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THEME SECTION

# Effects of ocean acidification on marine ecosystems

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CONTENTS





# Introduction

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Although the potential for increased atmospheric $CO_2$ concentrations to affect ocean pH and marine calcification rates has been known for decades, the issue came to the fore following the Ocean in a High $CO_2$ World symposium (Orr et al. 2005a). Ocean acidification has recently been the subject of several high-profile publications (Caldeira & Wickett 2003, Orr et al. 2005b), comprehensive priority-setting assessments (Royal Society 2005, Kleypas et al. 2006), and numerous articles in the mass media. Despite the serious implications of ocean acidification for marine ecosystems, thorough scientific investigation of this problem is only just beginning.

It is accepted that average global ocean pH has declined over the 20th century and will continue to do so within the near future (Caldeira & Wickett 2005). It is also generally accepted that the pH in the global

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ocean has already fallen by 0.1 units and is likely to fall a further 0.3 units by 2050 and 0.5 units by 2100 (Caldeira & Wickett 2005). These predictions are relatively certain, in part because the geological feedbacks that could affect the decline in pH are too slow to have any real effect on a century timescale. The potential effects of this decline in pH, however, on marine organisms and ecosystems are poorly understood. We felt that it was worthwhile at this early stage to assemble articles that critically evaluate the current state of knowledge on this topic and make constructive suggestions for future research.

Past work on the biological effects of change in ocean pH has focused on acute exposure, rather than on slow and continuous decline in pH levels such as those expected under global climate change. Without an understanding of how such a slow and continuous decline in pH is likely to affect ocean ecosystems, we may miss important aspects of this global ocean pH change. To compound this uncertainty, recent research (Iglesias-Rodriguez et al. 2008, Gutowska et al. 2008, this Theme Section [TS]) reveals counter-intuitive, positive/neutral effects of acidification on some organisms and processes. These studies highlight a near universal issue arising in studies of broad environmental problems: that is, the diversity and complexity of responses by organisms make it difficult to form general predictions.

Faced with this complexity, the first article in this TS proposes a bold program which focuses on unraveling the fundamental physiological processes that underpin the diversity of observed responses (Pörtner 2008, this TS). The proposal for a focus on physiology will not necessarily meet with universal agreement; nevertheless, a deeper understanding of ocean acidification at a physiological level is essential for progress in understanding impacts that extend beyond the effects on calcification. It is also stimulating to reflect on potential unifying principles that may underlie organisms' responses to temperature, $CO_2$ and oxygen, and thus affect community structure. This approach already leads to the inference that higher invertebrates and other organisms with high metabolism and well-developed acid/base regulation may withstand acidification better than the lower invertebrates (see Ishimatsu et al. 2008, this TS, Gutowska et al. 2008).

Using functional genomics is another way to derive an increased mechanistic understanding of responses to acidification. This in turn can lead to more general understandings as outlined by Hofmann et al. (2008, this TS). Although the focus of their paper is on laboratory studies of biomineralization, the approach could be applied to other potential physiological responses and could lead to diagnostic tools that can be used in the field (DeLong & Karl 2005).

Rost et al. (2008, this TS) review the methodologies that have been used to date to investigate effects of pH on phytoplankton. They report that differences in experimental design and methods may underlie the sometimes contradictory results. Importantly, these authors provide a framework for future experimental studies that may help eliminate these problems. Shifting from the laboratory to the field, Balch & Fabry (2008, this TS) review current approaches to estimate changes in pelagic calcification *in situ* and propose a program to quantify the effects of acidification on calcification on the global scale.

Most of the research to date on the effects of ocean acidification has focused on calcifying organisms, in particular structure-forming organisms such as corals. The rise of $CO_2$ in ocean waters leads to more corrosive conditions for calcifying organisms, making it more difficult for them to build and maintain their carbonate skeletons. Also, the threatened status and ecological importance of coral reefs inevitably brings attention to their responses to acidification. It is widely recognized that the saturation state of carbonates has a major influence on calcification at species and community levels (Kleypas & Langdon 2006). Atkinson & Cuet (2008, this TS), however, point out a number of biological and ecological factors that can influence this relationship and propose a research program to address the uncertainties. Lough (2008, this TS) discusses the recent shift from growth-based indicators towards geochemical indicators of coral response to environmental conditions, and makes the point that growth records remain a rich source of information and should not be forgotten in the continuing investigation of coral response to acidification and temperature changes. Andersson et al. (2008, this TS) combine a review of extant knowledge and model calculations to predict faster than expected changes in community structure, particularly at high latitudes, linked mainly to the differences in solubility among different forms of carbonate skeletons. These papers together illustrate that much remains to be done, even in the best-studied part of the acidification puzzle.

Comparatively little attention has been devoted to the impact of acidification on other ecosystem components and processes. A critical question here is the potential effect of acidification on early life stages of marine invertebrates. These larval and juvenile stages may be particularly sensitive, in part because they form their internal skeletons out of amorphous calcite which is more soluble than other forms of carbonate. Kurihara (2008, this TS) reviews the current state of knowledge on the effects of acidification on the reproduction and early life stages of marine invertebrates, to reveal just how little we know about this crucial issue, and to sketch a way forward. Dupont et al. (2008,

this TS) offer disquieting evidence that populations of a major keystone species of the North Atlantic may be severely disrupted through the effects of probable future acidification levels on its larval stages. The impact of ocean acidification on marine fish is reviewed by Ishimatsu et al. (2008) who identify the scarcity of studies using realistic pH levels under conditions of prolonged exposure, and urge new research along these lines. The TS closes with a paper by Gutowska et al. (2008) which reports counter-intuitive responses of a cephalopod species to very high $CO_2$ levels, neatly illustrating the deep uncertainties within this major environmental issue.

This TS covers a broad range of issues, approaches and taxonomic groups, but there were certainly areas we were not able to cover. Many authors discuss the potential for genetic adaptation to rapid ocean acidification, and this remains a topic of great importance; however, little progress has been made in this path of research. There are few analyses based on evolutionary thinking (although the study of Collins & Bell 2004 is often cited). Another gap is the integration of the information into models that can help us apprehend higher levels (community, ecosystem) responses to acidification. There is still uncertainty as to what types of models and modeling studies are needed to integrate extant knowledge and extrapolate possible future states of the ecosystem; whether it is just a question of adding incrementally to the existing ecological-biogeochemical models used extensively for global change research (Hood et al. 2006), or whether we need new approaches or different model structures. Interestingly, these are also gaps that were identified in the recent reports to the Royal Society and US funding agencies (Royal Society 2005, Kleypas et al. 2006). Hopefully, the next reviews and syntheses of this rapidly evolving field will include more work in these critical areas.

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# 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 $CO_2$ uptake 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 $H_2CO_3$, 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 bioclimate. 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 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.

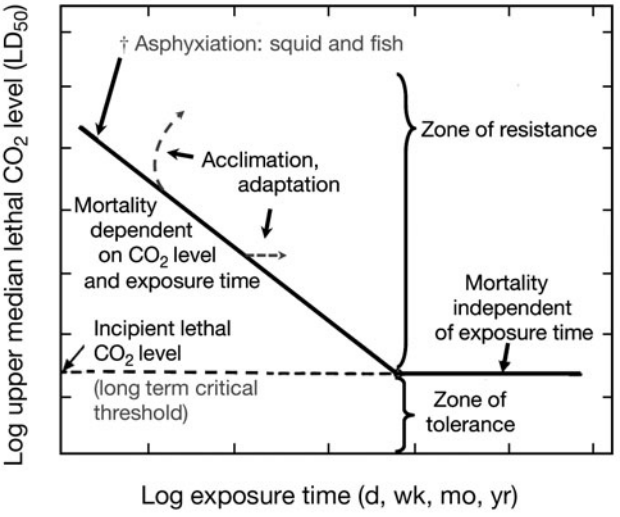


Fig. 1. Mortality in animals corresponding to exposure time and concentration of ambient $CO_2$ (conceptual considerations, after Pörtner et al. 2005). Priorities among effective mechanisms in causing mortality likely shift between short-term exposure to high concentrations (hampering oxygen supply) 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

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

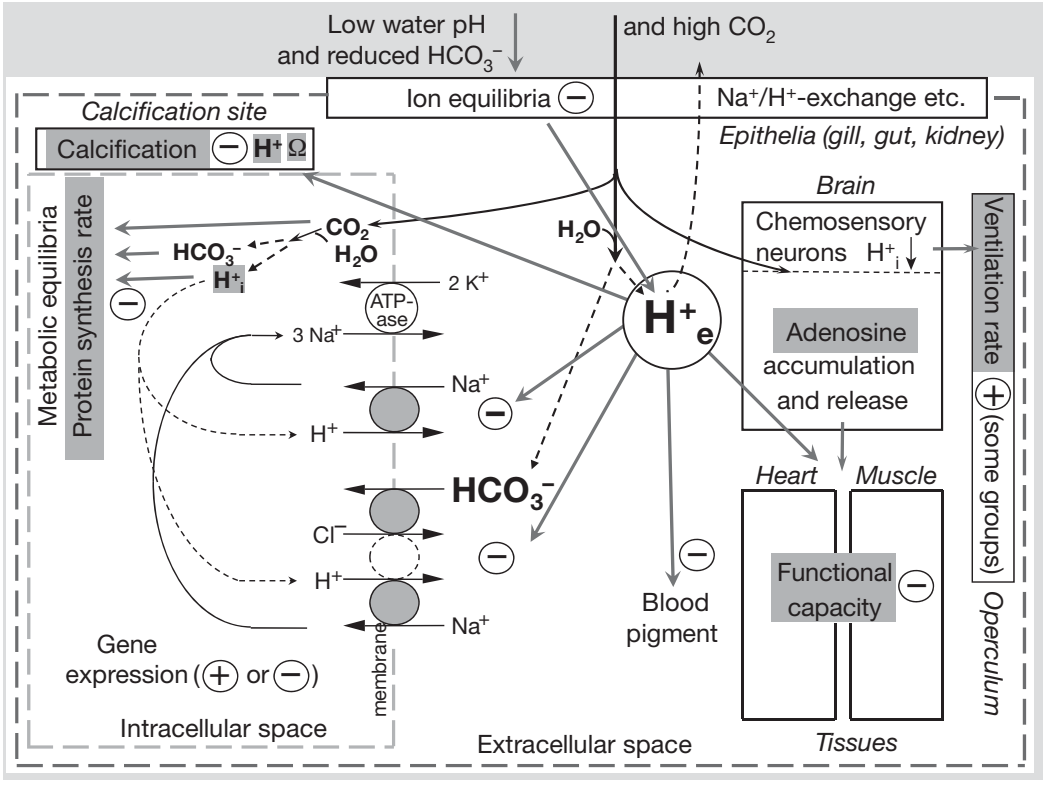


Fig. 2. Overview of processes and mechanisms affected by $CO_2$ in a generalized water-breathing animal, emphasizing a key role for extracellular pH in defining sensitivity to ocean hypercapnia and acidification (after Pörtner et al. 2005). As with thermal sensitivity, the first line of hypercapnia tolerance 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 $K^\star_{sp}$ is the solubility product

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

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 $\Omega$ (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

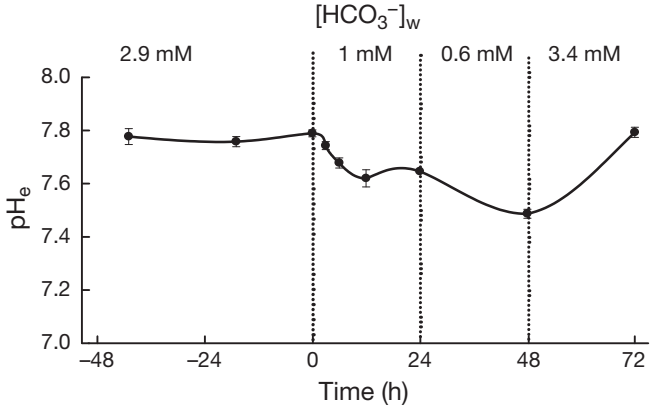


Fig. 3. Body fluid extracellular pH ($pH_e$) in a lower marine invertebrate, the sipunculid *Sipunculus nudus*, depending on water bicarbonate levels ($[HCO_3^-]_w$) at constant levels of gaseous $CO_2$ (author's unpubl. data). The data emphasize a rapid response to changing water physicochemistry and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

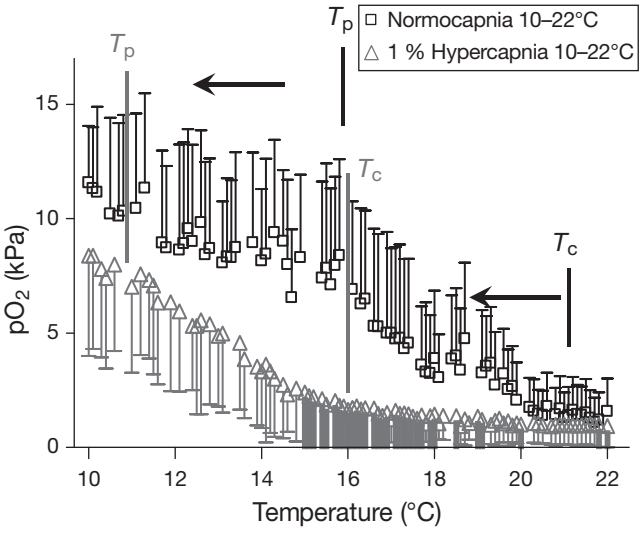


Fig. 4. Heat tolerance of the edible crab *Cancer pagurus* under normo- and hypercapnia (after Metzger et al. 2007). Discontinuities in the curve depicting arterial oxygen tensions ($pO_2$) under normocapnia were identified as indicators of thermal limits (upper pejus temperature, $T_p$, according to 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.

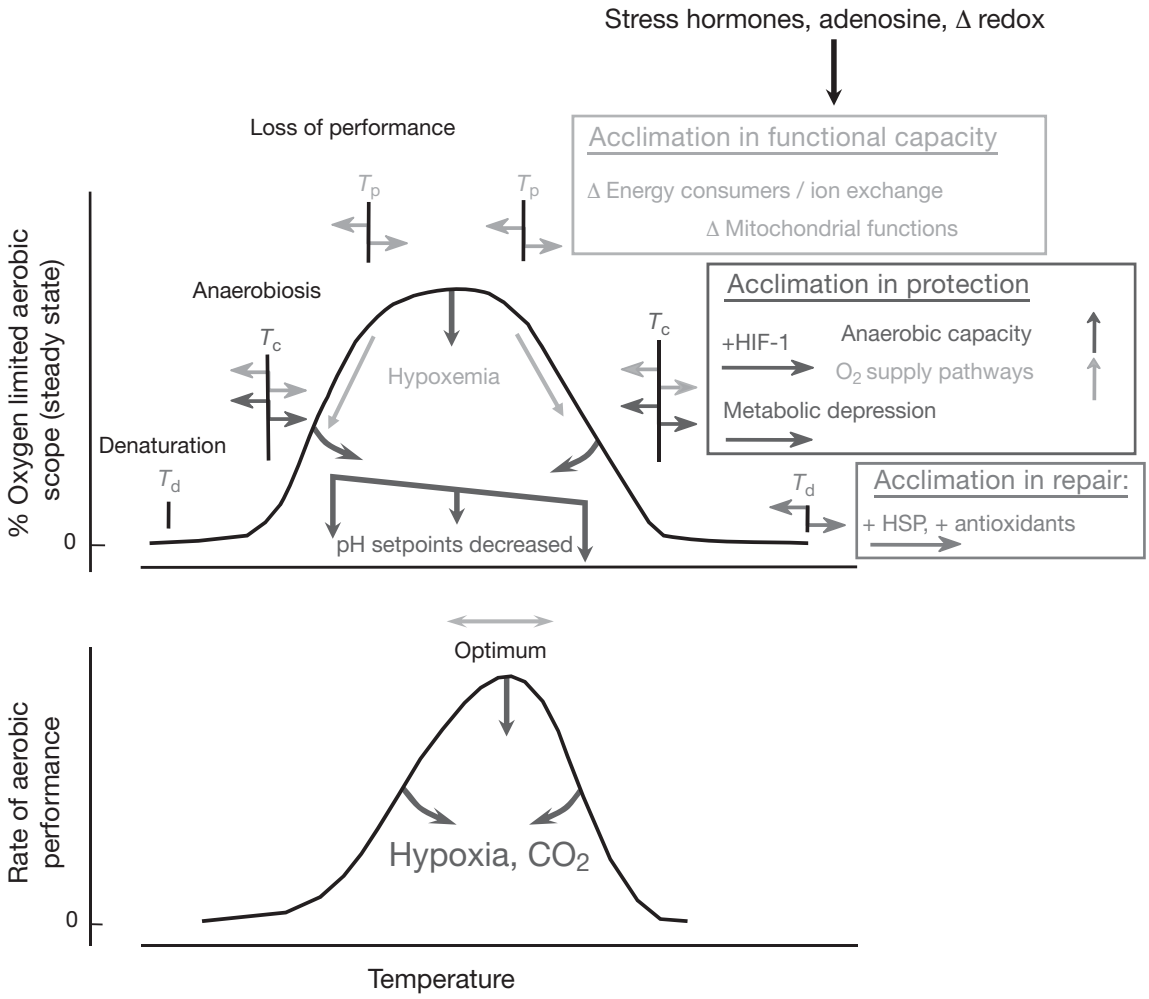


Fig. 5. Conceptual model of how ocean acidification, hypoxia and temperature extremes interact mechanistically, based on the oxygen and capacity limitation concept of thermal windows. The model indicates the hierarchies (upper panel) of functional limitation (beyond pejus temperatures, $T_p$), hypoxemia, 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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# Using functional genomics to explore the effects of ocean acidification on calcifying marine organisms

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ABSTRACT: As the research community attempts to forecast the effects of ocean acidification on marine ecosystems, a critical element is a clear understanding of the effects of ocean acidification on an individual organism's physiology. This article explores how the use of genomics-based tools that measure gene expression — DNA microarrays and quantitative PCR — can assist in this effort and reveal aspects of how calcifying marine organisms will respond to ocean acidification. More specifically, what stands to be gained from this approach is an understanding of the direct effects of ocean acidification and whether organisms have sufficient physiological plasticity to adapt to the altered $CO_2$ conditions. We provide a brief overview of biomineralization processes in corals and sea urchin larvae, and then link these pathways to ways in which gene expression analysis can reveal physiological responses and mechanisms, and further, can define new testable hypotheses. In addition, we review the resources available and strategies that might be taken for each of 2 study organisms, stony corals and sea urchins. Finally, we suggest strategies for gene expression profiling in organisms that differ in availability of genomic resources.

KEY WORDS: Ocean acidification · Biomineralization · Microarrays · Calcification · Coral · Gene expression · Genomics · Sea urchin



## INTRODUCTION

As the research community explores the effects of ocean acidification on marine ecosystems (Royal Society 2005, Kleypas et al. 2006), a key link to forecasting the effects of this altered seawater chemistry is understanding the response at the organismal level. A potentially productive path for the ocean acidification research community is to leverage genomics tools (Box 1) to understand the mechanisms that might be driving altered skeleton formation in marine calcifying organisms, and in addition, to reveal whether potential compensation in the key pathways for biomineralization and other processes is possible.

Genomics approaches have been solidly integrated into the general field of ecology. Notably, transcriptomics — the measurement of all mRNAs in a biological sample, usually performed with a microarray — has recently emerged in marine ecology (Hofmann et al. 2005). Notably, since microarrays have been used to assess the physiological responses of organisms to abiotic environmental conditions (Gracey 2007), they also have the potential to highlight pathways that are changing in response to elevated $CO_2$.

There are many barriers to success in using microarrays or other methods to profile gene expression (e.g. quantitative PCR [qPCR]), but they generally narrow down to whether there is sufficient DNA sequence available for a particular species to support the construction and use of a microarray or the design of gene-specific primers for qPCR. Fortunately, there is significant movement in the field as more libraries and platforms are available for ecologically and economically important marine species. Additionally, in the ab-

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Box 1. Quantitative PCR & microarrays. Several tools exist to quantify the expression of gene transcripts within organisms. Two promising techniques for assessing effects of ocean acidification are described

**Quantitative PCR (qPCR).** A modification of the standard PCR in which cDNA is quantified after each round of amplification (real-time) as opposed to end-point analysis. qPCR determines the relative starting quantity of messenger RNA (mRNA) in a sample with high resolution and precision and enables the researcher to quantify relative gene expression in a cell or tissue type at a particular time. This technique focuses on a single to few genes at a time and consequently is a more targeted approach to identifying mechanisms or describing a particular physiological pathway. qPCR is also used to verify DNA microarray results for a smaller set of genes. For qPCR, sufficient sequence information is preferred to create gene-specific primers.

**Microarrays.** The power of the microarray technology is in its scale. This technique allows for the simultaneous quantification of thousands of mRNAs in a given sample and therefore enables the researcher to profile the expression of genes involved in a large number of physiological pathways in a single step. Microarrays consist of glass slides spotted with up to tens of thousands of 'features': short segments of single DNA sequences in high density. Using competitive hybridization of 2 alternatively labeled samples to the feature spots, microarrays measure the relative abundance of thousands of mRNAs in a 'control' vs 'experimental' sample. While microarrays lack the detailed resolution of qPCR and can be technically more challenging to implement, the capacity to profile an organism's genome-wide response to a particular environmental condition makes this tool invaluable to ecological genomics. Below we outline the two primary microarray technologies used for gene expression analysis.

*cDNA microarray:* For non-model systems with little to no available sequence information, it is possible to construct a library of all potentially expressed mRNA transcripts found within individuals of the target species. These cDNAs can then be spotted as 'features' on a microarray. cDNA microarrays are inexpensive to manufacture, but require considerable time and effort to develop the underlying cDNA library.

*Oligonucleotide microarray:* Where sufficient sequence information exists, such as for model organisms or those with completely sequenced genomes, it is possible to select sequences from a database and simply order an array of synthesized gene-specific oligonucleotide 'features'. While oligo arrays offer a more sensitive and reproducible microarray technology for genome-wide transcript profiling, they are considerably more expensive than cDNA arrays and may be limited in their capacity for cross-species hybridizations.

sence of a sequenced genome, there are strategies that will allow some level of gene expression analysis in almost any organism (see Table 1). In this article we hope to highlight the utility of gene expression profiling and its potential to provide deeper insight into mechanisms of biomineralization in important marine calcifiers. We briefly outline the techniques, suggest candidate calcifying marine organisms that are currently most fruitful for this pursuit, and highlight how gene expression profiling can serve as a powerful tool to examine the response of organisms to ocean acidification regardless of whether a genome database exists or not.

## CANDIDATE STUDY ORGANISMS

Certainly one of the obvious initial questions is: Which marine organisms best support using gene expression profiling to address cellular- and molecular-level mechanisms in ocean acidification scenarios? Another is: Which species are critical to study due to the urgency of the ocean acidification problem? Since these approaches are significantly facilitated by access to DNA sequence information, a ranking of organisms by the depth of genomic and molecular resources is perhaps one of the first steps to consider. As we see it, amongst marine organisms, there are 5 excellent candidates: the purple sea urchin *Strongylocentrotus purpuratus*, scleractinian corals, oysters, limpets and coccolithophorids. A microarray-based approach has already been used in the study of calcification in coccolithophorids (Quinn et al. 2006). For the calcifying marine invertebrates, genomics resources are available in the form of sequenced and annotated genomes (Sea Urchin Genome Sequencing Consortium 2006) or excellent microarray resources are in place (Forêt et al. 2007, Jenny et al. 2007, Desalvo et al. 2008). Other strategies are available for investigators interested in non-model but ecologically critical species. Specifically, the design of PCR primers is possible given the available sequence data in various databases (Table 1). Additionally, efforts to obtain sequence data for critical species such as pteropods in high latitude seas are underway using pyrosequencing (G. Hofmann & V. Fabry unpubl. data), and highly feasible given the increasing availability of affordable high-throughput sequencing and its proven utility in the study of ecologically important questions (Vera et al. 2008).

However, for the purpose of this article, we will focus on how to apply functional genomics to the question of the effects of ocean acidification on sea urchins, due to the availability of the data in the sequenced genome, and, secondly, for stony corals given their ecological importance in biomineralization in coral reef ecosystems.

## EVIDENCE FOR THE IMPACT OF OCEAN ACIDIFICATION: WHERE TO START

For our purposes, it would be useful to first identify the cellular mechanisms involved in biomineralization,

Table 1. Candidate genes in marine calcifying organisms that currently lack a sequenced genome

| Gene for: | Function | Organism | GenBank accession no. |
|---|---|---|---|
| Nacrein or nacrein-like proteins | Thought to play a role in the regulation of calcium carbonate ($CaCO_3$) crystal formation in mollusk shells | Oyster | D83523, AB252484, AB252480 |
| | | Scallop | AB252482 |
| | | Snail | AB073680 |
| Chitin synthase (ArCS-1p) | Involved in chitin deposition in the mollusk shell during nacre formation | Pen shell | DQ081727 |
| Perlustrin | Believed to play a role in the nucleation and/or the growth of $CaCO_3$ crystals | Abalone | P82595 |
| Lustrin A or lustrin | Control the morphology and packing of $CaCO_3$ crystals by becoming occluded in the mineralized composite during shell formation | Abalone | AF023459, DQ298402 |
| Perlucin | Believed to play a role in the nucleation and/or the growth of $CaCO_3$ crystals | Abalone | P82596 |
| Perlinhibin | Involved in the inhibition of $CaCO_3$ crystal growth and dissolution | Abalone | P85035 |
| Shell matrix proteins | Control the morphology and packing of $CaCO_3$ crystals by becoming occluded in the mineralized composite during shell formation | Scallop | AB073617 |
| | | Mussel | AY364453 |
| Pearlin or pearlin-like proteins | Control of nucleation of the first layer of oriented calcite/aragonite in deposition of the abalone shell and flat pearl | Oyster | AB020779, AB159512, AB094512 |
| | | Pen shell | AF145215 |

an exercise that will highlight the types of genes that could be driving the observed changes in biogenic calcification in our 2 study organisms, sea urchins and stony corals (Fig. 1). Although numerous experimental studies have demonstrated that elevated $CO_2$ has a sub-lethal impact on organismal, developmental and physiological features in marine calcifying organisms (Fabry et al. 2008, Guinotte & Fabry 2008, Doney et al. 2009), very little is known about the cellular-level mechanisms that alter these processes in response to elevated $CO_2$ conditions. Additionally, since marine calcifiers have different forms of the biomineral calcium carbonate (Lowenstam & Weiner 1989), we expect the responses to vary by taxon. Thus, a taxonomically broad effort, encompassing a variety of calcifiers, will capture individual responses that can integrate to reveal impacts on ecosystem-level processes.

## Sea urchins

Due to its status as a model organism for development, the purple sea urchin has emerged as the marine invertebrate with the deepest genomic resources. Combined with a developed view of how biomineralization occurs in sea urchin embryos (Wilt 2002), the opportunity to use genomics approaches to explore the expression of genes involved in biomineralization are rich (Livingston et al. 2006). When considering how to begin these studies, we have identified a suite of genes that, if targeted, can reveal considerable detail into how ocean acidification and elevated $CO_2$ will impact biomineralization and skeleton formation in larval and adult sea urchins (A. Todgham & G. Hofmann unpubl. data). These 3 classes are (1) genes for proteins in the organic matrix, (2) genes for transporters in membranes, and (3) genes coding for carbonic anhydrase, an enzyme that drives $CO_2$ elimination in cells (Fig. 1).

If, in this first-cut analysis, we focus on the effect of $CO_2$ on the process of spicule formation in the sea urchin larvae, we would examine genes that are involved in biomineralization during skeletogenesis. In sea urchins, the spicule is formed by primary mesenchyme cells (PMCs) where the PMCs act as a cytoplasmic sheath around the forming spicule (Fig. 1). Gene expression in the PMCs is thought to be involved in calcium transport where calcium is transported from the external seawater, modified in the PMC cytoplasm, and then moved via exocytosis into the extracellular space around the forming spicule. In addition to genes involved in calcium transport to form calcite, there are proteins that facilitate precipitation of calcium in the spicule and there are also 45 proteins that have been identified in association with the spicule. Although the roles of all these proteins are not known, some of them are well known, e.g. SM30 is embedded in the mineral phase of the spicule and SM30 and SM50 have high expression rates at the growing nascent tips of larval spicules (Wilt 2002). Should sea urchin larvae be able to compensate for the impact of $CO_2$ on biomineral-

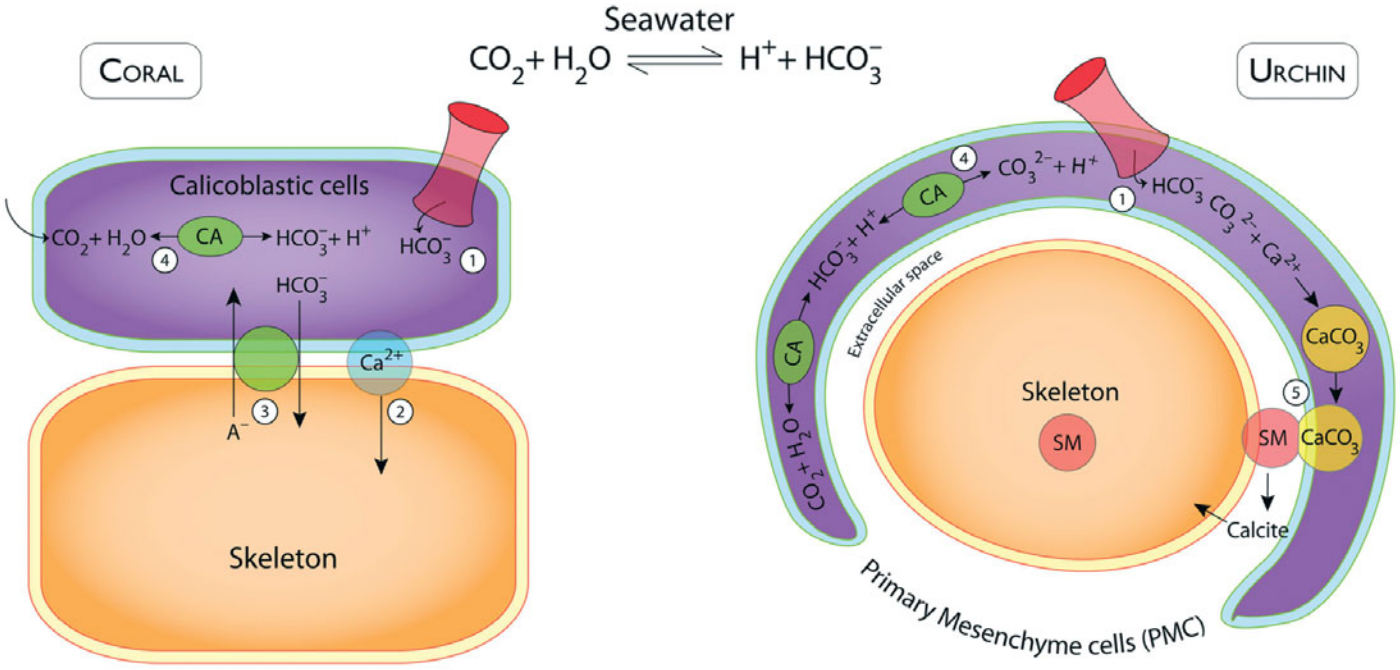


Fig. 1. Cellular pathways involved in calcification and skeleton biogenesis in sea urchins and corals. This schematic shows the cell types that form the skeleton of the coral polyp and the sea urchin larvae. The numbers within indicate general classes of genes that would be likely targets for study, or that may be predicted to exhibit changes in expression patterns, given their role in biomineralization. For corals, the calicoblastic cell forms the skeleton, and in sea urchin larvae, the primary mesenchyme cells (PMCs) form a cytoplasmic syncitium around the growing spicule (skeleton rod in the larvae). The genes encoding the following proteins are part of the general process of biomineralization: 1, various transmembrane transporters for $HCO_3^-$ and calcium from surrounding seawater; 2, transmembrane transport of calcium from the cytoplasm of the calicoblastic cell to the extracellular space where the calcium is mineralized at the skeleton; 3, anion exchangers that transport carbonate to the site of calcification; 4, action of carbonic anhydrase (CA), an enzyme that converts $CO_2$ in the cell; 5, in sea urchins, certain spicule matrix proteins (SM) are involved in directing biomineralization in the extracellular space between the PMC and the growing skeleton. Sea urchin drawing after Wilt (2002)

ization, one might predict that there would be changes in the expression of these genes. Notably, recent studies have shown sub-lethal effects of $CO_2$ on skeleton formation in sea urchins (Kurihara & Shirayama 2004), and gene expression studies indicate that expression patterns of spicule matrix proteins change with $CO_2$ levels (A. Todgham & G. Hofmann unpubl. data).

## Corals

Because a fully sequenced genome is not available for a species of stony coral, the resources are not as advanced as they are for sea urchins. However, there are DNA sequence data available for numerous species (reviewed in Forêt et al. 2007), microarray and qPCR studies are very achievable (de Boer et al. 2007, Schwarz et al. 2008, Mayfield et al. 2009), and this effort could easily be turned to questions of the impact of ocean acidification.

For corals, skeleton formation requires the transport of calcium and dissolved inorganic carbon (DIC) from seawater to the site of calcification at the epithelium of the calicoblastic cells (Fig. 1) of a coral polyp to form aragonite, a calcium carbonate mineral that makes up the skeleton in combination with the organic matrix (reviewed in Allemand et al. 2004). Although our 'gene targeting' approach is less clear-cut due to the complexities of coral skeletogenesis, ocean acidification impacts on biogenic calcification in corals can be examined by looking at active processes that are driven by a protein or a transport mechanism (Fig. 1).

In terms of calcium transport, early work indicated that calcium is delivered to the site of calcification by transcellular transport (reviewed in Gattuso et al. 1999, Cohen & McConnaughey 2003). Recent research supports these earlier studies and measured intracellular gradients of calcium that suggested the active, transcellular transport of calcium (Marshall et al. 2007). Calcium channels have been found in the calicoblastic epithelium and a goal would be to target the expression of these genes (Zoccola et al. 1999).

For the carbon source, benchmark research first showed that the carbonate in the skeleton can originate from 2 carbon sources, either from metabolic $CO_2$ or from soluble carbonate in external seawater. More recent research has focused on the source of carbon for coral skeleton formation and has pointed towards cel-

lular processes of interest. For example, the role of carbonic anhydrase has recently been the focus of biochemical research and this enzyme activity is found in tissues and in the organic matrix of an azooxanthellate coral (S. Tambutté et al. 2007). In addition, immunochemical methods have shown that calicoblastic cells are secreting components of the organic matrix (Puverel et al. 2005, 2007) and some of these matrix proteins have been cloned (Fukuda et al. 2003). Taken together, these accumulating experimental observations, and studies further describing the tissue–skeleton interface, argue for an active role of calicoblastic cells in the physiological process that controls calcification of the coral skeleton (E. Tambutté et al. 2007), and that, for example, carbonic anhydrase expression would be a good target of study. Most importantly, if more genes are explored in this endeavor, it will be possible to get a physiological fingerprint of the response of corals to ocean acidification and have a more comprehensive view of calcification. This endeavor is underway as more genomic resources for corals become available, a situation that will lead to clearer understanding of the skeletogenesis in corals in general, and then how this process will respond to ocean acidification at the molecular and cellular level.

## GLOBAL PHYSIOLOGICAL RESPONSE TO OCEAN ACIDIFICATION

It should not be forgotten that a transcriptomics approach also affords the investigator a view of many metabolic processes, not just the activity of those genes involved in biomineralization. In many ways, this 'discovery' aspect of the genomics approach supplies a platform on which future hypotheses, and a search for mechanism, can be built. Most importantly, this perspective will provide a more complete understanding of whether marine calcifiers have the physiological plasticity to compensate for the effects of ocean acidification and continue to build skeletons under future $CO_2$ conditions. Microarray expression profiling has been used in numerous studies on non-model organisms to reveal patterns of physiological response to environmental factors (Gracey 2007) and this approach has revealed important transcriptional responses to environmental stressors in non-model marine organisms (Podrabsky & Somero 2004, de la Vega et al. 2007, Kassahn et al. 2007, Kultz et al. 2007, Teranishi & Stillman 2007, Place et al. 2008). In an ocean acidification scenario, one notable organismal function that would be perturbed is acid/base balance (Pörtner et al. 2005). Organismal studies have shown an effect of $CO_2$ on acid/base balance in calcifiers such as sea urchins (Miles et al. 2007). Thus, the study of acid/base balance in marine organisms is an example of how gene expression profiling might reveal genes that are changing, or steps in metabolic pathways that are being altered, in response to a changing abiotic environment. Finally, recent studies on coral larvae have identified genes that are involved in the cross-talk between the algal symbionts and the invertebrate host (de Boer et al. 2007). Such studies could be extended to assess the effects of ocean acidification on the algal–coral symbioses from a more global perspective, i.e. whether the association of coral with their *Symbiodinium* sp. symbionts will change as a function of different seawater chemistry.

## SUMMARY

The application of genomics approaches to the question of the impact of ocean acidification will likely develop as fast as the resources become available. With the increase in the use of cross-species hybridizations (Buckley 2007), there is the opportunity to extend these resources without making gene chips for every species under study. Overall, gene expression profiling gives us a powerful tool to begin to understand how the physiology of marine calcifying organisms is likely to change in the face of a more acidic ocean. Targeted studies of individual species are significant in that each calcifier's response will vary and thus the ecosystem-level impact will be transduced through the physiology of key species. Although gene expression is but one technique (there are other approaches in systems biology, e.g. proteomics or metabolomics), there is great potential to learn about the complexity of the compensatory responses in calcification and other metabolic pathways under ocean acidification conditions. Additionally, transcriptome profiling and its ability to reveal subtle, complex patterns will be a powerful approach to tease apart interacting stressors such as the synergistic effects of ocean acidification and warming, the 'double jeopardy' scenario within global climate change. Overall, the use of functional genomics will contribute to 2 important unknowns in the effort to forecast the effects of ocean acidification on marine ecosystems: (1) What are the basic organismal responses to the predicted levels of $CO_2$? and (2) Will marine calcifying organisms have sufficient plasticity to build skeletons in a high-$CO_2$ world?

