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THEME SECTION

Biological responses in an anthropogenically modified ocean

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INTRODUCTION

Understanding the responses of ocean biota to a complex matrix of cumulative anthropogenic change

Philip W. Boyd[1,*], David A. Hutchins[2]

[1]NIWA Centre for Chemical and Physical Oceanography, Department of Chemistry, University of Otago, Dunedin 9012, New Zealand

[3]Department of Biological Sciences, University of Southern California, Los Angeles California 90089, USA

ABSTRACT: Oceanic conditions are changing at an unprecedented rate, and these anthropogenically driven changes will intensify into the future. The marine biota will encounter a complex shifting matrix of simultaneous environmental changes—including temperature, pH/pCO_2, nutrients, light, and oxygen—which will be further compounded by concurrent regional and local anthropogenic impacts, such as altered freshwater runoff regimes or biomass harvesting. We are only beginning to grasp the complexity of these interactive changes on ocean biota. To understand the pronounced and/or nonlinear effects of cumulative environmental stresses on organismal fitness and ecosystem functioning, the marine global-change research community can profit from the large body of existing evidence from freshwater lakes or polluted aquatic systems. We explore how the complex environmental changes will affect the biota from primary producers to higher trophic levels in both nearshore and open ocean waters, and conclude by proposing new approaches to address the formidable challenges of this research field. This Theme Section on 'Biological responses in an anthropogenically modified ocean' presents a set of papers that highlights the multiplicity of factors that will alter major biogeochemical and ecological frameworks, and raises awareness of the complexities involved in disentangling the combined effects of global, regional and local anthropogenic change on marine food webs.

KEY WORDS: Climate change · Cumulative environmental stress · Biological responses

Introduction

A major challenge for oceanographers in the 21st century will be to identify, quantify and understand how the changing climate will impact ocean biota in both coastal and offshore waters. Taking up the challenge will enable scientists to make better predictions of how climate change will alter oceanic ecosystems, biogeochemistry and resources, and hence provide projections on the magnitude of changes to ecosystem services, biogeochemical feedbacks to climate change, and food security.

Evidence about the nature and extent of changes to the ocean has come from global ocean and coupled ocean–atmosphere modelling experiments (Boyd & Doney 2002, Sarmiento et al. 2004), time-series observations (Dore et al. 2009) and biological manipulation experiments (Riebesell et al. 2000, Boyd et al. 2008). Together, these 3 strands provide initial verification of changing oceanic conditions (Roemmich et al. 2012); and insights into how altered conditions will affect the physiological performance of biota (Hutchins et al. 2009).

*Email: pboyd@chemistry.otago.ac.nz

To date, the focus of manipulation experiments has been on perturbing one environmental property, such as pH (Gattuso & Hansson 2011). These invaluable studies have helped to define the many ramifications of altering even just one component of the ocean, along with the physiological, ecological and biogeochemical consequences. However, it is increasingly evident from more complex manipulation studies that this traditional reductive approach cannot capture a truly prognostic view of future ecosystem changes. Climate-change mediated shifts in multiple environmental properties often exert non-linear and counterintuitive controls over many aspects of ocean biota (Fu et al. 2008, Rose et al. 2009a). To date, the implications of these complex changes for ocean biota remain largely uninvestigated (Boyd 2011).

Examination of freshwater, terrestrial, and ecotoxicological studies reveals a diverse literature on multiple environmental stressors from the 1980s onwards (Calow 1989), and which explore concepts such as cumulative environmental stress (Breitberg et al. 1998). Puzzlingly, with a few exceptions (Crain et al. 2008), there has been little awareness in the oceanographic community of this valuable repository of ideas and concepts, and virtually no attempts to link what marine scientists term global (climate change), regional (e.g. atmospheric pollutants) and local (e.g. point source runoff) anthropogenic 'stressors' (hereafter referred to as 'drivers', see 'Definitions' below) and their potentially combined effects on ocean biota. There is much to be learnt from this wider literature. For example, Breitberg et al. (1999) concluded that:

> While many approaches are similar to those used to examine the effects of a single stress, studying the effects of multiple stressors usually requires a more complex experimental design and/or statistical methods to separate out often subtle and interacting effects.

In the present review, we first revisit and revise the definitions used in the wider literature; summarise the main concepts from the prior research on multiple drivers; and explore the environmental heterogeneity of marine versus other systems across a range of scales. We then discuss key topics such as the differential susceptibility of organisms to environmental drivers, and current approaches to multiple driver research, before highlighting the findings of the 7 papers — from phytoplankton to higher trophic levels — that comprise this Theme Section. Finally, we advocate new approaches to help develop research into multiple drivers.

We offer this Theme Section to highlight both recent achievements and remaining gaps in our knowledge of how multiple environmental drivers may affect future marine food webs. Inevitably, there are important subjects that are not covered here, including (but not limited to) environmental effects on key groups such as heterotrophic bacteria, zooplankton, and corals, as well as emerging experimental and analytical methodologies including molecular biology. In many cases, these subjects have been addressed elsewhere (e.g. Steinberg 2012), as they are key components of our expanding knowledge of how marine biota will respond to global change.

Definitions

The literature on multiple stressors provides useful definitions, for example Calow (1989), that can be modified to better align them with the growing climate change issue.

There is a tendency to refer to alteration of environmental properties as biological 'stressors' (Breitburg et al. 1998), as the studies in the wider literature often focussed solely on detrimental effects such as acid rain. There is recent experimental evidence that climate change perturbations, such as higher oceanic CO_2 concentrations, may result in beneficial effects for some organisms, e.g. diazotroph N_2 fixation rates (Hutchins et al. 2007), but detrimental effects to others, e.g. coccolithophore calcification rates (Riebesell et al. 2000). Indeed, any major change in oceanic conditions will create not only 'losers', but also 'winners' who can best adapt to the altered environment (Nogales et al. 2011). Thus, the generic term 'driver' is a more accurate descriptor than 'stressor' when discussing the effects of global anthropogenic change on ocean biota. Furthermore, the terms 'synergism' and 'antagonism' are often used to describe the interplay among multiple environmental drivers (MEDs), but often their use without further qualification (see 'Synergisms and antagonisms — what we have learnt') has caused confusion. Here, we present key definitions to help clarify some of these issues:

(1) Driver — An environmental change that results in a quantifiable biological response, ranging from stress to enhancement.

(2) Stressor — An environmental change that decreases organismal fitness.

(3) Enhancer — An environmental change that increases organismal fitness.

(4) Additive—A biotic response to 2 or more interacting factors that equates to the sum of their individual effects (sensu Folt et al. 1999).

(5) Multiplicative—A biotic response to 2 or more interacting factors which significantly exceeds the sum of their individual effects (sensu Folt et al. 1999).

(6) Synergism—A positive feedback interaction between MEDs that is multiplicative, and requires an intrinsic metric such as a quantitative measure of organismal fitness or a physiological property.

