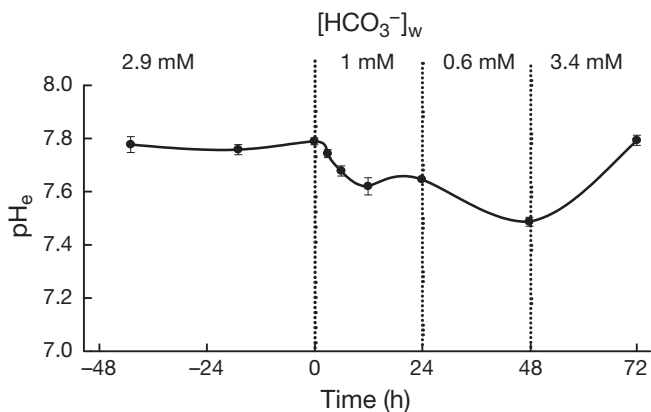
(Deigweiher et al. 2008) and indicate significant acclimation capacity to long term hypercapnia in fish.

Overall, while current emphasis is on the sensitivity of calcifiers to ocean hypercapnia, they are likely sensitive not because they are calcifiers but because at the same time, they are sessile, hypometabolic organisms that display a poor capacity to regulate their systemic acid–base status and, mainly, extracellular pH.

METHODS CRITIQUE FOR STUDIES OF CALCIFICATION AND ACID–BASE REGULATION

As outlined above, the available data indicate that acid–base status and the capacity to regulate and compensate for acid–base disturbances are crucially important in setting sensitivity to ocean hypercapnia. As a consequence, studies of calcification or other processes affected by ocean acidification need to investigate the organism in steady state with respect to internal parameters like extracellular pH which modulate those rates. Studies of calcification that do not consider steady-state acid–base regulation will not support long term predictions of calcification rates. On long time scales, over periods of weeks or months, acclimation or adaptation may shift the mechanisms and setpoints (steady-state values) of acid–base regulation and may thereby compensate for the CO_2-induced acid–base disturbance and its effect on physiological processes, including calcification.

In this context, physiological (including biomedical) sciences and oceanography have both met the challenge to precisely quantify relevant physicochemical parameters defining acid–base status of body fluids and ocean water. Due to the parallel and independent evolution of these fields, they have developed comparable but different strategies to do so. It is beyond the scope of this opinion paper to review the respective methodologies. From a physiological point of view it is crucial to analyse acid–base parameters in water and body fluids by use of the same techniques, for reliable estimates of effective acid–base parameters within and outside the body and for analyses of associated ion gradients across epithelia. In the fields of medical and comparative physiology this has traditionally been done by use of glass electrodes for analyses of pH and, after adequate modification, of pCO_2 (Egginton et al. 1999). Quantification of proton equivalent ion exchange has been carried out through assays of titratable alkalinity in water or urine, through continuous pH recordings in water (glass electrodes) or analyses of total CO_2 in water and body fluids. Continuous monitoring of intracellular pH is possible by use of ^{31}P-NMR (nuclear magnetic resonance), whereas a set of homogenate techniques reliably quantifies acid–

base parameters in tissues (Pörtner 1990, Pörtner et al. 1990).

Calcification rates are frequently analysed from changes in water acid–base status through the alkalinity anomaly technique (Smith & Key 1975, Gazeau et al. 2007). The consideration of interfering metabolic and acid–base regulation processes casts some doubt on the absolute rates determined. Metabolism and the associated net rates of proton or base production influence water alkalinity and may have to be taken into account. Protein metabolism causes net proton release and thus a potential overestimation of calcification rates. Under those circumstances, and with the methods used, any CO_2 or pH effects on metabolism (Pörtner 1995) including the consecutive proton-equivalent ion exchange between animals and water may thus mimic changes in calcification.