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# Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions

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ABSTRACT: Despite their microscopic size, marine phytoplankton are responsible for about half of the global primary production and represent the basis of the marine food web. This diverse group of organisms drives important biogeochemical cycles, exporting massive amounts of carbon to deep waters and sediments, and strongly influencing ocean–atmosphere gas exchanges. Anthropogenic climate change will result in significant alterations in the marine environment over the next 100 yr and beyond. The increase in atmospheric $CO_2$ has already caused significantly higher aquatic $CO_2$ concentrations and lower pH values ('ocean acidification') than in pre-industrial times. Rising temperatures will also impact surface ocean stratification, which in turn will affect the surface-water light regime and nutrient input from deeper layers. Phytoplankton will be affected by these environmental changes in many ways. In this article we assess the possible responses of different phytoplankton groups with regard to the expected physico-chemical changes. In addition to summarizing laboratory and field studies, we outline the current understanding of the underlying mechanisms that cause processes such as photosynthesis, calcification, and nitrogen fixation to be sensitive to ocean acidification. We describe different approaches to manipulate carbonate chemistry (e.g. acid/base or $CO_2$ addition), discuss their potential to simulate future ocean acidification, and allude to common problems in experiments caused, for instance, by high biomass or the use of buffers. In addition to guidelines for $CO_2$ perturbation experiments, we argue that it is essential to look at multiple environmental factors in combination with $CO_2$, to aim for process-understanding rather than correlation, and to assess a wider diversity of phytoplankton species both in laboratory and field studies.

KEY WORDS: Ocean acidification · $CO_2$ manipulations · Photosynthesis · Carbon acquisition · Calcification · Nitrogen fixation



## GLOBAL CHANGE IN THE MARINE ENVIRONMENT

The Earth's climate has undergone major changes over geological time scales, shaping the structure and productivity of ecosystems and the proliferation or disappearance of species. Biological activity has in turn directly affected climate by driving many of the global elemental cycles. Phytoplankton has played a central role in mitigating and amplifying climate change in the past and may have contributed to stabilizing the climate by influencing the partitioning of climate-relevant gases between the ocean and atmosphere (Schlesinger 2005). Changes in environmental conditions are presently occurring at an unprecedented rate due to large-scale perturbations induced by human activities. For the past 10 million yr the atmospheric partial pressure of $CO_2$ ($pCO_2$) has most probably remained $<300$ µatm (Berner 1990, Pearson & Palmer 2000) and fluctuated between 180 µatm in glacial and 300 µatm in interglacial times over the last 800 000 yr (Petit et al. 1999, Lüthi et al. 2008). With the beginning of the industrial revolution, $CO_2$ emissions from the burning of fossil fuel and changes in land use led to atmospheric $CO_2$ concentrations well above the upper limit of the last several million years. At present, the $pCO_2$ has reached about

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380 µatm and is expected to rise to 750 µatm by the end of this century (IPCC Scenario IS92a; Houghton et al. 2001) or even values >1000 µatm (Raven et al. 2005, Raupach et al. 2007).

Such changes are altering the physico-chemical conditions in the marine environment. Changes in atmospheric $pCO_2$ will directly affect the carbonate system of the surface ocean, since atmosphere and surface ocean exchange $CO_2$ on time scales of several months (Zeebe & Wolf-Gladrow 2001). As $CO_2$ dissolves in the surface ocean it reacts with water to form carbonic acid ($H_2CO_3$), which dissociates to bicarbonate ($HCO_3^-$), carbonate ions ($CO_3^{2-}$), and 'protons' ($H^+$). As a consequence of this chemical reaction, the ocean can take up large amounts of $CO_2$ and store it as dissolved inorganic carbon (DIC), which is the sum of the concentrations of these carbon compounds. Currently, <1% of DIC remains in the form of dissolved $CO_2$ (including tiny amounts of $H_2CO_3$), while the rest is in the form of $HCO_3^-$ (~90%) or $CO_3^{2-}$ (~9%). With increasing atmospheric $pCO_2$, DIC will increase and the equilibrium of the carbonate system will shift to higher $CO_2$ and $HCO_3^-$ levels, while $CO_3^{2-}$ concentration and pH will decrease. These changes in carbonate chemistry, often referred to as 'ocean acidification', are already occurring and are expected to intensify in the future. The projected increase in atmospheric $pCO_2$ to about 750 µatm by the end of this century is estimated to almost triple surface water $CO_2$ concentrations relative to preindustrial values. Concomitantly, seawater $CO_3^{2-}$ concentrations and pH will drop by 50% and 0.4 units, respectively (Fig. 1; Wolf-Gladrow et al. 1999, Caldeira & Wickett 2003). It should be noted that this change in pH corresponds to a 2.5-fold increase in the $H^+$ concentration. The lower $CO_3^{2-}$ concentration will lead to a reduction of the saturation level for carbonates such as calcite or aragonite. These changes in carbonate chemistry will affect phytoplankton in general and certain processes in particular. Depending on the underlying process, the sensitivity to carbonate chemistry may be strongly modified by temperature, light, and nutrient availability. This is important to consider because other environmental conditions are likely to also change within the framework of global change.

The increase in atmospheric greenhouse gases like $CO_2$ has caused global average temperatures to increase over the last century, especially in the past

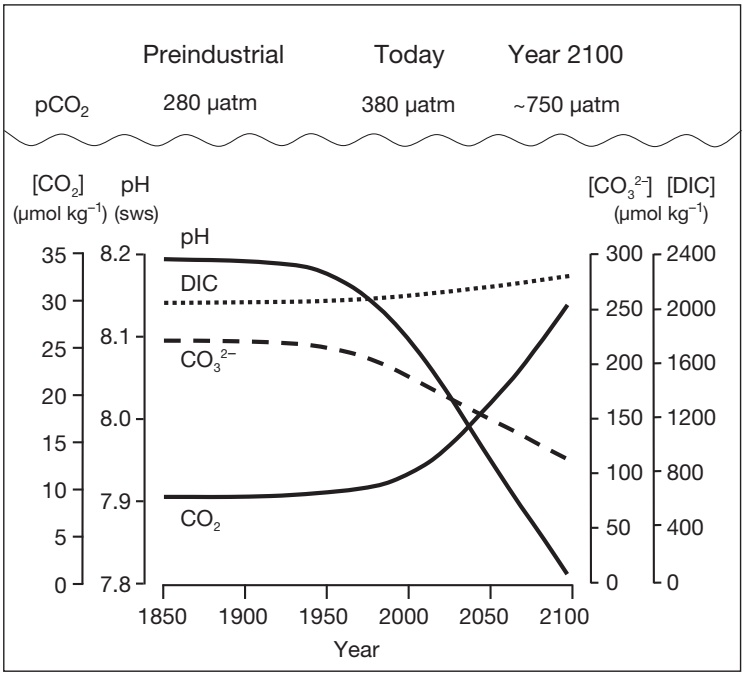


Fig. 1. Predicted changes in the surface ocean carbonate system in response to changes in atmospheric $pCO_2$ assuming the IS92a Scenario. Modified after Wolf-Gladrow et al. (1999)

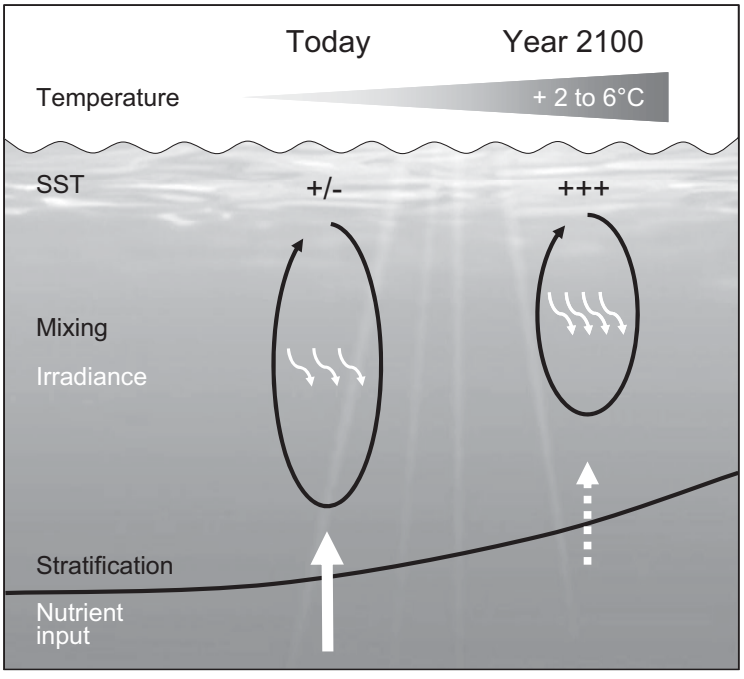


Fig. 2. Putative changes in sea surface temperatures (SST) in response to global warming and its effect on stratification, nutrient and light availability. Modified after Rost & Riebesell (2004)

few decades. The predictions for the future temperature rise range from 2 to 6°C until the end of this century, and, independent of uncertainties in magnitude, the changes will be most pronounced at high latitudes (IPCC 2007). The temperature increase will increase stratification of the surface ocean and this can, in turn affect phytoplankton. Enhanced upper ocean stratification reduces nutrient supply from deeper layers and increases light availability due to shoaling of the upper mixed layer (Fig. 2; Sarmiento et al. 2004). Such changes have opposing impacts on the productivity of phytoplankton, and the overall effect will vary in different oceanic provinces: further extension of nutrient-limited, low-productivity regions such as subtropical gyres (Bopp et al. 2001, Behrenfeld et al. 2006), and increases in productivity in high-latitude regions currently light-limited owing to pronounced vertical mixing can be expected (Bopp et al. 2001, Doney 2006).

The physico-chemical changes described above will inevitably affect phytoplankton in numerous ways. Despite the complexity of these responses, they can be divided into physiological and ecological aspects, i.e. changes in the rates of processes and shifts in the dominance of species (Falkowski et al. 1998, Boyd & Doney 2002). Depending on which species or groups are affected in what manner, these changes have the potential to alter productivity and to feedback on biogeochemical cycles. With respect to the latter, phytoplankton can be distinguished into phytoplankton functional types. *Silicifiers* (mainly diatoms) play a major role in determining the vertical fluxes of silicate and organic carbon. *Calcifiers* (mainly coccolithophores), on the other hand, affect the carbon cycle through the production of calcium carbonate and its impact on seawater alkalinity. *Diazotrophs* ($N_2$-fixing cyanobacteria) influence marine productivity by altering the availability of reactive nitrogen. Several recent studies have found that key species from these groups are in fact sensitive to changes in carbonate chemistry.

In the following we describe the different responses of these phytoplankton groups to changes in carbonate chemistry and illustrate our current (or lack of) knowledge of the underlying mechanisms causing the sensitivity in key processes such as photosynthesis, calcification, or nitrogen fixation. Our aim is not to compare the results of the individual studies in detail, but rather to point out general observations and apparently contradictory results. Furthermore, we will describe common approaches to simulate ocean acidification in experiments and discuss potential problems of these manipulations as we believe much of the controversy in the literature might be caused by different protocols.

## SENSITIVITY OF PHYTOPLANKTON TO CARBONATE CHEMISTRY

### Photosynthesis and carbon acquisition

Assessing effects of rising atmospheric $CO_2$ on phytoplankton requires an understanding of the photosynthetic processes that provide energy for growth and any other downstream process. Photosynthesis involves a series of reactions that start with capturing light energy, converting it into ATP and the reductant NADPH, and using these compounds to fix $CO_2$ in the Calvin-Benson cycle (Falkowski & Raven 2007). As a consequence, photosynthesis and subsequent processes are primarily affected by light, but also by $CO_2$ availability. The inherent $CO_2$ sensitivity in photosynthesis is largely the result of the primary carboxylating enzyme, Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). This ancient and highly conserved enzyme, which evolved during times of elevated atmospheric $CO_2$ and low $O_2$ levels (Falkowski & Raven 2007), is characterized by low affinities for its substrate $CO_2$ and a susceptibility to a competing reaction with $O_2$.

Despite differences in these catalytic properties of RubisCO, the generally poor substrate affinities for $CO_2$ (i.e. high half-saturation constants [$K_M$] with values from 20 to 185 µmol $l^{-1}$; Badger et al. 1998) impose constraints on carbon assimilation under the low $CO_2$ concentrations present in seawater (5 to 25 µmol $l^{-1}$). To alleviate the risk of carbon limitation, most microalgae have thus developed different mechanisms that enhance $CO_2$ concentration in the close vicinity of RubisCO (Badger et al. 1998, Thoms et al. 2001). Over the past 2 decades, significant progress has been made towards understanding $CO_2$-concentrating mechanisms (CCMs). Many microalgae have been shown to possess complex CCMs that involve the uptake of $CO_2$ and/or $HCO_3^-$, as well as various isoforms of the enzyme carbonic anhydrase (CA), which accelerate the otherwise slow interconversion between these carbon species. Processes that minimize $CO_2$ efflux from the cell are also important components of an efficient CCM. For details on the different CCMs we refer to reviews by Giordano et al. (2005), Price et al. (2007), and Roberts et al. (2007).

The extent to which various species operate these CCMs is still poorly understood, but the few existing studies on marine phytoplankton suggest that species differ in efficiency and regulation of their CCMs (e.g. Burkhardt et al. 2001, Tortell & Morel 2002, Rost et al. 2003, Trimborn et al. 2008). In general, species relying on diffusive $CO_2$ uptake or those with inefficient CCMs (i.e. low apparent affinities for inorganic carbon) are highly $CO_2$ sensitive in photosynthesis and

thus may directly benefit from the increase in $CO_2$. Those species operating highly efficient CCMs are at, or close to, rate-saturation under present-day $CO_2$ concentrations. The latter species can nevertheless benefit in the future, since a down-regulation of the CCM under elevated $CO_2$ levels may allow for optimized energy and resource allocation. The capability for regulation is generally important to consider, as it permits phytoplankton to adjust CCM activity to the actual demand, which also explains the strong modulation of $CO_2$ sensitivity by light or nutrient availability. The observed species-specific differences in CCMs imply that changes in the carbonate chemistry may have profound effects on phytoplankton communities, e.g. by directly affecting the productivity of ecosystems or influencing the species assemblage and succession. In fact, several laboratory and field studies have observed $CO_2$ effects on photosynthesis and downstream processes in various phytoplankton taxa.

## Coccolithophores

Coccolithophores have been in the focus of discussion about the consequences of ocean acidification research because of their remarkable sensitivity in processes such as photosynthesis and calcification. In species such as *Emiliania huxleyi*, photosynthesis was found to be well below saturation under present-day carbonate chemistry and, hence, photosynthesis generally increases under elevated $CO_2$ levels (Paasche 1964, Nielsen 1995, Riebesell et al. 2000, Berry et al. 2002, Zondervan et al. 2002, Rost et al. 2003, Leonardos & Geider 2005, Iglesias-Rodriguez et al. 2008). The strong $CO_2$ sensitivity of photosynthesis is consistent with the low-affinity CCM observed in *E. huxleyi* (Rost et al. 2003). Apart from these laboratory studies, $CO_2$ effects on photosynthesis have also been observed in natural communities that were dominated by *E. huxleyi* (Engel et al. 2005, Riebesell et al. 2007, Schulz et al. 2008). It should be noted, however, that the dependency of photosynthesis on $CO_2$ concentration is not straightforward and seems to be modified by light and nutrient supply (for reviews see Rost & Riebesell 2004, Zondervan 2007).

Laboratory experiments (Riebesell et al. 2000, Zondervan et al. 2002, Sciandra et al. 2003), as well as mesocosm studies (Dellile et al. 2005), suggest that calcification by coccolithophores will be reduced in response to ocean acidification. The changes in calcification rates under elevated $pCO_2$ have been related to the concomitant decrease in carbonate ion concentration and thus calcite saturation levels, but other entities of the carbonate system such as the pH may also be responsible for the observed relationship. A reduction in the degree of calcification is assumed to put coccolithophores at an ecological disadvantage, suggesting a rather 'grim future' for this group of phytoplankton. Although this view is widely accepted, there are also other lines of evidence and many open questions.

First of all, most of our current understanding on the process and sensitivity of calcification, as well as photosynthesis, stems predominantly from studies on *Emiliania huxleyi* and the closely related *Gephyrocapsa oceanica*. Both species belong to a lineage of rather atypical coccolithophores in terms of structure, physiology, and ecology (Sáez et al. 2003). A study by Langer et al. (2006) with the globally important $CaCO_3$ producers *Coccolithus pelagicus* and *Calcidiscus leptoporus* showed that species-specific differences in the sensitivity to carbonate chemistry do exist. While in *C. leptoporus* an optimum curve was observed with maximum calcification rates at present-day $CO_2$ levels, calcification rates did not vary significantly with $pCO_2$ in *C. pelagicus*. In both species, photosynthetic carbon fixation rates remained constant at $CO_2$ levels ranging between 150 and 920 µatm. Also challenging the general view are the recent findings by Iglesias-Rodriguez et al. (2008), who observed a stimulation in calcification rate of *E. huxleyi* under elevated $CO_2$.

In view of these apparently contradictory findings it is essential to unravel the process of calcification, which is not completely understood (for review see Brownlee & Taylor 2004). Moreover, the fate of coccolithophores can only adequately be predicted when we have revealed the function(s) of calcification and understand the consequences of different degrees of calcification. The latter remain enigmatic, since reduced calcification rates do not alter growth or photosynthesis in *Emiliania huxleyi* (Herfort et al. 2004, Rost & Riebesell 2004, Trimborn et al. 2007). Independent of the discussed changes in process rates, coccolithophores may benefit from increasing stratification, since they favor moderately stratified conditions (for review see Tyrrell & Merico 2004). Floristic shifts are already occurring (Smyth et al. 2004) and will be important to consider when assessing the fate of coccolithophores.

## Diatoms

Regarding this important group of phytoplankton, many studies have investigated the influence of light or nutrients, but very few have focused on the potential effect of ocean acidification. While earlier studies suggested that large diatoms were limited by $CO_2$ supply in the contemporary ocean (Riebesell et al. 1993), subsequent studies found that many diatoms, especially bloom-forming ones, were capable of compensating

for low $CO_2$ supply through the use of highly regulated and efficient CCMs (Burkhardt et al. 2001, Rost et al. 2003, Trimborn et al. 2008). With respect to the process of silification, diatoms also do not appear to be particularly $CO_2$ sensitive (Milligan et al. 2004). Field studies have demonstrated, however, that different $CO_2$ levels caused shifts in the dominance of diatom species in the phytoplankton assemblage of the Equatorial Pacific (Tortell et al. 2002), as well as of the Southern Ocean (Tortell et al. 2008). In the latter study, elevated $CO_2$ concentrations led to an increase in phytoplankton productivity and promoted the growth of larger chain-forming diatoms.

While the effect of $CO_2$ on photosynthesis and growth may yet be small in diatoms, at least when compared to other taxa, the predicted changes in stratification and, thus, light and nutrient availability (Fig. 2) will certainly affect this group strongly. Thriving in turbulent waters with high nutrient concentrations, diatoms will possibly suffer under enhanced stratification in most regions. In higher latitudes, however, diatoms may benefit, since the projected reduction in mixing may alleviate light limitation and thereby increase the productivity. Future studies on diatoms should therefore investigate carbonate chemistry effects in combination with nutrient and light availability.

### Cyanobacteria

$N_2$-fixing cyanobacteria support a large fraction of total biological productivity in tropical and subtropical areas and exert, over long time scales, a significant influence on the global carbon cycle by providing a major source of reactive N to the water column (Falkowski 1997, Gruber & Sarmiento 1997). A number of recent studies (Barcelos e Ramos et al. 2007, Hutchins et al. 2007, Levitan et al. 2007) have investigated the effect of elevated $CO_2$ on the bloom-forming cyanobacterium *Trichodesmium*, species of this genus are responsible for much of the marine $N_2$ fixation (Capone et al. 1997). All 3 studies observed a strong increase in photosynthesis, $N_2$ fixation, and even division rates under elevated $CO_2$ levels. The magnitude of these $CO_2$ effects exceeds those previously seen in other photoautotrophs and would, if representative for the natural environment, have large implications for the future ocean. The processes responsible for the strong $CO_2$ sensitivity are currently unknown.

Both carbon uptake and fixation and nitrogen fixation are key processes, which compete for energy and reductive power (Berman-Frank et al. 2001). Since cyanobacteria possess RubisCOs with very low $CO_2$ affinities ($K_M$ values from 105 to 185 µmol $l^{-1}$; Badger et al. 1998), increasing $CO_2$ levels could favor this group, either by directly increasing the carboxylation efficiency of RubisCO or, indirectly, by reducing the energy costs of their CCMs. Recent results revealed changes in CCM efficiency under elevated $CO_2$ and point to improved resource allocation between photosynthesis, carbon acquisition, and $N_2$ fixation (Kranz et al. 2009). Significant uncertainties remain, however, as to the degree of sensitivity for $CO_2$, the modulation by other environmental factors (light, P, or Fe), and whether the observed responses can be generalized to other important diazotrophic species (Montoya et al. 2004). In addition to the $CO_2$ stimulation, the magnitude of marine $N_2$ fixation may also increase due to the expansion of oligotrophic regions to higher latitudes as a consequence of increased warming, stratification, and the concomitant changes in nutrient levels (Boyd & Doney 2002). On the other hand, Breitbarth et al. (2007) predict the overall $N_2$ fixation to decrease, despite wider distribution of *Trichodesmium* spp., because temperatures will rise above the optimum for $N_2$ fixation in some areas.

Although $CO_2$ effects have also been investigated in a number of non-diazotrophic cyanobacteria (for review see Price et al. 2007), most of these studies compare unnaturally high (2 to 5% $CO_2$) with ambient $CO_2$ levels. Currently, there is little information available on the sensitivity of this group to more realistic $CO_2$ scenarios. A recent study by Fu et al. (2007) observed higher rates of growth and photosynthesis in *Synechococcus* spp. when grown at 750 µatm $CO_2$. *Prochlorococcus* spp. remained unaffected by elevated $CO_2$ in the present study. Such species-specific difference in $CO_2$/pH sensitivity could lead to shifts in community structure. Our current knowledge is, however, based on too few studies (and species). In view of the potential ecological and biogeochemical implications, investigation of diazotrophic and other cyanobacteria is clearly a research priority.

## EXPERIMENTAL DESIGN

Despite addressing similar questions and working with the same species, studies often have yielded different and sometimes even contradictory results. While some of the discrepancy might reflect the variability in physiology, a significant part most probably results from differences in the experimental design regarding growth conditions, as well as methodology, specifically, how the response is measured. Here, we describe the most common ways to manipulate the carbonate chemistry, explain how it is affected by physiological processes, and point to pitfalls associated with $CO_2$ perturbation experiments.

The marine carbonate system comprises $CO_2$, $HCO_3^-$, $CO_3^{2-}$, $H^+$, $OH^-$, and several weak acid–base systems of which borate-boric acid [$B(OH)_3$, $B(OH)_4^-$] is the most important. For an accurate description, 2 different components are of particular interest because they are conservative in the sense that their concentrations do not change with temperature or pressure. As introduced earlier, DIC is the sum of all dissolved inorganic carbon species, while total alkalinity (TA) equals $[HCO_3^-] + 2\,[CO_3^{2-}] + [B(OH)_4^-]$ + minor components and reflects the excess of proton acceptors over proton donors with respect to a zero level of protons (for details see Dickson 1981, Wolf-Gladrow et al. 2007). Analytically, TA is determined by the titration of seawater with a strong acid and thus can also be regarded as a measure for the buffering capacity of seawater. If 2 components of the carbonate system are known (for example, $CO_2$ and $H^+$ or DIC and TA), all other components can be calculated for seawater with typical nutrient concentrations for a given temperature, salinity, and pressure (more information is necessary for solutions with different compositions, e.g. high nutrient concentrations, unusual ionic compositions, organic buffers). Changes in any single component due to physical or biogeochemical processes lead to changes in several if not all other components. In other words, it is impossible to vary a single component of the carbonate system while keeping all other components constant. This interdependency in the carbonate system is important to consider when performing $CO_2$ perturbation experiments.

### Carbonate chemistry manipulations

To adjust different $pCO_2$ values, the carbonate system can be manipulated in various ways, which are depicted in Fig. 3. Like in the natural system, the carbonate chemistry can be altered by equilibrating seawater with gas mixtures of different $pCO_2$. The $CO_2$

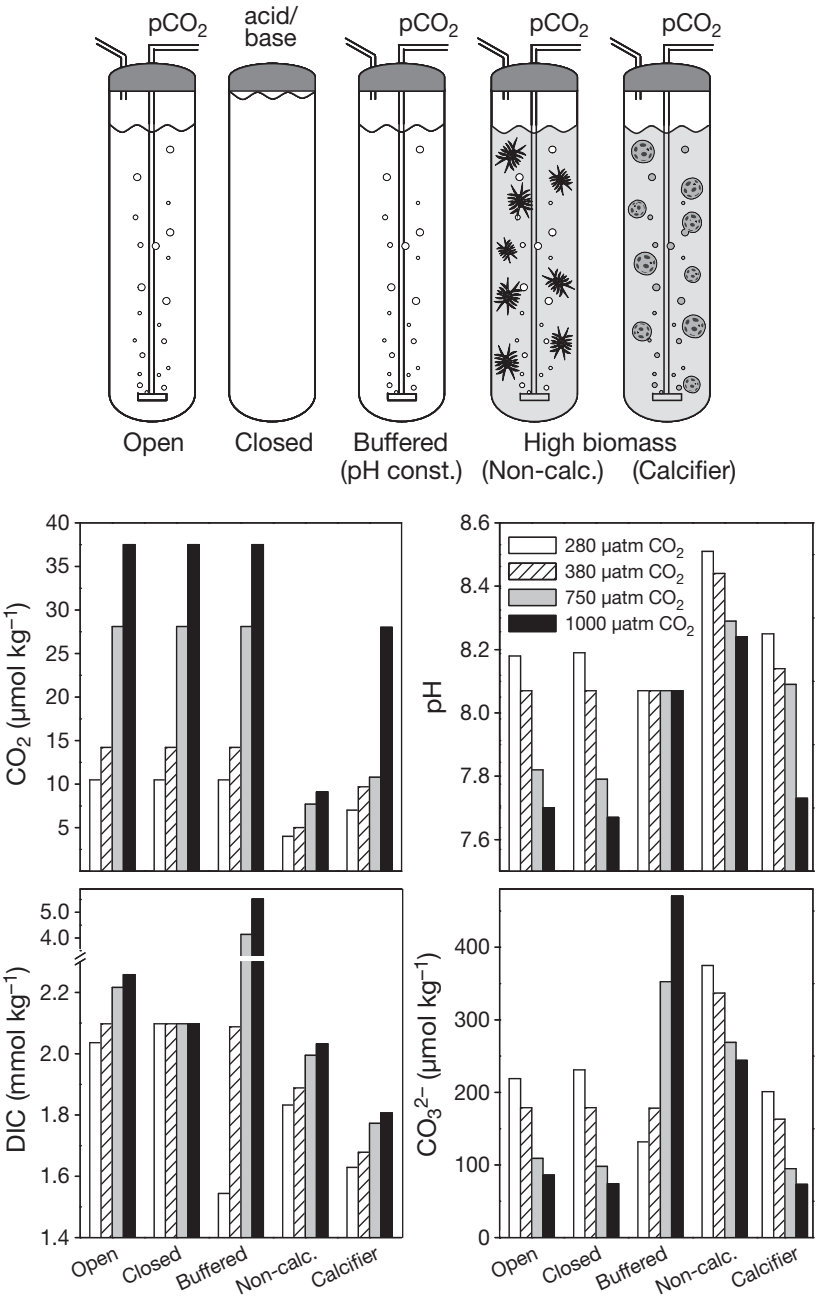


Fig. 3. Different carbonate chemistry manipulations and the effect of high biomass in laboratory experiments. Top panel illustrates the manipulation systems; bottom panels show the respective carbonate chemistry. The open system reflects the future scenario of ocean acidification, while, in the closed system, pH is altered by acid or base addition to closed bottles without headspace, where dissolved inorganic carbon (DIC) remains constant. In a buffered system, the pH is kept constant by means of organic buffers, which prevent the typical shifts in carbon speciation with changes in $pCO_2$. The example for high biomass of a non-calcifier reflects a situation in which organic matter production by phytoplankton exceeds the $CO_2$ supply by bubbling, here causing DIC levels to be reduced by 10% with respect to the initial equilibrated conditions. In the case of a calcifier, we assume equal molar quantities of $CaCO_3$ to be precipitated with the biomass. In both cases, biological activity causes strong deviations from the desired carbonate chemistry (compare with open system), which is discussed in the subsection 'Effect of biology'. For all calculations, temperature = 15°C and salinity = 35

exchange in such an 'open system' is driven by differences in atmospheric ($pCO_2$) and aquatic partial pressure ($PCO_2$) until an equilibrium is established. According to Henry's law, the equilibrium $CO_2$ concentration is dependent on a temperature- and salinity-dependent solubility coefficient. Consequently, $CO_2$ concentrations will increase with increasing atmospheric $pCO_2$ but also with decreasing temperature or decreasing salinity through changes in solubility. When seawater is manipulated in this way, the carbon speciation, pH, and DIC are affected, while TA remains constant (Fig. 3). Note that the relative changes in DIC are an order of magnitude smaller than the relative changes in the aquatic $CO_2$ or $CO_3^{2-}$ concentrations. To investigate the effect of ocean acidification, manipulations with the 'open system' reflect future changes in the carbonate chemistry and thus will be used as a reference for further comparisons.

Another commonly used perturbation approach is the addition of strong acid (HCl) or base (NaOH) to a 'closed system', for instance a bottle filled without headspace. Such manipulation directly alters the pH and hence the DIC speciation, resulting in higher or lower $CO_2$ concentrations (Fig. 3). Since these experiments are performed in gas-tight bottles, the shift in equilibrium concentrations and hence aquatic $PCO_2$ cannot result in concomitant DIC changes by $CO_2$ release or invasion. Addition of a strong acid or base is an easy way to realize the large relative changes in $H^+$, $CO_2$, and $CO_3^{2-}$ concentrations also typical for the natural system, while accepting small deviations in those quantities that show much smaller relative changes (DIC, $HCO_3^-$) or no change at all (TA). The 'closed system' has advantages when working with phytoplankton that are sensitive to continuous bubbling. To combine this with the advantage of an 'open system' (i.e. more realistic simulation of ocean acidification), the media can be equilibrated with different $pCO_2$ prior to the inoculation of cells. Alternatively, an increase of $CO_2$ at constant TA can also be achieved without bubbling by adding equimolar amounts of HCl and $NaHCO_3$.

In order to elucidate particular mechanisms it can make sense to manipulate the carbonate system in a different way than that previously described. In order to separate $CO_2$ effects from pH effects, for instance, one may change $CO_2$ while keeping pH constant by addition of a certain ratio of $Na_2CO_3$ and $NaHCO_3$ (for details see Zeebe & Wolf-Gladrow 2001). More commonly, organic buffers (e.g. Tris, Bicine, HEPES) have been added to keep pH constant while changing $CO_2$ concentrations (Fig. 3). Although often used in the attempt to keep the desired carbonate chemistry more stable, this approach causes large deviation from the 'natural' system and complicates the calculation of the carbonate chemistry via TA. When buffered seawater is aerated over a range of $pCO_2$, the changes in DIC are much larger than in natural seawater and the $CO_3^{2-}$ concentration increases with $pCO_2$. In terms of assessing the effects of ocean acidification this, buffered system is clearly the wrong approach.

Studies often present and compare their results on the basis of $CO_2$ or pH, and take these quantities as a 'proxy' for the rest of the carbonate chemistry. This can be troublesome for 2 reasons: (1) investigations often do not report which of the 4 different pH scales (NBS, free, total, and seawater scale) has been used, and these can deviate by >0.1 units. If these differences are ignored, the corresponding error in calculated $pCO_2$ can easily be >100 µatm (for details see Zeebe & Wolf-Gladrow 2001); (2) in view of the different approaches used to manipulate pH or $CO_2$, it is not adequate to report only 1 quantity. Hence, for a thorough comparison of studies, the full description of the carbonate chemistry has to be provided. It is also advisable to over-constrain the carbonate chemistry (by measuring >2 quantities of the carbonate system) to allow for cross-checks. In this respect, developing protocols for standardizing procedures is important.

## Effect of biology

As we examine the effects of carbonate chemistry on physiological processes, we have to be aware that these processes possibly alter the initial carbonate chemistry of the experiment significantly (Fig. 4). During photosynthetic carbon fixation, for instance, $CO_2$ and DIC decrease while pH increases (respiration causes the reverse reaction). During calcification $CaCO_3$ is precipitated, thereby reducing DIC and TA in a molar ratio of 1:2 (carbonate dissolution causes the reverse reaction). Since TA is more strongly affected than DIC, the chemical equilibrium shifts towards higher $CO_2$ concentrations and a lower pH.

When working with high cell densities, these processes can severely shift experimental conditions (Fig. 3). The latter is especially true for closed systems, but, even working in an open system, the biological drawdown of inorganic carbon can quickly exceed the $CO_2$ supply (taking into account the slow air–water gas exchange) and thus cause a shift in carbonate chemistry. This problem is more pronounced when processes such as calcification decrease TA, which cannot be restored by increasing the $CO_2$ supply. It is important to mention here that, depending on the ratio of photosynthesis to calcification, this can elevate, decrease, or even maintain the desired $pCO_2$, but always at reduced concentrations of DIC. In view of the high cell densities reported in some studies, the poten-

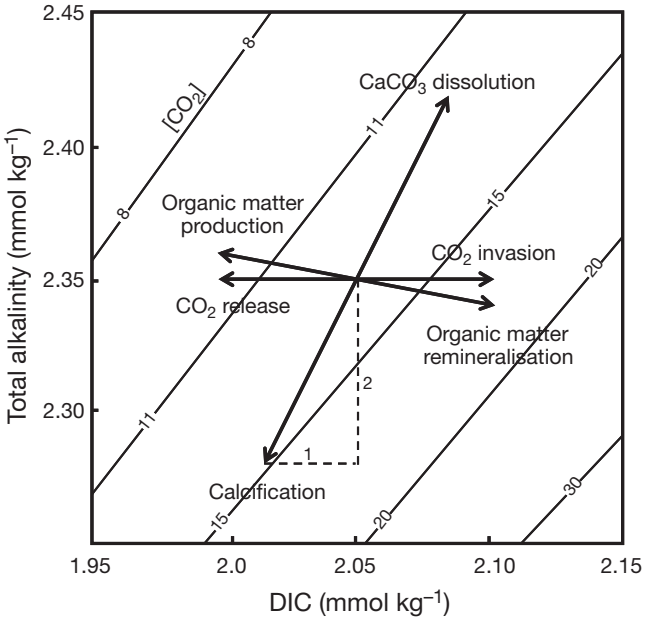


Fig. 4. Effect of various processes (arrows) on dissolved inorganic carbon (DIC) and total alkalinity (TA) that occur in culture experiments. Lines indicate levels of constant dissolved $CO_2$ (in µmol kg$^{-1}$) as a function of DIC and TA. Invasion of atmospheric $CO_2$ into seawater (for instance by bubbling with elevated $pCO_2$) increases DIC, while release of $CO_2$ to the atmosphere has the opposite effect; TA remains constant in the 2 cases. Calcification reduces DIC by 1, and TA, by 2 units, thereby driving the system to higher $CO_2$ levels and lower pH. DIC changes associated with organic matter production and remineralization are caused by photosynthesis and respiration, respectively. The small changes in TA reflect nitrate assimilation and remineralization assuming Redfield stoichiometry. Modified after Zeebe & Wolf-Gladrow (2001)

tial shifts in the experimental carbonate system may often be larger than described in our examples.

To ensure well-controlled experimental conditions, it is therefore crucial to work with low cell densities. This important canon of studying carbonate chemistry effects has often been overlooked, also due to other requirements such as the need for sufficient biomass for analysis. It should be mentioned that certain incubation techniques are better suited to work with low cell densities (e.g. semi-continuous or dilute batch) while others tend to have higher cell densities (e.g. chemostats or classical batch). Despite being more challenging, the goal of working with low biomass should be taken on more earnestly in future studies.

## CONCLUSIONS AND RESEARCH DIRECTIONS

While uncertainties regarding the magnitude of physico-chemical changes in the marine environment remain, we are only starting to understand how phytoplankton will respond. To answer the question regarding who will be the 'winners' and 'losers' of global change, future research must cover complementary issues on different processes and scales, ranging from the level of individual studies to community efforts in field research. In the following we summarize our main conclusions and outline future research priorities:

(1) Carbonate chemistry manipulations: future laboratory studies should aim to mimic environmental conditions as closely as possible. This relates to realistic $pCO_2$ levels and manipulations, but also cell densities and their influence on carbonate chemistry. Few papers currently offer details on carbonate chemistry, irradiance, and other ancillary data. In order to be able to compare studies and to deal with controversial findings, it will therefore be critical to develop standard protocols.

(2) Multiple environmental factors: most experiments have examined $CO_2$/pH effects in isolation from other environmental factors, typically using saturating light and ample nutrient supply. Since light and/or nutrient availability have been shown to strongly modify the $CO_2$/pH sensitivity of phytoplankton (and these are also conditions predicted to change), future experiments should look at multiple variables in combination with $CO_2$ and assess their interactive effects.

(3) Process-based understanding: an empirical relationship between growth conditions and response of a phytoplankton species is a necessary first step to estimate the potential impact of certain environmental variations on organisms, ecosystems, and the cycling of elements. However, growth conditions are often correlated or anti-correlated in experiments (e.g. $CO_2$ and pH), and thus it is not possible to assign the observed response to a single environmental parameter. Future studies should therefore go beyond the descriptive level and unravel the underlying mechanism(s) for the observed responses. Such process-understanding will allow for extrapolation to other species or growth conditions and therefore significantly improve our predictive capabilities.

(4) Diversity in responses: thus far, studies have focused on a limited number of model species. With respect to coccolithophores, for example, only 4 species have been tested to date. Moreover, their different life cycle stages (haploid and diploid), which display different morphologies and modes of calcification, may differ completely in their responses to $CO_2$/pH. Regarding cyanobacteria, the mismatch between investigated species and overall diversity is even larger. Future studies should therefore acknowledge the diversity in phytoplankton groups and also include other relevant species.