(7) Antagonism—A negative feedback interaction between MEDs that is multiplicative, and requires an intrinsic metric.

(8) Acclimation—Short-term change resulting from a physiological response at the individual organismal level (sensu Falkowski & LaRoche 1991).

(9) Adaptation—Long-term evolutionary change resulting from natural selection at the population level (sensu Falkowski & LaRoche 1991).

These definitions must be employed in a context-dependent manner. For example, at the cellular level they may refer to effects on processes such as gene transcription and translation, whereas at the community level they may refer to effects on biodiversity, and at the biome level to integrative properties such as major biogeographical patterns.

It is also crucial to introduce appropriate qualifiers when using these terms. The terms synergism and antagonism are potentially confusing, since it is essential to define whether they are being employed in a mechanistic- or an outcome-based sense. In other words, are they referring to mechanistic interactions between the drivers, or to the resultant net outcome for the organism? An example of the former is the antagonistic interaction between ocean acidification (OA) and warming, whereby lowered CO_2 solubility at higher ocean temperatures partially offsets CO_2-driven increases in OA (Hare et al. 2007). An illustrative biological outcome-based antagonism is evidence that increases in coral calcification rates due to warming could partially counter the negative effects on calcification of decreasing carbonate ion concentration due to OA (Lough & Barnes 2000).

A related semantic issue with these terms is the need to specify whether a positive or negative connotation is intended. For example, in discussing a hypothetical antagonistic effect, A + B = C, a negative connotation would be if C is a cumulative deleterious outcome of the 2 detrimental factors A and B. Crain et al. (2008) defined antagonism in this sense when examining a collation of marine experimental studies on what they termed 'multiple stressors'. However, if C is less detrimental (than A or B alone) to the organism because of an 'antagonism' between the 2 negative factors A and B, then the net effect is a positive one for the organism. This use of 'antagonism' was employed by Didham et al. (2007) in a study of the cumulative effects of habitat loss and invasive species.

Synergisms and antagonisms—What have we learnt?

The logistical and interpretational issues of conducting and then interpreting the findings of a study in which MEDs are manipulated (Fig. 1) make research into the cumulative influence of MEDs daunting. The potential interplay between MEDs and the likelihood of non-linearities due to amplification and/or diminution, relative to the effects of a single driver, adds greatly to the potential complexity of the study. Again, the prior literature has much to offer: Folt et al. (1999) defined antagonism and synergism and introduced the concepts of their additive and multiplicative effects. This study emphasized the difference between multiplicative effects that arise collectively from MEDs, and effects that are largely

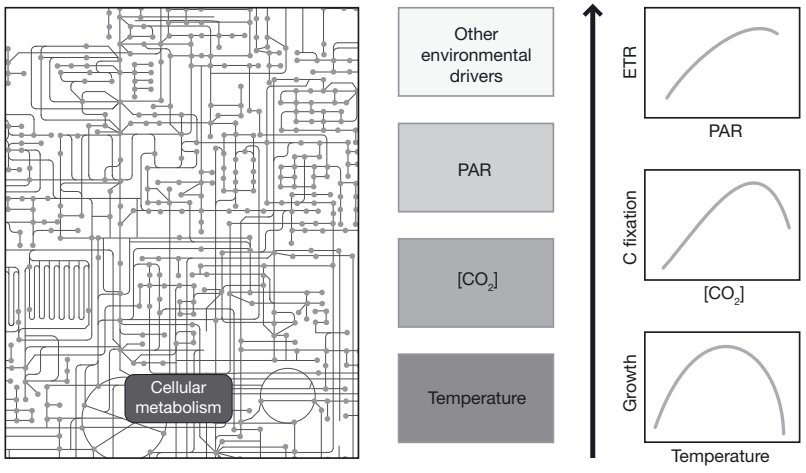

Fig. 1. Cumulative environmental stress (vertical arrow, central panels) results from both the individual and interactive effects of altered environmental conditions (central panels) on a range of the biochemical pathways (left hand panel, based on iPath, Letunic et al. 2008) of a cell, and leads to alteration of fundamental physiological processes including carbon fixation and growth (right hand panels). ETR: electron transfer rate, PAR: photosynthetically active radiation

the result of a single over-riding driver among many. Crain et al. (2008) conducted a meta-analysis of a suite of marine studies that had investigated the biological effects of MEDs, and reported that certain experimental approaches are more likely to introduce a skew towards either an antagonism or a synergism. For example, they found that studies employing 3 stressors exhibited about twice the frequency of true synergistic outcomes compared to studies using only 2 variables. Darling & Côté (2008) in a meta-analysis of experimental studies in which mainly 2 drivers were manipulated, reported that the incidence of synergisms and antagonisms was less than expected. Both these studies suggest that the likelihood of synergisms and antagonisms when 3 or more factors are altered could be high indeed.

Environmental heterogeneity

The effects of MEDs on biota will be influenced by environmental heterogeneity, the characteristics of the terrestrial or aquatic systems they reside in. Hence, any intercomparison of biological responses to alteration of MED properties across systems must examine their intrinsic differences. For example, the size of the open ocean and its relative isolation due to the 'buffer' of nearshore waters means that it is unlikely to be impacted by allochthonous materials such as terrestrial pollutants, compared to coastal waters and lakes. Thus, global and regional drivers as opposed to local ones will dominate in the open ocean (Fig. 2). Ocean chemistry often differs in fundamental ways from that of freshwater, e.g. carbonate chemistry in the ocean is highly buffered (Dickson 1992) and hence pH variability is less than in most freshwater systems (Toupin 2005, Hofmann et al. 2011). Environmental heterogeneity differs between the ocean and land, with implications for how the biota will respond to environmental change (Reusch & Boyd in press).

The geographic realm of influence of drivers will also influence environmental heterogeneity, e.g. the overlap of global and local drivers, as this sets how many drivers are acting concurrently on the biota (Fig. 2). Our understanding of how MEDs can result in cumulative stress for the resident biota (Fig. 1) has mainly come from studies of local drivers, e.g. point source disturbances such as warming (Schiel et al. 2004). Recently, increasing awareness of the many ramifications of climate change has enhanced our knowledge of how global anthropogenic drivers, such as hypoxia, will influence the biota (Gruber 2011). However, only a few studies have considered both local and global anthropogenic drivers and their joint influence (Darling & Côté 2008).

Regional drivers are often associated with the role of the atmosphere as a conduit between terrestrial and aquatic systems (Fig. 2). Hence we have drivers such as acid rain (Bouwman et al. 2002), nitrogen supply to the coastal ocean (Seitzinger & Sanders 1997, Duce et al. 2008), and long-range delivery of fossil-fuel pollutants to offshore waters such as the western subtropical Atlantic (Sholkovitz et al. 2009). The interplay among MEDs may alter environmental heterogeneity and hence exacerbate the cumulative stress on biota (Fig. 2). However, in some cases, the interactions between local and global drivers may partially negate each other (Fig. 2).