PERSPECTIVES: INTEGRATING THERMAL, HYPOXIA AND HYPERCAPNIA RESPONSES

Ocean acidification occurs in concert with ocean warming and an increased frequency of hypoxia events. Recent work demonstrated that knowing the thermal window of performance of a species is crucial in defining sensitivity to the warming trend (Pörtner & Knust 2007). Future studies need to address effects of ocean hypercapnia and acidification within and beyond the limits of the baseline thermal window of a species, considering its capacity to thermally acclimate or adapt. The focus should be on measures of performance, metabolism and calcification in animals that have reached new acid–base equilibria during longer term exposures. Sensitivities to temperature and CO_2 integrate in such a way that elevated CO_2 levels enhance the sensitivity of organisms to thermal extremes. This occurs through reductions in tissue functional capacities including those involved in oxygen supply (Pörtner et al. 2005, Metzger et al. 2007). Considering the mechanisms affected by CO_2 (Fig. 2) it appears that a shift of acid–base status, including a shift of extracellular pH, likely reduces the functional capacity of affected mechanisms and of the whole organism in due course. As a result, pO_2 levels in the body fluids fall and, upon warming, reach limiting levels earlier than during normocapnia (Fig. 4). A narrowing of thermal windows results and the effect observed suggests a large sensitivity of the width of thermal windows to CO_2. Such effects would be corroborated by increasing hypoxia events in the oceans. Conversely, if elevated CO_2 levels or hypoxia cause a narrowing of thermal windows, this also means that exposure to thermal extremes will enhance sensitivity to elevated CO_2 levels or hypoxia.
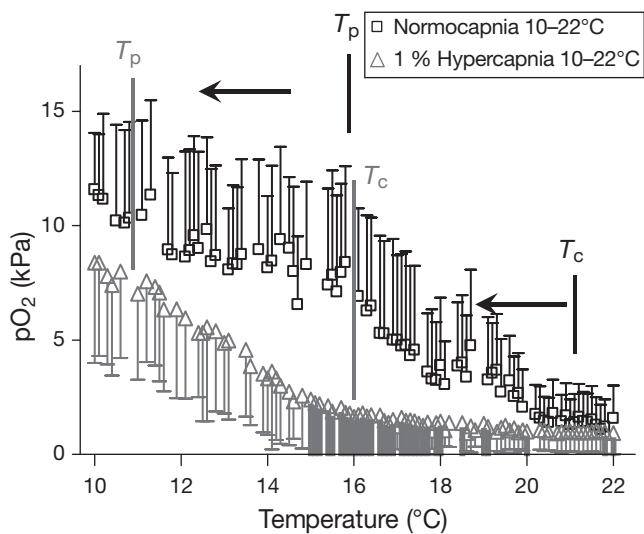
Fig. 4. Heat tolerance of the edible crab *Cancer pagurus* under normo- and hypercapnia (after Metzger et al. 2007). Discontinuities in the curve depicting arterial oxygen tensions (pO$_2$) under normocapnia were identified as indicators of thermal limits (upper pejus temperature, T_p, according to Frederich & Pörtner 2000) reflecting onset of a loss in ecologically relevant performance and fitness (Pörtner & Knust 2007). Highly elevated CO$_2$ levels (1% hypercapnia) cause heat tolerance to decrease dramatically by about 5°C. Similarly, the general lowering of haemolymph pO$_2$ under hypercapnia causes a downward shift of upper critical temperatures (T_c) by about 4.5°C. Assuming a symmetric thermal window the data reflect a high sensitivity to CO$_2$ and shrinkage of the thermal window by more than 80%. Temperature-dependent biogeographical ranges of marine animals may thus respond to even moderately elevated CO$_2$ levels (Pörtner et al. 2005)

This paper presents a set of hypotheses for a comprehensive mechanistic framework which brings the individual effects of the factors temperature, CO$_2$ and hypoxia together into an integrative picture of climate sensitivity at organismal level (Fig. 5). The mechanistic scheme illustrates how virtually all mechanisms relevant in setting and shifting thermal windows will be affected through the exacerbation of hypoxemia (hypoxia in body fluids) under the effects of ambient hypercapnia or hypoxia. Both factors cause a decreased pH regulation capacity and setpoint of acid–base regulation, and will likely do so to the largest extent where temperature extremes are already causing hypoxemia. Thermal windows and sensitivities differ between species co-existing in the same ecosystem. Through differences in sensitivities, some of these effects will cause changes in species interactions and thereby functional shifts observed in ecosystem level processes.