(5) Acclimation versus adaptation: in all classical experimental work, the different growth conditions are

imposed rather quickly and experiments last days to weeks and perhaps months; hence, they only deal with the effect of *acclimation* (i.e. the plasticity of organisms to react to environmental conditions without genetic changes). Given that global change occurs gradually over decades, it is likely that evolution results in species that are genetically and phenotypically different from the contemporary population. Future studies should focus on this important aspect of *adaptation*, which has yet not adequately been addressed (however see Collins & Bell 2004, Collins et al. 2006).

(6) Community level: as laboratory studies with mono-specific cultures lack interactions within or between trophic levels, it will be difficult to draw robust conclusions for whole ecosystems. Possible ways to assess community responses are on-deck perturbations (Tortell & Morel 2002, Tortell et al. 2008) and mesocosms (Engel et al. 2005, Schulz et al. 2008). In addition to these perturbations, comparisons of phytoplankton communities in regions with differences in carbonate chemistry, noting that variations in the contemporary ocean are as high as the changes associated with the projected doubling of $pCO_2$, may improve our understanding of how phytoplankton will respond in the future at the ecosystem level.

(7) Quantitative predictions: models are critical for the integration of results from laboratory and field. Ecosystem models based on more detailed understanding of physiological and ecological responses to changes in $CO_2$ and other relevant quantities coupled with general circulation models may lead to quantitative predictions of changes of the global carbon cycle and help to constrain the wide spectrum in future climate scenarios.

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# Ocean acidification: documenting its impact on calcifying phytoplankton at basin scales

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ABSTRACT: In this paper, we evaluate several approaches to discern the impact of ocean acidification on calcifying plankton, over basin scales. We focus on estimates of the standing stock of particulate inorganic carbon (PIC) associated with calcifying plankton since it is thought that these organisms will be the most sensitive to ocean acidification. Chemical techniques provide the greatest accuracy and precision for measuring the concentration of PIC in seawater, but basin-scale chemical surveys are formidably expensive due to the high costs of ship time and analytical instrumentation. Optical techniques, while not yet as precise as chemical methods, provide the opportunity to rapidly sample over much greater spatial scales, with large numbers of samples contributing to each PIC determination (which reduces the SE of each mean determination). Optical measurements from autonomous platforms (buoys and gliders) will provide important depth resolution of PIC, which is otherwise not accessible to ocean color satellites. We propose a strategy for future PIC measurements that employs both optical and chemical measurements on the same water samples. This will ensure adequate knowledge of the PIC backscattering cross-section, critical for satellite PIC determinations at basin scales.

KEY WORDS: Coccolithophores · Ocean acidification · Calcium carbonate · Calcite · Coccolith · Ocean backscattering



## TIME AND SPACE CONSIDERATIONS OF OCEAN ACIDIFICATION

Ocean acidification resulting from anthropogenic $CO_2$ will likely take place over basin scales since anthropogenic $CO_2$ is globally dispersed throughout the atmosphere but with notable spatial bias (Conway et al. 1994). Moreover, the impact of ocean acidification will vary by latitude due to the low $CO_3^{2-}$ concentrations in cold, polar waters as well as increased concentrations of $CO_2$ associated with recently upwelled water, also prevalent at high latitudes (Orr et al. 2005). Ideally, methods to study the impact of ocean acidification should match the global scale of its influence.

Sampling frequencies for detecting the impact of ocean acidification should be at least annual, in order to discern the complicating effects of other well-known climate phenomena such as El Niño that cause strong interannual variability in global weather patterns and marine productivity. Seasonal aliasing also could be significant, however (e.g. due to temperature effects on $CO_2$ equilibrium as well as seasonal variability in phytoplankton primary production and community respiration). We could resolve seasonal variability with a sampling frequency of 8 times $yr^{-1}$, (i.e. the Nyquist frequency, the minimal frequency with which one could unambiguously represent the data without aliasing; Nyquist 1928).

By far, one of the biggest predicted biogeochemical effects of ocean acidification will be on the global ocean carbonate cycle. The global standing stock of particulate inorganic carbon (PIC) depends on calcium carbonate production and dissolution, both of which are expected to be affected by ocean acidification. In the pelagic ocean, PIC is contributed by a host of marine organisms, including: coccolithophores, calcifying dinoflagellates (e.g. *Thoracosphaera* sp.), foraminifera, pteropods, and diverse larval species of calci-

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fying invertebrates. The relative contribution of these groups varies in space and time, but on a global basis, coccolithophores are one of the most important producers of biogenic $CaCO_3$.

The effect of climate change and ocean acidification on coccolithophores will be difficult to predict unequivocally. On the one hand, a lower pH and carbonate ion concentration might reduce calcification in the bloom-forming coccolithophores *Emiliania huxleyi* and *Gephyrocapsa oceanica* (Riebesell et al. 2000, Zondervan et al. 2002), but the response does not appear to be uniform across all coccolithophorid species(Langer et al. 2006). On the other hand, changes in climate (warming and precipitation) could enhance vertical density stratification, which is known to encourage *E. huxleyi* growth over other phytoplankton species such as diatoms (Tyrrell & Taylor 1996). The purpose of this essay is to discuss techniques for large-scale assessment of the impact of ocean acidification on the standing stock of suspended PIC, which is a good overall proxy of the ocean carbonate cycle. The chemical and optical methods we will discuss have been used primarily to assess small PIC particles such as coccolithophores and calcifying dinoflagellates. We will discuss the various methods and end by outlining an overall strategy for detecting basin-scale changes in PIC.

## MEASURING THE STANDING STOCK OF PIC

### Chemical techniques

The standing stock of PIC can be estimated over basin scales using chemical analyses of particulate calcium measured from ships. A number of techniques are available to estimate particulate calcium including X-ray fluorescence (Hurd & Spencer 1991, Twining et al. 2004), and atomic absorption (AA) (including flame AA, graphite furnace AA, and inductively coupled plasma AA [ICPAA]) (Cheng et al. 2004) or inductively coupled plasma mass spectrometry (ICPMS) (Platzner et al. 2008). Background seawater concentrations of calcium (~0.1 mM) present a challenge to measuring particulate calcium, since dissolved $Ca^{++}$ in seawater must be rinsed away completely in order to accurately discern nanogram quantities of particulate calcium. This is usually accomplished by carefully rinsing filters with buffered potassium tetraborate tetrahydrate (Fernández et al. 1993). Moreover, with AA, corrections for background seawater can be made by simultaneous measurement of sodium. Profiles of particulate calcium made using AA have shown excellent precision and consistent decreases in particulate calcium concentrations with increasing depth over the top 500 m (Sherrell et al. 1998), suggesting removal of PIC particles (see also Bishop et al. 1980, 1986). Such chemical observations, along with a plethora of other evidence, have led to the speculation that calcium carbonate particles are dissolving above the lysocline (Milliman et al. 1999).

Chemical techniques such as ICPAA, ICPMS, and even X-ray fluorescence are highly accurate and precise for measuring particulate calcium, yet seasonal, basin-scale ocean surveys, such as WOCE (World Ocean Circulation Experiment)- or GEOSECS (Geochemical Ocean Section Study)-style decadal surveys, would be prohibitively expensive using such techniques. It should be noted that, unless a large volume of water is sampled for particulate calcium, relatively large, rare PIC particles from foraminiferal tests, pteropod shells, and calcareous invertebrate larvae may be undersampled. This highlights an advantage of high-volume, *in situ* filtration units (Bishop et al. 1985, Thomalla et al. 2008). Nevertheless, distinct advantages of the chemical techniques are the accuracy and precision associated with the laboratory analyses and the high resolution of vertical profiles of PIC. It should be noted, however, that compared to particulate organic carbon, relatively few data exist on the vertical distribution of PIC (Bishop et al. 1980, Balch & Kilpatrick 1996, Sherrell et al. 1998, Balch et al. 2000, Poulton et al. 2006) and chemical techniques remain the most accurate way to quantify PIC.

### Optical measurements on small volumes

Optical approaches for estimating PIC are based fundamentally on the strong refractive index of calcium carbonate relative to water (1.19) (Broerse et al. 2003), which is significantly greater than relative refractive index of pure biogenic silica (1.06) (Costello et al. 1995) or non-minerogenic phytoplankton (1.05 to 1.06) (Ackleson & Spinrad 1988). The high relative refractive index means that calcium carbonate is an intense scatterer of light. Calcium carbonate also is highly birefringent (it rotates the plane of polarized light by 90°), a property used by micropaleontologists for decades to enumerate biogenic and lithogenic mineral particles (Fig. 1A).

Recently, a technique to document particle birefringence has been adapted to estimate PIC by adding polarizing filters to a transmissometer (Guay & Bishop 2002). It is calibrated using purified mineral suspensions of diatomaceous earth and calcareous sediments. The technique shows promise. One possible limitation is that other organic molecules also can be highly birefringent. For example, in observations of thousands of samples with polarization microscopy, we have observed that zooplankton carapaces, certain

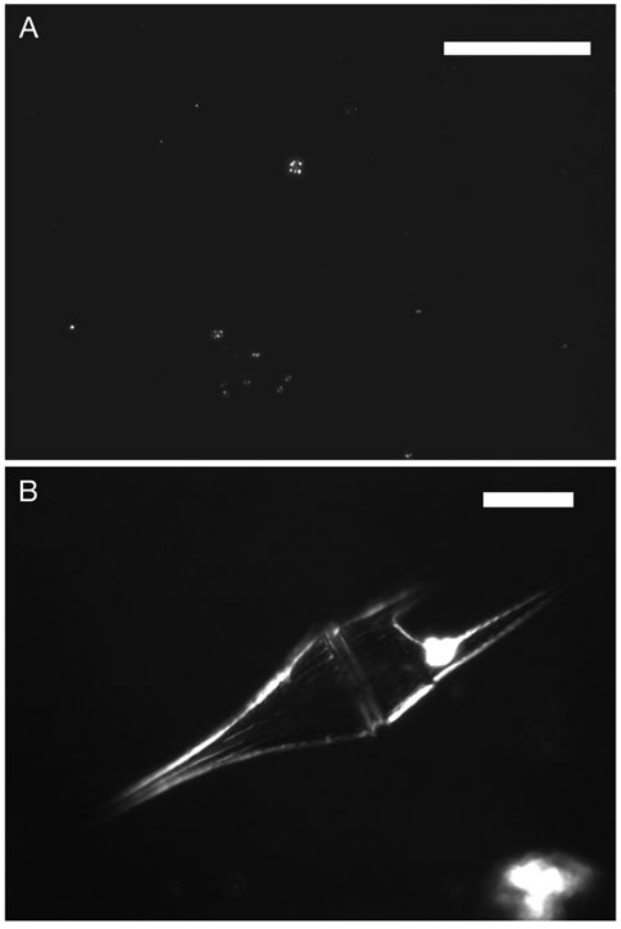


Fig. 1. (A) Birefringence microscopy image of detached coccoliths and plated coccolithophores of various species taken from the north Atlantic Ocean (30.462° N, 19.467° E; 2 m depth) on 26 September 2004 during Atlantic Meridional Transect cruise 15 aboard the RRS 'Discovery'. The technique for preparation of the sample was according to Haidar & Thierstein (2001). Scale bar = 30 µm. Micrograph was made using 1.00 s exposure time. (B) Birefringence microscopy image of a dinoflagellate of the genus *Ceratium* taken from the north Atlantic Ocean (45.599° N, 20.275° E; 5 m depth) on 26 June 2005 during Atlantic Meridional Transect cruise 16 aboard the RRS 'Discovery'. Slide preparation same as in (A). Scale bar = 30 µm. Exposure time was 1.34 s. All other settings identical to those used for the image in (A). Panel (B) is presented as an example of a non-calcifying cell that is birefringent. Birefringent organic debris is relatively common in natural samples, thus the presence of such particles must be included in the calibration of the birefringence technique for particulate inorganic carbon (PIC) quantification

dinoflagellate thecae, as well as generic detritus can be birefringent (e.g. Fig. 1B). This would mean that in field samples, organic detritus could potentially lower the accuracy of birefringence-based PIC estimates. Nonetheless, provided the above transmissometer technique is calibrated with suspensions of naturally occurring particles (including naturally occurring PIC and organic detritus), such errors should be easily quantifiable. This technology has been adapted for use on autonomous drifters (Bishop et al. 2004), which provides useful information on the standing stock of PIC over the entire water column and at mesoscale spatial domains, over periods of days to months. It would be especially useful for quantifying PIC in coccolithophore blooms.

Optical backscattering of PIC can also be used to estimate the standing stock of PIC. Acid-labile backscattering represents the backscattering that disappears following the lowering of seawater pH to <5.8. This technique lends itself to semi-continuous measurements aboard ships, in which backscattering and pH are measured continuously in seawater from the ship's non-contaminated seawater system. Every 2 min, the pH is lowered using a weak acid to dissolve PIC. Once the pH stabilizes at the lower value, optical backscattering is re-measured with a light-scattering photometer (which samples the optical volume scattering function). The difference between total and acidified backscattering measurements represents 'acid-labile backscattering', $b_b'$. By using the same photometer for acidified and unacidified measurements, this eliminates inter-instrument calibration issues and causes only minor spatial aliasing in the $b_b'$ measurement (Balch et al. 2001).

The spatial resolution of this method, at typical ship speeds, is about 1 km, the same resolution as satellite ocean color measurements. Adding a second sensor (one for raw seawater and one for constantly acidified seawater) would allow this spatial scale to be reduced further, but at the expense of increased errors due to sensor inter-calibration. Estimates of $b_b'$ can be calibrated to PIC concentration as a power function, which typically explains about 77 to 85% of the variance in PIC concentration, in non-bloom and bloom waters alike (Balch et al. 2001). Drops in the explained variance can be attributed to differences in particle backscattering cross-section for different-sized calcium carbonate particles (Balch et al. 1999).

### Optical techniques and remote sensing

While optical scattering by PIC occurs in all directions (forward and backward), it is the strong backward scattering ($b_b$) of PIC that is critical to its being remotely sensed from space (Gordon et al. 1988). Remote sensing reflectance at a given wavelength, λ, is a function of both absorption, a(λ), and backscattering, $b_b(\lambda)$, and varies as $b_b(\lambda)/a(\lambda)$. Coccolith PIC has barely measurable absorbance (Balch et al. 1991), thus the presence of PIC principally elevates $b_b$, thus increasing reflectance.

One complication in the optical remote sensing of PIC is that the relationship between $b_b(\lambda)$ or total integrated scattering ($b(\lambda)$) versus PIC concentration is not necessarily the same for different-sized PIC particles. For example, optical scattering per unit PIC (otherwise known as the scattering cross-section, $b^*$, in units of $m^2$ $(mol\ PIC)^{-1}$) is orders of magnitude lower for a large particle like a pteropod than for a small coccolith (Balch et al. 1996). Moreover, the backscattering cross-section, $b_b^*$, shows moderate variability between different species of coccolithophores (Balch et al. 1999). This means that enhanced backscattering in the ocean caused by PIC is mostly due to small PIC particles like coccoliths and is negligible for larger particles such as foraminifera and pteropods. Moreover, this illustrates an important limitation of optical PIC determinations (whether based on scattering or birefringence) since the scattering cross-sections of small coccoliths are not constant but have some degree of variability, and layering of coccoliths, such as around cells, can cause nonlinear variability in their volume-scattering properties.

Information on the backscattering cross-section of field PIC particles has been critical for development of a coccolithophore remote-sensing algorithm, which is fundamentally a backscattering algorithm (Gordon et al. 2001, Balch et al. 2005) (Fig. 2A). These algorithms are not just for high-reflectance coccolithophore blooms but can be used in non-bloom situations as well. While remote-sensing algorithms for PIC are less precise than chemical PIC measurements, regional and temporal binning of satellite data allows time/space averages to be calculated with estimated SEs well below the concentration of PIC in seawater (e.g. the SE for 36 km, monthly binned data is 6.67 nM PIC; Balch et al. 2005). A frequency plot of ship and satellite-derived PIC concentration demonstrates similar concentration ranges in the Atlantic Ocean from 50° N to 40° S (Fig. 2B). Plots of satellite-derived PIC concentrations versus ship-based values are highly statistically significant ($p < 0.001$), although they account for only about 63% of the total variance for a linear fit (SE: 0.069 µM) or 25% of the total variance for a power fit (SE: 0.33 log units) (Fig. 2C). This result is to be expected, especially in oligotrophic waters in which there are other particles that affect the average background backscattering. In coccolithophore blooms, the relative precision of the PIC determination is higher due to the fact that PIC dominates all other particle backscattering (increased signal-to-noise ratio).

Along-track comparisons of satellite versus ship-derived PIC concentrations show regions of consistent satellite bias (Fig. 2D,E) probably associated with differences in water mass and particle types and mean changes in the background backscattering. Recent empirical algorithms also have been developed to estimate coccolithophore calcification from satellite, based on sea surface temperature, chlorophyll, PIC, day length, and depth (Balch et al. 2007). Determination of both PIC standing stock and production rate allows the estimation of PIC turnover times, which are relatively fast, typically on time scales of ~5 d (Balch & Kilpatrick 1996, Balch et al. 2000, Poulton et al. 2007).

Another complication to remote sensing of PIC is that most of the reflectance from the surface ocean originates from the top 2 optical depths of the euphotic zone (i.e. waters above the depth where irradiance exceeds 13.5% of surface irradiance) (Gordon & McCluney 1975). Indeed, the reflected light emanating from the sea is heavily weighted to the top optical depth (i.e. water above the depth of 37% irradiance penetration). Phytoplankton inhabit the euphotic zone, usually defined as the zone where irradiance is ≥1% of surface irradiance. Since light disminishes exponentially with depth, the euphotic zone encompasses 4.6 optical depths ($\ln(0.01) = -4.6$). Thus, passive remote sensing techniques only 'see' the surface depths of the euphotic zone, and miss the deeper phytoplankton populations. This suggests that *in situ* measurements (whether from glider, buoys, or ships) will be important for resolving the deep PIC not discernible from satellite. It should be noted, however, that despite this restriction of satellite optical measurements to the top optical depths, satellite images of surface phytoplankton chlorophyll have consistently demonstrated remarkably coherent, basin-scale distributions, over time scales of days to years (McClain et al. 2004). Such rapid evaluation of surface phytoplankton variability across the globe can only be achieved through satellite remote sensing.

## A STRATEGY FOR THE DETECTION OF LONG-TERM, BASIN-SCALE VARIABILITY IN PIC

Chemical and optical techniques for measuring PIC have important biases. Chemical measurements allow better depth resolution, but they require labor-intensive, ship-based sampling. This limits the numbers of samples that can be taken, thereby limiting the spatial resolution and frequency of sampling. Optical estimates have the advantage of large sample sizes and rapid sampling frequency, but lower precision and limited depth resolution. The latter will require deep *in situ* measurements or statistical modeling of the PIC profile, just as others have modeled the relationship between surface chlorophyll and integrated chlorophyll for the determination of euphotic-zone phytoplankton biomass (Morel 1988, Platt et al. 1988, Balch et al. 1992). Similarly, Mitchell (1996) found that chlorophyll algorithms had to

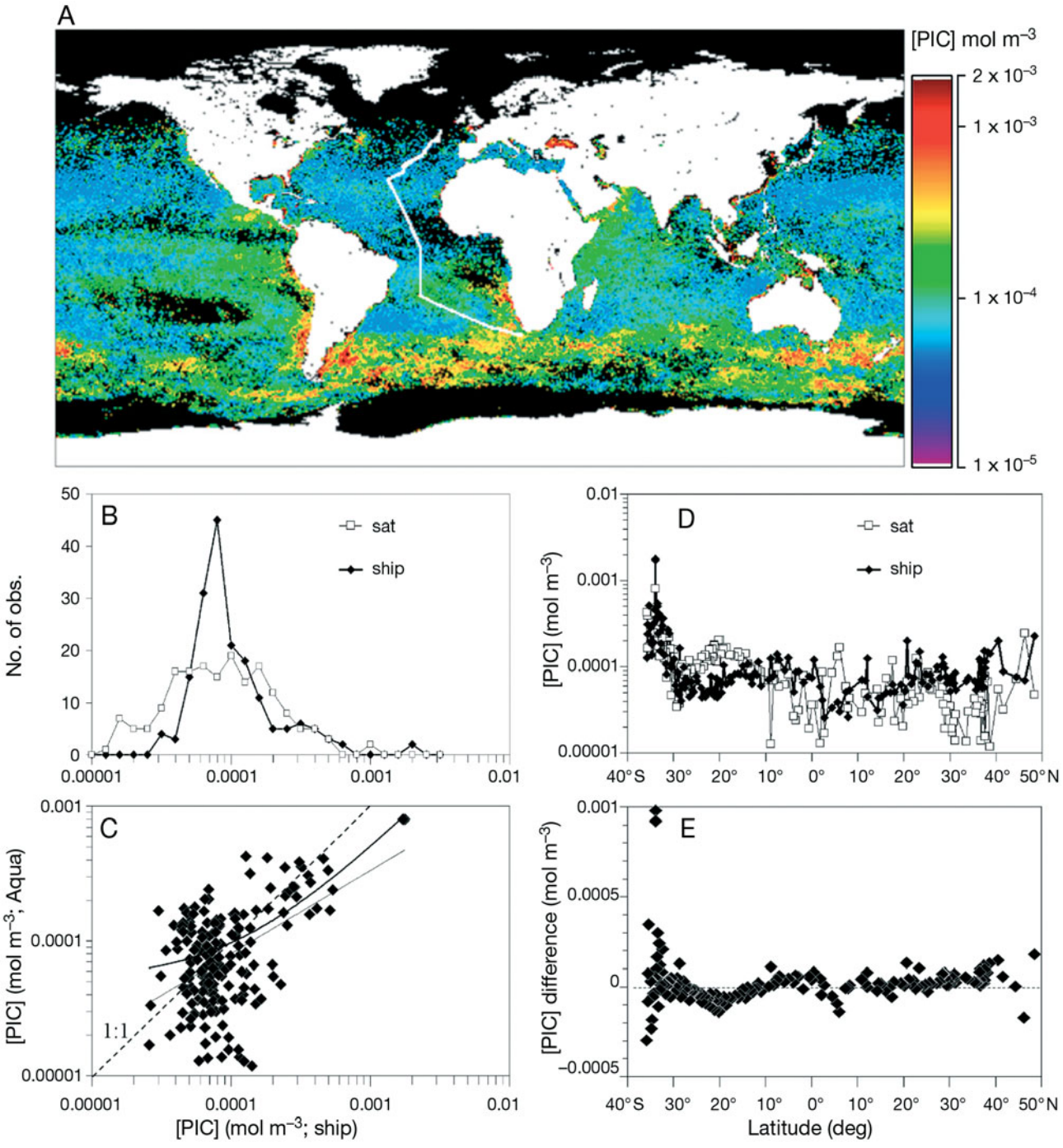


Fig. 2. (A) Global monthly MODIS-Aqua composite image of particulate inorganic carbon (PIC) concentration for November 2005, as determined using the merged 2-band/3-band PIC algorithm (Gordon et al. 2001, Balch et al. 2005). Color bar for PIC concentration shown on right side. Transect of Atlantic Meridional Transect cruise 17 (50° N to 40° S) aboard the RRS 'Discovery' shown as white line. (B) Frequency plot of PIC concentration values for both ship (◆) and satellite (□) measurements. Results demonstrate similar overall distributions; median values differ by 0.9% (satellite greater), average values differ by 16% (ship greater) and the modes differ by 11% of each other (ship greater). SDs of the data distributions are 0.375 log units for the satellite results and 0.302 log units for the ship data. (C) Monthly-binned satellite-derived PIC values plotted against surface PIC values measured aboard ship. Dashed line represents the 1:1 relationship. Bold upper line represents the best-fit line ($y$ [± $6.94 \times 10^{-5}$] = 0.4548 [± 0.026] $x + 5 \times 10^{-5}$ [± $6.2 \times 10^{-6}$]; $r^2 = 0.63$; $F = 295$; $p < 0.001$). Lower fine line represents the best-fit power function ($y$ [± 0.326] = 0.0237 [± 0.0199] $x^{0.6173\ [\pm\ 0.0816]}$; $r^2 = 0.25$; $F = 57$; $p < 0.001$). Equation values in square brackets are the SEs of each preceeding coefficient. (D) PIC concentration as a function of latitude. Symbols as in (B). (E) Difference in PIC concentration (ship–satellite) shown in (D), as a function of latitude

be adjusted for geographic variability in the absorption cross-section of chlorophyll, and likewise, PIC algorithms may have to incorporate the variability in the PIC-specific backscattering cross-sections in different parts of the global ocean. Insufficient data currently exist to resolve whether there are coherent trends in $b_b^*$ in space and time in the sea, but such trends, if they exist, would provide invaluable information for the global remote sensing of PIC. This information will be critical for assessing whether coccolithophores are changing their geographic distribution as a function of ocean acidification.

To document the effect of ocean acidification on the global carbonate cycle, methods are needed to accurately extrapolate point measurements over basin scales. We believe that the best strategy will involve a combined chemical and optical approach, using multiple platforms. That is, time-series sites should routinely measure PIC profiles using both chemical and optical techniques. Chemical techniques will provide accurate estimates of PIC standing stock (highly temporally resolved but spatially limited). Such measurements would probably be done aboard ships. Parallel optical analysis of PIC in the same shipboard water samples will provide the information needed to calibrate optical PIC algorithms (whether for in-water or satellite-derived optical measurements).

Ships and fuel are increasingly expensive and other platforms should be considered in this strategy such as profiling buoys (Argo Science Team 1998, Alverson & Baker 2006) and gliders (Schofield et al. 2007); gliders can extend long-term coverage of the ocean, on time scales of months, spatial scales of 500 to 1000 km and depth scales of thousands of meters. Profiling floats have been successfully equipped with optical instrumentation (Son et al. 2006, Boss et al. 2008) as well as instrumentation to estimate particulate organic carbon (Bishop et al. 2004). Outfitting floats with PIC birefringence sensors (Guay & Bishop 2002) or acid-labile backscattering sensors (Balch et al. 2001) should be straightforward, provided that there is a robust field calibration. Gliders are now available 'off the shelf', equipped with highly sophisticated radiometers, absorption, and backscattering sensors. Fundamentally, the critical link to interpret PIC data from autonomous platforms will be the quality of the calibration between PIC concentration and optical properties.

While PIC remote sensing only works for the smallest calcite particles (i.e. coccolithophores and coccospheres), standing stock measurements of PIC associated with foraminifera and pteropods will be critical for understanding the other major components of the global PIC standing stock. Observations of these heterotrophic organisms will have to extend much deeper than the euphotic zone due to their wide-ranging depth preferences and will require sampling methods and volumes appropriate for their larger sizes and typically lower concentrations in sea water, relative to coccolithophores.

## NEW DEVELOPMENTS

There are some new developments for documenting the carbonate cycle over large spatial scales. Techniques for measuring the inorganic carbon system in seawater (e.g. total alkalinity, dissolved inorganic carbon concentration, $pCO_2$, and pH) have improved sensitivity, accuracy, and precision (DeGrandpre et al. 2002, Nemzer & Dickson 2005, Martz et al. 2006, Seidel et al. 2006), and the availability of certified reference materials (CRMs) has been critical to achieving high-quality, reproducible measurements (Lamb et al. 2001, Sabine et al. 2002a, Chung et al. 2003, Dickson et al. 2003). *In situ* PIC dissolution rates have been estimated by combining total alkalinity increases (corrected for the effect of salinity and nitrate) in a water parcel with age estimates obtained from chlorofluorocarbon (CFC-11 and CFC-12) concentrations or carbon-14 ages (for deeper waters where CFC-11 and CFC-12 were not detectable) (Feely et al. 2002, 2004, Chung et al. 2004, Berelson et al. 2007). Specific details of this calculation can be found in Feely et al. (2004). Such alkalinity-derived estimates of PIC dissolution in the upper ocean, which integrate over large spatial and temporal scales, suggest that the aragonite and calcite saturation horizons in several regions are shoaling as a result of oceanic uptake of atmospheric $CO_2$ (Feely et al. 2002, 2004, Sabine et al. 2002b, Chung et al. 2003, 2004, Berelson et al. 2007) and the reported increase in alkalinity (Sarma et al. 2002) implies that enhanced dissolution of PIC particles has already occurred. While the areas of undersaturation with respect to aragonite and calcite appear to be expanding in several areas (Feely et al. 2004), there is currently no long-term monitoring effort to track and quantify PIC dissolution fluxes. Because increased PIC dissolution in the upper water column enhances the ability of the oceans to absorb anthropogenic $CO_2$, it is an important feedback in the carbon cycle.

There are 2 recent optical advances worthy of note. One new development is the use of range-gated light detection and ranging (LIDAR) techniques for understanding the vertical distribution of optically scattering particulate matter throughout the euphotic zone. This has been accomplished from aircraft (Chekalyuk 2006, Cowles et al. 2006) and more recently from satellite sensor (Koziana et al. 2006, Y. Hu pers. comm.). While the technology is still young, such

measurements will add the depth dimension to remotely sensed PIC measurements, thereby providing the means to directly measure backscattering over the water column and vertically integrate PIC instead of relying on statistical information to extend surface measurements to depth.

Another new development concerns the multi-angle imaging spectroradiometer (MISR) instrument on board the NASA Terra satellite platform. This instrument measures radiance at 9 different angles (–70° to 70° from nadir), at 4 bands (blue, green, red, and near-infrared), in a narrow, 250 km swath, and with a 9 d global repeat time. While it is primarily designed to look at atmospheric properties, with careful atmospheric correction, it can be used to examine whether the reflectance coming from the sea surface is isotropic (the same in all directions). There is some preliminary evidence (J. Martonhik & D. Diner pers. comm.) that MISR can detect differences in the angular dependence of reflectance associated with turbid coccolithophore blooms versus non-coccolithophore dominated waters. Certainly laboratory evidence based on optical volume-scattering measurements in coccolithophore cultures (Voss et al. 1998) verified that the volume-scattering function of coccolithophores is flatter in the backwards direction. It is not known whether the space-based observation of more isotropic backwards scattering in a coccolithophore blooms is true for all phytoplankton or is unique to coccolithophores. This clearly deserves more work.

## CONCLUDING REMARKS

A combination of in-water (i.e. ship, autonomous vehicle, buoy) optical and chemical measurements, plus satellite optical measurements, will be needed to fully discern the effect of ocean acidification on the standing stock of PIC, across ocean basins, and over seasonal to decadal time scales. Such results will be important for modeling the ocean biosphere, albedo (Tyrrell & Taylor 1996, Tyrrell et al. 1999), and DMS production (Malin et al. 1993, Matrai & Keller 1993), just to name a few examples. Chemical observations of PIC will remain key for the documentation of ocean acidification, particularly through global-scale research programs (like WOCE or GEOSECS). 'Decadal repeat' chemical measurements of PIC will accurately assess long-term change in the carbonate cycle associated with ocean acidification. Estimation of basin-scale changes in the carbonate cycle over sub-decadal time scales, however, will best be achieved through dedicated optical sampling with autonomous buoys (e.g. the ARGO program; Balmaseda et al. 2007) and ocean color satellites.

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# Possible effects of ocean acidification on coral reef biogeochemistry: topics for research

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ABSTRACT: This paper is a short review of recent literature on how ocean acidification may influence coral reef organisms and coral reef communities. We argue that it is unclear as to how, and to what extent, ocean acidification will influence calcium carbonate calcification and dissolution, and affect changes in community structure of present-day coral reefs. It is critical to evaluate the extent to which the metabolism of present-day reefs is influenced by mineral saturation states, and to determine a threshold saturation state at which coral communities cease to function as reefs.

KEY WORDS: Ocean acidification · Climate change · Coral reefs · Biogeochemistry



## INTRODUCTION

Ocean acidification is the progressive increase in hydrogen ions ($H^+$) in the world's oceans as a result of rising partial pressure of atmospheric carbon dioxide ($CO_2$), i.e. a decrease in seawater pH, where pH = $-\log_{10}[H^+]$; see Dickson (1984) for a review of the different pH scales for seawater. As atmospheric carbon dioxide has increased as a result of the burning of fossil fuels, increasing amounts of $CO_2$ have entered the ocean and reacted with water (Sabine et al. 2004). When $CO_2$ gas reacts with water, carbonic acid is formed and the ocean becomes progressively more acidic ($CO_2 + H_2O = H_2CO_3 = HCO_3^- + H^+ = CO_3^{2-} + 2H^+$), driving the $CO_2$ chemical equilibrium toward $CO_2$ and $HCO_3^-$, reducing $CO_3^{2-}$, the carbonate ion.

Several oceanic feedback loops buffer pH, but presently these buffering mechanisms are considered relatively small, and will not counteract the falling pH over the next 100 yr (Andersson et al. 2006, 2007). $CO_2$ gas influx and efflux between oceans and atmosphere are large terms in the overall oceanic carbon budget, with substantial errors (Houghton 2007). Nevertheless, the scientific community has observed an estimated decrease of 0.1 pH units in the surface ocean in the last 100 yr and current trends in atmospheric $CO_2$ partial pressure project a further change of 0.3 to 0.4 pH units over the next 100 yr (Sabine et al. 2004, Orr et al. 2005). The above calculations are based on a stable total alkalinity. Thus, over the next 100 yr, $CO_2$ gas dissolved in tropical oceans is expected to increase 200 to 250%, and $CO_3^{2-}$ is expected to decrease 35 to 50%, reducing the saturation state of seawater with respect to calcium carbonate minerals (Orr et al. 2005).

The saturation state of seawater for a mineral ($\Omega$) is a measure of the thermodynamic potential for the mineral to form or to dissolve; specifically it is the product of the concentrations (or activities) of the reacting ions that form the mineral ($Ca^{2+}$ and $CO_3^{2-}$), divided by the product of the concentrations of those ions when the mineral is at equilibrium ($K_{sp}$), that is, when the mineral is neither forming nor dissolving:

$$\Omega = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}} \quad (1)$$

when $\Omega > 1.0$, the formation of the mineral is thermodynamically favorable; when $\Omega < 1.0$, the dissolution of the mineral is favorable. Aragonite $\Omega$ ($\Omega_{arag}$) of surface seawater is expected to decrease throughout the tropics from the present-day values of 3 to 3.5 to 2 to 2.5 in 100 yr; and the ratio of dissolved $CO_2$ gas to $CO_3^{2-}$ will increase by a factor of 4 (Orr et al. 2005).

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Based on principles of thermodynamics, the rate of formation of carbonates is positively correlated to Ω; this pervading principle has been central to biological and chemical production, distribution and dissolution of carbonates in the oceans (Feely et al. 2004, Zachos et al. 2005), as well as tropical coastal seas (Broecker et al. 2001, Morse et al. 2003). Thus, there has been a long standing observation that the distribution of coral reefs is highly correlated to $\Omega_{arag}$ in the ocean, implying that the limits of coral reef formation may not be controlled solely by temperature, light, salinity or substrate availability (Grigg 1982, Smith & Buddemeier 1992, Buddemeier & Fautin 1996). Further investigations have explicitly stated that $\Omega_{arag}$, together with light and temperature, set boundaries for coral reef biogeography (Kleypas et al. 1999a). The present paper will discuss the effects of ocean acidification on coral reefs from the above estimates of changes in pH and $CO_2$ equilibria.

There are 2 possible major effects of the changing ratio of $CO_2$ gas to $CO_3^{2-}$ on coral reefs: (1) changes in organism and community rates of calcification and dissolution, and (2) changes in relative metabolism of autotrophs, cyanobacteria and bacteria, which in turn have the potential to alter community structure and biogeochemical cycles.

## CALCIFICATION

### Impact of decreasing saturation state on organismic calcification rates

Saturation state has been shown to affect growth in calcifying green algae, crustose coralline algae and corals (reviewed by Kleypas & Langdon 2006). The first estimates of total pre-industrial to 2100 reef calcification decreases were 17 to 40% (Gattuso et al. 1999, Kleypas et al. 1999b). With increasing sea surface temperature (SST) and reduced Ω, there is concern that coral reefs may soon reach a threshold of 'no return', losing corals and other calcifiers, becoming dead carbonate platforms covered in macro-algae (Hoegh-Guldberg et al. 2007). Several studies have observed thresholds of coral growth and coral reef development at Ω = 3.0 to 3.3 based on the geographic distribution of coral reefs (Kleypas et al. 1999b, Guinotte et al. 2003, Hoegh-Guldberg et al. 2007, Buddemeier et al. 2008). Considering the enormity of the postulated impact, it is crucial to continue research into how Ω influences the growth of a variety of different taxa. There are many questions remaining to be answered, however, before thresholds of growth/calcification can be established. For example, responses of coral to Ω vary between experiments (see Langdon & Atkinson 2005, their Fig. 9). Thus, we suggest several avenues of research (next subsections), all towards understanding how Ω affects the basic calcification mechanism, and trying to establish some confidence in a particular value of Ω at which reefs might degrade.

### Saturation state versus co-varying parameters

Although coral calcification is unquestionably influenced by Ω, it is not clear whether calcification is also responding to other co-varying parameters, such as pH, $HCO_3^-$ or $pCO_2$. For example, in some cultures of *Emiliania huxleyi* (a carbon-limited coccolithophore) grown under high $pCO_2$ and high nutrients, inorganic and organic carbon production and cell size were enhanced despite the decrease in calcite Ω ($\Omega_{calc}$) (Iglesias-Rodriguez et al. 2008). Thus, concerns have arisen regarding experiments using only HCl to decrease pH, without further addition of bicarbonate to offset the drop in total alkalinity. Achieving reduced pH by $CO_2$ bubbling or by combinations of both acid and bicarbonate to maintain constant alkalinity, is thought to better mimic future $CO_2$ scenarios.