The degree of environmental heterogeneity, and how it varies spatially (Helmuth et al. 2010) and temporally (Garcia Molinos & Donohue 2010), determines rates and modes of acclimation or adaptation for the biota; for instance, highly variable environ-

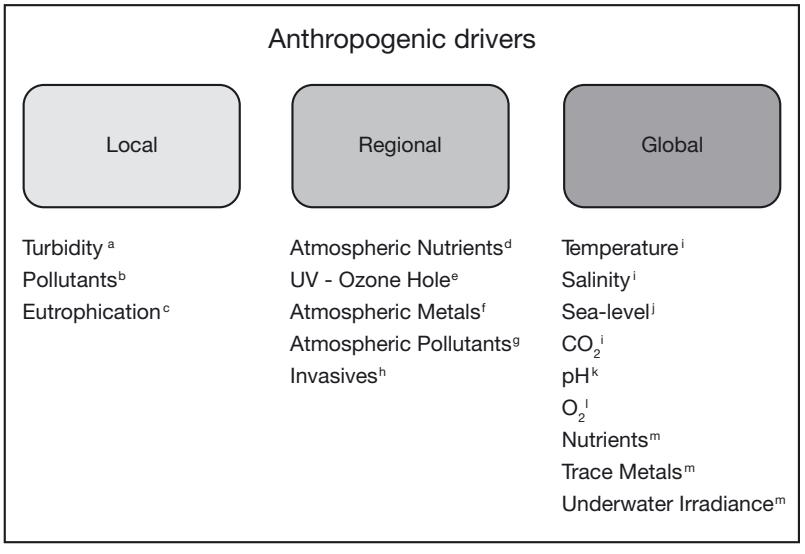

Fig. 2. The number of anthropogenic drivers increases when local, regional and global processes are considered together. The interplay among some drivers may serve to offset each other—for example, less upwelling nutrient supply versus more atmospheric nutrient supply to surface waters. Sources: [a]Boyd (2011), [b]Doney (2010), [c]Seitzinger & Sanders (1997), [d]Duce et al. (2008), [e]Cullen & Neale (1997), [f]Paytan et al. (2009), [g]Sholkovitz et al. (2009); [h]Jaspers et al. (2011), [i]Sarmiento et al. (2004), [j]Church & White (2006); [k]Caldeira & Wickett (2003), [l]Stramma et al. (2010), [m]Boyd et al. (2010)

ments may place a premium on phenotypic plasticity. Another fundamental environmental difference between land and ocean is that the latter is dominated by microbes that are primarily free-drifting forms. These microbial communities comprise a very diverse community which may have built-in redundancy with respect to ecological and biogeochemical functions in a changing climate—the so-called insurance theory (Sogin et al. 2006). Such differences between systems in both environmental heterogeneity and ecosystem structure and function can make comparing trends in responses of the biota to altered MEDs problematic.

Interactive environmental controls on ocean biota

The control of primary producers by physical and chemical factors has historically been a major oceanographic research theme. The potentially growth-limiting environmental factors include nutrient supply, temperature, iron availability, and light. Early studies implicitly assumed that only a sole limiting factor was operative at a time, a concept known as the Liebig limitation (de Baar 1994). It is now recognized that multiple factors often simultaneously co-limit primary production (Arrigo 2005) and include iron and light (Sunda & Huntsman 1997), and iron and silicate (Hutchins et al. 2002). In the last decade, studies have tested how MED's such as pCO_2, warming, and changing iron availability may affect the base of pelagic food webs, and importantly, explicitly interpreting these multivariate interactions in a global change context (Fig. 3). Warming and iron-enrichment are shown to synergistically amplify the growth and productivity of antarctic phytoplankton communities (Rose et al. 2009a). Similarly, raising CO_2 and temperature together strongly stimulates coccolithophore growth in the North Atlantic, but concurrently depresses calcification (Feng et al. 2009), confounding the wider biogeochemical implications.

Such interactive global change effects are less well-documented for higher trophic levels in pelagic systems, partly because large, active animals are often more difficult to manipulate experimentally than the phytoplankton. However, Rose et al. (2009b) observed that warming and OA together had a negative effect on microzooplankton grazer abundance in North Atlantic waters. Rossoll et al. (2012) found that diatom cells grown at high pCO_2 inhibited growth and reproduction in copepod grazers, due to a severely reduced essential fatty acid content. Rosa & Seibel (2008) demonstrated a negative interaction between OA, warming, and hypoxia, on the physiology and distribution of a top predator, the jumbo squid *Dosidicus gigas.* A major difference between biota at low and high trophic levels is that the former are subject to a wider range of MEDs (Fig. 3).

Differential susceptibility to drivers across trophic levels

Most research to date, on the effects of MEDs has focused on species or strains within a sole trophic level (Crain et al. 2008); for example OA studies have largely been conducted on calcifying primary producers (Gattuso & Hansson 2011). Such findings need to be put into a wider context by addressing how the concurrent alteration of MEDs influences different trophic levels and ecosystem structure and functioning. This will introduce a further level of complexity to what are already challenging experiments; nevertheless, a continued emphasis only on one trophic level is inadequate to further our understanding of this topic at the ecosystem level (for

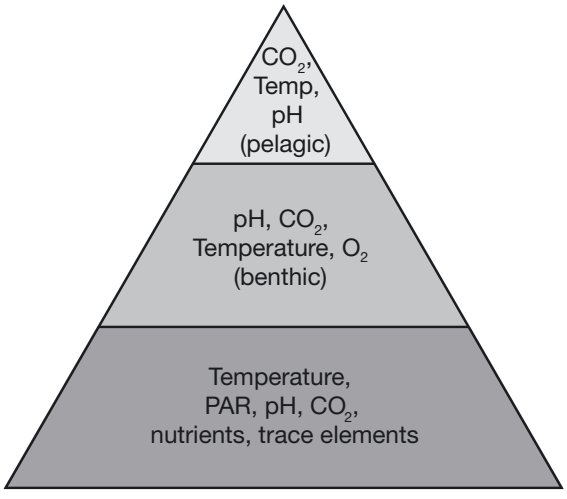

Fig. 3. The base of most food webs is influenced by a greater number of environmental conditions than those that influence the higher trophic levels, and thus by the complex interplay of many factors (Darling & Côté 2008) under changing environmental conditions. Higher trophic levels (middle and apex of pyramid), in contrast, are embedded within a trophodynamic structure and may be vulnerable to the effects of differential susceptibilities of lower (prey) and higher (predators) trophic levels to altered environmental conditions. At higher trophic levels, pelagic organisms in relatively well-oxygenated waters may be affected by fewer environmental conditions (and their alteration under a changing climate) than benthic organisms. PAR: photosynthetically active radiation

recent thematic reviews, see Brose et al. 2012 and Caron & Hutchins in press).