Comparable to thermal limitation (Pörtner 2002), efforts to understand sensitivity of marine animals to

CO$_2$ should include studies at a high organisational level, especially with respect to the intact organism and the mechanisms involved. This includes studying the patterns of acid–base regulation and hypoxemia as well as the capacity to regulate extracellular acid–base status and mainly extracellular pH, at extreme temperatures for an analysis of the background of temperature-dependent CO$_2$ or hypoxia sensitivity and, vice versa, CO$_2$- and oxygen-dependent thermal sensitivity.

While larval and juvenile stages may be more sensitive when effects of hypercapnia are studied in isolation (Ishimatsu et al. 2004, 2005) these relationships may become more complicated when temperature effects are considered. The temperature signal is currently the strongest signal eliciting ecosystem change, due to physiological impacts and the limited thermal windows of individual species (e.g. Pörtner & Knust 2007). The available data indicate that (1) thermal extremes affect large individuals first and (2) a thermally variable environment favours species with smaller individuals including juveniles, due to their wider windows of thermal tolerance (e.g. Pörtner et al. 2008). If CO$_2$ exacerbates these relationships by narrowing thermal windows this would favour smaller body sizes (and their wider thermal windows) even more and further constrain the size range of a species. Constant CO$_2$ conditions may thus favour larger body sizes. The synergistic interactions between temperature and CO$_2$ thus have implications for how the sensitivity of a species to global change depends on body size (allometry). While sensitivity to CO$_2$ per se may be highest in early life stages of many organisms, thermal stress also impacts the largest individuals of a species. With their already constrained thermal windows, they may then also become more sensitive to the synergistic effects of CO$_2$. Once again, the regulation of extracellular acid–base status may be crucial in this context as efficient pH regulation and its temperature-dependent characteristics are limited to within the thermal window of a species (e.g. Sommer et al. 1997).