The extent to which increased dissolved inorganic carbon (DIC) can counteract the effect of decreasing Ω on coral calcification is considered moderate (Kleypas & Langdon 2006) because (1) $HCO_3^-$ (the substrate for photosynthesis) will increase only about 14% under doubled $pCO_2$ conditions; (2) increased $pCO_2$ is usually assumed to have little or no effect on photosynthesis (Reynaud et al. 2003, Schneider & Erez 2006); (3) it is not evident that an increase in photosynthesis will necessarily lead to increased calcification (discussed by Kleypas & Langdon 2006). Some studies have, however, shown an enhancement of coral growth (calcification) after an increase in DIC (Marubini & Thake 1999, Schneider & Erez 2006, Herfort et al. 2008), suggesting that the ambient DIC concentration of seawater may limit the calcification rates of hermatypic corals. In some experiments, an increase in DIC concentration also resulted in an increase in photosynthesis (Herfort et al. 2008, Marubini et al. 2008). Marubini et al. (2008) recently reported that *Stylophora pistillata* nubbins grew faster in bicarbonate-enriched seawater independent of pH conditions (pH 7.6 to 8.2). Thus, it is essential that studies of coral calcification and acidification report details of the DIC parameters and not only pH.

### Mechanism of coral calcification

Inadequate understanding of the mechanism of coral calcification limits our ability to provide an accurate prediction of the effect of increasing atmospheric $CO_2$

(Gattuso et al. 1999). It is still not well understood how the elemental composition and physical chemistry of the external environment interacts with biological control under different saturation state conditions (Cohen et al. 2006). In hermatypic corals, the supply of $Ca^{2+}$, as well as $HCO_3^-$ derived from host tissue respiration (via a carbonic anhydrase), are biologically controlled (Allemand et al. 2004). Geochemical models (e.g. Adkins et al. 2003, Gaetani & Cohen 2006, Sinclair & Risk 2006), however, have considered diffusion of $CO_2$ across the calicoblastic epithelium, and passive entry of seawater, e.g. through pericellular channels (see Cohen & McConnaughey 2003). The level of control of skeletal organic compounds (the organic matrix synthesized by the calicoblastic cells) over the chemistry and growth of coral skeleton is also a topic of debate (Meibom et al. 2007). Thus, considering these models of coral calcification, there is still some question as to why external concentrations of carbonate should have such a strong effect on calcification.

Even though calcification observed under light is greater than that under dark conditions (reviewed by Gattuso et al. 1999), the relationship between calcification and Ω appears to have a similar slope in both the light and the dark (Ohde & Hossain 2004, Schneider & Erez 2006). One interpretation of this evidence is that a simple diffusion pathway must exist, possibly revealing an increase in the flux of bicarbonate from enhanced light respiration (see discussion in Marubini et al. 2008). Based upon morphological evidence, the calicoblastic cell layer is regarded as a 'tight' epithelium, reducing the ability of $Ca^{2+}$ and $CO_3^{2-}$ to diffuse away via a paracellular route (Clode & Marshall 2002). An increase in outward diffusion of carbonate when external carbonate is low cannot be dismissed, however. A decrease in pH may affect different cellular processes, e.g. anionic permeability (Gattuso et al. 1999), or pH regulation during the calcification process (Marubini et al. 2008). Indeed, $H^+$ ions produced during calcium carbonate precipitation ($HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+$) are removed from the calcification sites by an energy-dependent carrier ($Ca^{2+}$-ATPase), thereby increasing Ω (see reviews by Cohen & McConnaughey 2003, Allemand et al. 2004).

Studies are needed that focus on the physico-chemical characteristics of the sub-calicoblastic space under different saturation state conditions, or on actual morphological features connecting the calicoblastic cells to seawater. For example, Tambutté et al. (2007) report that some tissues calcifying at the highest rates in *Stylophora pistillata* consist only of ectodermal cell-layers separated by mesoglea. It would be informative to determine whether these observations give more clues about the mechanism of coral calcification. The magnitude of the response to low Ω seems to be constant between coral species (Marubini et al. 2003). There should be, however, further effort to compare taxa with differing sensitivities to external saturation state.

### Interaction with other parameters

Hermatypic coral calcification is a strong function of light (Gladfelter 1984, Allemand et al. 2004), shows temperature optima (Marshall & Clode 2004), and is affected by nutrients (Tanaka et al. 2007) and particulate feeding (Houlbrèque et al. 2003), yet there are no studies on the effects of Ω that adequately control all 4 of these variables. The magnitude of the effect of Ω on calcification increases with increasing light and temperature (Marubini et al. 2001, Reynaud et al. 2003). When nutrients are added to the waters surrounding corals, however, the corals become less sensitive to Ω (Atkinson et al. 1995, Langdon & Atkinson 2005, selected data from their Fig. 5), perhaps because symbiotic dinoflagellates (*Symbiodinium* spp.) enhance calcification by providing the biochemical precursors of the organic matrix (Muscatine et al. 2005). Nutrient loading, typical of that seen in the field, increased the Ω threshold for calcification of a mixed coral community from 1.5 to 1.0 (recent experiments, unpublished, Fig. 1). This coral community dominates in Hawaii, and calcifies at $\Omega < 3.0$,

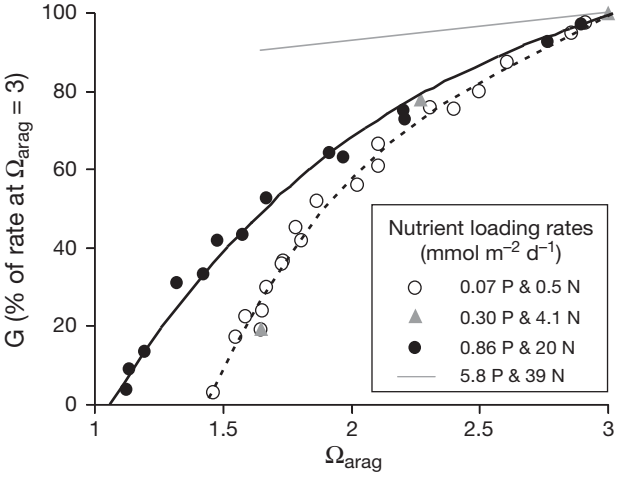


Fig. 1. Effect of nutrient loading on coral calcification rate (G). Coral communities (0.3 $m^2$; *Montipora capitata*, *Porites compressa* and *Pocillopora damicornis*) were placed in a wave flume with equal light, gross primary production, water motion and initial aragonite saturation state ($\Omega_{arag}$). Nutrient loading was varied from extremely low values to those representative of field conditions. The closed black circles (black line) represent communities that were given natural nutrient loading rates and the open circles (dashed line) are low nutrient conditions. The grey triangles and the grey line are data from Langdon & Atkinson (2005) for a nutrient loading experiment with the same species. Data are normalized to $\Omega_{arag} = 3.0$ for intercomparison. The nutrient loading rates are in the key

contrary to calcification threshold values stated in Buddemeier et al. (2008). Also, nutrient loading as particles often increases skeletal growth (Bongiorni et al. 2003, Houlbrèque et al. 2003, Shafir et al. 2006), but no study to date has assessed the possible interactions between feeding and Ω. It is our suspicion that many experiments have been performed in low light, relatively starved conditions, with extremely low pH. Future studies should report nutrient and particulate loading as well as optimal light intensities in order to assess the nutritional status of the corals. More research is needed on the effects of Ω, under natural field conditions, for a variety of coral reef calcifying organisms (Schneider & Erez 2006). These are difficult experiments and require improved facilities to control the environmental variables. Data on a variety of taxa may then be synthesized to present a threshold at which corals do not grow (and at which changes in community structure may prove to be unavoidable).

Anthony et al. (2008) reported increased sensitivity of corals to bleaching as a result of acidified seawater conditions. One possible explanation for the loss of zooxanthellae is that high $CO_2$ reduces the efficiency of RuBisCO (maximum activity at pH 9) to react with oxygen radicals, leaving more oxygen radicals to stimulate bleaching in coral tissue. Other interpretations may relate to the greater efficiency of *Symbiodinium* spp. under carbon-rich conditions, and the resulting compensatory reduction in symbiont number (i.e. bleaching). These observations are contrary to published experiments and observations in which corals were grown at very low pH (7.2 to 7.8) without bleaching (Atkinson et al. 1995, Marubini & Atkinson 1999). Clearly, there appears to be a diversity of responses from corals that requires further investigation.

## Community calcification

Are communities of corals showing effects of Ω in the field? This question was addressed by Kinsey in the 1970s. He tried to find some latitudinal variation in calcification, correlating with mean temperature and Ω, and instead developed the concept of standard metabolism and reef zonation, controlled largely by hydrodynamics (Kinsey 1979, 1985). Kinsey made a strong case that community structure and corresponding zonation gave characteristic rates of calcification (reviewed in Atkinson & Falter 2003). Calcification rates vary by an order of magnitude within a coral reef, from areas that have rich coral communities to other areas that are flat and just consist of sand. The higher the 3-dimensional relief of the benthic community, the greater is primary productivity and calcification. In general, daily calcification rates are about 15 to 20% of the gross primary production (Kinsey 1985, Gattuso et al. 1999). Thus, coral reef calcification and coral calcification are strongly a function of gross productivity, even at the scale of a polyp (Al-Horani et al. 2005). It is now assumed that reef calcification is proportional to both gross primary productivity and Ω (Nakamura & Nakamori 2007), but it is not clear to what extent Ω actually affects a whole reef. When a lack of storm damage allows the maintenance of high productivity community structure, by lack of storm damage, rising sea-level and warming temperatures may be more important than small changes in Ω. These issues are clearly important and should be the focus for future research.

Evidence of an effect of Ω on community calcification in the field is still scarce. In a seasonal study, calcification was correlated to Ω (Silverman et al. 2007), however, Ω was also correlated to seasonal changes in temperature, light and nutrients, and it was difficult to identify the forcing parameter. The model for calculating calcification was also based on salinity changes between nearshore and offshore sampling sites and made all variables dependent. Yates & Halley (2006), using a large closed chamber on a reef flat in Hawaii, reported rates of dissolution and calcification as a function of Ω. Their results were quite variable and it was difficult to set a single Ω threshold value. They showed an enhancement of calcification at high Ω, but it is not clear in their study whether calcification was responding to increased productivity or elevated Ω within the chamber. Studies on growth of large *Porites* spp. in the Great Barrier Reef show both inter-decadal increases and decreases in calcification per surface area (Cooper et al. 2008). These data are difficult to interpret on an ecological basis (see Lough 2008, this Theme Section). As a single coral head grows larger (more convoluted and oblique to the light field) light absorption per area decreases (Stambler & Dubinsky 2005). This effect probably reduces calcification per surface area of the coral, even though calcification per planar area of reef may be increasing. There is large variability in environmental light quantity and quality due to daylength, cloud-cover and water turbidity, thus changes in calcification rate per surface area of coral tissue can change daily, seasonally, and even within decades, making it difficult to link coral growth to changes in temperature and Ω.

One of the key issues underlying our understanding of the impact of ocean acidification is how to evaluate the impact of Ω on reef calcification under field conditions. Field studies must obviously involve measurement of light, productivity and respiration. We suggest a comprehensive experiment involving several reefs with different Ωs, to determine how the relationship between primary productivity and calcification is

affected or controlled by temperature, Ω and nutrient input. If calcification is decreasing with respect to Ω, then on a large reef scale, independent of detailed community structure, we should be capable of producing a derivative calcification ratio, G:P (slope of calcification = *f*(gross primary production)) related to Ω. With present technology, it is possible to evaluate calcification and productivity on large reef scales, combining all zones and communities by using a combination of *in situ* measurements, hydrodynamic models and remote sensing products. *In situ* chemical measurements and accurate hydrodynamic models (e.g. Lowe et al. 2008) can be used to measure calcification rates of large areas of coral reefs and remote sensing products are being developed for reef productivity (Hochberg & Atkinson 2007).

### Dissolution of calcium carbonates

The solubilities of high-Mg carbonates from natural reef environments are not well determined (Morse et al. 2006). These carbonates are abundant on reefs and form a significant part of the framework, thus, it is important to determine the Ω or pH at which these minerals will dissolve. There are conflicting views as to the extent to which the Ω of overlying seawater can influence dissolution in sediments. Manzello et al. (2008) showed increased dissolution in the Eastern Tropical Pacific, suggesting that this is from low-pH upwelled water, while Andersson et al. (2007) showed high-Mg calcites were dissolving in Bermuda from natural changes in Ω, but with little difference compared to other places; they pointed out the influence of naturally low pH in sediments. A primary direction of research is to understand the dissolution of these carbonates in a natural system.

Bio-erosion by endolithic phototrophs, which inhabit every available carbonate substrate, should also be better quantified. The endolithic chlorophyte *Ostreobium quekettii* increased its depth of penetration under $pCO_2$ of 750 µatm (Tribollet et al. in press), suggesting increased biogenic carbonate dissolution under high $pCO_2$.

## EFFECTS ON BIOGEOCHEMICAL CYCLES, AND COMMUNITY STRUCTURE SHIFTS

### Carbon and nutrients

It is very likely that increased $CO_2$ will alter the relative growth and efficiency of different groups of organisms (Phytoplankton, Riebesell et al. 2007; Cyanophytes, Levitan et al. 2007; Seagrass, Palacios & Zimmerman 2007). For example, the net photosynthetic rates of epilithic coralline algae decreased in 750 µatm $pCO_2$, while endolithic communities remained constant (Tribollet et al. 2006). Increased $pCO_2$ may stimulate growth of algae that do not have carbon-concentrating mechanisms (Kaplan & Reinhold 1999). It is usually assumed that macro-algae as a group will exhibit little photosynthetic response to increasing $pCO_2$, because most of them possess carbon-concentrating mechanisms. However, some species are carbon-limited with the current levels of dissolved inorganic carbon in seawater (see references in Zou 2005). It is also suggested that the energy used for carbon-concentrating mechanisms can be used for growth when $pCO_2$ is high (Levitan et al. 2007).

Faster growth under high $pCO_2$ may increase C:N:P ratios of macro-algae, further providing relatively low quality food to herbivores. Uptake of phosphate and nitrogen compounds are generally under hydrodynamic control (Atkinson & Falter 2003), thus it is unlikely that increased net production would stimulate increased nitrogen uptake. If this response occurs, then coral reefs may shift to higher export of organic matter, deposition and bacteria remineralization in back-reef areas, creating zones of anoxia. There may also be more export of dissolved organic matter. This scenario would suggest less carbon of higher quality moving up and through the foodweb. On the other hand, it is also quite possible that increased growth of nitrogen fixing cyanobacteria may enhance nitrogen fixation (Levitan et al. 2007). Nitrogen fixation can be a large source of nitrogen to some reefs, thus an increase in nitrogen fixation may further enhance photosynthetic efficiencies and net production. There may be major shifts in the biogeochemistry of reefs, yet we know very little how nutrient cycles are presently coupled to carbon cycles, nor how different groups of algae compete for scarce nutrients.

### Community structure

The recruitment rate and growth of crustose coralline algae is severely inhibited under elevated $pCO_2$, suggesting changes in benthic community structure may occur owing to the impact of ocean acidification on recruitment and competition for space (Kuffner et al. 2008, Jokiel et al. 2008). At a shallow coastal site in the Mediterranean where vents of volcanic carbon dioxide reduce seawater pH (pH 7.8 to 7.9), non-calcareous algae proved to be resilient to naturally high $pCO_2$, replacing typical rocky shore communities (pH 8.1 to 8.2) with >60% cover of Corallinaceae (Hall-Spencer et al. 2008). In the mesocosm experiment performed by Jokiel et al. (2008), however,

the space made available from the reduction in crustose coralline algae cover was not colonized by non-calcifying algae (e.g. turfs). Any advantage of non-calcifying algae under high $pCO_2$ could be offset by increased herbivory (Jokiel et al. 2008). With reductions in crustose coralline algae, coral recruitment may be affected (Hoegh-Guldberg et al. 2007), but coral spawning and recruitment were not affected under elevated $pCO_2$ (Jokiel et al. 2008). Some scleractinian coral species were also found to survive from decalcification as polyps in the laboratory, including normal gametogenesis (Fine & Tchernov 2007).

These experiments are just the beginning to our understanding of the complex response of coral reefs to ocean acidification. Undoubtedly, ocean acidification will create major shifts in community structure that will certainly affect communities of grazers. We suggest developing an enclosed high-$pCO_2$ natural coral reef mesocosm, in which synergistic effects of different organisms responding to changes in water chemistry can be observed. In this way, organismal calcification, growth and competition can be compared with changes in community structure. Also direct measurements and observations of calcium carbonate mineral dissolution in sediments can be achieved.

## RECOMMENDATIONS FOR RESEARCH

A coordinated research effort is required to understand and ascertain whether a decrease in Ω will alter the community structure and function of coral reefs. It is now accepted that dissolved inorganic carbon species are very important chemical parameters of the function of a variety of key taxa comprising coral reefs, but our understanding is limited and quantification almost non-existent. The next challenge is to understand how both organismal and community metabolism interact with dissolved inorganic carbon chemistries. This effort will require a new generation of experimental facilities and instrumentation for reefs. Some suggestions for research directions are to:

(1) continue to evaluate the effects of bicarbonate and carbonate ions on growth and calcification of key taxa, under environmental realistic conditions of light, temperature, nutrients and dissolved inorganic carbon;

(2) study the morphology of carbonate calcification, looking for the structural detail at the sites of calcification;

(3) develop an improved model for coral calcification;

(4) conduct studies on high-Mg carbonate solubility constants for naturally occurring carbonates on coral reefs;

(5) develop several natural coral reef mesocosms, complete with sediments, to observe whole system changes and community structure competition:

(6) develop a program to evaluate the relationships between community metabolism and calcification at several coral reefs with different Ωs, and test whether present naturally-varying Ω drives community calcification rates;

(7) expand efforts in monitoring basic $CO_2$ parameters on a number of coral reefs worldwide, in conjunction with basic community structure data. These do not have to be continuous but must span decades;

(8) study effects of pH on a variety of algae, including endoliths, as well as key species of nitrogen fixing cyanobacteria.

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# Coral calcification from skeletal records revisited

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ABSTRACT: Skeletal growth records in annually banded massive coral skeletons are an underexploited archive of coral responses to environmental changes. Average linear extension and calcification rates in Indo-Pacific *Porites* are linearly related to average water temperatures through 23 to 30°C. Assessing long-term trends in *Porites* extension and density requires caution as there is evidence of an age effect whereby in earlier growth years corals will tend to extend less and form a higher density skeleton than in later years. This does not appear to affect calcification rates. Coral growth characteristics at 2 of 3 reefs in the central Great Barrier Reef provide evidence of a recent decline. This is of concern, although the exact causes cannot be identified. International efforts are required to make full use of both coral growth histories and geochemical tracers contained in massive coral skeletons to understand the nature and significance of recent trends and their possible links with environmental changes such as ocean chemistry, warming tropical oceans and increased frequency of coral bleaching events.

KEY WORDS: Coral calcification · *Porites* · Temperature · Climate · Great Barrier Reef



## INTRODUCTION

The discovery of alternating dense and less dense bands in the calcium carbonate ($CaCO_3$) skeletons of massive corals and their confirmation as annual by autoradiography (Knutson et al. 1972) and radiometric techniques (Macintyre & Smith 1974, Moore & Krishnaswami 1974) opened the door to the 'vast storehouses of information about chemical and physical changes of waters in which they grew' (Moore & Krishnaswami 1974, p. 274). These annual density bands are apparent when slices of coral skeleton, taken perpendicular to the main vertical growth axis of the colony, are X-rayed. Knowing the date of collection of the sample, well-displayed annual band pairs, consisting of a dense and less dense band per year, can be counted back through time to provide a chronology of coral growth.

Starting from this basis, 2 main types of dated records have been obtained from massive corals. The first is growth data, i.e. annual linear extension rate, average annual skeletal density and, combining these, the mass of $CaCO_3$ deposited per year (calcification rate). Hiatuses in coral growth and unusual banding patterns, such as 'stress' bands, can also be seen on X-rays of coral slices. The second derives from geochemical composition analyses of the calcium carbonate skeleton: a wealth of isotopic and geochemical tracers are incorporated into the skeleton during growth (known as 'inclusive' records) and are measured in samples removed from along major growth axes of the coral.

In the 36 yr since the discovery of annual density bands, nearly 800 papers have been published describing analyses of records obtained from massive coral skeletons. In the first 15 yr after their discovery, the majority (60%) of papers examined the annual bands as records of coral growth. In the most recent 15 yr period, however, the vast majority of papers (80%) have dealt with analyses of inclusive records. This change in focus is also reflected in recent reviews by the almost exclusive emphasis on proxy climate and environmental records provided by geochemical tracers from corals (Gagan et al. 2000, Cole 2003, Felis & Patzold 2003, Correge 2006, Grottoli & Eakin 2007). The annual density banding

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pattern appears to have been relegated to the role of an initial visualisation tool for identifying transects for subsequent geochemical analyses and to assist in establishing a chronology. Does this change in focus mean that coral growth records now provide little useful information?

There is now a variety of experimental, modelling and theoretical evidence that coral calcification rates (and those of other marine calcifying organisms) will decrease as the oceans continue to absorb part of the excess atmospheric $CO_2$ produced by anthropogenic activities (Royal Society 2005, Kleypas et al. 2006). In this article, I consider how annual growth records from massive coral skeletons, the commonly used Indo-Pacific *Porites* spp., can contribute to identifying the possible consequences of increasing ocean acidification and warming water temperatures for a major marine calcifying organism. I present

- An update on the spatial temperature control of average *Porites* growth characteristics
- Evidence for possible age effects on coral growth records that could confound detecting long-term trends
- Evidence for recent coral growth changes from 3 reefs in the central Great Barrier Reef (GBR), Australia.

## MEASURING CORAL GROWTH VARIABLES

Three variables describing coral growth can be obtained from the annual density banding pattern: (1) how much the coral is extending each year—i.e. the linear extension rate measured between annual density minima or maxima (mm $yr^{-1}$); (2) average annual skeletal density (g $cm^{-3}$); and (3) the calcification rate (g $cm^{-2}$ $yr^{-1}$)—i.e. the multiple of the first 2 variables, which provides the mass of $CaCO_3$ skeleton deposited per year. These are typically obtained from skeletal slices (~7 mm thick) cut along the plane of the vertical growth axis of a coral core or colony.

The most commonly reported coral growth variable is the linear extension rate. This can be measured directly from X-ray positive prints of skeletal slices with the annual bandwidth defined as the linear distance between equivalent parts of adjacent annual density band pairs (e.g. Hudson 1981). A variety of techniques have been used to measure the less commonly reported skeletal density variable. These include the destructive technique of removing sections of skeleton and determining the weight and volume, and hence the density (e.g. Highsmith 1979, Carricart-Ganivet et al. 2000) and the following non-destructive techniques: 'photo' or 'optical' scanning of the coral X-ray with appropriate $CaCO_3$ standards to obtain absolute skeletal density (e.g. Aller & Dodge 1974, Buddemeier et al. 1974, Grigg 1981, Helmle et al. 2002); computerized tomography (CT) scanning of a coral slice (Logan & Anderson 1991, Bessat & Buigues 2001); and gamma densitometry (Fig. 1), which measures the attenuation through the thickness of a coral slice of a beam of gamma photons (e.g. Chalker & Barnes 1990, Draschba et al. 2000) and has been shown to produce comparable measurements to the optical technique (Carricart-Ganivet & Barnes 2007). Once measurements of linear extension and skeletal density have been obtained, it is simple to produce annual calcification rates.

Unless indicated otherwise, all material used in the following analyses were from the Australian Institute of Marine Science collection of small coral colonies and coral cores (see Lough et al. 1999) and some recently collected short (~50 cm length) cores from *Porites* corals growing in shallow-water (< 10 m) environments of the Great Barrier Reef (GBR), Australia. Dated skeletal growth variables were obtained by gamma densitometry of the coral slices (Lough & Barnes 1997, 2000). For comparative analyses of growth records from different corals, each dated coral series was first standardized by dividing by the long-term mean of

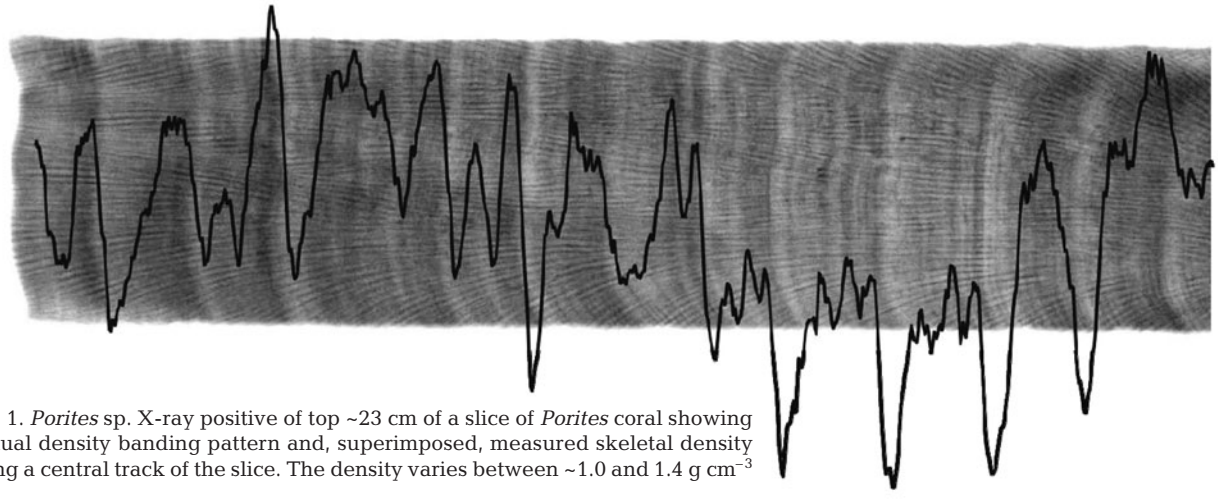

Fig. 1. *Porites* sp. X-ray positive of top ~23 cm of a slice of *Porites* coral showing annual density banding pattern and, superimposed, measured skeletal density along a central track of the slice. The density varies between ~1.0 and 1.4 g $cm^{-3}$

each series. This allowed comparisons of relative trends amongst corals with differing average skeletal growth parameters. Standard linear regression techniques were used to examine relationships between variables and through time.

## TEMPERATURE CONTROL ON AVERAGE *PORITES* GROWTH CHARACTERISTICS

Average coral growth rates in *Porites* from 44 Indo-Pacific reefs were analysed by Lough & Barnes (2000). This dataset has been expanded to 49 reefs with the addition of growth data for 15 short *Porites* cores from 4 sites in the Arabian Gulf (~28° N, 50° E) (Poulsen et al. 2006) and 11 *Porites* colonies from Lihir Island, Papua New Guinea (~3° S, 153° E) (J. M. Lough unpubl. data). For the 49 sites, as reported previously (Lough & Barnes 2000), average skeletal density was inversely related to linear extension rate and calcification rate ($r^2$ = 0.57, p < 0.000; $r^2$ = 0.35, p < 0.000, respectively) and linear extension is the main source of variability in calcification rate ($r^2$ = 0.94, p < 0.000).

Even with the addition of new data for 5 sites (including Lihir Island with the warmest, of all the 49 sites, average annual sea surface temperature [SST] of 29.5°C) there is no change in the significant linear relationship between average annual SST and *Porites* growth characteristics found by Lough & Barnes (2000); average linear extension increases ~3 mm $yr^{-1}$ and average calcification by ~0.33 g $cm^{-2}$ $yr^{-1}$ for each 1°C rise in average SSTs (Fig. 2).

Average linear extension and calcification rates in the massive coral *Porites* are significantly linearly related to average SST. This spatially derived relationship is evident based on corals growing in average water temperatures between ~23 to 30°C. Earlier evidence of increasing coral extension and calcification rates obtained from long coral cores (covering the past 200 to 250 yr) that matched observed temperature increased suggested that, at least initially, some corals may respond to global warming by increasing their growth rates (Lough & Barnes 2000, Bessat & Buigues 2001). This neglects 3 other possible responses of coral growth to the enhanced greenhouse effect. The first of these is reduced or impaired growth as a result of more frequent mass coral bleaching events, though massive *Porites* tend to be more thermally tolerant than branching species (Marshall & Baird 2000). Several recent *Porites* coral cores from the Great Barrier Reef did, however, show growth hiatuses associated with the 1998 and 2002 (Berkelmans et al. 2004) mass coral bleaching events on the GBR (J. M. Lough pers. obs.). The second response is the reduced coral growth as a result of changing ocean chemistry reducing the ability of marine calcifying organisms to form their skeletons (Kleypas et al. 1999), and the third possibility is a non-linear response of coral calcification rates to rising water temperatures with calcification reaching a plateau and then declining at higher temperatures (Jokiel & Coles 1977, Marshall & Clode 2004, Kleypas et al. 2005, Cooper et al. 2008).

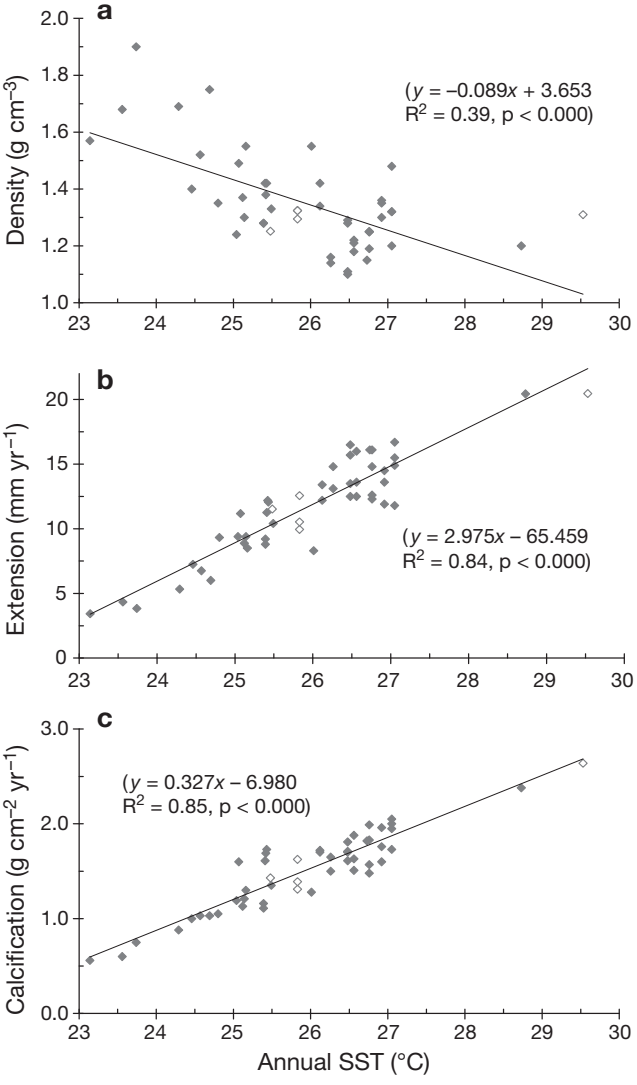


Fig. 2. *Porites* spp. *Porites* growth data averaged across colonies from each of 49 reefs vs. annual average sea surface temperature (SST) for (a) density, (b) extension and (c) calcification. Linear regressions also shown. Open diamonds are data for 4 sites in the Arabian Gulf and 1 site at Lihir Island

## AGE EFFECTS ON CORAL GROWTH RECORDS

Tree-ring widths typically exhibit an age effect with the young tree producing wider annual rings which progressively decrease in width as the tree ages (Fritts 1976). This 'growth curve' artefact has to be removed

before using tree-ring width chronologies for dendroclimatic reconstructions. There has, to date, been no systematic analyses of possible age effects on growth records from massive corals, though Lough & Barnes (1997) noted 'a tendency for higher density to be associated with lower extension rates during the early parts' of 35 long cores from the GBR.

To test for possible age effects on coral growth parameters, growth variables were taken from 43 long *Porites* cores with at least 100 yr of record. The earliest start year was 1572 and the latest 1900. The cores were from inshore, mid-shelf and offshore reefs between ~10° to 24°S on the GBR. All 43 series were then set to start in Year 1, regardless of the actual start year of the record. This start year was the earliest dated year in each core, which was not necessarily the first year of growth of the coral. The 43 series were then averaged for successive 10 and 20 yr periods and tested for significant linear trends with age.

Analysis of age effects in these 43 long-core records all scaled to start in the same year confirms the earlier observation of Lough & Barnes (1997) (Fig. 3). Extension rate showed a significant increase through time though modulated by multidecadal variability. Skeletal density showed a more marked and significant decrease in, at least, the first 100 yr of growth. Average extension rate in Years 61 to 80 and 81 to 100 were significantly higher than in the first 20 yr of record. Average skeletal density in all 20 yr periods up to Years 81 to 100 were significantly lower than in the first 20 yr of record. There was, however, no significant trend in calcification rate associated with colony age.

Application of skeletal growth records to the detection of changes associated with environmental trends, such as decreasing aragonite saturation state and warming water temperatures, requires that the coral's growth characteristics do not change with colony age. Evidence presented here shows that there is an age effect on skeletal density and, to a lesser extent, on linear extension rate in long-lived *Porites*, but not on calcification rate. This is important as it indicates: (1) long-term trends in coral calcification are not biased by age effects, (2) a trend of decreasing skeletal density on its own could potentially be associated with an age effect, but (3) a trend of decreasing density and decreasing extension are unlikely to be associated with an age effect.

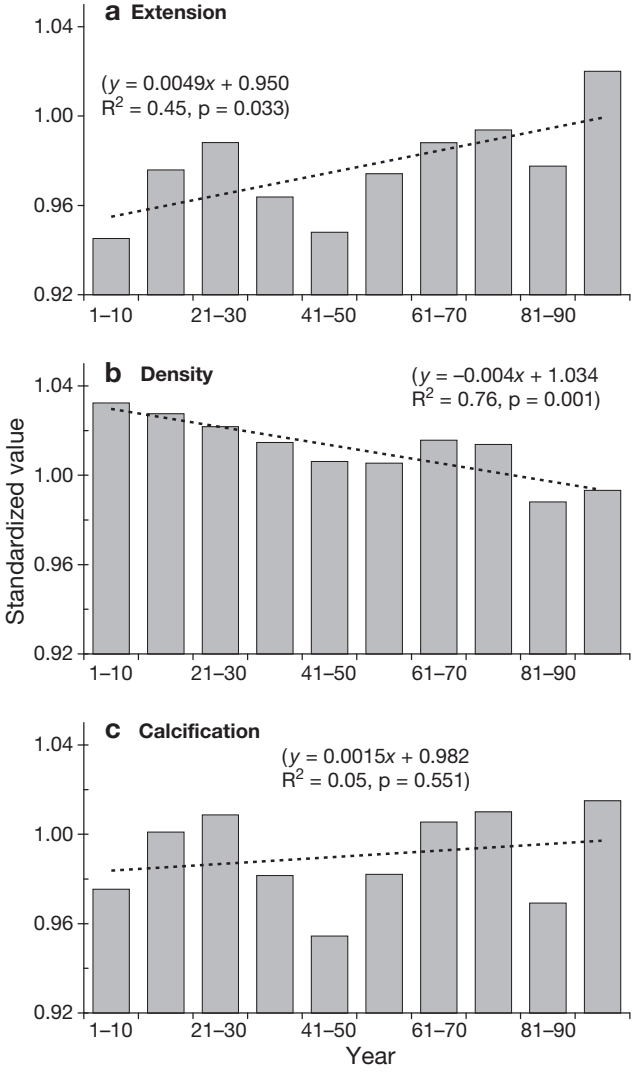


Fig. 3. *Porites* spp. Standardized growth variables for 43 coral cores averaged for 10 yr periods for (a) extension, (b) density and (c) calcification with all cores starting from Year 1 regardless of actual age. Linear regressions also shown

## RECENT CORAL GROWTH CHANGES IN THE CENTRAL GBR

To examine recent changes in coral growth characteristics, coral growth records were examined from 3 reefs in the central section of the GBR: Pandora Reef, an inshore reef (based on between 9 to 25 coral records); Rib Reef, a mid-shelf reef (8 to 27 records); and Myrmidon Reef, an offshore reef (12 to 25 records). Standardized series of linear extension, skeletal density and calcification were averaged for 5 yr periods from 1961 to 1965 through 2001 to 2005 and compared to similarly averaged SST data (Rayner et al. 2003).