Several studies have revealed differential susceptibility to MED's both within trophic levels (different species, e.g. coccolithophores, Langer et al. 2006; N_2 fixers, Hutchins et al. unpubl.) and across trophic levels (e.g. temperate coastal calcifiers, Hurd et al. 2011). The ecological influence of such differential susceptibility amongst primary producers on higher trophic levels is difficult to gauge (Smayda 2011), whereas studies that include several trophic levels provide a more holistic view of the potential trophodynamic effects of a changing climate (Helmuth et al. 2010). Such differential vulnerability to change may result in counter-intuitive findings. For example, the ecological outcome of increased UV stress was enhanced primary productivity of benthic diatom communities, due to their grazers being more susceptible to UV damage (Bothwell et al. 1994). Marine ecologists can learn much from how entire ecosystems are perturbed across entire lakes (Carpenter et al. 2011), or in coastal waters using mesocosms (Riebesell 2004). Such studies enhance our understanding of the extent and nature of the 'ecological surprises' that may result from a changing climate (Lindenmayer et al. 2010). Other ecologically relevant topics which may influence or result from differential vulnerability across trophic levels, but which are beyond the scope of this Introduction, include: biodiversity and ecosystem dynamics (Vinebrooke et al. 2004); altered species distributions through migration (Parmesan et al. 1999) and invasion (Jaspers et al. 2011); and the nature of competitive versus facilitative relationships between organisms (Bulleri 2009).

Present day approaches to multiple-driver research

Current approaches focus mainly on perturbation studies in which MEDs are manipulated, e.g. temperature, CO_2 and light (Feng et al. 2009). This complex approach arises from prior simpler studies in which a sole driver was manipulated, such as pH (Riebesell & Tortell 2011). MED experiments require additional diagnostics (Fig. 1), relative to single-driver manipulations, to enhance the interpretative skills needed when several MEDs are being manipulated concurrently. Such studies are beginning to reveal the complex interplay and feedbacks between what have previously been considered to be relatively simple relationships between an individual driver and a particular physiological process, e.g. OA and phytoplankton calcification (Fig. 4).

Other studies are using the diagnostic power of '-omics' to probe changes resulting from such MED manipulations (Matallana-Surget et al. 2012) but so far they have mainly focused on the effects of chronic stress responses to toxins. A few such studies have been able to link multiple 'omics'—transcriptomics, proteomics or metabolomics via bioinformatics and modelling (Steinberg 2012). These provide a more complete picture of how environmental manipulations affect the biota, and also address issues such as acclimation to altered environmental conditions (Dyhrman et al. 2012). Other recent developments in manipulation experiments are studies conducted with microbes over many generations (i.e. years), as opposed to the brief span (weeks) normally employed in such experiments. Lohbeck et al. (2012) used a long-term approach to reveal that coccolithophores can adapt micro-evolutionarily to altered CO_2/pH conditions. Another approach has focused on competitive exclusion experiments by comparing short-term (weeks) competitive dominance experiments using a 'naïve' natural dinoflagellate commu-

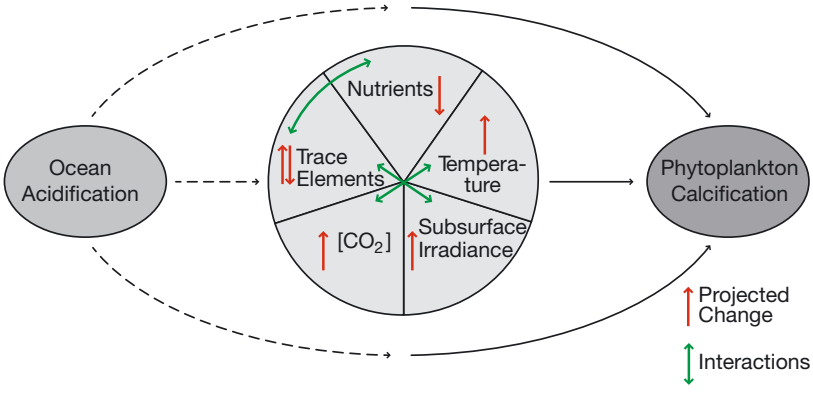

Fig. 4. Interplay between ocean acidification (OA) and phytoplankton calcification, when MEDs are considered. OA alters calcification (Riebesell et al. 2000), but under the MEDs scenario, OA influences some of the drivers (dashed lines), and additional drivers can influence calcification (solid lines). For example, OA affects nutrient and trace element availability (Royal Society 2005), and oceanic CO_2 concentrations and speciation (Royal Society 2005), whereas other environmental drivers influence calcification (CO_2: Royal Society 2005; temperature: Boyd et al. 2010; phosphate: Dyhrman et al. 2006, and light: Paasche 2001). Red arrows: the projected changes in ocean properties due to climate change (Boyd et al. 2010), green arrows: examples of synergistic and/or antagonistic effects of MEDs on the biota (Boyd et al. 2010). Confounding biological feedbacks on environmental factors (e.g. high CO_2 may enhance N_2 fixation and hence boost nutrient inventories, Hutchins et al. 2009) are not included in this schematic

nity with the outcome of competition between the same species in analogous artificial communities after conditioning each species in clonal cultures under high CO_2 conditions for ~1 yr (Tatters et al. in press). Below, in 'The future, new approaches', we examine how today's methods can be supplemented.

Summary of the Theme Section

For this Theme Section on 'Biological responses in an anthropogenically modified ocean', we have assembled a group of papers that examine how MEDs influence and affect key organisms and processes in the marine environment. The authors have taken on the difficult task of assembling and synthesizing the (often fragmentary) knowledge about how the effects of MEDs simultaneously in flux may differ from marine science's traditional reductionist emphasis on one factor at a time.

The coccolithophores have been at the forefront of both research advances and controversy in the study of the effects of OA. However, arguably less is known about their potential responses to MEDs than for any other phytoplankton group (Boyd et al. 2010). Raven & Crawfurd (2012, this volume) tackle this topic by examining how MEDs affect this keystone functional group to global change. To do this, they draw on evidence from sources ranging from paleo-oceanography, through field-oriented process studies, to laboratory investigations using molecular and physiological methods. They conclude that more studies incorporating multivariate experimental designs and genetic methods of assessing the potential for adaptation are needed before we can fully understand the responses of the coccolithophores to MEDs.

Gao et al. (2012, this volume) address a topic that has, surprisingly, not been widely examined previously: How will rising CO_2 and OA interact with solar radiation to affect phytoplankton? They review the literature on the individual effects of OA, warming, and changes in irradiance (i.e. photosynthetically active- and ultra violet-radiation). Next they describe the few studies that address interactions between these MEDs, and conclude with suggestions to move this field forward in the future.