As a general conclusion, these relationships and their implications at an ecosystem level need to be investigated with a wide range of organisms from various habitats. With the currently available data it is unclear whether these relationships have already started to affect species and ecosystems, for example through a narrowing of biogeographical distribution ranges. It appears most likely that such integrative effects will be the first to be observed in the field and bring with them the need to then disentangle the contribution of CO$_2$, hypoxia and temperature as well as their synergistic interaction in causing those effects.
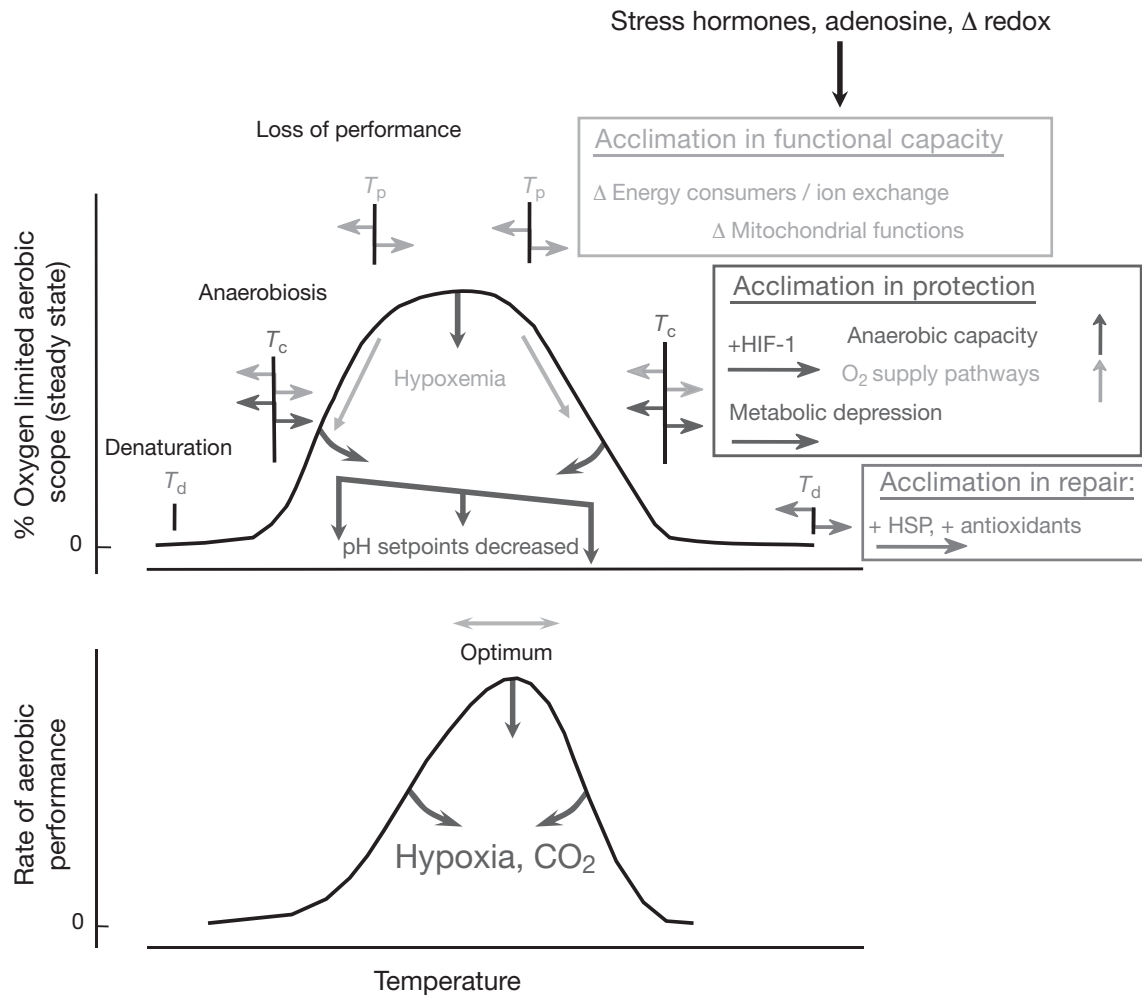
Fig. 5. Conceptual model of how ocean acidification, hypoxia and temperature extremes interact mechanistically, based on the oxygen and capacity limitation concept of thermal windows. The model indicates the hierarchies (upper panel) of functional limitation (beyond pejus temperatures, T_p), hypoxemia, anaerobic metabolism and protection through metabolic depression (below and beyond critical temperatures, T_c) and denaturation as well as repair (beyond denaturation temperatures, T_d). Optimized oxygen supply to tissues between low and high pejus temperatures (upper panel), combined with the kinetic stimulation of performance rates by warming, supports a performance optimum (i.e. an optimum of aerobic scope) close to upper pejus temperature (lower panel). Systemic (e.g. stress hormones, adenosine) and cellular signals (e.g. hypoxia inducible factor HIF-1α, and redox status) associated with temperature-induced hypoxemia contribute to the acclimation response (horizontal arrows in upper panel), which leads to a shift in thermal tolerance limits and thus windows. Ambient hypoxia and elevated CO_2 levels both cause lower performance optima and a narrowing of thermal windows (arrows attached to curves in both panels), through lower systemic oxygen tensions and shifted setpoints of acid–base regulation including the deviation of pH from the typical linear temperature-dependent decline (pH scale not shown) (upper panel). Details of the signalling pathways involved are not depicted. Modified from Pörtner & Knust (2007)

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