For Pandora Reef (Fig. 4a–c) there was a significant decrease through time in linear extension and calcification but no significant trend in skeletal density. There were no significant trends in any of the 3 growth variables at Rib Reef, although linear extension and calcification were notably lower in the most recent 5 yr period (Fig. 4d–f). At Myrmidon Reef, there was a significant decrease through time in skeletal density and calcification rate and although extension also de-

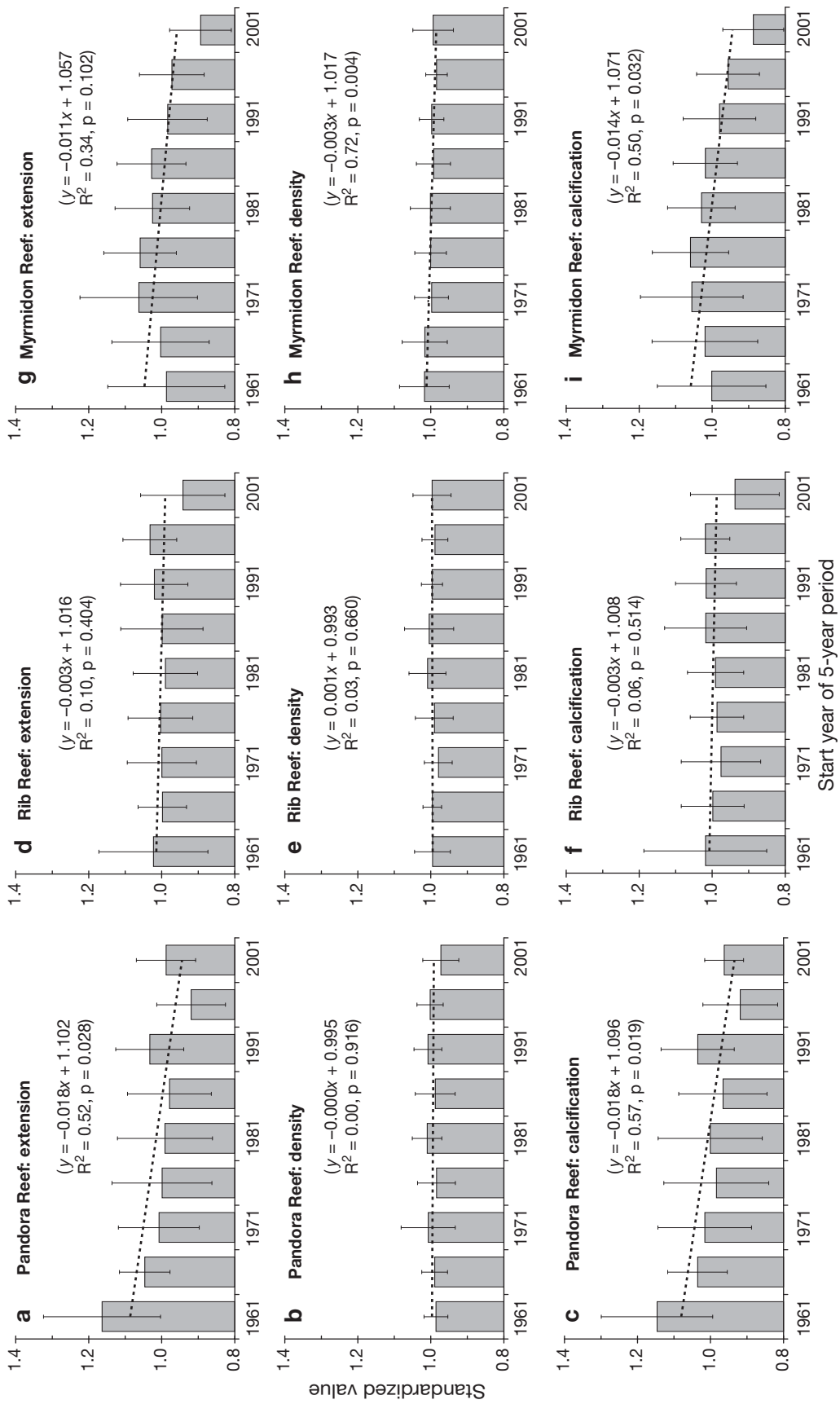


Fig. 4. *Porites* spp. Standardized growth variables (± SD) averaged for 5 yr periods (1961–1965 to 2001–2005) for (a–c) Pandora Reef, (d–f) Rib Reef and (g–i) Myrmidon Reef. Linear regressions also shown

creased, the trend was not significant (Fig. 4g–i). Although there was a certain amount of variability in the 5 yr averages (indicated by the overlapping error bars in Fig. 4) there was evidence of significant decline in calcification rates over the most recent 45 yr period at an inshore (Pandora) and an offshore (Myrmidon) reef in the central GBR. Average annual SSTs on the GBR have significantly warmed since the late 19th century (Lough 2007) and, based on analysis of proxy SST records obtained from massive coral skeletons (Hendy et al. 2002) updated to the present (Lough et al. 2006), are probably the warmest in, at least, the past ~250 yr. In the central GBR there has been significant warming over the 45 yr period corresponding to the examined coral growth records (Fig. 5). Applying the equations linking average annual SST and skeletal extension and calcification rates (Fig. 1) to the observed change in SST between 1961 to 1965 and 2001 to 2005, would give, if only SST was driving coral growth, an increase in extension and calcification rates ~12 to 13%. The observed changes are, however, decreases in linear extension and calcification by ~15 to 16% at Pandora Reef and by 9 to 11% at Myrmidon Reef.

Evidence of enhanced calcification rates in long *Porites* cores from the GBR (Lough & Barnes 2000) only provided data from 1780 through 1979. Results presented here for an inshore (Pandora) and an offshore (Myrmidon) reef in the central GBR, and recently published analyses for 2 nearshore regions in the northern GBR (Cooper et al. 2008), that include growth data subsequent to 1979 show, however, a recent decline in coral growth characteristics. An apparent recent decline in *Porites* growth in the Arabian Gulf was also noted by Lough et al. (2003). In all of these studies, there has been significant warming of ocean temperatures that may have been expected to enhance growth. The exact causes of these declines cannot be identified at present (see Cooper et al. 2008) nor can they, at present, be directly related to lower aragonite saturation state. They may also be evidence of a thermal control on coral calcification rates that have reached an optimum and have now started to decline. There is, however, now some disturbing field evidence, from this study and Cooper et al. (2008) for recent declining growth in massive *Porites*.

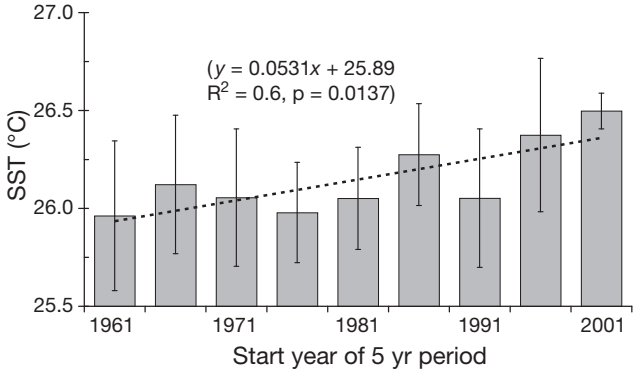


Fig. 5. Average 5 yr (1961–1965 to 2001–2005) sea surface temperature (SST) (± SD) at 18.5° S, 146.5–147.5° E. Data from HadlSST (Rayner et al. 2003). Linear regression also shown

## CONCLUSIONS

The skeletal growth histories contained in massive coral skeletons can make a significant contribution to assessing coral responses to environmental changes. This is particularly important in an era of rapidly changing global climate, warming oceans, and changing global ocean chemistry, in addition to local stresses to coral reefs. Massive coral skeletons containing annual density bands provide dated coral growth histories that can be exploited to assess the consequences of environmental changes (as originally envisaged by Knutson et al. 1972), including progressive ocean acidification. These sources of coral growth histories can be used to determine base-line growth rates and natural variability prior to anthropogenic changes to coral reef environments and global warming, and help detect current changes. Routine examination of coral growth characteristics in conjunction with geochemical analyses of the same material can greatly enhance the environmental information obtained from coral archives. These retrospective monitors of coral reef environments are at present underexploited. There are, for example, a large number of coral cores collected in recent years primarily for the analyses of geochemical records and reconstruction of past oceanic climates and environments (www.ncdc.noaa.gov/paleo/index.html). Rarely are details of coral growth provided, yet, at the very least, annual linear extension rates can be readily measured from X-rays of coral slices or determined from high-resolution sampled geochemical records with annual cycles (similar to obtaining extension rates from skeletal density). There are also several collections of massive coral colonies, some of which have been analysed in terms of coral growth characteristics (e.g. Hudson 1981, Lough et al. 1999, Carricart-Ganivet & Merino 2001, Dodge & Helmle 2003, Halley & Hudson 2007). There is an urgent need to ensure that this valuable material is not lost and that the information on coral growth rates is obtained from these under-used archives. This requires a coordinated international effort to both identify what material is available, to ensure it is appropriately curated and encourage the routine measurement of coral skeletal growth records in concert with geochemical analyses.

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# Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers

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ABSTRACT: Future anthropogenic emissions of $CO_2$ and the resulting ocean acidification may have severe consequences for marine calcifying organisms and ecosystems. Marine calcifiers depositing calcitic hard parts that contain significant concentrations of magnesium, i.e. Mg-calcite, and calcifying organisms living in high latitude and/or cold-water environments are at immediate risk to ocean acidification and decreasing seawater carbonate saturation because they are currently immersed in seawater that is just slightly supersaturated with respect to the carbonate phases they secrete. Under the present rate of $CO_2$ emissions, model calculations show that high latitude ocean waters could reach undersaturation with respect to aragonite in just a few decades. Thus, before this happens these waters will be undersaturated with respect to Mg-calcite minerals of higher solubility than that of aragonite. Similarly, tropical surface seawater could become undersaturated with respect to Mg-calcite minerals containing ≥12 mole percent (mol%) $MgCO_3$ during this century. As a result of these changes in surface seawater chemistry and further penetration of anthropogenic $CO_2$ into the ocean interior, we suggest that (1) the magnesium content of calcitic hard parts will decrease in many ocean environments, (2) the relative proportion of calcifiers depositing stable carbonate minerals, such as calcite and low Mg-calcite, will increase and (3) the average magnesium content of carbonate sediments will decrease. Furthermore, the highest latitude and deepest depth at which cold-water corals and other calcifiers currently exist will move towards lower latitudes and shallower depth, respectively. These changes suggest that anthropogenic emissions of $CO_2$ may be currently pushing the oceans towards an episode characteristic of a 'calcite sea.'

KEY WORDS: Ocean acidification · Calcification · Carbonate dissolution · Mg-calcite · High latitude · Aragonite · Saturation state · Calcite sea



## INTRODUCTION

Continuous anthropogenic emissions of $CO_2$ to the atmosphere and uptake of part of this $CO_2$ by the oceans will result in a continuous decline in surface seawater calcium carbonate saturation state ($\Omega$; see Appendix 1) and a decrease in pH, often termed ocean acidification (e.g. Broecker et al. 1971, Bacastow & Keeling 1973, Kleypas et al. 1999a, Caldeira & Wickett 2003, Andersson et al. 2005, Orr et al. 2005). On timescales of several thousands of years, much of the $CO_2$ originating from anthropogenic activities will be absorbed by the oceans and ultimately neutralized by dissolution of sedimentary calcium carbonate minerals (Broecker et al. 1971, Archer et al. 1998). However, on shorter timescales, the only way to slow down significantly or prevent future ocean acidification is to reduce the emissions of $CO_2$ from human activities to the atmosphere. At this time, because of the current global political and socio-economic situation, a large reduction in $CO_2$ emissions is highly unlikely (Clarke et al. 2007). Therefore, surface seawater pH will continue to

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decline, with all the ecological implications of such a change in a major earth surface system carbon reservoir, until emissions are reduced and the atmospheric $CO_2$ concentration stabilizes.

Ocean acidification has raised serious concerns about the potential effects on marine organisms and ecosystems, especially those organisms producing shells, tests or skeletons out of calcium carbonate ($CaCO_3$). In particular, the fates of tropical coral reefs and scleractinian corals have received most of the attention in the ongoing ocean acidification debate (e.g. Kleypas et al. 1999a, 2006, Marubini et al. 2003, Hoegh-Guldberg et al. 2007). Much less attention has been given to marine calcifiers depositing calcium carbonate minerals containing significant proportions of magnesium ions, i.e. Mg-calcite (Borowitzka 1981, Mackenzie & Agegian 1989, Gao et al. 1993, Kuffner et al. 2008), and calcifying organisms living in high latitude and/or cold-water environments (Orr et al. 2005, Guinotte et al. 2006, Roberts et al. 2006, Turley et al. 2007). In either situation, these organisms exist under conditions more corrosive to their shells and skeletons than organisms depositing less soluble mineral phases or organisms living at lower latitudes. The solubility of Mg-calcite minerals with a significant mole percent (mol%) $MgCO_3$ is greater than the solubility of aragonite and calcite, and the seawater saturation state with respect to carbonate minerals is lower in high latitudes than at low latitudes. The same is true for deep waters compared with shallow waters. Thus, it is likely that Mg-calcite, high latitude and cold-water calcifying organisms will be the first responders to ocean acidification and will serve as 'canaries' to the potential detrimental consequences of this process.

The objective of the present investigation is to evaluate how the surface seawater carbonate saturation state may change in different environments and with respect to Mg-calcite minerals as a result of future ocean acidification. In the second part of this study we evaluate these changes numerically under a business-as-usual (BAU) $CO_2$ emissions scenario and review the results in terms of the potential consequences to marine calcifiers and carbonate environments.

## OVERVIEW OF MARINE CALCIFICATION AND Mg-CALCITE MINERALS

Marine calcifying organisms are an important component of almost all ecosystems, ranging from warm tropical to cold high latitude waters and also the deep sea. In fact, carbonate sediments will accumulate and reflect their presence in the sedimentary column, where they are not significantly exported, dissolved or diluted by terrigenous clastics or other sedimentary material (Chave 1967). Most of the calcifying taxa found in tropical and subtropical regions are also found in colder environments and include corals, mollusks, coralline algae, foraminifera, bryozoans, echinoderms and crustaceans (Chave 1954). There are even high latitude and cold-water corals and coral ecosystems that produce extensive calcium carbonate structures comparable with shallow tropical reefs (e.g. Rogers 1999, Freiwald et al. 2004, Freiwald & Roberts 2005, Roberts et al. 2006). There are also both warm- and cold-water, shallow-living calcifiers including coralline algae, echinoderms and bryozoans that deposit Mg-calcite of variable composition that can contribute significantly to regional carbonate budgets (Chave 1954). Furthermore, planktonic calcifiers, such as coccolithophorids, pteropods, foraminifera and heteropods, play disproportionally important roles in temperate and high latitude marine environments relative to their role in warmer subtropical/tropical environments. Coccolithophorids and pteropods are the major pelagic producers of calcite and aragonite, respectively, and account for a significant proportion of the particulate organic and particulate inorganic carbon exported from the surface ocean to the deep ocean both regionally and globally (e.g. Iglesias-Rodríguez et al. 2002, Jin et al. 2006).

The occurrence of magnesium in marine skeletal hard parts was first documented by Silliman (1846). Almost a century later while studying mollusk shells, Bøggild (1930) recognized a relationship between magnesium content and skeletal carbonate mineralogy. Bøggild (1930) distinguished among 3 different compositional carbonate phases that occurred in nature: low Mg-aragonite, low Mg-calcite (<4% $MgCO_3$), and high Mg-calcite (>4% $MgCO_3$). At first it was commonly believed that the magnesium in high magnesian calcite skeletons was present in the form of the mineral dolomite. This assumption was disproved by Chave (1952), who demonstrated that calcium ions were replaced by magnesium ions in the calcite lattice, shrinking it, and forming a partial solid solution at low temperatures between calcite and dolomite. Mg-calcites are essentially isomorphs of calcite, but compared with pure calcite the substitution of calcium ions with the much smaller magnesium ions in a completely random fashion causes variations in the mineralogical structure, such as carbonate anion and cation positional disorder (e.g. Reeder 1983, Bischoff et al. 1987, Tribble et al. 1995). As a result, under present earth surface temperature and pressure conditions, Mg-calcite minerals are metastable relative to nearly pure calcite and dolomite, i.e. thermodynamically we would not expect their existence, but they persist owing to kinetic constraints (Goldschmidt 1983, Mackenzie et al. 1983).

A wide range of marine calcifers produce shells, tests or skeletons containing various amounts of magnesium in calcite. The most common and probably also the most important Mg-calcite producers containing significant mol% $MgCO_3$ are the red coralline algae, benthic foraminifera, bryozoans and echinoderms, but other groups of organisms such as crustaceans, molluscs, annelid worms, calcareous sponges, barnacles and brachiopods also deposit Mg-calcite of varying composition (Chave 1954, Morse & Mackenzie 1990). Mg-calcite-producing marine calcifiers make up a significant proportion of the total biomass of calcifying organisms on coral reefs and in shoal water environments although aragonite-producing organisms (e.g. scleractinian corals, green algae and certain molluscs) are the most important calcifiers in these environments. However, in many coral reefs, coralline algae of high magnesian calcite compositions are the major framework and cementing taxa. Based on more than 700 sediment samples from different tropical and subtropical neritic environments, the relative proportions of aragonite, Mg-calcite and calcite (including low Mg-calcite) were found to be 63, 24 and 13%, respectively (Land 1967). The average Mg-calcite composition of carbonate sediments in the same environments contains about 13 to 15 mol% $MgCO_3$ (Morse & Mackenzie 1990).

The mechanisms controlling the magnesium content of marine calcitic skeletons are poorly understood and exert different influences on different organisms. Marine calcifiers depositing aragonite contain almost none or very little magnesium (<1 mol%) and the same is true for calcite producers, which are mostly represented by pelagic calcifiers, such as certain species of coccolithophorids and foraminifera. Among organisms depositing Mg-calcite of various compositions, ranging from a few mol% to as much as 30 mol%, there are distinct differences between different species. Clearly, there is a strong taxonomic control on the magnesium content of calcitic skeletons (Chave 1954). Also, the magnesium content of marine calcifiers depositing Mg-calcite is seen to decrease as a function of increasing latitude (Fig. 1). Thus, the Mg content of skeletal hard parts covaries with changes in environmental variables such as temperature, light and seawater carbonate saturation state, all of which decrease with increasing latitude (Chave 1954, Mackenzie et al. 1983). This variation in magnesium content has been attributed to variation in growth rate (Moberly 1968), which is not only a function of temperature and seawater carbonate saturation state, but also energy availability (i.e. food), and in phototrophic organisms (or organisms dependent on phototrophic symbionts) it is also a function of photosynthetic activity and consequently light and nutrient concentration (Mackenzie et al. 1983). The magnesium content of shallow water Mg-calcite cements follows a similar latitudinal trend as biogenic phases, and a similar trend has also been observed as a function of depth and consequently decreasing seawater carbonate ion concentration and temperature (Schlager & James 1978, Mackenzie et al. 1983, Videtich 1985).

Experimental results have clearly demonstrated that the rate of calcification in marine calcifiers is directly related to the seawater carbonate saturation state (e.g. Gattuso et al. 1999, Langdon et al. 2000, Marubini et al. 2003). Furthermore, Mackenzie & Agegian (1989) showed that the growth rate of *Porolithon gardineri*, measured as linear extension and the magnesium content were directly correlated to seawater carbonate saturation state and temperature. Other studies have demonstrated that the magnesium content of calcitic skeletons varies as a result of changes in the Mg to Ca ratio in seawater (Stanley et al. 2002, Ries 2006, see also Mackenzie et al. 1983 for a synthesis of data on Mg to Ca ratio of solution versus precipitate Mg-calcite composition), but this is only important on timescales of millions of years when significant changes of this ratio in seawater occurred (Guidry et al. 2007). However, a change in Ca and Mg concentrations and the ratio of these ions essentially implies a change in the seawater saturation state with respect to Mg-calcite phases, which also could be accomplished by a change in the carbonate ion concentration (Appendix 1). Lower $CO_3^{2-}$ concentration and consequently lower carbonate saturation state favour Mg-calcite deposits of lower magnesium content both kinetically and thermodynamically (Mackenzie et al. 1983). Lower seawater carbonate saturation states also result in increas-

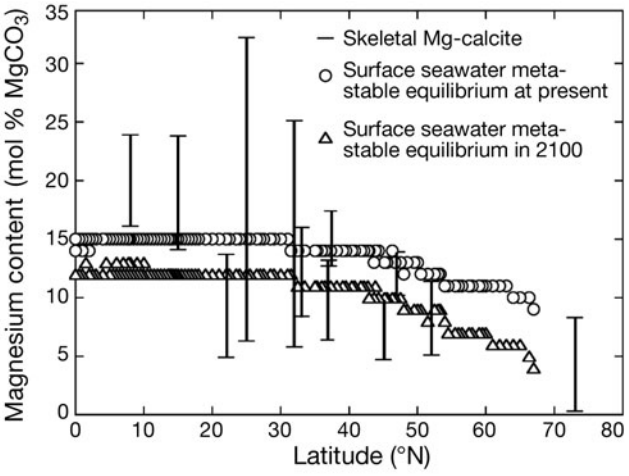


Fig. 1. Range of magnesium content of calcitic skeletons as a function of latitude (Chave 1954). The Mg-calcite phase in metastable equilibrium with the surface seawater according to the 'minimally prepared' solubility curve (see text) is also shown at present time and in the year 2100 under a business-as-usual (BAU) $CO_2$ emissions scenario (IS92a)

ing dissolution rates of metastable carbonate mineral phases subjected to undersaturated conditions.

One of the most controversial and highly debated problems related to the Mg-calcite minerals is that of their solubility (see Morse et al. 2006 for a detailed discussion and references therein). In general, biogenic Mg-calcites with a significant mol% $MgCO_3$ are more soluble than both calcite and aragonite, and the approximate Mg-calcite composition with the same solubility as aragonite ranges from 8 to 12 mol% $MgCO_3$ depending on the experimental solubility curve adopted. There are essentially 2 different experimental solubility curves for biogenic Mg-calcites that are referred to as either the biogenic 'minimally prepared' (Plummer & Mackenzie 1974) or the biogenic 'cleaned' solubility curve (e.g. Bischoff et al. 1987), and each is differentiated by the extent of preparation of the experimental materials. At this time, it is not fully understood which solubility curve most accurately reflects the behaviour of biogenic Mg-calcite minerals in the natural environment (Morse et al. 2006), although suggestions in favour of the use of the 'minimally prepared' solubility curve have been made in the literature (Bischoff et al. 1993, Tribble et al. 1995). One of the problems in determining the solubility of biogenic Mg-calcites arises from their heterogeneous nature and inclusion of impurities other than Mg, such as $H_2O$, $OH^-$, $HCO_3^-$, $SO_4^{2-}$ and other ions, which commonly are found in biogenic skeletons. Furthermore, another problem arises from the fact that a true equilibrium cannot be established between a given Mg-calcite and a solution (Garrels & Wollast 1978). In the initial stages of experimental dissolution studies, the dissolution process occurs congruently, but becomes incongruent as the solution becomes supersaturated with respect to a Mg-calcite phase of lower magnesium content, which then starts to precipitate. To overcome this problem, experimentalists have extrapolated data from the congruent step to infinite time, making the assumption that this represents equilibrium at a condition referred to as stoichiometric saturation for the solid solution.

## FUTURE CHANGES IN SEAWATER CARBONATE SATURATION STATE AND EFFECTS ON CALCIFIERS

### Methodology

In the present calculations, surface seawater saturation states with respect to calcite, aragonite, and 18, 15 and 12 mol% Mg-calcite were calculated as follows. Average salinity (S) and total alkalinity (TA) were extracted for the chosen temperature regimes from a global model developed by M. Jeffries at the Bermuda Institute of Ocean Sciences (BIOS) based on data from the World Ocean Circulation Experiment (WOCE) and the Global Ocean Data Analysis Project (GLODAP) (M. Jeffries & N. Bates unpubl. data). The following conditions for 3 typical environments were determined: tropical/subtropical (temperature = 25 ± 1°C, S = 35.53 ± 0.87, TA = 2333.8 ± 51.5 µmol $kg^{-1}$), temperate (temperature = 13 ± 1°C, S = 34.51 ± 0.88, TA = 2284.1 ± 50.0 µmol $kg^{-1}$) and high latitude (temperature = 4 ± 1°C, S = 33.82 ± 0.45, TA = 2271.0 ± 28.3 µmol $kg^{-1}$). Saturation state with respect to Mg-calcite mineral compositions were calculated based on both the biogenic 'minimally prepared' and biogenic 'cleaned' solubility curves using total ion activity coefficients defined by Millero & Pierrot (1998) (Appendix 1). Assuming equilibrium between the atmosphere and the surface ocean with respect to the partial pressure of $CO_2$ ($pCO_2$), dissolved inorganic carbon parameters were calculated using CO2SYS (Lewis & Wallace 1998) and stoichiometric carbonic acid system constants defined by Mehrbach et al. (1973) and refit by Dickson & Millero (1987). Future projections until the year 2100 were based on the Intergovernmental Panel on Climate Change IS92a $CO_2$ emissions scenario (IPCC 2001). Since increasing temperature counteracts the effect of rising $pCO_2$ on seawater carbonate saturation state, the effect of increasing surface seawater temperature (SST) on this parameter was also evaluated. The fossil fuel intensive emissions scenario A1FI was adopted in this simulation to evaluate the maximum effect of increasing temperature during the present century.

### Results

At present , surface seawater saturation state with respect to carbonate minerals decreases with increasing latitude owing to decreasing $CO_3^{2-}$ concentration and temperature (Figs. 1 & 2). Typical tropical/ subtropical surface seawater is currently supersaturated with respect to calcite and aragonite by 550 and 360%, respectively (Fig. 2). Temperate surface seawater is supersaturated with respect to these mineral phases by 370 and 230%, respectively, and typical high latitude surface seawater is supersaturated by 260 and 160%, respectively (Fig. 2). Based on the biogenic 'minimally prepared' solubility curve (Plummer & Mackenzie 1974), saturation state calculations show that average tropical/subtropical surface seawater is close to a metastable equilibrium state with 15 mol% Mg-calcite and, consequently, undersaturated with respect to Mg-calcite phases with higher magnesium content. The same calculations show that average high latitude and temperate surface seawater is currently undersaturated

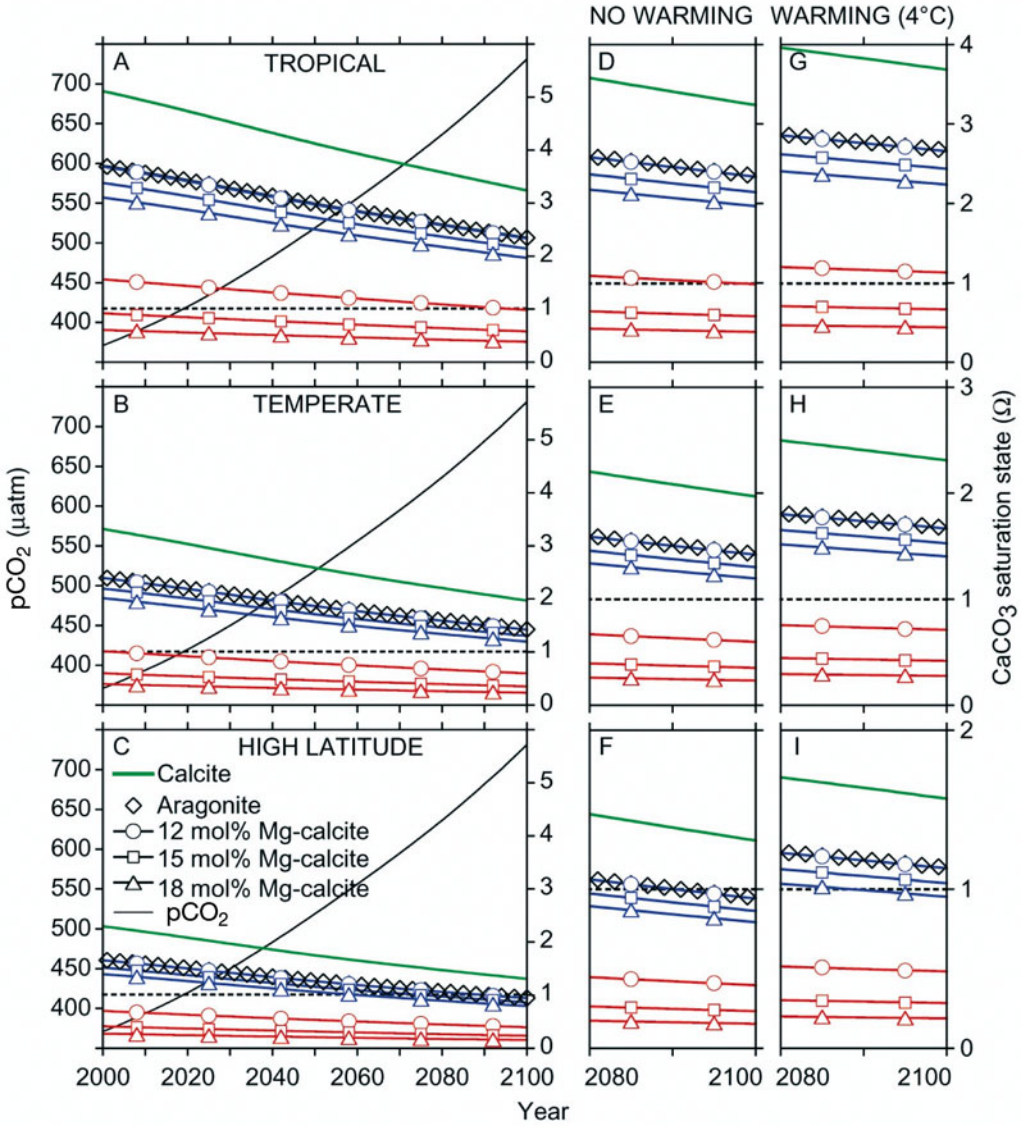


Fig. 2. Surface seawater $pCO_2$ (partial pressure of $CO_2$) and carbonate saturation state with respect to calcite, aragonite, and 12, 15 and 18 mol% Mg-calcite for a typical tropical/subtropical (temperature = 25 ± 1°C, salinity [S] = 35.53 ± 0.87, total alkalinity [TA] = 2333.8 ± 51.5), temperate (temperature = 13 ± 1°C, S = 34.51 ± 0.88, TA = 2284.1 ± 50.0) and high latitude (temperature = 4 ± 1°C, S = 33.82 ± 0.45, TA = 2271.0 ± 28.3) ocean environment during the 21st century. The calculations were forced by an increase in $pCO_2$ following the IS92a $CO_2$ emissions scenario (IPCC 2001) and assuming instantaneous equilibrium between the atmosphere and the surface ocean. TA, S and temperature were assumed constant in panels (A) through (F). In panels (G) through (I), the calculations were forced by an increase in global mean temperature following the A1FI $CO_2$ emissions scenario, resulting in an increase in temperature of ~4°C from the year 2000 to 2100 (IPCC 2001). Years 2080 to 2100 are highlighted in panels (D) through (I) to demonstrate the effect of warming on the seawater carbonate saturation state. The seawater saturation state with respect to biogenic Mg-calcite minerals was calculated based on the solubility curve of both the 'minimally prepared' (red lines; Plummer & Mackenzie 1974) and the 'cleaned' (blue lines) biogenic carbonate material (Bischoff et al. 1987, 1993)

with respect to 12, 15, and 18 mol% Mg-calcite (Fig. 2). In contrast, calculations based on the biogenic 'cleaned' solubility curve (e.g. Bischoff et al. 1987, 1993) suggest that surface seawater in all climatic regions is currently well supersaturated with respect to these Mg-calcite compositions (Fig. 2).

In the present model scenario, the surface seawater saturation state with respect to carbonate minerals will decrease in all environments owing to uptake of anthropogenic $CO_2$. Numerical simulations show that tropical/subtropical and temperate surface seawater will remain supersaturated with respect to calcite and aragonite by the year 2100. However, average high latitude surface seawater could become undersaturated in the second half of this century with respect to aragonite and, thus, become undersaturated with respect to

Mg-calcite phases that are more soluble than aragonite prior to this (see Orr et al. 2005 for a detailed evaluation on the effects of anthropogenic $CO_2$ on surface seawater aragonite saturation). According to the biogenic 'minimally prepared' solubility curve (Plummer & Mackenzie 1974), surface seawater in all climatic regions will be undersaturated or at metastable equilibrium with respect to a 12 mol% Mg-calcite and phases of greater magnesium content by the year 2100 (Fig. 2). High latitude surface seawater could be in metastable equilibrium with a Mg-calcite phase containing as little as 4 to 5 mol% $MgCO_3$ by 2100 (Fig. 1). In contrast, calculations based on the biogenic 'cleaned' solubility curve (e.g. Bischoff et al. 1987, 1993) show that only high latitude surface seawater will become undersaturated with respect to Mg-calcite phases containing 12 mol% and higher $MgCO_3$ during the 21st century (Fig. 2). A potential increase in SST of approximately 4°C during the 21st century will affect the final saturation state index with respect to aragonite in year 2100 by 0.32 Ω units in tropical/subtropical environments, 0.25 Ω units in temperate environments and 0.19 Ω units in high latitude environments. Thus, the effect of warming on the seawater aragonite saturation state (and other carbonate mineral phases) is small compared with the decrease in this variable owing to uptake of anthropogenic $CO_2$ and ocean acidification (Fig. 2).

## Discussion

Clearly, to predict accurately the timing of when seawater becomes undersaturated with respect to a particular Mg-calcite phase owing to ocean acidification, it is necessary to determine more accurately the solubility and kinetic behaviour of these mineral phases in the natural environment and under conditions of increasing $CO_2$. Nevertheless, the seawater saturation level for many of these mineral phases is lower than that of aragonite and the degree of saturation is decreasing in most marine environments owing to rising atmospheric $CO_2$ and ocean acidification. Surface seawater is likely to become undersaturated with respect to aragonite (its solubility is well characterised) at high latitudes in a matter of decades (Fig. 2; Orr et al. 2005); thus, seawater will be undersaturated with respect to many Mg-calcite phases prior to this time.

The observed trend of decreasing Mg content in calcitic skeletons as a function of increasing latitude is in all probability partly a direct reflection of the slower growth rate of these organisms due to decreasing seawater carbonate saturation state and colder temperatures. Although the Mg content is highly variable and affected by additional factors (see previous discussion), changes in seawater chemistry owing to uptake of anthropogenic $CO_2$ will most probably result in a decrease in the average Mg composition of Mg-calcite-producing organisms in all environments, despite the warmer SSTs of the future. The same could be true for inorganic precipitates, such as carbonate cements. As a result, the average magnesium content of contemporary carbonate sediments will decrease because of the lower magnesium content in the source material, i.e. marine calcifiers and inorganic precipitates, but also owing to the selective dissolution of highly soluble Mg-calcite phases (Andersson et al. 2005, 2007, Morse et al. 2006).

Despite uncertainties in terms of the Mg-calcite solubility curve as a function of $MgCO_3$ content, it is probable that many Mg-calcite-secreting organisms and contemporary carbonate sediments currently exist or soon may exist close to a metastable equilibrium with the seawater in which they are immersed (Fig. 1). Thus, a small alteration of the seawater carbonate saturation state owing to $CO_2$ uptake will result in these organisms and sediments being immersed in seawater undersaturated with respect to these mineral phases. The same is true for organisms depositing aragonite and living in high latitude or cold-water environments, such as pteropods and cold-water corals, which soon could also be immersed in seawater undersaturated with respect to this mineral phase (Orr et al. 2005, Guinotte et al. 2006, Turley et al. 2007; Fig. 2). Undersaturated seawater conditions certainly imply that unprecedented challenges and alterations to the function, structure and distribution of calcifying organisms and carbonate ecosystems exposed to these conditions will occur. It is improbable that, under such conditions, marine calcifers could sustain themselves as they do today. Hence, as high latitude environments progressively become increasingly acidic and undersaturated with respect to aragonite, the highest latitude at which cold-water corals and other calcifiers are found is likely to move progressively towards lower latitudes. A similar situation has been proposed with respect to tropical aragonitic corals as the aragonite saturation state reaches a 'critical' threshold, below which these organisms cannot thrive (Kleypas et al. 1999b, Guinotte et al. 2003). Furthermore, as anthropogenic $CO_2$ penetrates deeper into the oceans, the seawater saturation horizons (the depth at which $\Omega = 1$; Appendix 1) with respect to various carbonate mineral phases will become shallower (Feely et al. 2004). Consequently, the maximum depth at which deep water corals and other calcifying organism are found will shoal (Guinotte et al. 2006, Turley et al. 2007). If significant dissolution takes place above the chemical lysoclines of the biogenic carbonate minerals (Milliman et al. 1999), anthropogenic $CO_2$ may not have to penetrate

as deep into the ocean as currently thought to begin to dissolve carbonate phases at depth. The timing of these changes depends on the rate at which anthropogenic $CO_2$ is transported into the interior of the oceans, but this may already occur in certain environments.

Lower seawater carbonate saturation state most certainly implies slower rates of calcification for most marine calcifiers and possibly a lessened ability to compete for space and other important resources (e.g. Kleypas et al. 1999a, 2006, Andersson et al. 2005, Kuffner et al. 2008). As a result, the relative proportion of non-calcifying organisms to calcifying organisms may become increasingly larger in the near future than is seen today. Lower carbonate saturation state also favours deposition of less soluble mineral phases (Mackenzie et al. 1983). Thus, organisms depositing stable carbonate minerals, such as calcite and low Mg-calcite could become increasingly dominant under future increasingly acidic seawater conditions. Some organisms currently depositing metastable carbonate phases such as aragonite and high Mg-calcite may not be able to sustain themselves under such conditions (Kuffner et al. 2008), but others could possibly persist through deposition of carbonate minerals of greater stability. For example, Stolarski et al. (2007) discovered that corals known to deposit aragonite actually secreted calcite during an episode of the Cretaceous Period, but the controlling mechanisms are unknown. Ries et al. (2006) observed similar results for corals growing in artificial seawater of variable magnesium to calcium ratio, thus, changing the composition of the mineral phase favoured to precipitate based on thermodynamic and kinetic principles. Other evidence suggests that some corals could survive detrimental acidic seawater conditions in a non-calcifying state (Fine & Tchernov 2007).

It is noteworthy that the ecological and compositional changes for marine calcifiers and ecosystems discussed here (as a result of ocean acidification and decreasing carbonate saturation state) are similar to those changes inferred from the carbonate sedimentary record, which showed that the transitions from aragonite to calcite seas favoured the deposition of carbonate minerals of increasing stability in both skeletal and non-skeletal precipitates (e.g. Mackenzie & Pigott 1981, Sandberg 1983, Stanley & Hardie 1998, Stanley et al. 2002). Mackenzie & Pigott (1981) and Sandberg (1983) first noted oscillations in the primary mineralogy of ooids and marine cements between calcite and aragonite during the Phanerozoic Eon. Sandberg (1983) referred to these oscillations as aragonite-inhibiting or aragonite-facilitating episodes and Mackenzie & Pigott (1981) referred to them as oscillatory and submergent tectonic modes. Later these episodes have been referred to as aragonite or calcite seas. Stanley & Hardie (1998) subsequently demonstrated that the dominant mineralogy of biogenic skeletal carbonate deposits also varied between calcite (and low Mg-calcite) and aragonite mineralogy (and high Mg-calcite) during this time period, which correlated well with Sandberg's (1983) and Mackenzie & Pigott's (1981) episodes. These oscillations are commonly associated with climatic episodes, sea level fluctuations, plate tectonic modes and carbonate–silicate weathering cycles, involving changes in seawater Mg to Ca ratio, atmospheric and seawater $CO_2$ concentrations and, consequently, seawater carbonate saturation state (Sandberg 1983, Morse & Mackenzie 1990, Stanley & Hardie 1998, Guidry et al. 2007). Although seawater composition and chemistry were significantly different during these episodes relative to the present (Guidry et al. 2007), decadal to centurial scale ocean acidification owing to the absorption of $CO_2$ in the oceans from the burning of fossil fuels and land use changes and consequent changes in the seawater carbonate saturation state could push the Earth towards an episode with carbonate environments similar to that of a calcite sea. Mg-calcite, high latitude and cold-water marine calcifiers, which already exist on the margin in terms of seawater carbonate saturation state, will be the first responders to this major transition. The consequences to Mg-calcite-producing organisms and sediments are most certainly drastic, but the timing is uncertain because we do not fully understand the solubilities and kinetic behaviour of these mineral phases in the natural environment. This lack of knowledge of these phenomena warrants further study.