Hoffmann et al. (2012, this volume) use the large body of research on trace element biogeochemistry from the last 20 years to predict future trends in the cycles of iron, zinc, copper, and other metals. They note that although single drivers affecting trace metal sources, speciation, solubility and biogeochemical cycling have been investigated to some extent, little information exists on the cumulative effects of a suite of MEDs comprising OA, warming, and hypoxia. They discuss the potential interplay between these MEDs in both high- and low-latitude regimes, and advocate the need for careful scrutiny and standardization of methodology in experiments combining both trace metal and MEDs.

Fu et al. (2012, this volume) consider the interactive effects of MEDs on environmentally destructive toxic and harmful algae. Due to worldwide human health impacts and economic damage caused by harmful algal bloom groups such as dinoflagellates, the effects of many individual factors including nutrients, light, temperature, CO_2, and salinity have been examined in a well-established literature. However, oceanographers are just beginning to consider how coincident shifts in such MEDs may affect harmful algal blooms in the context of changing estuarine, coastal and oceanic environments.

Litchman et al. (2012, this volume) highlight the utility of the ecological niche concept in understanding the long-term responses of phytoplankton to global change. They make the case that trait-based niche models can be extended into multiple dimensions in order to describe the adaptation of algal functional groups to a changing matrix of MEDs. Development of such new trait-based models may have the ability to better predict the adaptive trajectories of key phytoplankton species in response to selection by MEDs, and Litchman et al. (2012) make a convincing case for their application to future studies in a variety of conceptual and experimental contexts.

A major question about global-change impacts is how the critical global biogeochemical processes may respond to simultaneous forcing from MEDs. Passow & Carlson (2012, this volume) address this question for a key component of of the carbon cycle, the storage of carbon in the deep ocean by sinking biogenic particles. They argue that current knowledge gaps concerning the combined impacts of MEDs preclude firm predictions of whether oceanic carbon storage via the biological pump will increase or decrease in the future. They offer a way forward, though, by suggesting that better-constrained regional models of the biological pump can be integrated to obtain a holistic picture of global trends in future ocean carbon uptake..

The review by Pörtner (2012, this volume) uses the concept of oxygen and capacity dependent thermal tolerance (OCLTT) as a tool to understand the integrated responses of organisms to MEDs. Pörtner argues that OCLTT can be applied broadly enough

to address the impacts of climate change and other anthropogenic drivers like pollution over scales ranging from physiological to ecological. The linkage between aerobic capacity and the thermal tolerance range of organisms in a variety of habitats may offer unique insights into their climate change responses as individuals and populations, as well as their roles in ocean ecosystems.

The future—New approaches

To begin to understand how an anthropogenically modified ocean affects the biota, we should learn from the last decade of studies into climate change effects on biota, in particular the research stemming from the OA research community, which to date has been the vanguard (Boyd 2011). OA research has advanced many facets of an acidifying ocean, but in comparison, the MED research represents a major logistical challenge (see Fig. 4), due to the numbers of permutations that must be explored for a wide range of species and trophic levels. There is a strong likelihood that individual labs will develop tangential research trajectories unless there is well-implemented international coordination, e.g. the complex nature of carbonate chemistry and its manipulation resulted in many studies being conducted using different protocols (Hurd et al. 2009) and hence, obtaining a consensus view is difficult.

The production of a best practice guide, e.g. by OA researchers (Riebesell et al. 2010) is one way to tackle this major challenge for the MEDs community. A complementary approach is to use the resources of a relatively large scientific community to conduct experiments in a systematic manner, as has been done for some major projects (Human Microbiome Project Consortium 2012). Boyd et al. (unpubl.) have recently conducted such a community-wide study using a pre-agreed experimental protocol for a study (across 8 laboratories) on thermal reaction norms for phytoplankton species spanning the global ocean. There have been calls for similar community-wide initiatives to look at the ecological ramifications of climate change in the ocean (Nogués-Bravo & Rahbek et al. 2011).

Experimental evolutionary biology is another potentially powerful tool in MED research to better tackle issues such as acclimation (plasticity) versus adaptation to global change in aquatic organisms (Piersma & Drent 2003). This tool is increasingly being applied to understand long-term responses of organisms to selection by single factors such as warming (Huertas et al. 2011) and CO_2 (Collins & Bell 2004). This new oceanographic research emphasis by experimental environmental biology has, however, yet to attempt to address multivariate global change issues. The difficulties of understanding the short-term physiological responses of marine species to multiple drivers relative to single drivers pale in comparison to the bewildering complexities of disentangling long-term evolutionary responses to many simultaneous changes to MEDs. Rigorous attribution of observed adaptive trends with their own synergisms and antagonisms, and prediction of the consequences for organismal reproductive and competitive success, will require marine global-change scientists to make a quantum leap forward conceptually and logistically. Fortunately, a new toolbox of genomic, transcriptomic, and proteomic methods (Dyhrman et al. 2012, Steinberg 2012) is revolutionising our science by allowing us to capture and understand holistic organismal responses to a changing ocean.

Climate-change modelling approaches will also be powerful tools to inform the experimentalists of the zones in which the overlap of local, regional and global anthropogenic change will be particularly large. Specialised modelling of ecosystem structure and interactions, cellular physiology, and evolutionary adaptation to change will also play an essential role in directing research. Close collaborations between experimentalists, observationalists and biogeochemical modelers can greatly improve our predictions of the global (e.g. Hutchins et al. unpubl.) and regional (Boyd et al. 2011) consequences of MEDs.

For MED research on phytoplankton, there is the issue of the thousands of phytoplankton species (Smayda 2011), and strains (Iglesias-Rodriguez et al. 2006), and the impossibility of investigating how each will respond to complex patterns of environmental change. OA research has revealed that different coccolithophore species have markedly different responses to lowering pH (Langer et al. 2006), as do strains of some of these calcifying species, such as *Emiliania huxleyi* (Langer et al. 2009). Strain-specific responses are also documented for CO_2 effects on N_2-fixing cyanobacteria *Trichodesmium* and *Crocosphaera* (Hutchins et al. unpubl.), as well as on the widely distributed eukaryotic picoplankton species *Ostreococcus tauri* (Schaum et al. unpubl.). Taxon-specific differences in the ability to adapt to warming have been demonstrated across a range of phytoplankton species (Huertas et al. 2011). These findings point to the difficulty in selecting a phyto-

plankton functional type, as has been the approach traditionally used in biogeochemical models (Hood et al. 2006). Alternatively, these results may indicate that there is sufficient redundancy across the phytoplankton assemblage that will make the assemblage malleable to such complex patterns of environmental change. However, the ecological and biogeochemical ramifications of such redundancy—for example a small calcifier or N-fixer being replaced by a larger one—are issues that still need to be resolved (Joint et al. 2011).

One approach to circumvent the 'paralysis of the plankton' in research terms would be to examine a subset of species/strains (or in the case of microbes, using metabolic functions, Dinsdale et al. (2008), Burke et al. (2011)), across a range of biomes to explore how such functionality responds to environmental change This has been attempted for the higher trophic levels with respect to temperature by Pörtner & Knust (2007) and Helmuth et al. (2010). Such a unifying theory might be built around the physicochemical constraints that impose as to how far cell physiology (for example diffusion rates of nutrients, Kiørboe 2008), phytoplankton functional traits (Litchman et al. 2012) or cell biochemistry (Makarieva et al. 2008) can be altered by a complex matrix of changing conditions. These approaches would link well with the outputs from climate change models. By thoughtfully debating and subsequently adopting such research guide- and time-lines, in the coming decade a coordinated and integrated ocean global change research community should be able to significantly advance these themes studying the responses of the biota in an anthropogenically modified ocean.

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Contribution to the Theme Section 'Biological responses in an anthropogenically modified ocean'

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Environmental controls on coccolithophore calcification

John A. Raven[1,*], Katharine Crawfurd[2]

[1]Division of Plant Sciences, University of Dundee at the James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

[2]Department of Biological Oceanography, Royal Netherlands Institute for Sea Research (Royal NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands

ABSTRACT: Coccolithophores are major contributors to global marine planktonic calcification, and in nature coccolithophores are invariably calcified through almost all of their life cycle. The response of calcification to environmental factors is essential in understanding the persistence of coccolithophores through at least 220 million years of changing global environments, and their prospects for current environmental change. So far the responses examined have been at the level of acclimation rather than adaptation in evolution. Variation in results of CO_2 manipulation experiments can be tentatively attributed to variation among genotypes rather than differences in experimental procedure. Comparisons of methods using the same genotype, and of several genotypes using a single method, suggest significant variation among genotypes. The general response is a decreased particulate inorganic carbon (PIC) to particulate organic carbon (POC) ratio in higher than present CO_2 concentrations and vice versa for lower CO_2 concentrations. Fewer studies have investigated the effect of other environmental factors. Decreased availability of phosphorus and, to a lesser extent, nitrogen, as well as decreasing photosynthetically active radiation (PAR) down to a certain low value increase PIC:POC, while variable results have been found for changes in ultraviolet radiation (UVR). Many of these results can be accommodated by considering the restriction of calcification to the G1 phase of the cell cycle and the length of this phase under different growth conditions. Fewer studies have investigated the interactions among environmental factors which change with increased CO_2 and increasing sea surface temperature; the shoaling of the thermocline will increase the mean PAR and UVR whilst decreasing nitrogen and phosphorus availability. More studies of these interactions, as well as of genetic adaptation in response to changed environmental factors, are needed.

KEY WORDS: Calcification · Coccolithophores · Carbon dioxide · Phosphorus · Nitrogen · Photosynthetically active radiation · Temperature · Ultraviolet radiation

INTRODUCTION

In order to review the literature concerning calcification in coccolithophores, it is interesting to first note that the function, or selective advantage, that coccoliths provide is still unknown. We will explore the evidence for the various hypotheses proposed. The mechanisms involved in calcification are also still under investigation, so to work towards a process-based understanding, we will summarise the current evidence. From this base we will then examine the evidence from numerous studies and attempt to draw out systematic responses to environmental variables. There is much scope for future research in this area, and we will try to expose areas of particular interest.

Coccolithophores (Prymnesiophyceae: Haptophyta) are calcified planktonic primary producers found in both coastal and oceanic regions where they often form blooms. At least 150 species are known (West-

*Email: j.a.raven@dundee.ac.uk

broek et al. 1993, Winter & Siesser 1994), and within these formal species, strains with unique physiology and morphology have been characterised. Coccolithophores have existed for at least 220 million years. This minimum age for the origin of coccolithophores comes from the fossil record (Falkowski et al. 2004), with the origin of heterococcoliths at least 215 million years ago and of holococcoliths at least 185 million years ago (Medlin et al. 2008). In the Cretaceous, they were more cosmopolitan and diverse than present-day species, which are largely dominant in warm, stratified, nutrient-poor oceanic waters (Brand 1994). In the present ocean, coccolithophores are believed to account for at least half of the 80 to 120 Tmol particulate inorganic carbon (PIC) produced each year in the marine pelagial (Degens & Ittekkot 1986, Westbroek et al. 1993, Balch et al. 2007, Berelson et al. 2007, Broecker & Clark 2009). A further 21% of the marine pelagial $CaCO_3$ is deposited by foraminiferans (Langer 2008), some of which are symbiotically photosynthetic. The other photosynthetic $CaCO_3$ producers in the marine pelagial are certain dinoflagellates (Gadd & Raven 2010) and cyanobacteria such as *Trichodesmium* (Kranz et al. 2010), but these only make very minor contributions to global pelagial $CaCO_3$ precipitation. The high density of the liths, with the present depth of the calcite lysocline and the occurrence of organic coatings, means that much of the calcite produced is exported to the deep ocean, constituting the carbonate pump (Westbroek et al. 1993, Balch et al. 2007, Berelson et al. 2007, Broecker & Clark 2009, Lebrato et al. 2010). There seem to be no coccolith-specific estimates, but the total PIC flux sinking below 2000 m may be as much as 50 Tmol C yr^{-1} (see Table 3 of Berelson et al. 2007). This consists predominantly of liths which have become associated with other particulate organic matter, e.g. faecal pellets and transparent exopolymeric particles (Pedrotti et al. 2012), and are effective ballast for export to the deep ocean, fuelling the biological pump of organic carbon (Rost & Riebesell 2004, Biermann & Engel 2010). While ballasting of particulate organic carbon (POC) by PIC increases the atmosphere-to-ocean CO_2 flux by decreasing mineralisation in the surface ocean, it also decreases PIC dissolution in the surface ocean, leaving a larger fraction of the CO_2 generated in PIC production in the surface ocean which decreases the atmosphere-to-ocean flux of CO_2. Note that calcite, the form of $CaCO_3$ in coccoliths, is only two-thirds as soluble as aragonite (Mucci 1983).

We begin by reflecting on the possible function(s) of coccoliths and the formation of hetero- and holococcoliths. The interactions of coccolithophores with environmental change are then discussed, including experimental methods for investigating calcification responses.

FUNCTIONS OF COCCOLITHS

Despite the efforts made to discover the function of calcification, none of the various hypotheses are supported by sufficient evidence to have been fully accepted. Table 1 summarises the hypotheses proposed and references providing evidence for and against them. These hypotheses will be discussed briefly (see also Table 1) but, due to insufficient evidence, for the purposes of this review we assume that coccoliths provide some benefit to the cell which outweighs the cost of production.