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**Appendix 1.** Calculating seawater carbonate saturation state ($\Omega$)

A general expression for the calculation of seawater saturation state with respect to carbonate minerals is: $\Omega = \{Mg^{2+}\}^{x}\{Ca^{2+}\}^{(1-x)}\{CO_3^{2-}\}/K_x$, where brackets {} represent ion activities, $x$ is the mol fraction magnesium ions, and $K_x$ is the equilibrium constant with respect to the particular carbonate phase ($K_x$ = IAP, ion activity product at equilibrium).

Ion activities are not easily measured and most commonly seawater saturation state with respect to calcite and aragonite are calculated using ion concentrations (denoted by square brackets []) and stoichiometric solubility products ($K_{sp}^{*}$ = ICP, ion concentration product at equilibrium): $\Omega = [Ca^{2+}]\,[CO_3^{2-}]/K_{sp}^{*}$.

In contrast, stoichiometric solubility products with respect to Mg-calcite minerals have not been determined and the saturation state with respect to these mineral phases has to be calculated based on ion activities. The ion activity ($a$) is calculated based on the observed ion concentrations ($C$) multiplied by the total ion activity coefficient ($\gamma_T$), which has been determined experimentally or from theory (e.g. Millero & Pierrot 1998): $a = \gamma_T \times C$. Since a true equilibrium cannot be achieved with respect to Mg-calcite minerals, $K_x$ represents a metastable equilibrium state obtained from what has been referred to as stoichiometric saturation (Thorstenson & Plummer 1977; a term not equivalent to the definition of the stoichiometric solubility product, see for example Morse et al. 2006 and references therein).

If $\Omega > 1$ with respect to a particular mineral phase, the seawater is supersaturated with respect to that phase, and if $\Omega < 1$, the seawater is undersaturated. When $\Omega = 1$ this implies that the seawater is in thermodynamic equilibrium with respect to that mineral phase, i.e. the forward and backward reaction (dissolution and precipitation) are equal to one another. Thermodynamically, one would expect net dissolution if a mineral phase were immersed in a solution undersaturated with respect to that mineral phase, and the opposite, i.e. net precipitation, if the solution were supersaturated. This is strictly not the case because of kinetic constraints and inhibition by various components present in seawater.

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# Effects of $CO_2$-driven ocean acidification on the early developmental stages of invertebrates

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ABSTRACT: $CO_2$ emissions arising from the burning of fossil fuels have altered seawater chemistry far more rapidly than the Earth has previously experienced, and the rate and extent of this change are expected to affect shallow water marine organisms. The increased $CO_2$ diffuses from the atmosphere into ocean surface waters, resulting in increased partial pressure of $CO_2$, and reduced [$CO_3^{2-}$] and pH. The $CO_2$-driven ocean acidification leads to a decrease in calcium carbonate ($CaCO_3$) saturation state in the ocean surface waters and has potential impacts on calcifiers. The present study focuses on the effects of ocean acidification on early developmental and reproductive stages of calcifiers, both of which are believed to be the most vulnerable stages to environmental change within a life cycle. Laboratory experiments revealed that ocean acidification has negative impacts on the fertilization, cleavage, larva, settlement and reproductive stages of several marine calcifiers, including echinoderm, bivalve, coral and crustacean species. There appear to be significant ontogenetic impacts and species-specific differences in tolerance to the high $CO_2$ levels. The conclusion is that future changes in ocean acidity will potentially impact the population size and dynamics, as well as the community structure of calcifiers, and will therefore have negative impacts on marine ecosystems. Further studies are needed to evaluate the potential impacts on non-calcifiers, as well as the synergistic impacts of ocean acidification and climate change. Studies should also focus on the adaptive capability of marine organisms, which will be crucial to the ability to forecast how marine organisms and ecosystems will respond to the world's oceans as they warm and acidify.

KEY WORDS: $CO_2$ · Ocean acidification · Seawater chemistry · Calcifiers · Early development · Reproduction · Rapid environmental change



## INTRODUCTION

Approximately one-third of the $CO_2$ that has entered the atmosphere over the past 100 yr has been absorbed into ocean surface waters and has resulted in the elevation of partial pressure of $CO_2$ ($pCO_2$) in seawater and reduction of seawater pH (Caldeira & Wickett 2003, Royal Society 2005, German Advisory Council on Global Change 2006, Denman et al. 2007). One biological impact of ocean acidification is its effect on calcifiers, because seawater acidification results in a decrease of [$CO_3^{2-}$], thereby reducing the calcium carbonate ($CaCO_3$) saturation state, which is determined by [$CO_3^{2-}$][$Ca^{2+}$] / *K*sp (*K*sp is the stoichiometric solubility of $CaCO_3$; Kleypas et al. 2006). Of the 2 major biologically secreted forms of $CaCO_3$ in modern calcifiers, aragonite is more soluble than calcite (Zeebe & Wolf-Gladrow 2001). Orr et al. (2005) reported that high-latitude surface oceans will become undersaturated with respect to aragonite by the year 2050, and lead to aragonite shell dissolution (Feely et al. 2004, Orr et al. 2005). Recent studies have shown that the calcification rate of calcifiers, such as corals, coccolithophores, foraminiferans and bivalves, decreases with increasing $pCO_2$, even in seawater supersaturated with respect to $CaCO_3$ (Gattuso et al. 1998, Riebesell et al. 2000, Bijma et al. 2002, Kleypas et al. 2006, Gazeau et al. 2007). Additionally, increased $pCO_2$ may also have complex effects on the physiology, growth and reproductive success of marine calcifiers. Indeed, recent studies have

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demonstrated that adult calcifiers exposed to hypercapnia suffer from physiological stress in addition to reduced calcification (Pörtner et al. 2004, Michaelidis et al. 2005, Miles et al. 2007, Spicer et al. 2007). To understand the effect of ocean acidification at a population level, however, it is important to focus on the most sensitive life cycle stages to environmental change. Usually these are early developmental and reproductive stages, during which environmental requirements are often more specific and acute than at other stages (Thorson 1950). Indeed mortality of marine invertebrates, including benthic calcifiers, exceeded 90% during early life stages in their natural habitat according to Gosselin & Qian (1997).

There are a number of different life cycle stages of benthic calcifiers, such as fertilization, cleavage, planktonic larva, settlement, metamorphosis, juvenile, adult and reproductive stages, which are possibly affected differently by high $pCO_2$ (Fig. 1). The first deposition of $CaCO_3$ is known to occur during the larval stage, as in echinoderms and bivalves, or during the settlement stage, as in corals and barnacles. Hence, these stages are highly susceptible to the potential effects of ocean acidification. Beckerman et al. (2002) suggested that environmental conditions experienced during early development can have profound effects on the subsequent performance of individuals and cohorts. Indeed, Green et al. (2004) showed that the low $CaCO_3$ saturation state may explain the exponential losses of juvenile bivalves and the low recruitment transition from the pelagic larval phase to the benthic juvenile phase. Therefore, effects of ocean acidification on larval survival rate, as well as reproduction rate, will directly influence the population abundance, distribution and community structure. To evaluate the impact of ocean acidification on calcareous organisms at a community level, the present paper focuses on the effects of high $pCO_2$ on early developmental stages including fertilization, cleavage, hatching, larva, settlement and reproductive stages of calcifiers.

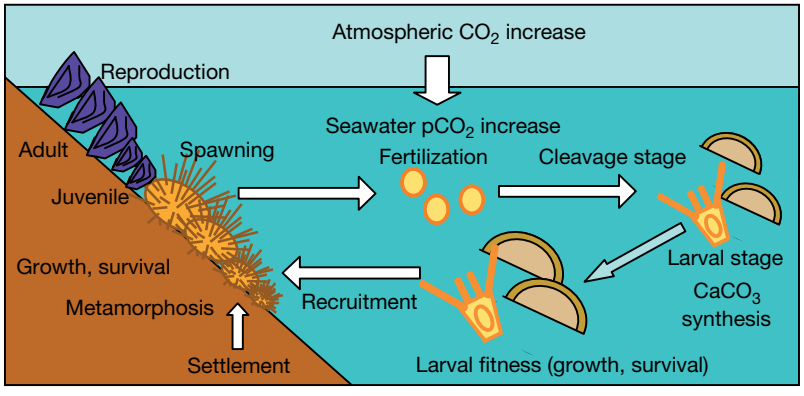


Fig. 1. Different life-cycle stages of benthic calcifiers, including reproduction, fertilization, planktonic larva, settlement, metamorphosis, juvenile and benthic adult stages, that are potentially affected in different manners by ocean acidification

## EFFECTS ON FERTILIZATION, CLEAVAGE AND HATCHING STAGE

The fertilization rate of sea urchins decreased with increasing $pCO_2$ concentration (360 to 10 360 µatm, pH 8.1 to 6.8) in eggs of both *Hemicentrotus pulcherrimus* (Fig. 2; $r_s = 0.74$, $p < 0.001$) and *Echinometra mathaei* (Fig. 2; $r_s = 0.88$, $p < 0.001$; Kurihara & Shirayama 2004a,b). However, the impact of increasing $pCO_2$ on fertilization differed between females, as revealed by the large SDs (Fig. 2), possibly reflecting a degree of genetic variation for $CO_2$ tolerance within populations. Additionally, in contrast with the linear decrease of fertilization rate in high $pCO_2$ seawater, the fertilization rate decreased at pH levels only <7.0 when seawater was acidified with HCl (Fig. 2; Kurihara & Shirayama 2004a,b). Effects of low pH using mineral acids on sperm motility have been well studied for sea urchins. Christen et al. (1983) demonstrated that sperm motility was suppressed at pH < 7.0. Polyspermic fertilization was also reported in *Anthocidaris crassispina* sea urchin eggs fertilized at pH 7.0 (Kobayashi 1971). Recently, Havenhand et al. (2008) found that sperm swimming speed and percent sperm motility of the sea urchin *Heliocidaris erythrogramma* exposed to 1000 µatm $pCO_2$ (pH 7.7) seawater decreased compared to controls. These results suggest again that high $pCO_2$ may affect egg fertilization more strongly than mineral acids. One of the reasons for this difference is likely to be the diffusion capability of $CO_2$ and protons. Ion transport is an energy (ATP)-consuming process (Heisler 1993), whereas molecular $CO_2$ directly diffuses across the biological cell membrane far faster than protons (Gutknecht et al. 1977), and hence $CO_2$ can readily enter into eggs or sperm and decrease the intracellular pH. Since the intracellular pH of sea urchin eggs is known to rise after insemination (Lopo & Vacquier 1977) and trigger the initiation of embryonic development (Johnson et al. 1976), in addition to the impact on sperm motility, the low intracellular egg pH may prevent fertilization and subsequent development.

The fertilization rates of marine bivalves, the oyster *Crassostrea gigas* and the mussel *Mytilus galloprovincialis* were unaffected in 2000 µatm

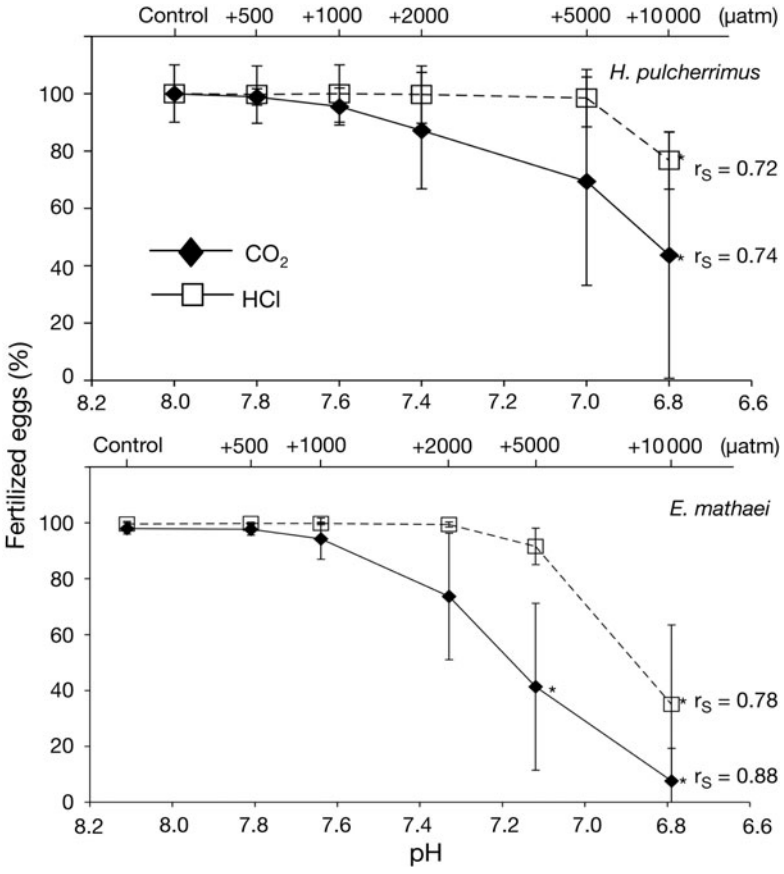


Fig. 2. *Hemicentrotus pulcherrimus* and *Echinometra mathaei*. Fertilization rate of eggs fertilized under 6 different pH conditions. Seawater was acidified with $CO_2$ or HCl; 6 and 3 batches were used for *H. pulcherrimus* and for *E. mathaei*, respectively. Error bar: SD; $r_s$: Spearman's rank correlation coefficient; *: significant difference compared to control (Tukey-Kramer, $p < 0.05$)

$pCO_2$ (pH 7.4) seawater (Kurihara et al. 2007, Kurihara et al. unpubl. data), whereas Desrosiers et al. (1996) reported that polyspermic fertilization in the giant scallop *Placopecten magellanicus* increased at seawater pH < 7.5. Additionally, during the scallop embryonic stage, the time to complete the first cleavage was shortest at pH 8.2 and increased with decreasing pH. Similarly, the cleavage speed of sea urchin embryos *Hemicentrotus pulcherrimus* and *Echinometra mathaei* slowed with decreasing pH (Kurihara & Shirayama 2004a,b). When embryos of the sea urchin *Sphaerechinus granularis* were reared in seawater acidified with HCl or $H_2SO_4$, mitotic abnormalities were induced at pH < 6.5 (Pagano et al. 1985a,b, Cipollaro et al. 1986). Incubating zygotes in seawater acidified by mineral acids reduces protein synthesis (Grainger et al. 1979). Such impacts on protein synthesis and mitotic activity probably decrease growth and cleavage rates.

Both hatching and nauplius survival decrease with increasing $pCO_2$ in the copepods *Acartia erythraea*, even though negative impacts were significant only at $pCO_2$ levels higher than those projected to occur in the future ocean (Kurihara et al. 2004a,b). Similarly, Mayor et al. (2007) also demonstrated a decrease of hatching success in the copepod *Calanus finmarchicus* only at 8000 µatm $pCO_2$ (pH 6.9). When *A. tsuensis* eggs were reared under 2000 µatm $pCO_2$ (pH 7.3) until they developed into adults, survival, growth and morphology were unaffected at all stages (Kurihara & Ishimatsu 2008). Additionally, the hatching rate was unaffected during ensuing generations (0 to 2 generations).

## EFFECTS ON LARVAL DEVELOPMENT

The larval development of several calcifiers is affected by elevations of seawater $pCO_2$. When *Hemicentrotus pulcherrimus* and *Echinometra mathaei* embryos were reared under 6 different $CO_2$ concentrations until they developed to the pluteus larval stage, larval and arm sizes were significantly smaller with increasing $pCO_2$ and their morphology, principally the larval skeletogenesis, tended to be abnormal (Fig. 3a to f; Kurihara & Shirayama 2004a,b). Similarly, the larval shells of *Crassostrea gigas* and *Mytilus galloprovincialis* were strongly affected by high $pCO_2$ conditions (Fig. 3g to k). When oyster eggs were reared under 1000 µatm $pCO_2$ (pH 7.8), though $CO_2$-treated larvae were completely shelled, they showed malformations such as convex hinges (Fig. 3h), which are typical criteria to identify abnormal development of veliger larvae in embryotoxicology bioassays (His et al. 1997). When oyster eggs were reared under 2000 µatm $pCO_2$ (pH 7.4), >70% of the $CO_2$-treated larvae were either completely non-shelled, or only partially shelled (Fig. 3i), and only 4% of $CO_2$-treated embryos developed into normal 'D-shaped' veliger larvae by 48 h after fertilization, in contrast to about 70% successful development in control embryos (Fig. 3g; Kurihara et al. 2007). A negative impact of 2000 µatm $pCO_2$ (pH 7.4) was also observed in *M. galloprovincialis* larvae. Though all $CO_2$-treated mussel larvae were completely shelled in contrast with oyster larvae, larval size was about 20% smaller than that of larvae from the control conditions and showed morphological abnormalities such as convex hinges, protrusion of mantle and malformed shells (Fig. 3i,k; Kurihara et al. in press).

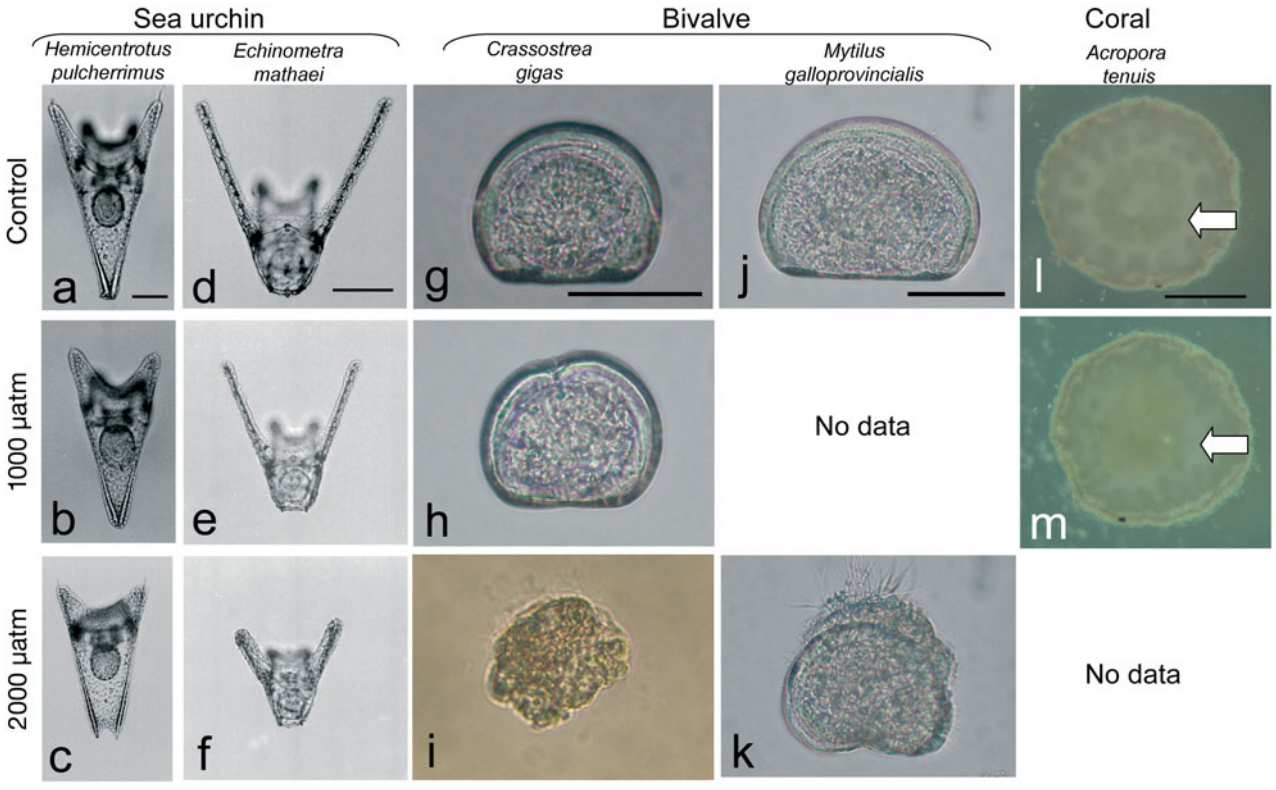


Fig. 3. Larval or polyp morphology of sea urchins *Hemicentrotus pulcherrimus* (a to c) and *Echinometra mathaei* (d to f), bivalves *Crassostrea gigas* (g to i) and *Mytilus galloprovincialis* (j,k), and the coral *Acropora tenuis* (l,m) incubated in the control (a,d,g,j,l), 1000 µatm $pCO_2$ (b,e,h,m) and 2000 µatm $pCO_2$, (c,f,i,k). Scale bars = 50 µm (a to j), 500 µm (l,m); the bars in (a,d,g,j,l) apply to the panels of the whole column

All these results suggest that high $pCO_2$ affected larval skeleton and shell synthesis. To evaluate the mechanism of this effect, I have recently examined the effect of high $CO_2$ (1000 and 2000 µatm $pCO_2$ / pH 7.7 and 7.45) on the expression of the gene related to spicule elongation (SM50) (Peled-Kamar et al. 2002), and of the gene that regulates the direction of crystal growth (SM30) in embryos of the sea urchin *Hemicentrotus pulcherrimus*. No effect was observed on the expression of these genes, even though spicule size and morphology of larvae were affected (Kurihara et al. unpubl. data). Further experiments evaluating effects on other proteins such as msp130, known to be related to $Ca^{2+}$ transportation (Farach-Carson et al. 1989), will help clarify effects on calcification.

Encounter and clearance rates of food particles depend on larval body size, and, therefore, smaller larvae are more prone to starvation (Anger 1987, Strathmann 1987, Hart & Strathmann 1995). Simkiss & Wilbur (1989) pointed out that the $CaCO_3$ structures have vital functions for calcified larvae, such as defense against predation, as well as roles in feeding, buoyancy control and pH regulation. Predation is generally considered to be the most important cause of larval mortality (Morgan 1995). Research to date on ocean acidification strongly suggests that it will lead to a reduction in fitness and survivorship of sea urchin and bivalve larvae due to both size reduction and disruption of $CaCO_3$ skeletogenesis.

## EFFECTS ON LARVAL SETTLEMENT

Mortality and shell dissolution rates of the bivalve *Mercenaria mercenaria* juveniles were significantly higher in $CaCO_3$-undersaturated conditions at the sediment–seawater interface than in supersaturated conditions (Green et al. 2004). They also demonstrated that the mortality rates were higher for small size classes (0.2 and 0.3 mm) than for larger individuals (1.0 and 2.0 mm). To examine the effect of ocean acidification on the settlement and the subsequent growth of coral polyps, eggs of the coral *Acropora tenuis* were reared under control and 1000 µatm $pCO_2$ (pH 7.6) conditions for 2 wk. In contrast with sea urchin and bivalve larvae, coral was unaffected by high $pCO_2$ until the larval stage. An impact of $CO_2$, however, was observed after settlement, while they developed into the polyp stage. The morphology of the $CO_2$-treated polyp endoskeleton was disturbed and malformed

compared to the radial pattern of control polyps (Fig. 3l,m). When hatched embryos of the marine shrimp *Palaemon pacificus* were cultured until settlement stage under 2000 µatm $pCO_2$ seawater (pH 7.6), no significant effect was observed on planktonic larval stages; however, $CO_2$-treated metamorphosing and settling juveniles were significantly smaller than in the control (2-way repeated-measures ANOVA; Fig. 4). Relatively small perturbations in initial populations of settling marine bivalves have been shown to induce large alterations in adult populations (Gosselin & Qian 1997, Hunt & Scheibling 1997). Hence, the impact of ocean acidification on settlement stages may well have profound ecological implications for their populations.

## EFFECTS ON REPRODUCTION

While effects of hypercapnia on fish reproduction have been studied to some extent (Ishimatsu et al. 2005), less is known for invertebrates. Some recent studies suggest that ocean acidification exerts negative impacts on invertebrate reproduction. Siikavuopio et al. (2007) reported that gonad growth was reduced by 67% when the green sea urchin *Strongylocentrotus droebachiensis* was exposed to high $pCO_2$ (pH 6.98) for 56 d. When the sea urchin *Hemicentrotus pulcherrimus* was reared under 1000 µatm $pCO_2$ (pH 7.8) for 10 mo, gonad development was delayed, and the spawning period was shortened to almost half that of the control (Kurihara et al. unpubl. data). The marine shrimp *Palaemon pacificus* cultured under 1000 µatm $pCO_2$ (pH 7.9) seawater for 30 wk showed reduced reproduction compared to the control (Kurihara et al. 2008). On the other hand, egg production of all copepods studied (e.g. *Acropora steueri*, *A. erythraea* and *A. tsuensis*) was not affected when reared under the high $pCO_2$ projected to occur in the future ocean (>2000 µatm $pCO_2$; Kurihara et al. 2004a,b, Kurihara & Ishimatsu 2008). Consequently, although some organisms appear less sensitive to elevated $pCO_2$, ocean acidification would directly affect the population size of several calcifiers.

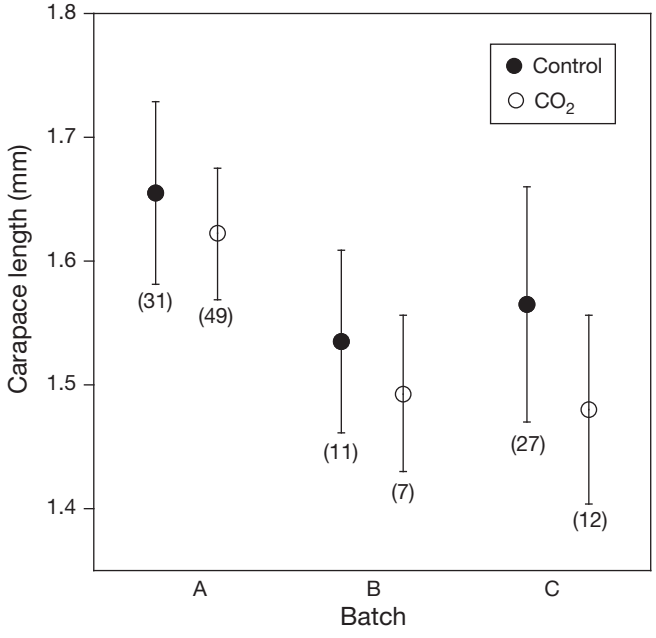


Fig. 4. *Palaemon pacificus.* Carapace length of a just settled marine shrimp juvenile reared under control and 2000 µatm $pCO_2$. Three different batches (A to C) were used for the experiment. The size of shrimp in $CO_2$ seawater was significantly smaller than that of control (2-way repeated-measures ANOVA). Number of shrimp shown in parentheses. Error bars: SD

## ONTOGENIC IMPACTS OF HIGH $CO_2$

Table 1 lists the effects of low pH condition (by addition of $CO_2$ or mineral acids) on the early developmental stages of marine calcifiers and their adult stages. The data indicate that ocean acidification has negative impacts on both larval and adult stages of corals, mollusks, echinoderms and crustaceans. Although data are limited for direct comparison of $CO_2$ tolerance between larval and adult stages, larvae appear to be more sensitive than adults. For example, whereas calcification of oyster adults reared under 2000 µatm $pCO_2$ (pH 7.4) decreased by about 50%, approximately half of the oyster larvae completely lacked a shell when cultured under the same $pCO_2$ concentration (Table 1; Gazeau et al. 2007, Kurihara et al. 2007). Although adult oyster shells are mainly composed of calcite (Stenzel 1964), oyster larval shell is completely formed of aragonite. Since the solubility of aragonite is higher than that of calcite, the $CaCO_3$ shells of bivalve larvae are probably affected more severely than those of adults. Additionally, although the growth and size of the adult sea urchin *Hemicentrotus pulcherrimus* was not affected when cultured for 10 mo under 1000 µatm $pCO_2$ (pH 7.8), the larval size of *H. pulcherrimus* was significantly reduced compared to the control when reared under 860 µatm $pCO_2$ (pH 7.8) for 3 d. Larvae of bivalves such as *Crassostrea gigas* and *Mercenaria merceneria* and also sea urchins such as *Paracentrotus lividus* and *Strongylocentrotus purpuratus* are known to initially deposit amorphous calcium carbonate (ACC), with a solubility 30 times larger than that of aragonite (Breãeviç & Nielsen 1989, Weiss et al. 2002, Addadi et al. 2003, Politi et al. 2004). For larval shells of bivalves, the ACC transformed into aragonite, and then to calcite in adult oysters, or into a mixture of aragonite and calcite in adult mussels (Hubbard et al.

Table 1. Effects of low pH condition (by addition of $CO_2$ or mineral acids) on the early developmental stages and adults of marine calcifiers. Organisms that are impacted at $CO_2$ concentrations expected to occur in the future ocean (380~2000 µatm $pCO_2$ / pH 8.2~7.3) are given in **bold**. However, most of the studies evaluating effects of acidification on bivalves used mineral acids and not $CO_2$. ppmv: parts per million by volume

| Taxon | $CO_2$ (ppmv) or Acid | pH | Exposure period | Effect | Source |
|---|---|---|---|---|---|
| ***Acropora tenuis*** | $CO_2$ 1000 | 7.6 | 14 d | Reduced growth of polyp size | Present study |
| ***Crassostrea gigas*** **(larva)** | $CO_2$ 2000 | 7.4 | 48 h | Inhibition of shell synthesis, reduced larval size | Kurihara et al. (2007) |
| | $CO_2$ 1000 | 7.8 | 48 h | Shell malformation | Kurihara et al. (unpubl. data) |
| ***C. gigas*** **(adult)** | $CO_2$ 698–2774 | 8.07–7.55 | 2 h | Decreased calcification rate | Gazeau et al. (2007) |
| | $H_2SO_4$ | 6.0–7.5 | 30 d | pH < 7.0, reduced feeding, growth, size, shell weight | Bamber (1990) |
| ***Mytilus galloprovincialis*** **(larva)** | $CO_2$ 2000 | 7.4 | 6 d | Shell malformation, reduced larval size | Kurihara et al. (in press) |
| ***M. galloprovincialis*** **(adult)** | $CO_2$ 5000 | 7.3 | 3 mo | Reduced growth, metabolism rate | Michaelidis et al. (2005) |
| *Mytilus edulis* (young, adult) | $H_2SO_4$ | 6.0–8.0 | 30 d | pH < 7.0, growth, feeding depression, shell dissolution | Bamber (1990) |
| *M. edulis* (adult) | $CO_2$ 421–2351 | 8.13–7.46 | 2 h | Decreased calcification rate | Gazeau et al. (2007) |
| *Crassostrea virginica* (larva) | HCl | 6.0–9.25 | 12 d | pH < 6.25, increased mortality rate; x pH < 6.75, decreased growth rate | Calabrese & Davis (1966) |
| *Mercenaria mercenaria* (larva) | HCl | 6.0–9.25 | 10 d | pH < 6.25, increased mortality rate; x pH < 6.75, decreased growth rate | Calabrese & Davis (1966) |
| *M. mercenaria* (juvenile) | $CO_2$ 50 000 | 7.1 | 21 d | Shell dissolution | Green et al. (2004) |
| ***Venerpis decussata*** **(adult)** | $H_2SO_4$ | 3.5–8.2 | 8–30 d | pH < 7.5, shell dissolution; pH < 7.0, feeding inhibition; pH < 6.1, 50% mortality | Bamber (1987) |
| ***V. decussata*** **(juvenile, 3–4 mm)** | | | | pH < 6.4, 50% mortality | Bamber (1987) |
| ***Placopecten magellanicus*** **(egg)** | Unknown | 7.0–9.0 | 5 h | pH < 7.5, polysperm, slow cleavage speed | Desrosiers et al. (1996) |
| *Acartia steueri* (adult) | $CO_2$ 2000–10 000 | 7.4–6.8 | 8 d | pH < 6.8, decreased egg production | Kurihara et al. (2004a,b) |
| *Acartia erythraea* (egg, larva) | $CO_2$ 2000–10 000 | 7.4–6.8 | 2 d | Increased nauplius mortality, hatching rate | Kurihara et al. (2004a,b) |
| *A. erythraea* (adult) | $CO_2$ 5000–10 000 | 7.0–6.8 | 8 d | pH < 7.0, decreased egg production | Kurihara et al. (2004a,b) |
| *Acartia tsuensis* (egg, larva) | $CO_2$ 2000 | 7.4 | 9 d | No effect | Kurihara et al. (2008) |
| *A. tsuensis* (adult) | $CO_2$ 2000 | 7.4 | 27 d | No effect | Kurihara et al. (2008) |
| *Calanus finmarchicus* (egg) | $CO_2$ 8000 | 6.95 | 72 h | Decreased hatching success | Mayor et al. (2007) |
| *C. finmarchicus* (adult) | $CO_2$ 8000 | 6.95 | 5 d | Decreased egg production | Mayor et al. (2007) |
| ***Palaemon pacificus*** **(egg, juvenile)** | $CO_2$ 2000 | 7.6 | 23~36 d | Decreased settling size | Present study |
| ***P. pacificus*** **(adult)** | $CO_2$ 1000, 2000 | 7.9, 7.6 | 30,15 wk | Decreased survival, growth, egg production | Kurihara et al. (2008) |
| **Antarctic krill (egg, larva)** | $CO_2$ 1000, 2000 | 7.7, 7.4 | 26 d | Decreased hatching success | Kurihara et al. (unpubl. data) |
| ***Hemicentrotus pulcherrimus*** **(egg, larva)** | $CO_2$ 860–10 360 | 7.8–6.8 | 3 d | pH < 7.8, skeletal malformation, reduced larval size, fertilization decrease with increasing $CO_2$ | Kurihara & Shirayama (2004a,b) |
| ***H. pulcherrimus*** **(adult)** | $CO_2$ 560 | 7.9 | 6 mo | Decreased survival, growth rate | Shirayama & Thornton (2005) |
| | $CO_2$ 1000 | 7.8 | 8 m | Decreased reproduction, no effects on survival | Kurihara et al. (unpubl. data) |
| ***Echinometra mathaei*** **(egg, larva)** | $CO_2$ 860–10 360 | 7.8–6.8 | 3 d | pH < 7.8, skeletal malformation, reduced larval size | Kurihara & Shirayama (2004a,b) |
| ***E. mathaei*** **(adult)** | | | | Fertilization decrease with increasing $CO_2$ | Kurihara & Shirayama (2004a,b) |
| ***Paracentrotus lividus*** **(egg, larva)** | HCl | 6.5–8.5 | 48 h | pH < 7.5, skeletal malformation; pH < 7.7, morphological abnormality; Mitotic abnormality with decreasing pH | Pagano et al. (1985a,b) |
| *Sphaerechinus granularis* (egg, larva) | HCl, $H_2SO_4$ | 5.5–8.0 | 5 h | pH < 6.5, total metaphase blockage | Cipollaro et al. (1986) |

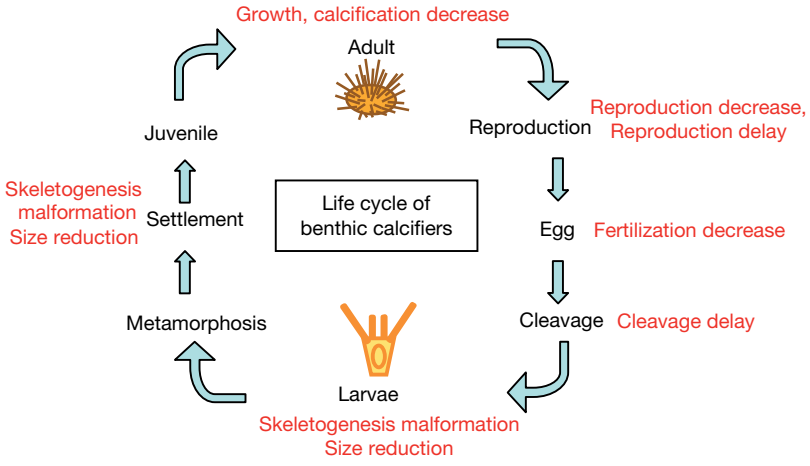


Fig. 5. Summary of $CO_2$ effects at different life cycle stages of benthic calcifiers under $CO_2$ concentrations that are expected to occur in the future ocean (380~2000 µatm $pCO_2$ / pH 8.2~7.3). Although the magnitude of $CO_2$ tolerance may differ between species and life stages, effects of high $CO_2$ are proposed for several different life stages, including reproduction, egg, cleavage, larva, settlement and adult stages

1981). Similarly, ACC in sea urchin larvae transformed into high magnesium calcite (Mg-calcite, >4 mol% $Mg^{2+}$ substituting for $Ca^{2+}$) over a period of hours to days (Addadi et al. 2003, Politi et al. 2004). A recent study predicts that the stoichiometric solubility of Mg-calcite can exceed that of aragonite (Morse et al. 2006). Studies evaluating whether or not other calcifiers also use ACC as a transient precursor phase in their larval stages are very limited (Addadi et al. 2003). However, since research shows that both mollusks and echinoderms, on 2 separate phylogenetic branches, initially precipitate ACC before less soluble forms during later life stages, it is highly probable that this strategy is widespread among marine calcifiers. Further studies evaluating the ontogenic impacts of high $pCO_2$ concentration on calcifiers are anticipated.

## CONCLUSIONS AND PERSPECTIVES

As discussed above, $CO_2$ is expected to impact the life cycles of benthic calcifiers in different ways under increasing levels (380~2000 µatm $pCO_2$/ pH 8.2~7.3). The effects of high $pCO_2$ in seawater are anticipated to occur in several different life stages, including egg, cleavage, larva, settlement, juvenile and adult stages, which are consequently likely to impact the distribution and abundance of benthic calcifiers (Fig. 5). Impacts on fertilization and reproduction can directly affect population size, and decreased calcification at larval and settlement stages is considered to affect their fitness and increase mortality. Cumulative effects across different life stages may lead to species extinctions.

$CO_2$ tolerance seems to differ between life stages (e.g. larva and adult). Additionally, the vulnerable stages can also differ between species. For example, although the larval stage of sea urchins and bivalves seemed to be most vulnerable to high $pCO_2$, the settlement stage was the most severely affected in corals and marine shrimps. This can be partially explained by the fact that most echinoderms and mollusks start shell and skeleton synthesis at their larval stage, whereas corals start at the settlement stage. The present study also demonstrates that there are significant differences in the tolerance within and between different species (Table 1). Although most calcifiers were affected at $pCO_2$ values >1000 µatm (pH 7.9~7.7), copepods appear less sensitive to elevated $pCO_2$ conditions. The fertilization rate of *Echinometra mathaei* was observed to be more affected than that of *Hemicentrotus pulcherrimus* at the same $pCO_2$ level (Fig. 2). Therefore, it is possible that the community structure of calcifiers will change in the future ocean. Additionally, the impact of ocean acidification may also differ between organisms that live at different latitudes. Adding studies of Antarctic and Arctic species will be important given that the saturation states of aragonite and calcite decrease faster at high versus low latitudes (Orr et al. 2005).

Most calcifiers, such as corals, echinoderms, bivalves and crustaceans, play important roles in coastal ecosystems as keystone species, bioturbators and ecosystem engineers (Suchanek 1985, Gutiérrez et al. 2003). They are also socio-economically important as food sources and for industries such as tourism. On a global scale, $CaCO_3$ plays a role in regulating the oceanic carbon cycle (Feely et al. 2004). For example, marine mollusks are estimated to produce about 50 to 1000 g $CaCO_3$ $m^{-2}$ $yr^{-1}$ (Beukema 1982, Gutiérrez et al. 2003). For coral reef, the rate of calcification is approximately 10 kg $CaCO_3$ $m^{-2}$ $yr^{-1}$ (Chave et al. 1975). Given the importance of marine calcifiers to these processes, influences on their population size and composition will potentially cause negative impacts to coastal ecosystems, which, consequently, may even affect the whole oceanic ecosystem.

In contrast with marine calcifiers, effects of ocean acidification on non-calcifiers are poorly described. The present study reveals that elevated atmospheric $CO_2$ not only affects calcification, but also several other biological processes, such as fertilization, reproduction and physiology. There is a critical need for information on the effect of ocean acidification on non-calcifiers.

Additionally, in order to accurately assess the ecological impact of atmospheric $CO_2$, studies evaluating the synergetic impacts of ocean acidification and global warming on the early life and reproductive stages should be emphasized due to the vulnerability of these stages to environmental change. Impacts of global warming on the early life and reproductive stages have been studied to some extent. Foster (1971) mentions that larvae generally require a narrower temperature range for development compared to adults. O'Connor et al. (2007) demonstrated that temperature affects larval dispersal distance, with the implication that a warming ocean may influence population connectivity and structure. Svensson et al. (2005) demonstrated that unpredictable spring temperatures could lead to the mismatching of larval release with spring phytoplankton blooming, and reduce their recruitment. Thus, the interactive effect of $CO_2$ and temperature on early development and reproductive stages is a high priority for future studies.

Finally, a better understanding of the mechanisms behind $CO_2$ impacts on organisms and processes of biological adaptation and evolution is very important for any attempt to accurately forecast how marine organisms and the ecosystem will respond to ocean acidification. Most of the data gathered on the effects of ocean acidification (e.g. Table 1) highlight the impact of high $pCO_2$ (low $[CO_3^{2-}]$ and $CaCO_3$ saturation state) on both internal and external $CaCO_3$ skeletogenesis, even in seawater supersaturated with $CaCO_3$. Nevertheless, the mechanism behind this phenomenon is still obscure, because several studies have suggested that the major source of dissolved inorganic carbon for calcification is $HCO_3^-$ derived from the surrounding seawater or converted by metabolic $CO_2$ rather than $CO_3^{2-}$ (Tanaka et al. 1986, Furla et al. 2000, McConnaughey & Gillikin 2008). This may be partially explained by the indirect effect of decreased metabolic rate due to high $pCO_2$, since the respiration rate of several marine animals is observed to decrease under high $pCO_2$ (Langenbuch & Pörtner 2004, Michaelidis et al. 2005). Another possible explanation is that the extracellular fluid (where calcification takes place) of calcifiers becomes undersaturated for $CaCO_3$ even in $CaCO_3$ supersaturated seawater. The extracellular pH of most marine organisms is generally lower than that in the surrounding seawater (e.g. bivalve mantle hemolymph, pH 7.4~7.6), whereas $[Ca^{2+}]$ is similar to that of seawater (9 to 10 mM; Omori et al. 1988). When invertebrate calcifiers, such as bivalves and sea urchins, are exposed to high $pCO_2$ conditions, the hemolymph pH shows a permanent reduction (Michaelidis et al. 2005, Miles et al. 2007), suggesting that extracellular pH can become undersaturated even with a slight increase in seawater $pCO_2$.

On the basis of future climate scenarios, it is predicted that 15 to 37% of species and taxa will become extinct by 2050 (Thomas et al. 2004). However, it remains to be determined whether marine organisms will be able to adapt to a rapidly changing ocean environment. Recent research has revealed that organisms could evolve within decades in response to strong pressures, which Stockwell et al. (2003) termed 'contemporary evolution'. However, the capacity of marine organisms to adapt to increased seawater $pCO_2$ is unclear. Collins & Bell (2004) have performed the only study to examine the possible adaptation to an increased $CO_2$ concentration by an organism, the green alga *Chlamydomonas reinhardtii.* However, the relatively long generation length of marine calcifiers, such as echinoderms, bivalves and corals, which is an important factor for the evolutionary potential of a species, makes 'rapid evolution' of most calcifiers unlikely in response to the changes in the ocean environment (Berteaux et al. 2004).

Meanwhile, recent palaeontological studies have demonstrated that during the Paleocene-Eocene thermal maximum (PETM), when atmospheric $CO_2$ increased at the rate of 0.2 GtC $yr^{-1}$ within <10 000 yr, catastrophic extinctions of 35 to 50% of benthic foraminiferan species occurred (Thomas 1998, Gibbs et al. 2006). It is also worth mentioning that the present anthropogenic rate of $CO_2$ emission is 8 GtC $yr^{-1}$, which is 16 times the rate during the PETM interval (Gibbs et al. 2006). Though further information is urgently needed on genetic variation, genetic response and adaptation of marine organisms in a high $CO_2$ world, the present data suggest that deleterious impacts on marine calcifier populations are very likely to occur in the future ocean.

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

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# Near-future level of $CO_2$-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*

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ABSTRACT: The world's oceans are slowly becoming more acidic. In the last 150 yr, the pH of the oceans has dropped by ~0.1 units, which is equivalent to a 25% increase in acidity. Modelling predicts the pH of the oceans to fall by 0.2 to 0.4 units by the year 2100. These changes will have significant effects on marine organisms, especially those with calcareous skeletons such as echinoderms. Little is known about the possible long-term impact of predicted pH changes on marine invertebrate larval development. Here we predict the consequences of increased $CO_2$ (corresponding to pH drops of 0.2 and 0.4 units) on the larval development of the brittlestar *Ophiothrix fragilis*, which is a keystone species occurring in high densities and stable populations throughout the shelf seas of northwestern Europe (eastern Atlantic). Acidification by 0.2 units induced 100% larval mortality within 8 d while control larvae showed 70% survival over the same period. Exposure to low pH also resulted in a temporal decrease in larval size as well as abnormal development and skeletogenesis (abnormalities, asymmetry, altered skeletal proportions). If oceans continue to acidify as expected, ecosystems of the Atlantic dominated by this keystone species will be seriously threatened with major changes in many key benthic and pelagic ecosystems. Thus, it may be useful to monitor *O. fragilis* populations and initiate conservation if needed.

KEY WORDS: Climate change · Ocean acidification · Echinoderms · Larval development · $CO_2$ · Brittlestar · Calcification · Skeletogenesis



## INTRODUCTION

Recent global models predict that pH at the ocean surface will fall by an estimated 0.2 to 0.4 units by the year 2100 largely due to human-driven emissions of $CO_2$ (Caldeira & Wickett 2003, 2005, Royal Society 2005, Cao et al. 2007); work by Doney et al. (2007) suggests that this may be exacerbated by anthropogenically released sulphur and nitrogen, especially in coastal waters. These predicted changes in ocean pH are greater, and far more rapid, than any that have been experienced in the past 300 million yr, and the ability of marine organisms, populations and ecosystems to adapt to this unprecedented environmental modification is largely unknown.

Available estimates suggest that rates of calcification in marine organisms have decreased by 11 to 44% since pre-industrial times (Andersson et al. 2005), and will fall to 60% during the 21st century (Kleypas et al. 2006). The calcium carbonate shells or skeletons of many planktonic organisms make them susceptible to dissolution in acidic waters, their degree of susceptibility being dependent not only on pH and carbonate saturation, but also on the crystalline form of calcium carbonate used (aragonite being ~2× more soluble than calcite; Royal Society 2005. Experiments on organisms

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as varied as corals, coralline algae, molluscs, foraminiferans and coccolithophorids have all documented reduced capacity for biomineralization at high $pCO_2$ and its associated low pH (e.g. Kleypas et al. 2006).

Despite this recent work, the impacts of $CO_2$-driven acidification on the delicate embryonic and larval stages that are essential for recruitment and population maintenance of many marine invertebrate taxa have been largely ignored. To date, only the works of Kurihara and others (Kurihara & Shirayama 2004, Kurihara et al. 2004, 2007, Kurihara & Ishimatsu 2008) have focussed on these early life-history stages. These authors have shown significant deleterious effects of $CO_2$-induced acidification on larval development and survival in echinoderms, crustaceans and molluscs. In 2 sea urchin species, fertilization rate decreased with pH but significant effects were only observed when the acidification was severe (pH 6.95 and 7.13 depending on the species). Acidification also induced a decrease in body length at Day 3, with significant effect at pH 7.6 to 7.7 (Kurihara & Shirayama 2004, Kurihara et al. 2004).

Echinoderms are appropriate model organisms as they play major roles in ecosystems as keystone predators and grazers (Paine 1966, Estes & Palmisano 1974), as bioturbators and remineralizers (Ambrose et al. 2001), and as food sources for commercial fish (e.g. *Limanda limanda*; Duineveld & Noort 1986, Mattson 1992) and crustaceans (e.g. *Nephrops norvegicus*; Baden et al. 1990). Critically for this study, echinoderm larvae have been shown to form skeletal rods from an amorphous calcite crystal precursor, which is 30× more soluble than normal calcite (Politi et al. 2004). It is therefore likely that echinoderm larvae will be particularly susceptible to $CO_2$-induced decreases in ocean pH, and that this may result in compromised larval development and survival, possibly leading to developmental and/or recruitment failure.

Here we report the first detailed assessment of the effects of increased $CO_2$ on embryonic and larval stages of the echinoderm *Ophiothrix fragilis*, which is a keystone brittlestar species that occurs in high densities and stable populations throughout the shelf seas of northwestern Europe (Morgan & Jangoux 2005).

## MATERIALS AND METHODS

Specimens of *Ophiothrix fragilis* were collected using an Agassi trawl from a rocky substratum in the Gullmarsfjord in the vicinity of the Sven Lovén Centre for Marine Sciences, Kristineberg, Sweden, and were subsequently maintained in natural flowing seawater at 14°C. Individuals were collected during the period of sexual maturity between May and August 2007. Ripe individuals were identified by their obvious gonads (white testes; orange ovaries) visible through the extended walls of the bursae. Two males and 10 females were used for each fertilization. All 12 ind. were placed in a container of seawater, and males were slightly agitated by hand for a few seconds until the release of sperm, which subsequently induced the females to spawn (Morgan & Jangoux 2005).

Cleaving embryos (two-cell stage) were placed in 5 l aquaria filled with filtered seawater (FSW, taken from the sampling site) at a density of 10 $ml^{-1}$. The FSW was continuously aerated, and a 1 l volume was replaced every 3 d.

*Ophiothrix fragilis* gonads are most developed in May to July (George & Warwick 1985), with highest gonadal index in June and July (Lefebvre et al. 1999). The gametes are released from June to September depending on locality (Davoult et al. 1990, Lefebvre & Davoult 2000), although individuals can breed throughout the year in some populations (Ball et al. 1995). Larvae are affected by environmental and physical factors that are independent of the benthic environment experienced by adults. Adults are located at depths between 20 and 80 m, while larval life is pelagic. The planktonic larval phase lasts ~26 d and the larvae metamorphose into juveniles while still in the plankton (MacBride 1907). Larvae are present in the plankton over several months (Lefebvre & Davoult 2000), with the main recruitment occurring between the end of August and beginning of September (Davoult et al. 1990). Larvae are concentrated near the surface and are more abundant in the upper 15 m (Lefebvre & Davoult 1998, 2001).

During the period May to September, the pH in Gullmarsfjord decreases with depth (ranging between 8.33 and 7.97), but never falls below 8.07 in the upper 30 m where *Ophiothrix fragilis* larvae are concentrated (data from SMHI Database Svenskt Havrarkiv). Based on these data, we selected a range of seawater pH predicted to occur by the year 2100 ($\Delta pH \approx -0.2$ to $-0.4$ units; Caldeira & Wickett 2003, 2005), which we regulated by manipulation of environmental $CO_2$ levels. These treatments were control/natural seawater (pH = 8.1), pH 7.9 and pH 7.7. One 5 l aquarium was used for each of the 3 treatments. Cultures were maintained at 14°C, a salinity of 32‰ and alkalinity of 2.12 ± 0.02 mM as measured following Sarazin et al. 1999. After Day 2, larvae were fed daily with the red alga *Rhodomonas* sp. at a concentration of 150 µg C $l^{-1}$. Food concentration was checked using an Elzone 5380 particle sizing and counting analysis system and corrected daily (at this concentration, the pH had no impact on algal growth and/or survival). The entire experiment was repeated 3× (n = 3) using different batches of parental animals. pH was maintained in each aquarium using a computerised control system (AquaMedic) that regulated pH by the addition of pure gaseous $CO_2$ directly into the water to a resolution of 0.04 pH units.

Larval cultures were monitored daily. Each day, a subsample of 50 larvae was removed and fixed in 4% paraformaldehyde in FSW for later analysis. Density at time $t$ ($N_t$, larvae $l^{-1}$) was estimated by dividing the number of larvae (50) by the corresponding volume needed to collect this number of individuals. Instantaneous mortality was calculated as: $M_t = 1 - (N_t/N_{t-1})$. Larvae were photographed with a digital camera mounted on a dissecting microscope using polarised light to visualise the skeleton. Six morphometric parameters (see Fig. 2) were measured for each larva using LAS software (Leica). In addition, a symmetry index (SI = ratio of left to right overall length) was calculated. Images were processed using Adobe Photoshop.

Data were analysed using 1- and 2-way ANOVA, Scheffé's and Dunnett's tests, with Bonferroni correction. Canonical discriminant analysis was used to assess the impacts of pH and/or exposure time on morphometric parameters. The Shapiro-Wilk statistic W (Shapiro & Wilk 1965) was used to check the data for normality of distribution. When data were not normally distributed or showed heteroscedasticity, a logarithmic transformation was done following Sokal & Rohlf (1995). Analyses were performed using SAS/STAT (SAS Institute 1990). Percentages of abnormal larvae through time were analysed using the Bhattacharya (1967) method in order to estimate means and SEs, using FISAT II software (FAO-ICLARM Stock Assessment Tools).

## RESULTS

### Effects on survival

Survival in the controls (pH 8.1) was 29.5 ± 5.5% after 8 d (average equivalent mortality rate of 20% $d^{-1}$), in comparison to <0.1% in both low pH treatments (average equivalent mortality rate of 35 ± 10.8% $d^{-1}$ at pH 7.9 and 50.4 ± 10.5% $d^{-1}$ at pH 7.7). A significant mortality increase in the low pH treatments versus controls was first observed after 7 d at pH 7.9, and after 5 d at pH 7.7 (Fig. 1). After 25 d, control larvae still showed an overall survival rate of 10% (equivalent to a mortality rate of 9.1% $d^{-1}$).

### Effects on growth

Under our 'control' rearing conditions (pH 8.1, 14°C), larval development was complete after 25 d. The chronology of development followed the pattern described by Morgan & Jangoux (2005). After 24 h, 72% of the larvae had reached the 2-arm (posterolateral) stage (Figs. 2 & 3). The second pair of arms (post-oral) started to develop on the second day. By Day 3, the larvae had begun to feed on the supplied *Rhodomonas* microalgae. The 6-arm stage (anterolateral arms) was completed after 5 d and the 8-arm stage (post-oral arms) started on Day 7. A similar developmental series was observed at low pH but with 3 notable differences: (1) none of the larvae in the low-pH treatments reached the 8-arm pluteus stage, (2) a high proportion of the larvae raised at low pH were either abnormal or asymmetric (see 'Results; Effects on development'), and (3) despite similarity, the temporal dynamics of development was delayed at low pH, with larvae taking longer to reach the same developmental stage. Thus, 50% of the larvae in the control cultures were 4-armed after 1.83 d compared to 2.07 and 2.25 d at pH 7.9 and 7.7, respectively. Similarly, 50% of the control larvae were 6-armed after 5.42 d compared to 5.73 and 5.71 d at pH 7.9 and 7.7, respectively.

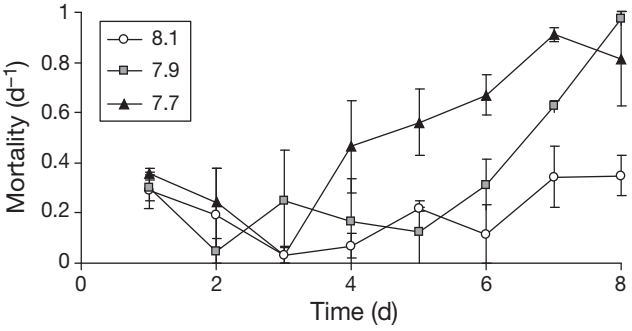


Fig. 1. *Ophiothrix fragilis.* Daily instantaneous mortality rates over time at the 3 tested pH values (n = 2). ANOVA showed significant effects of pH (df = 2, $F$ = 14.66, $p < 0.001$), time (df = 7, $F$ = 11.12, $p < 0.001$) and pH × time (df = 14, $F$ = 2.3, $p < 0.035$)

The impact of ocean acidification on larval and skeletal growth was assessed by measuring and comparing 7 morphometric parameters against 'normal'

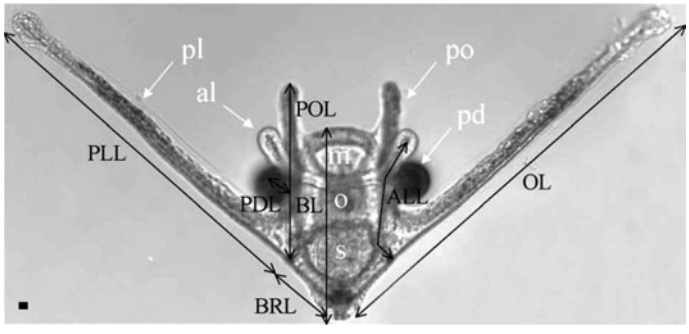


Fig. 2. *Ophiothrix fragilis.* Morphometric coordinates and morphology of the control 8-arm pluteus (Day 8, pH 8.1): al, anterolateral arm; ALL, anterolateral rod length; BL, body length; BRL, body rod length; pd, post-dorsal arm; m, mouth; o, oesophagus; PDL, post-dorsal rod length; pl, posterolateral arm; PLL, posterolateral rod length; po, post-oral arm; POL, post-oral rod length; OL, overall length; s, stomach. Scale bar = 10 µm

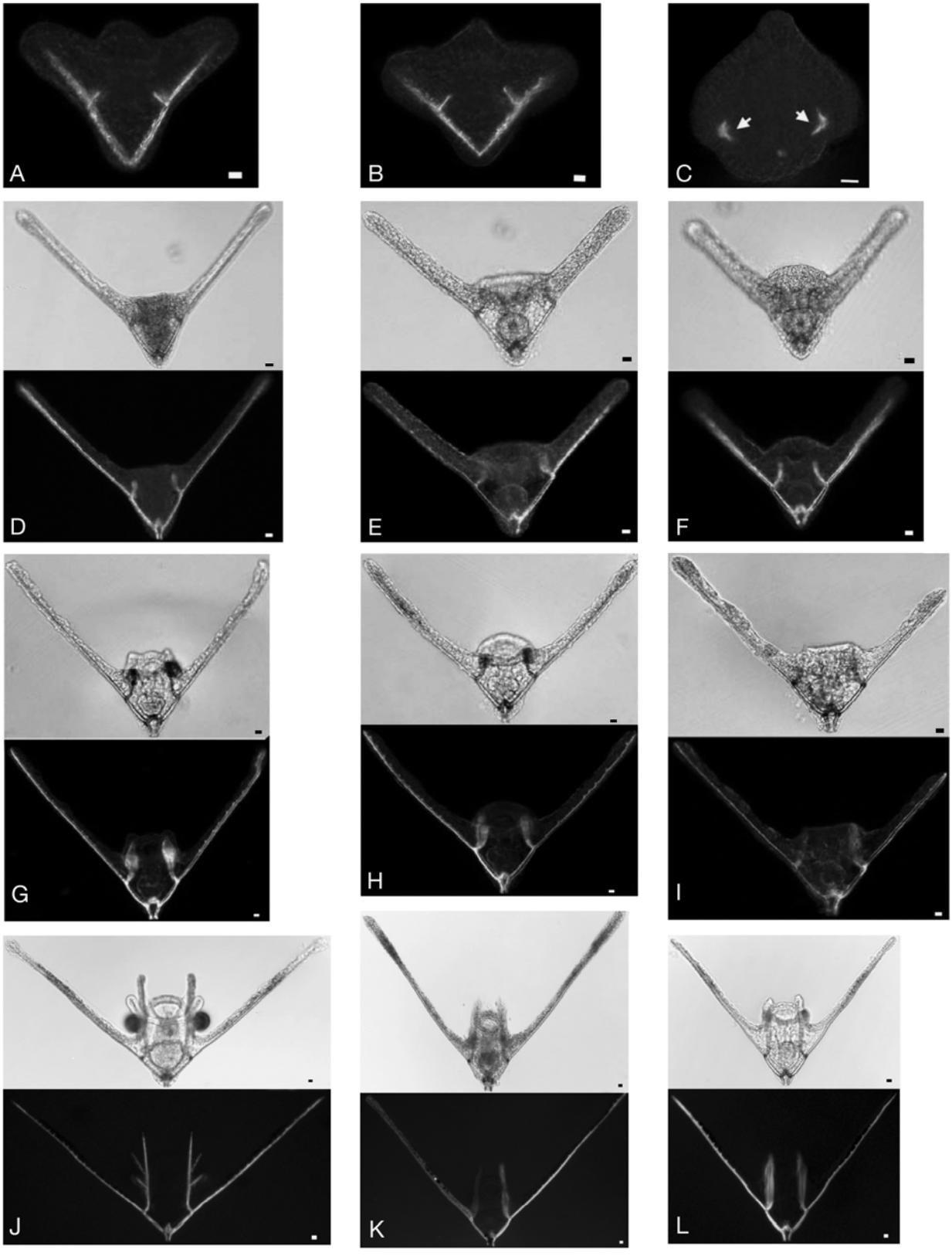


Fig. 3. *Ophiothrix fragilis*. Larval development at the 3 pHs used. First column (A, D, G, J), pH 8.1 (control); second column (B, E, H, K), pH 7.9; third column (C, F, I, L), pH 7.7. (A to C) Day 1, (D to F) Day 2, (G to I) Day 5, (J to L) Day 8. Dark panels: normal transmitted light; light panels: polarized light. Scale bars = 10 µm

larvae (Fig. 2). Abnormal and asymmetric larvae were excluded from this analysis. pH had no significant effect on anterolateral rod length (ALL, Fig. 4E) while differences were observed for other parameters. The most consistent differences were observed after Day 2 for body rod length (BRL, Fig. 4B), the rod being longer in the control than at pH 7.7. From Days 2 to 5, the larvae were more symmetric in the control than in those at low pH, even if the most asymmetric larvae (SI < 0.83) were not taken into account in this analysis. For the other parameters, some individual differences were observed, rods in the control being generally longer than at low pH (Fig. 4). Canonical discriminant analyses were performed on the morphometric para-

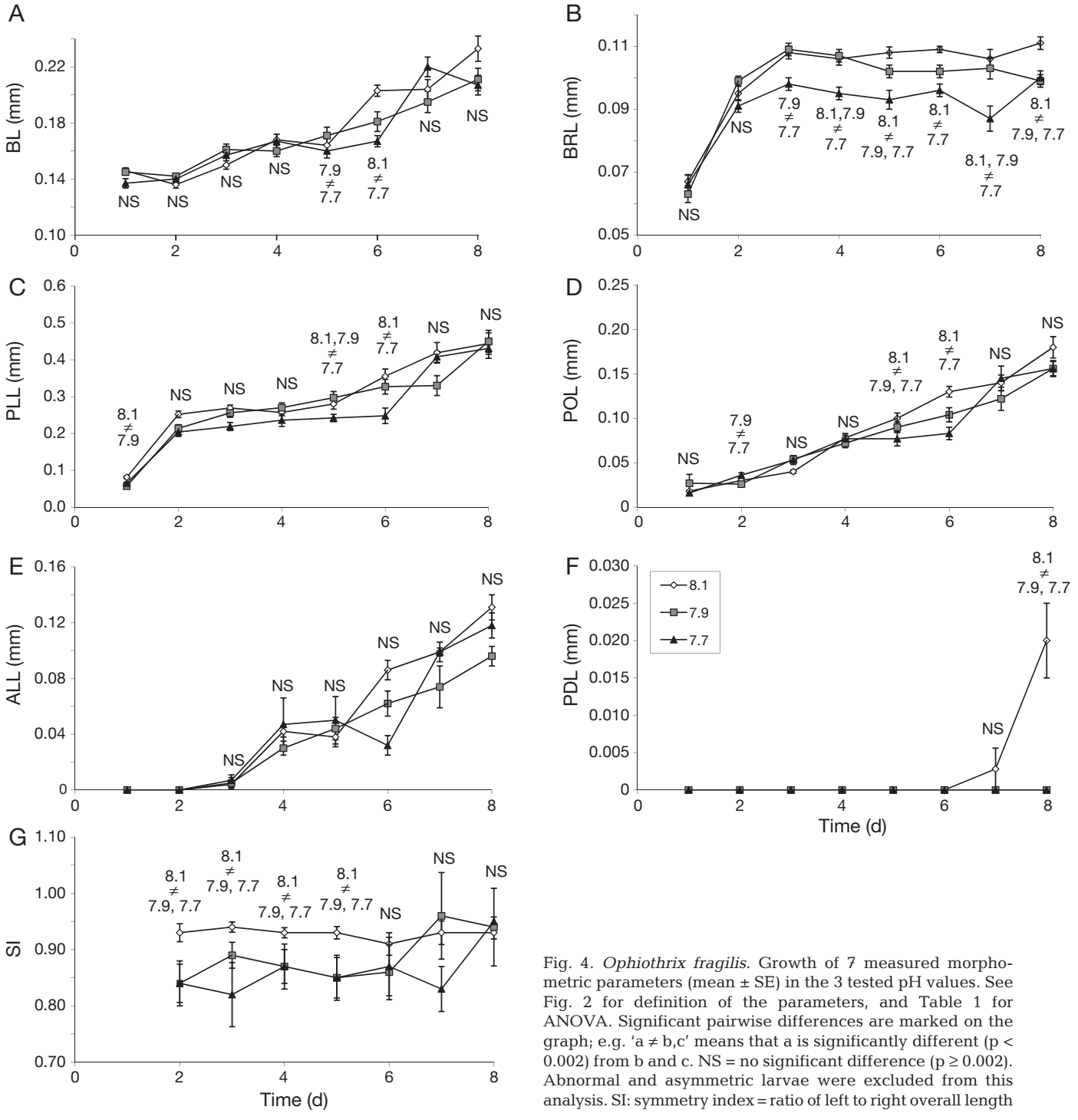


Fig. 4. *Ophiothrix fragilis.* Growth of 7 measured morphometric parameters (mean ± SE) in the 3 tested pH values. See Fig. 2 for definition of the parameters, and Table 1 for ANOVA. Significant pairwise differences are marked on the graph; e.g. 'a ≠ b,c' means that a is significantly different ($p < 0.002$) from b and c. NS = no significant difference ($p \geq 0.002$). Abnormal and asymmetric larvae were excluded from this analysis. SI: symmetry index = ratio of left to right overall length

Table 1. *Ophiothrix fragilis.* ANOVA of morphometric parameters as a function of pH and time. See Fig. 2 for definition of morphometric parameters. SI: symmetry index = ratio of left to right overall length

| Parameter | Source | df | *F* | p |
|---|---|---|---|---|
| BL | pH | 2 | 15.96 | <0.0001 |
| | Time | 7 | 72.44 | <0.0001 |
| | pH × Time | 14 | 4.76 | <0.0001 |
| BRL | pH | 2 | 30.34 | <0.0001 |
| | Time | 7 | 10.31 | <0.0001 |
| | pH × Time | 14 | 2.93 | 0.0003 |
| PLL | pH | 2 | 25.99 | <0.0001 |
| | Time | 7 | 102.82 | <0.0001 |
| | pH × Time | 14 | 3.07 | 0.0001 |
| POL | pH | 2 | 13.40 | <0.0001 |
| | Time | 7 | 152.15 | <0.0001 |
| | pH × Time | 14 | 5.34 | <0.0001 |
| ALL | pH | 2 | 1.04 | 0.36 |
| | Time | 7 | 0.81 | 0.58 |
| | pH × Time | 14 | 0.84 | 0.63 |
| PDL | pH | 2 | 10.51 | <0.0001 |
| | Time | 7 | 7.82 | <0.0001 |
| | pH × Time | 14 | 8.59 | <0.0001 |
| SI | pH | 2 | 10.35 | <0.0001 |
| | Time | 6 | 1.28 | 0.26 |
| | pH × Time | 12 | 1.05 | 0.40 |

meters to assess individual variation within the treatments at Days 1 and 4 (Fig. 5). At Day 1, all the larvae from the 3 pH treatments are clustered together indicating that they share similar body proportions. At Day 4, larvae raised at pH 7.7 are discriminated from those growing at pH 8.1 (all days) and pH 7.9 (Day 4). Larvae raised at pH 7.7 possessed proportions that were never observed in those raised at normal pH.

### Effects on development

A high proportion of the larvae raised at low pH were either abnormal (unable to develop into normal pluteus larvae; Fig. 6A) or asymmetric (Fig. 6B). The frequency of abnormal larvae through time followed a normal distribution (Fig. 7A). The highest proportion of abnormal larvae (calculated as the maximum of the normal distribution using the method of Battacharya [1967]) was observed after 3.7 ± 0.09 d at pH 7.7 and after 4.92 ± 0.07 d at pH 7.9. Abnormalities were completely absent in the control larvae.

A significant proportion of larvae showed asymmetry at low pH (Fig. 7B): after 2 d, 25 and 32% of normal larvae (i.e. no abnormalities) were asymmetric at pH 7.9 and 7.7 respectively. These percentages decreased throughout the larval period until the last 2 d, by which time very few individuals remained in culture.

## DISCUSSION

$CO_2$-driven acidification had a dramatic impact on survival and development of *Ophiothrix fragilis* larvae. After only 8 d, all larvae at reduced pH (7.9 and 7.7) were dead, whereas control larvae (pH 8.1) showed only 30% mortality (Fig. 1). This corresponds to a 12-fold increase in larval mortality rate, caused by $CO_2$-induced acidification. Even allowing for the possibility that our treatments may have elevated the sensitivity of larvae (due to stress, suboptimal feeds, laboratory conditions, etc.), our results imply that the levels of $CO_2$-induced acidification predicted to occur within the next 50 to 100 yr ($\Delta pH \approx -0.2$ to $-0.4$ units; Caldeira & Wickett 2003, 2005) could, at the very least, cause severe reductions in larval survival, and quite possibly completely eradicate *O. fragilis* populations with little potential for acclimation and/or adaptation.

If our oceans continue to acidify as expected, *Ophiothrix fragilis* larvae will not be able to escape from these deleterious conditions. Ophiopluteus larvae have low swimming capabilities (Mileikovsky 1971) and act as passive particles without diel vertical migration (Lefebvre & Davoult 1998, 2001). Adult populations show little interannual variability in density and partly act as metapopulations. While some populations are mainly self-sustaining, larval supply from neighbouring populations (larvae can disperse within 70 to 100 km by water displacement; Davoult et al. 1990) can exceed local retention in other populations (Lefebvre et al. 2003). Thus, even a local acidification event could impact *O. fragilis* populations on a wider scale.

*Ophiothrix fragilis* is a widely distributed species in the eastern Atlantic, from northern Norway to the Cape of Good Hope. It is a keystone and dominant species in many coastal communities (Lefebvre & Davoult 1997). It is also an essential component of the epibenthos that feed on phytoplankton and provide coupling between benthic and pelagic ecosystems in the English Channel. It can reach very high densities of up to 7000 ind. $m^{-2}$ (Davoult 1989, Migné & Davoult 1997, Davoult & Migné 2001) forming beds of considerable physical complexity with many crevices and shelters. In some beds where *O. fragilis* represents half of the biomass, up to 78 other species have been recorded (Warner 1971). *O. fragilis* also has a dominant role in nutrient exchanges between estuarine and coastal ecosystems (Lefebvre & Davoult 1997). For example, precipitation of calcium carbonate in skeletal ossicles is a source of carbon; for the English Channel community, *O. fragilis* provides as much as 35% of the phytoplankton carbon requirement (Migné & Davoult 1997, Migné et al. 1998). Stomach contents of most common predators also show that *O. fragilis* is an important food for many species (Warner 1971). If *O. fragilis* is threat-

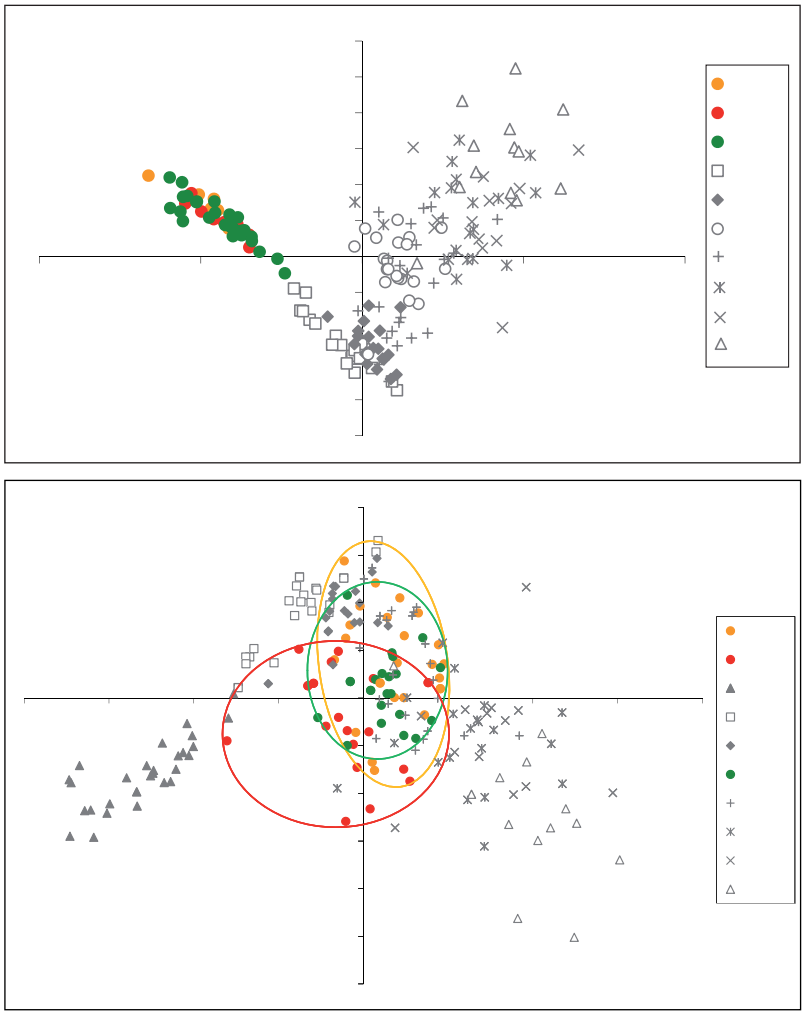

Fig. 5. *Ophiothrix fragilis.* Canonical discriminant analysis of the morphometric parameters used to separate the different pH treatments and time post fertilization (d). The data from Days 1 to 8 were used for the control pH 8.1 (8.1_1 to 8.1_8 in grey) when only the data from Day 1 (A) or Day 4 (B) were used for lower pH. (A) Day 1, no difference between the 3 treatments; (B) Day 4, larvae from pH 7.7 are discriminated from the other treatments and from larvae in the control. (A): can 1 = 0.73BL + 0.88BRL + 0.94PLL + 0.86POL + 0.8ALL + 0.3PDL; can 2 = 0.57BL + 0.31BRL – 0.01PLL + 0.48POL + 0.56ALL + 0.37PDL. (B): can 1 = 0.69BL + 0.82BRL + 0.89PLL + 0.85POL + 0.7ALL + 0.3PDL; can 2 = 0.58BL – 0.35BRL + 0.01PLL + 0.5POL + 0.54ALL + 0.41PDL

ened in the near future as suggested by our results, major changes in many key benthic and pelagic ecosystems of the Atlantic will likely occur. Thus, it may be useful to monitor *O. fragilis* populations and initiate conservation if needed.