The addition of liths, with higher density than other cell components, results in increased sinking rates (see Raven & Waite 2004, Biermann & Engel 2010). Nutrient limitation generally increases PIC:POC and hence cell density, bringing the cells into deeper waters with higher nutrient concentrations. However, cells in conditions which restrict the growth rate and cultures containing senescent cells may shed most or all of their liths (Paasche 2001). Decreased photosynthetically active radiation (PAR) reduces ballasting, slowing the rate at which cells move into deeper, low-PAR waters (Raven & Waite 2004).

One suggested function of coccoliths is physical restriction of virus infection (Raven & Waite 2004). However, Frada et al. (2008) found that, while the giant phycodnaviruses infect the diploid (heterococcolith-bearing) phase of *Emiliania huxleyi*, where they can be significant in terminating blooms, the haploid phase, without heterococcoliths, is immune and comprises a refuge from the viruses. However, this refuge is only temporary since the diploid phase is dominant in *E. huxleyi* and probably in other coccolithophores as well. Thus, the haploid phase is not the state in which *E. huxleyi* produces large populations in nature; the haploid phase may be involved in over-wintering (von Dassow et al. 2009).

Alternatively, cells infected by viruses or parasitoids may sink out, thus protecting the uninfected population, based on kin selection (Raven & Waite 2004). Hypothetically, infection may decrease the capacity of the protoplast to maintain a low density; however, this mechanism may apply more to large, vacuolate, silicified diatoms than to smaller coccolithophores with less vacuolation (Raven & Waite 2004). No experimental evidence supports this hypo-

Table 1. Potential functions of coccoliths. See 'Functions of coccoliths' in the main text for further details. PAR: photosynthetically active radiation; UV: ultraviolet

Hypothesis	Advantage	For	Against
Ballasting	Allows vertical migration to access nutrients	Paasche (2001), Raven & Waite (2004)	
Viruses	Affords protection	Raven & Waite (2004)	Frada et al. (2008) (holococcoliths vs. heterococcoliths)
Grazers	Affords protection	Nejstgaard et al. (1994)	Harris (1994)
PAR	Photoprotective at the surface	Braarud & Nordli (1952)	Paasche (1964), Paasche & Klaveness (1970), Nanninga & Tyrrell (1996), Houdan et al. (2005), Trimborn et al. (2007)
PAR	Focuses light to chloroplasts in deep water	Young (1994)	Nanninga & Tyrrell (1996), Raven & Waite (2004), Trimborn et al. (2007),
UV	Protective	Gao et al. (2009)	Gao et al. (2009) (not unequivocally for)
H^+ (hence CO_2) production	H^+ used to convert HCO_3^- to CO_2 or other uses of H^+, e.g. neutralising OH^- produced in assimilation of NO_3^- and SO_4^{2-}		Can occur, but not an obligate means of generating CO_2 for photosynthesis. Herfort et al. (2004), Leonardos et al. (2009)
Avoiding intracellular phosphate precipitation	Ca^{2+} precipitates HPO_4^{2-} and phosphate esters; calcification may have evolved to prevent this	Degens & Ittekkot (1986), Couradeau et al. (2012) reported intracellular carbonate deposits in an early-branching cyanobacterium, containing almost as much (Mg + Sr +Ba) as Ca	Low free Ca^{2+} in the cytosol predates coccolithogenesis by at least 2 billion years; low cytosolic free Ca^{2+} a problem for Ca transport from the plasmalemma to the coccolith-forming vesicle (Raven 1980, Sanders et al. 1999, Dodd et al. 2010)

thesis. The most obvious theory is that coccoliths deter grazers (Nejstgaard et al. 1994); however, coccolithophores have been found to be the preferred prey of copepods both in the laboratory (Sikes & Wilbur 1982, Harris 1994) and in mesocosms (Nejstgaard et al. 1994). Experimental evidence actually shows preferential grazing rates on lithed rather than naked cells by the heterotrophic dinoflagellate *Oxyrrhis marina* (Hansen et al. 1996).

Coccoliths may increase radiation scattering in surface waters with high photon flux densities. This would reduce photoinhibition of photosynthesis by PAR and ultraviolet radiation (UVR) as well as the damage by UVR. However, a similar lack of photoinhibition at high PAR is seen when comparing lightly and heavily calcified strains (Israel & Gonzalez 1996) or when the degree of calcification is experimentally reduced (Paasche 1964, Paasche & Klaveness 1970, Nanninga & Tyrrell 1996, Trimborn et al. 2007). However, Nielsen (1995) found a higher light-saturated rate of photosynthesis at a given inorganic carbon concentration in highly calcified cells than in cells with little calcification. Alternative evidence suggests that increased calcification in *Emiliania huxleyi* can increase photochemical quenching of excess excitation energy following a steep increase in PAR (Barcelos e Ramos et al. 2012). Coccoliths may also be able to focus PAR to the plastids in deeper waters where photosynthesis is PAR-limited (Nanninga & Tyrrell 1996, Raven & Waite 2004). However, in deeper waters, the ratio of diffuse (scalar) as opposed to direct (vector) radiation increases, making focussing of radiation by coccoliths more difficult. It is of interest that calcite is used in radiation focussing in extant ophiuroids and in extinct trilobites but in a sensory rather that an energetic role (Aizenberg et al. 2001). The effects of UVB radiation (UVBR) on calcification are discussed in this review but here it is sufficient to say that at least *Emiliania huxleyi* is very sensitive to UVBR and coccoliths do not seem to protect it (Peletier et al. 1996).

A hypothesis which has been discussed at great length is the role of calcification as an intracellular source of CO_2, based on the entry of HCO_3^- into the cells for calcification (Sikes et al. 1980, Brownlee et al. 1995a, Anning et al. 1996, Fabry et al. 2008, von Dassow et al. 2009, Mackinder et al. 2010). Within the coccolith vesicle, HCO_3^- is converted to calcium carbonate, releasing either CO_2 or H^+, depending on the equation used. These by-products must be

removed to the cytosol to prevent acidification of the coccolith vesicle inhibiting calcite formation (Brownlee et al. 1995a). The CO_2 form of this argument is expressed as:

$$2HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + CO_2 + H_2O \quad (1)$$

with the CO_2 consumed in photosynthesis. The alternative is to frame the argument in terms of the production of H^+ in calcification:

$$HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+ \quad (2)$$

with subsequent use of the H^+ to generate CO_2 from HCO_3^-:

$$H^+ + HCO_3^- \rightarrow CO_2 + H_2O \quad (3)$$

The sum of Eqs. (2) and (3) is identical to Eq. (1). The 2 mechanisms are experimentally indistinguishable, since intracellular carbonic anhydrase equilibrating the inorganic species with the H^+-OH^- is required when calcification and/or photosynthesis uses HCO_3^- as the form entering the cell. It must be acknowledged that the overall equation is an oversimplification, and varies with the pH of the intracellular compartment concerned.