Very few other workers have reported the impacts of $CO_2$-driven pH change on larval performance. The few studies available in the literature used ΔpH values much greater than those used here (Kurihara & Shirayama 2004, Kurihara et al. 2004, 2007). For example, in the oyster *Crassostrea gigas*, a reduction in pH of 0.7 units induced major morphological abnormalities in larvae (only 4 to 5% developed normally), and a significant decrease in the calcification rate (Kurihara et al. 2007). In the sea urchins *Hemicentrotus pulcherrimus* and *Echinometra mathaei*, ΔpH of −1.0 to −1.4 had significant negative impacts on fertilization rate, cleavage rate, developmental speed and larval size (Kurihara & Shirayama 2004, Kurihara et al. 2004). The high ΔpH values used in these studies correspond to much higher levels of acidification than predicted for the coming 2 centuries (Caldeira & Wickett 2005, Cao et al. 2007). Kurihara and colleagues also detected negative impacts on larvae at lower ΔpH values, although these effects were smaller possibly due to the shorter duration of their experiments (3 d, Kurihara & Shirayama 2004; 2 d, Kurihara et al. 2007). After comparable periods, our own experiments also showed nonsignificant declines in larval performance (Fig. 1). We can thus speculate statistically significant effects at smaller ΔpH values if Kurihara and co-workers had run their experiments for longer periods.

A key function of development is to put the right cells in the right places at the right time while simultaneously ensuring function and survival (Strathmann 2000). The calcite skeleton of larval brittlestars (and of echinopluteus larvae of sea urchins) has been proposed to confer several adaptive developmental benefits including maintenance of body shape (aids morphogenesis and feeding; Hörstadius 1939, Okazaki 1956, Pennington & Strathmann 1990); passive larval orientation (aids feeding and vertical migration; Pennington & Strathmann 1990); and defence against predators (Emlet 1983, but see Pennington & Strathmann 1990). Abnormal development of the skeleton would therefore be expected to have dramatic consequences for fitness, consistent with the results obtained here.

*Ophiothrix fragilis* larvae raised at low pH exhibited several developmental problems. A high proportion (>50% of the culture at Days 5 to 6, Fig. 7A) of abnormal larvae with none of the features of normal pluteus larvae (see Fig. 7 for examples) and a high proportion (Fig. 7B) of asymmetric larvae would result in problems with maintenance of normal larval orientation.

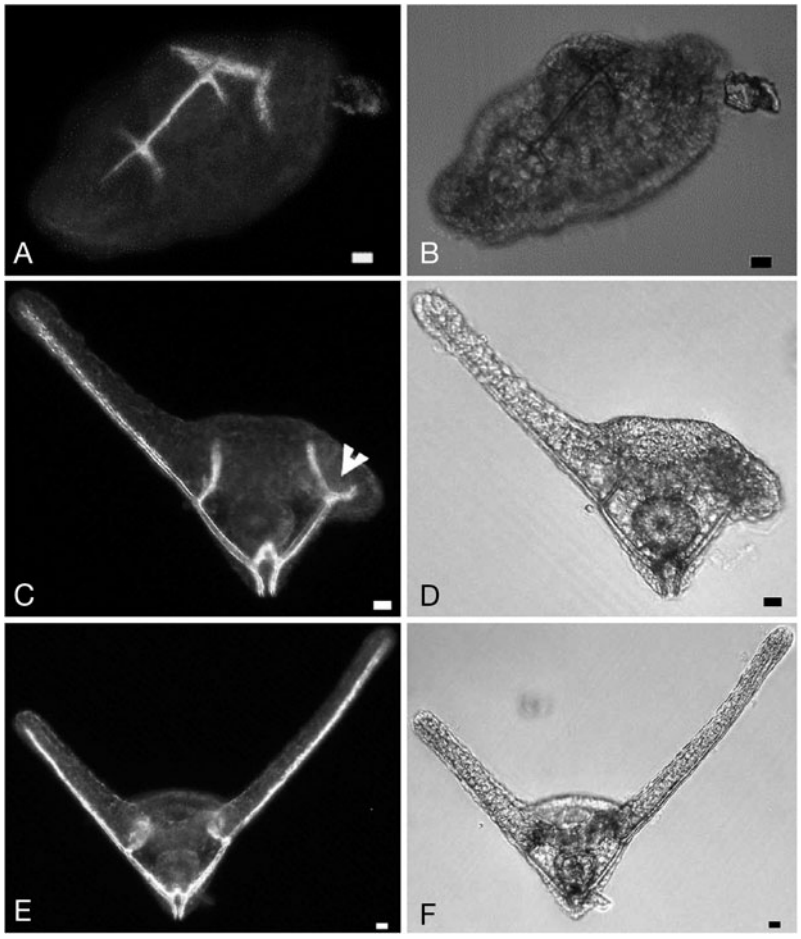


Fig. 6. *Ophiothrix fragilis.* Examples of abnormal (A,B) and asymmetric (C–F) larvae: (A,B) Day 2 larva at pH 7.7; (C,D) Day 2 pluteus at pH 7.7 with a reduced posterolateral rod (arrowhead); (E,F) Day 2 asymmetric pluteus at pH 7.9. (A,C,E) Under normal transmitted light; (B,D,F) under polarized light. Scale bars = 10 µm

Moreover, even larvae with normal shape (not abnormal or asymmetric) raised at low pH have different morphometric proportions than those raised at normal pH (Fig. 5). This may also have consequences for larval orientation and thus fitness and survival.

Our results showed marked increases in mortality in low pH treatments after 4 to 6 d (Fig. 1) — the stage at which the larvae started to feed — suggesting that mortality may be a consequence of compromised larval feeding performance at reduced pH as we observed in other species (S. Dupont & M. Thorndyke unpubl. data). Interestingly, the percentage of abnormalities decreased after Days 4 to 5 (Fig. 7), reflecting selective mortality.

It seems highly probable that pH-induced changes in skeletogenesis (abnormalities, asymmetry, morphometric changes) such as those observed here (Fig. 6) were due to the disruption of one or more molecular mechanisms involved in calcification (Livingston et al. 2006), in addition to interference with the basic chemistry of calcification. Moreover, ion transport mechanisms control asymmetry in sea urchins as they do in several vertebrate species (Hibino et al. 2006); these processes are highly sensitive to variations in pH (Mignen & Shuttleworth 2000).

Some authors have used guidelines published by the US Environmental Protection Agency to argue that a change of 0.2 pH units will be essentially unimportant for marine species (Loáiciga 2006). Even larger ΔpH ranges have been suggested to be 'environmentally safe' (Knutzen 1981). Yet few, if any, of the studies on which those conclusions were based had manipulated seawater pH (and carbonate saturation levels) by controlling $pCO_2$. There is still a critical shortage of environmentally relevant observations of the likely impacts of ocean acidification on marine species (Harley et al. 2006); however, current understanding of the relevant processes, in combination with experimental results (Langdon et al. 2000, Riebesell et al. 2000, Feely et al. 2004, Kurihara & Shirayama 2004, Shirayama & Thornton 2005, Berge et al. 2006, Kurihara et al. 2007, Miles et al. 2007) lends strong support to the assertion that such relatively small ranges of pH change should be considered as potentially harmful for marine biota (Caldeira & Wickett 2005).

Our data show that small changes in pH as low as the 0.2 unit decrease predicted for the coming few decades (Caldeira & Wickett 2003, 2005) can have dramatic consequences for larval development and survival of key species. Our results for the brittlestar *Ophiothrix fragilis* clearly show that such changes could threaten the long-term viability of the species. Whether other species of marine invertebrates are equally sensitive to such small pH shifts is unknown; there are no other strictly comparable data, although we argue above that the results of Kurihara & Shirayama 2004 are consistent with the results obtained here. Taxa from habitats that experience large natural pH shifts (e.g. algal bloom specialists such as planktonic copepods, or burrowing crustaceans and worms) are certainly likely to be better adapted to such changes. It has been suggested that this variability in sensitivity could have considerable implications for the diversity and functioning of communities as ocean pH declines (Royal Society 2005), placing some ecosystems more 'at risk' than others. If

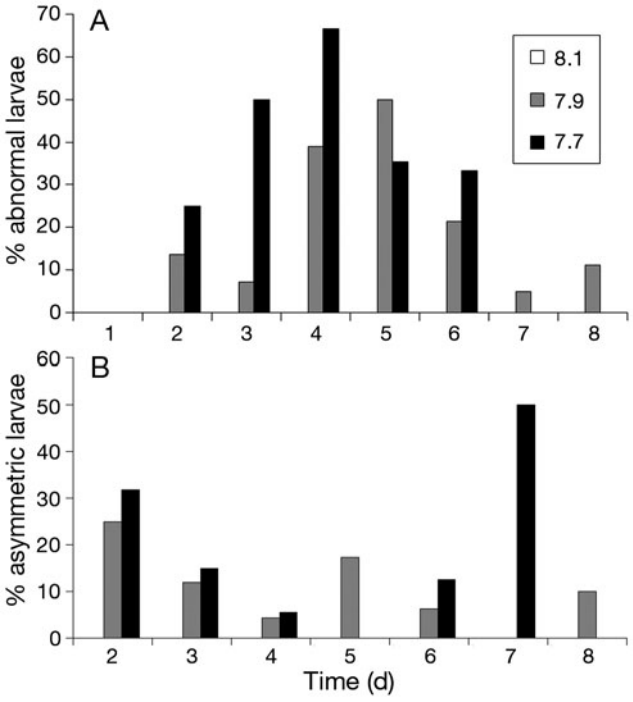


Fig. 7. *Ophiothrix fragilis.* Percentage of (A) abnormalities and (B) asymmetry in larvae raised at pH 8.1, 7.9 and 7.7 (data pooled from the different repetitions). By definition, no larva was asymmetric at pH 8.1 (controls). Note the absence of abnormalities at pH 8.1 (controls)

the pH continues to decrease as suggested by current models, we can then expect a strong selection for the more tolerant species and a major reorganisation at the ecosystem level.

We strongly echo the comments of Harley et al. (2006) that 'more research on the ecological implications of pH change is desperately needed' (Harley et al. 2006, p. 233). Experiments testing the impact of long-term exposure to small and environmentally relevant $CO_2$-induced decreases in pH should be conducted on other potential high-risk species such as echinoderms, molluscs and corals; more importantly, these experiments should be conducted on all life stages. Extinction does not require the instantaneous death of all individuals in a species. A decrease of as little as 1% per generation may reduce many animal populations to unsustainable densities in a little more than a century. Sublethal impacts of ocean acidification on egg production, fertilization success, larval development, larval dynamics and feeding, settlement success, metamorphic success and post-metamorphic survivorship will all influence the fitness and resilience of marine populations. Consequently, it is vital that future studies 'close the loop' by analysing the effects of acidification on all aspects of the life cycle, and over several generations, to assess acclimation, adaptive potential and adaptation of key species.

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# Fishes in high-$CO_2$, acidified oceans

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

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



## INTRODUCTION

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

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

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

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

[a]2 studies used both freshwater and seawater fish

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

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

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

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

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

## MORTALITY

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

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

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

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

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

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

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

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

## ENERGETIC COSTS OF LIVING IN HIGH $CO_2$ OCEANS

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

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

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

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

## OXYGEN CONSUMPTION

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

## GROWTH

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

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

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

[a]Transient significant increases at the onset of hypercapnia

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

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

## SKELETONS AND OTOLITH FORMATION

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

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

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

## SUMMARY AND RESEARCH NEEDS

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

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

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

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

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

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

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

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

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

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# Growth and calcification in the cephalopod *Sepia officinalis* under elevated seawater $pCO_2$

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ABSTRACT: Ocean acidification and associated changes in seawater carbonate chemistry negatively influence calcification processes and depress metabolism in many calcifying marine invertebrates. We present data on the cephalopod mollusc *Sepia officinalis*, an invertebrate that is capable of not only maintaining calcification, but also growth rates and metabolism when exposed to elevated partial pressures of carbon dioxide ($pCO_2$). During a 6 wk period, juvenile *S. officinalis* maintained calcification under ~4000 and ~6000 ppm $CO_2$, and grew at the same rate with the same gross growth efficiency as did control animals. They gained approximately 4% body mass daily and increased the mass of their calcified cuttlebone by over 500%. We conclude that active cephalopods possess a certain level of pre-adaptation to long-term increments in carbon dioxide levels. Our general understanding of the mechanistic processes that limit calcification must improve before we can begin to predict what effects future ocean acidification will have on calcifying marine invertebrates.

KEY WORDS: Ocean acidification · Calcification · Metabolism · Growth · Marine invertebrate · Cephalopod · *Sepia officinalis*



## INTRODUCTION

Anthropogenic carbon dioxide ($CO_2$) emissions are acidifying the world's oceans. While current ocean pH values are already more than 0.1 units below those of pre-industrial times, further increases in atmospheric $CO_2$ concentrations to values of 1500 to 2000 ppm could result in a drop of ocean pH of up to 0.8 units within the next 300 yr (Caldeira & Wickett 2003). Together with declining pH values, ocean carbonate ion ($CO_3^{2-}$) concentrations will decrease, which in turn will lead to a reduction of calcium carbonate saturation ($\Omega$) in seawater (Zeebe & Wolf-Gladrow 2001). As many marine organisms form shells or skeletons from calcium carbonate minerals (primarily aragonite or calcite), considerable attention has been devoted to studying calcification processes in response to seawater acidification. Surface ocean waters are currently supersaturated with respect to both calcite and aragonite. However, recent measurements and models predict that surface seawater calcium carbonate saturation states are decreasing globally (Feely et al. 2004). By the year 2050 it is predicted that high latitude regions will become undersaturated ($\Omega < 1$) with respect to aragonite ($\Omega_{arag}$) as a consequence of ocean acidification (Orr et al. 2005).

Most marine invertebrates respond negatively to elevated $CO_2$ concentrations. Many cnidarians, molluscs and echinoderms display reduced rates of calcification (Fabry et al. 2008). Interestingly, some of these organisms display strong linear relationships of calcification rate with the saturation of calcium carbonate ($\Omega$) (Fig. 1). The changes in calcification recorded over a 2 yr period in the Biosphere 2 mesocosm (Langdon et al. 2000; data replotted from their Table 4 in our Fig. 1) illustrate the high sensitivity of reef building communities to calcium carbonate undersaturation. Bivalve molluscs also react sensitively to decreasing pH and $\Omega_{arag}$. The work of Gazeau et al. (2007) shows that net calcification in the mussel *Mytilus edulis* decreases linearly with increasing $pCO_2$, and ceases when $pCO_2$ is above 1800 ppm (data replotted from their Table 1 in

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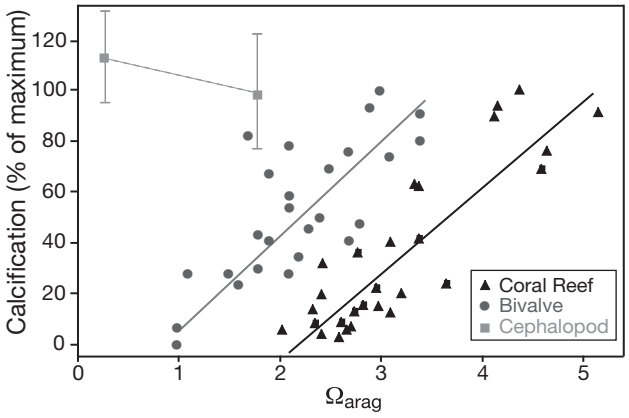


Fig. 1. The dependence of calcification on $CO_2$-dependent seawater calcium carbonate saturation ($\Omega_{arag}$) in marine invertebrates. Long-term coral reef data set recorded in the Biosphere 2 mesocosm (Langdon et al. 2000, data replotted from their Table 4), acute changes in *Mytilus edulis* (bivalve) calcification (Gazeau et al. 2007, data replotted from their Table 1), *Sepia officinalis* (cephalopod) calcification measured over 6 wk in this study (data are mean ± SD, n = 20). The highest calcification rates in the respective data sets were set at a value of 100 %

our Fig. 1). While the latter might be explained by external shell dissolution when $\Omega_{arag} < 1$, decreasing calcification at $\Omega_{arag} > 1$ may indicate that significant physicochemical control exists over calcification in mussels.

Marine invertebrates whose calcification processes are disturbed by elevated $CO_2$ are also characterised by comparatively low metabolic rates and activity levels. These factors may increase a marine organisms' sensitivity to ocean acidification, as suggested by Seibel & Walsh (2003). In response to this possibility, the present study explores the calcification and growth capacity of an active mollusc (cephalopod) with a high metabolic rate, the, European cuttlefish *Sepia officinalis*, under acidified conditions. Cuttlefish possess an internal aragonite shell ('cuttlebone', see Fig. 3) that serves as a structural support and, with the help of ion transport mechanisms, as a buoyancy control device (Denton & Gilpin-Brown 1961a). Interestingly, we find that *S. officinalis* does not reduce its growth or calcification rate when exposed to ~6000 ppm $CO_2$ for a period of 6 wk.

## MATERIALS AND METHODS

**Experimental animals.** *Sepia officinalis* egg clusters were collected in the Bay of Seine, Normandy, France, in May 2006 and 2007. Cuttlefish were hatched and raised at the Alfred-Wegener-Institute, Bremerhaven, Germany, in a closed recirculating system (20 $m^3$ total volume, protein skimmer, nitrification filter, UV disinfection unit (Sander), salinity 32 to 34, temperature (mean ± SD) 15 ± 0.1°C, pH 7.9 to 8.2, constant 12 h dark:12 h light cycle). Water quality parameters were monitored weekly and concentrations of ammonia and nitrite were kept below 0.2 and 50 mg $l^{-1}$, respectively. The cuttlefish were initially fed a daily diet consisting of live mysids *Neomysis integer* and progressively transitioned to feed exclusively on frozen brown shrimp *Crangon crangon*.

**Growth trials of *Sepia officinalis* under elevated $pCO_2$ conditions.** For the 2 growth trials, each group of 20 *Sepia officinalis* ind. was maintained in shallow PVC basins (20 × 40 × 60 cm). Basins drained into reservoir tanks where the seawater was pumped through a nitrifying biofilter (Eheim Pro 2) and past a 12 W UV sterilizer before being recirculated into the holding tanks. The total seawater volume of each system was approximately 300 l. Water values were maintained at <0.2 mg $l^{-1}$ ammonium and <40 mg $l^{-1}$ nitrite. Holding and reservoir tanks were continuously bubbled with the appropriate gas mixture supplied by an MKS gas controller (MKS, model GSV-19). Specific seawater conditions for the various incubations are given in Table 1. The pH was measured with a WTW 340i meter and SenTix81 electrode calibrated daily with National Bureau of Standards (NBS) buffers. Total dissolved inorganic carbon ($C_T$) was measured using a gas chromatographic method modified from Lenfant & Aucutt (1966) and Pörtner et al. (1990). Seawater carbonate chemistry parameters were calculated from $C_T$ and $pH_{NBS}$ with the software CO2SYS (Lewis & Wallace 1998) using the dissociation constants of Mehrbach et al. (1973) as refitted by Dickson & Millero (1987).

Throughout the duration of the growth trials, cuttlefish were fed ad libitum with live brown shrimp. The wet mass of shrimp consumed daily by each group was

Table 1. Seawater physiochemical conditions during 6 wk growth trials. NBS: National Bureau of Standards; $C_T$: total dissolved inorganic carbon; $pCO_2$: partial pressures of $CO_2$. Values (except aragonite saturation state, $\Omega_{arag}$) are mean ± SD

| Incubation group | Temperature (C°) | Salinity | $pH_{NBS}$ | $C_T$ (µmol $kg^{-1}$) | $pCO_2$ (ppm) | $\Omega_{arag}$ |
|---|---|---|---|---|---|---|
| Control | 16.32 ± 0.12 | 32.8 ± 0.5 | 7.94 ± 0.06 | 2047 ± 68 | 705 ± 101 | 1.47 |
| $CO_2$ ~4000 ppm | 16.37 ± 0.12 | 32.9 ± 0.4 | 7.23 ± 0.04 | 2451 ± 54 | 4271 ± 373 | 0.34 |
| Control | 17.45 ± 0.16 | 31.4 ± 0.4 | 8.01 ± 0.04 | 2104 ± 56 | 628 ± 60 | 1.78 |
| $CO_2$ ~6000 ppm | 17.43 ± 0.15 | 32.3 ± 0.6 | 7.10 ± 0.03 | 2583 ± 43 | 6068 ± 389 | 0.27 |

recorded. Cuttlefish wet masses and mantle lengths were determined weekly over a period of 6 wk. Slopes of the exponential growth curves were used to determine the daily increase in percent body mass. Gross growth efficiency (percent conversion of ingested shrimp into biomass) was calculated for each group on a weekly basis by dividing the weekly increase in animal wet mass (g) by the mass of the food consumed by that group over the same time interval (Forsythe et al. 2002).

Cuttlebone dry mass and calcium carbonate ($CaCO_3$) content were determined upon termination of the experiment. The organic matrix contributed only 5 to 8% of total cuttlebone dry mass in the size range of sampled individuals (data not shown), the remainder of the mass being $CaCO_3$ (aragonite). We determined $CaCO_3$ content by back-calculating from the dry mass of the remaining organic matrix after dissolution of the cuttlebone $CaCO_3$ fraction with 4 M HCl following Birchall & Thomas (1983). All samples were weighed on a precision balance (ME235S, Sartorius).

**Determination of standard metabolic rate under hypercapnia.** Standard metabolic rates (SMR) were determined using intermittent closed respirometry. Oxygen consumption rates (3 to 4 runs of approximately 20 min each) were obtained between 08:00 and 20:00 h to avoid peak night activity periods of the cuttlefish (Denton & Gilpin-Brown 1961b). Briefly, cuttlefish (mean ± SD; 10.4 ± 4.3 g, n = 6) were fasted for 24 h and then incubated in cylindrical perspex chambers (3 × 25 cm) for a period of 3 d during which time they were acutely exposed to hypercapnic conditions. The chambers were perfused with seawater using an Ismatec peristaltic pump (ISM 404B) and gas-tight Tygon tubing (T4406-23). Applied flow rates (100 ml $min^{-1}$) ensured chamber oxygen partial pressures of approximately 18 to 20 kPa between measurements. Seawater from the growth trial reservoirs was pumped through a UV sterilization unit and then used to perfuse the respiration chambers (see Table 1 for seawater values under control and hypercapnic conditions). Temperature was maintained at (mean ± SD) 16 ± 0.2°C by placing the 4 replicate chambers in a water bath fitted with a thermostat. Oxygen partial pressures were measured using a fiber optic oxygen sensing system (Oxy-4 Micro, PreSens) and needle-type optodes, incorporated into the closed loop. Data were recorded using software supplied by the manufacturer, and oxygen consumption rates were calculated from linear declines in chamber oxygen partial pressure.

**Statistical analyses**. Results were analyzed using GraphPad Prism 4. Unpaired *t*-tests were carried out to assess the significance of differences between incubation groups at $p < 0.05$. A linear regression analysis was used to determine whether oxygen consumption rates varied with exposure time. All values are expressed as means ± SD.

## RESULTS

No differences in soft-tissue growth performance were measured between cuttlefish incubated at ~4000 and ~6000 ppm $CO_2$ and controls (Table 2). Final average body mass for the cuttlefish incubated at ~4000 ppm $CO_2$ equaled 11.16 ± 1.40 g compared with 11.63 ± 1.39 g for the control group. In those incubated at ~6000 ppm $CO_2$ the corresponding mass was 23.06 ± 4.15 g compared with 24.15 ± 5.25 g in the controls. All 4 of the experimental groups grew at high rates typical of juvenile cephalopods (Forsythe et al. 1994, Melzner et al. 2005), increasing body mass exponentially at a rate of approximately 4% $d^{-1}$. There were no significant differences between the exponential curves used to calculate daily growth (Fig. 2). Gross growth efficiencies (GGE), calculated from weekly means, were also similar between the 4 incubation groups; the values ranged between 36.6 ± 6.2% and 39.5 ± 4.5%, and there were no significant differences (Table 2).

Standard metabolic rates of cuttlefish exposed acutely to ~6000 ppm $CO_2$ showed no significant increase or decrease over time ($F_{1,9} = 2.9$, $p > 0.1$; Fig. 3). Mean oxygen consumption values during the control period were 0.092 ± 0.004 µmol $O_2$ $g^{-1}$ $min^{-1}$, and after 24 h of $CO_2$ exposure were 0.088 ± 0.003 µmol $O_2$ $g^{-1}$ $min^{-1}$.

Growth of the calcified cuttlebone was determined both indirectly, from the mantle length of the cuttlefish, and directly, by measuring the amount of deposited $CaCO_3$. At the end of the trial period, there

Table 2. *Sepia officinalis.* Growth and calcification during each of 2 separate trials under elevated $CO_2$ conditions. Values are mean ± SD, n = 20 in each of the incubation groups

| Incubation group | Initial wet mass (g) | Initial mantle length (mm) | Final wet mass (g) | Final mantle length (mm) | Daily mass gain (%) | Gross growth efficiency (%) |
|---|---|---|---|---|---|---|
| Control | 2.69 ± 0.30 | 20.53 ± 0.14 | 11.63 ± 1.39 | 37.16 ± 1.88 | 4.0 | 36.6 ± 6.2 |
| $CO_2$ ~4000 ppm | 2.70 ± 0.33 | 20.71 ± 0.17 | 11.16 ± 1.40 | 36.33 ± 2.29 | 3.8 | 38.9 ± 3.6 |
| Control | 4.61 ± 1.01 | 27.83 ± 2.47 | 24.15 ± 5.25 | 52.84 ± 4.03 | 3.9 | 39.5 ± 4.5 |
| $CO_2$ ~6000 ppm | 4.50 ± 1.08 | 27.90 ± 2.39 | 23.06 ± 4.15 | 52.01 ± 4.76 | 3.7 | 39.4 ± 3.7 |

were no significant differences between the mantle lengths of control cuttlefish and those incubated at ~6000 ppm $CO_2$ (52.01 ± 4.76 mm versus 52.84 ± 4.03 mm, respectively), nor between the control and ~4000 ppm $CO_2$ incubated cuttlefish (37.16 ± 1.88 mm versus 36.33 ± 2.29 mm, respectively) (Table 2). During the 6 wk growth period all of the cuttlefish increased the mass of their cuttlebones by over 500% (Fig. 3). Interestingly, in the ~6000 ppm $CO_2$ growth trial, the $CO_2$ incubated animals incorporated significantly more $CaCO_3$ into their cuttlebones than did the control group, 0.80 ± 0.15 g versus 0.71 ± 0.15 g, respectively. Functional control of the cuttlebones (i.e. buoyancy regulation) did not appear to be negatively affected by low pH conditions.

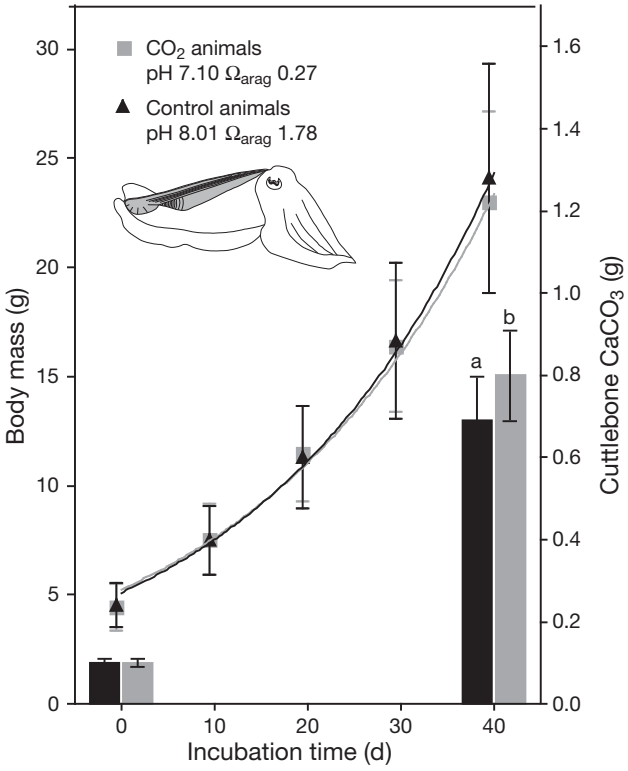


Fig. 2. *Sepia officinalis.* Growth (■▲, left *y*-axis) and calcification (bars, right *y*-axis) in the cuttlefish incubated under ~6000 ppm $CO_2$ (grey) and control conditions (black). For $CaCO_3$ accretion, means not sharing the same letter above bars are significantly different. Data are mean ± SD (n = 20). The calcified cuttlebone is shaded grey in the schematic drawing of *S. officinalis*

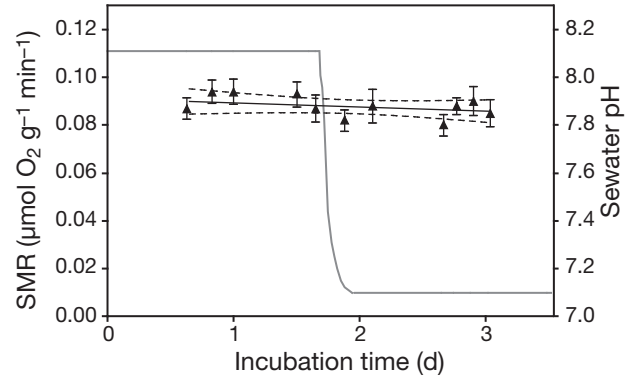


Fig. 3. *Sepia officinalis.* Standard metabolic rate (SMR) of cuttlefish during acute exposure to ~6000 ppm $CO_2$ (▲). Cuttlefish were placed in the chambers at Time = 0 and $CO_2$ exposure was started after 40 h of control measurements; the change in seawater pH (grey curve) reflects the time course of $CO_2$ exposure. Data are mean ± SD, n = 6

## DISCUSSION

The results of our growth trial show that at least 1 marine invertebrate species is capable of maintaining both metabolic rates and somatic growth performance at control levels during long-term exposure to significantly elevated seawater $CO_2$ concentrations.

### Growth

*Sepia officinalis* juveniles cultured at ~4000 and ~6000 ppm $CO_2$ grew at the same rate as did control individuals, gaining body mass at a rate of approximately 4% body mass $d^{-1}$ (Table 2). These growth rates closely correspond with results from previous work, where *S. officinalis* of similar size gained 3.5% body mass $d^{-1}$ at 17°C (Forsythe et al. 2002). Under both $CO_2$ conditions, there was no significant difference between control and treatment final wet mass gained during the 6 wk growth intervals. All cuttlefish more than quadrupled their body mass (Table 2). These results are in stark contrast to existing invertebrate growth studies under elevated $CO_2$. Michaelidis et al. (2005) found that under comparable $CO_2$ levels to our study, and over a growth period of 3 mo, shell length and soft body mass in the mussel *Mytilus galloprovincialis* were reduced by 55 and 70%, respectively (as calculated from their Fig. 3). Even more striking is the study reported by Shirayama & Thornton (2005) where significant differences in total body mass were measured in the sea urchin *Echinometra mathaei* and the gastropod *Strombus luhuanus* incubated under just 560 ppm $CO_2$ for half a year. Clearly, *S. officinalis* does not exhibit sensitivity to elevated $CO_2$ levels within the range of concentrations that elicits a negative response in most other invertebrates studied to date.

### Metabolism

Reduced growth performance in marine invertebrates under elevated $CO_2$ conditions has been suggested to

be a result of the organisms entering a state of metabolic depression (Pörtner et al. 2004). The cellular processes mediating metabolic depression have been extensively reviewed (Hand & Hardewig 1996, Guppy & Withers 1999, Storey & Storey 2007), and hypercapnia alone as an environmental stressor has been found to induce metabolic depression (Barnhart 1989, Rees & Hand 1990). Recent case studies on marine invertebrates support this conclusion; in *Sipunculus nudus* (Pörtner et al. 1998) and *Mytilus galloprovincialis* (Michaelidis et al. 2005) a decrease in metabolic rate in response to both acute and long-term hypercapnia exposure was accompanied by an uncompensated decrease in extracellular pH ($pH_e$). Working with an isolated muscle model, Pörtner et al. (2000) suggested that decreasing $pH_e$ slows down the rate of $H^+$ equivalent ion exchange between the extra- and intracellular space, and this in turn reduces the work load of $Na^+/K^+$-ATPase in maintaining the transepithelial electrochemical gradient. With this arrangement, organisms could effectively lower the energy requirements of acid–base regulation in their cells. However, they would still face new steady-state levels of decreased extracellular pH, elevated $pCO_2$ and $HCO_3^-$, which might have long-term effects on metabolic function (Reipschläger & Pörtner 1996). These could include changes in amino acid catabolism, with a preference towards net formation of metabolic bicarbonate for buffering (Langenbuch & Pörtner 2002). In combination with reduced rates of protein biosynthesis under low pH conditions (Smith et al. 1996, Reid et al. 1997, Langenbuch & Pörtner 2003), such processes would eventually limit somatic growth.

Metabolic depression is not evident in *Sepia officinalis* in response to acute $CO_2$ exposure, which matches the conserved growth rates observed in our study. Standard metabolic rates of around 0.09 µmol $O_2$ $g^{-1}$ $min^{-1}$ were maintained at a constant level during acute exposure to ~6000 ppm $CO_2$ (Fig. 2). The control metabolic rates we measured in *S. officinalis* match previously published values for similarly sized animals (Melzner et al. 2007a). A recent study working with the brittle star *Amphiura filiformis* also found no evidence of metabolic depression during long-term hypercapnic exposure under similar $CO_2$ levels (Wood et al. 2008). In fact, a significant increase in metabolic rate was found along with dramatic arm muscle wastage at an incubation pH of 7.3 (Wood et al. 2008). The catabolism of arm muscle to support elevated metabolic costs during hypercapnia, however, is indicative of a restructuring of the energy budget that significantly compromises long-term animal fitness.

In contrast, the cuttlefish in this study were not only capable of conserving growth and metabolic rates, but they also maintained their GGE at control levels under both ~4000 and ~6000 ppm $CO_2$ (Table 1). This suggests that the partitioning of their energy budget was conserved under hypercapnia, and that they did not simply ingest more food to maintain growth performance. Our GGE values, ranging from 36 to 39%, correspond with published values of 30 to 50% (Forsythe et al. 2002) for *Sepia officinalis* cultured at 17°C. A similar response is also known in fish, where metabolic rates and growth are not influenced even by high degrees of hypercapnia. Working with juvenile spotted wolffish *Anarhichas minor*, Foss et al. (2003) reported conserved growth rates, as well as food conversion efficiencies, at $CO_2$ concentrations up to 17 000 ppm $CO_2$. Fish are capable of maintaining growth rates under elevated $CO_2$ conditions because of their high ion transport and acid–base regulatory abilities. During acute hypercapnic exposure they rapidly increase $HCO_3^-$ levels in their blood, and are able to fully compensate their extracellular pH (Toews et al. 1983, Claiborne & Evans 1992, Larsen et al. 1997, Hayashi et al. 2004, Michaelidis et al. 2007). Thus, in contrast to most invertebrates, $pH_e$ is not depressed in fish during moderate, long-term hypercapnic exposure and, thus, does not influence potential reductions in metabolism and growth. The elevation of $HCO_3^-$ levels in response to hypercapnia-induced acidification is a response common to most organisms (Heisler 1989); however, the degree to which pH is compensated is dependent on ion-regulatory capacity and is species specific.

## Calcification

Not only does *Sepia officinalis* successfully acquire soft tissue mass under elevated $CO_2$ conditions, but it also maintains high calcification rates of its cuttlebone. *S. officinalis* is capable of calcifying under ~6000 ppm $CO_2$ and $\Omega_{arag}$ values of 0.27. Cuttlebone formation rate, as determined from mantle length measurements, was equal between all of the growth trial groups (Table 2). The cuttlebone is a fully internalized shell that is encased in a cuttlebone sac (Appellöf 1893), dorsally positioned along the anterior–posterior plane (see Fig. 3). When directly measured, total calcium carbonate accumulation in the cuttlebones of the ~6000 ppm $CO_2$ incubated individuals was actually found to be significantly higher than in the control group (Fig. 3). This puts *S. officinalis* in a unique position in relation to existing studies, since most invertebrates examined to date exhibit a negative influence of elevated $CO_2$ concentrations on calcification, and in some organisms there is a linear decrease of calcification rate with decreasing $\Omega_{arag}$ (Fig. 1). As far as we are aware, only one other study working with long-term hypercapnic exposure in invertebrates has shown in-

creased calcification rates under elevated seawater $CO_2$ levels (Wood et al. 2008).

Considering that calcification requires tight control of ionic composition and pH in the micro-environment at the deposition site (Weiner & Dove 2003), it seems likely that *Mytilus galloprovincialis*, and other invertebrates with low metabolic rates or low ion exchange capacities, are not capable of maintaining conditions favorable to mineral deposition under the acidification stress of hypercapnia. Findings of elevated calcium ions ($Ca^{2+}$) in *M. galloprovincialis* hemolymph, in combination with the previously mentioned uncompensated $pH_e$ reduction (Michaelidis et al. 2005), support such a hypothesis. In contrast, calcification at $\Omega_{arag} < 1$ in *Sepia officinalis* could be directly related to high, 'fish-like', ion regulatory capacities in this active invertebrate.

## SUMMARY

We conclude that marine ectothermic organisms with high metabolic rates (teleost fish, cephalopods) might be characterised by a certain level of pre-adaptation to acidification enabling them to grow and calcify under long-term elevated $CO_2$ conditions. By means of competition for similar resources, both fish and cephalopods have been forced into an active, high-power style of living (e.g. O'Dor & Webber 1986, 1991). During exercise, cephalopods are known to encounter $CO_2$ partial pressures >3000 ppm in their blood (Pörtner et al. 1991), which are values that are twice as high as those predicted for the world's oceans for the year 2300 (Caldeira & Wickett 2003). However, they are known to protect their blood from exercise-induced acidification by recycling octopine and associated protons in their mantle tissue (Pörtner et al. 1993). Since a stable blood pH is necessary for the proper function of their extracellular oxygen pigment hemocyanin (e.g. Melzner et al. 2007b), active cephalopods must possess a sophisticated ion transport machinery (and appropriate buffering systems) to cope with high, exercise-induced, $CO_2$ concentrations on a daily basis. Ongoing work on the blood acid–base parameters and the general ion regulatory ability of *Sepia officinalis* in response to hypercapnia will provide further insights.

Our work underlines the importance of improving our understanding of the processes responsible for biocalcification, growth and physiological homeostasis, when aiming towards predicting sensitivities of marine invertebrates to future climate change. The cuttlefish *Sepia officinalis* might, therein, serve as an important invertebrate model organism to identify specific biological mechanisms that promote tolerance to long-term reductions in seawater pH and calcium carbonate saturation.

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