A strict 1:1 stoichiometry of calcification and photosynthesis is clearly not applicable in the large number of cases in which the PIC:POC ratio is significantly different from 1:1 (Raven 2011a). These cases cannot be rescued by considering loss of photosynthate as (photo-) respired CO_2 or as dissolved organic matter or transparent exopolymeric particles (Raven 2011a, Pedrotti et al. 2012). This correction gives PIC per integrated net photosynthetic C accumulation which is lower than PIC:POC (Raven 2011a). This adjustment still yields cases in which generation of PIC produces more CO_2 than is consumed in photosynthesis as well as cases in which generation of PIC produces less CO_2 than is used in photosynthesis, or even produces no CO_2 (Raven 2011a). An interesting line of evidence for the lack of an obligatory coupling of photosynthesis to calcification comes from the growth of *Emiliania huxleyi* at low external [Ca^{2+}] which abolishes calcification yet leaves photosynthesis unaffected (Herfort et al. 2004, Trimborn et al. 2007). When the calcification rate exceeds the rate of photosynthesis, excess CO_2 or its equivalent as H^+ is excreted (Suffrian et al. 2011, Taylor et al. 2011). Conversely, when the photosynthetic rate exceeds that of calcification, additional CO_2 (Sikes et al. 1980) or HCO_3^- influx to the cell is required in addition to the CO_2 produced in calcification. These arguments speak against a widespread causal linkage of photosynthesis to calcification.

When the rate of calcification exceeds the rate of organic carbon production, there is an excess of CO_2 (Eq. 1) or H^+ (Eq. 2) relative to that needed to maintain intracellular acid–base balance.

This excess of H^+ could be used in converting HCO_3^- into CO_2 which is consumed in photosynthesis. A variety of methods have been used to clarify the carbonate species taken up and used in coccolithophore photosynthesis. Data from the membrane inlet mass spectrometer method suggest uptake of both CO_2 and HCO_3^- to supply photosynthesis in coccolithophores (Rost et al. 2003, 2007, Tchernov et al. 2003, Schulz et al. 2007), while isotope disequilibrium experiments suggest a predominant role for CO_2 entry (Sikes et al. 1980, Sikes & Wheeler 1982, Sekino & Shiraiwa 1994, but see Rost et al. 2007), and inhibitor studies indicate a significant role for HCO_3^- (Herfort et al. 2002). Examination of the rate of photosynthesis as a function of the concentration of inorganic carbon and of extracellular pH also suggests a predominant role for HCO_3^- entry (Paasche 1964, Buitenhuis et al. 1999). In the absence of this sink, H^+ could be lost from cells across the plasmalemma using the recently discovered plasmalemma H^+ channel (Suffrian et al. 2011, Taylor et al. 2011). The passive (energetically downhill) nature of this flux means that there is no direct energy input from metabolism to the H^+ transport. However, Raven (2011a) pointed out that energy input is needed to maintain the appropriate transplasmalemma electrical potential difference to maintain the H^+ efflux, and that this input is likely to be greater per unit calcification in a higher-CO_2 ocean. A similar H^+ channel has been found in a non-calcifying dinoflagellate (Smith et al. 2011). A complication in analysing the quantitative requirement for H^+ efflux is that H^+ is consumed when HCO_3^-, rather than CO_2, is the form in which inorganic carbon destined for photosynthesis enters the cell, and in acid–base balance following assimilation of NO_3^- and SO_4^{2-} (Raven 2011a). Ries (2011) also considered energetic constraints on calcification in a high-CO_2 environment.

HETEROCOCCOLITHS AND HOLOCOCCOLITHS

Coccolithophores produce characteristic coccoliths, comprising an organic template with crystalline calcite deposited on it in species-specific patterns (Braarud & Nordli 1952, Young 1994, Young & Henriksen 2003, Young et al. 2005) according to Eqs. (2) and (3). The process obviously requires calcium and bicarbonate ions; it also requires energy and, to con-

struct the synthetic and exocytotic apparatus, macro- and micronutrients. Once exocytosed, the calcite liths are exposed to ambient seawater, and the tendency for dissolution will be determined by the calcite saturation state of the seawater, which is determined by Eq. (4):

$$\Omega_{cal} = [Ca^{2+}]\,[CO_3^{2-}]/K_{sp} \quad (4)$$

where K_{sp} is the stoichiometric solubility product of calcite, which varies as a function of temperature, salinity and pressure (Mucci 1983, Zeebe & Wolf Gladrow 2001). In the modern ocean, $[Ca^{2+}]$ is considered as being constant (varies only with salinity), thus the carbonate ion concentration is the only real variable in this equation.

Coccolithophores typically have a diploid, heterococcolith-bearing phase, with calcified plates made up of complex crystal units in radial arrays. For many species, a haploid phase has been observed with either non-calcified organic scales, as in *Emiliania huxleyi,* or with a different calcification mode. The formation of holococcoliths, calcareous scales composed of many small identical euhedral crystallites (Frada et al. 2009), is the most common form of haploid biomineralisation in extant coccolithophores (Young et al. 2005). Studies have investigated gene expression by the 2 phases (von Dassow et al. 2009, Rokitta et al. 2011) and effects of CO_2 and their modulation by light (Rokitta & Rost 2012).

Heterococcoliths are formed internally in coccolith-forming vesicles which are part of the endomembrane system (Marsh 2003, Young & Henriksen 2003, Brownlee & Taylor 2004). Coupling to exergonic cell activities provides the energy which makes the depositional environment supersaturated with respect to calcite, and provides the organic template on which deposition occurs (Anning et al. 1996, Marsh 2003, Young & Henriksen 2003, Brownlee & Taylor 2004). The process of deposition is under more control by the organism than is the case for those organisms, e.g. coralline algae, which deposit $CaCO_3$ extracellularly (von Dassow et al. 2009, Mackinder et al. 2010, 2011, Raven 2011a).

The finished coccoliths are then externalised. The tendency for them to then dissolve is dependent on the saturation state in the boundary layer and may be reduced by the occurrence of a surface coating of organic material. Although holococcolith formation has not yet been well characterised, it is suggested that calcification still occurs within a delicate envelope or, less likely on grounds of comparative cell biology (Raven 1980), occurs rapidly just below the cell membrane (Young & Henriksen 2003). Von Dassow et al. (2009) showed for *Emiliania huxleyi* that transcript levels of genes whose products are involved in coccolithogenesis (Ca^{2+}, H^+ and HCO_3^- transporters) are expressed much more in the diploid (heterococcolith) than in the haploid (holococcolith) phase which has non-calcified coccoliths. The few studies concerning environmental effects on holococcoliths (Quintero-Torres et al. 2007, Fiorini et al. 2011a, Pedrotti et al. 2012) will be discussed in the relevant sections.

COCCOLITHOPHORES IN PAST AND PRESENT ENVIRONMENTS

Coccolithophores have experienced and thrived with significant variations in surface ocean chemistry, temperature and, through variations in the mixing depth, exposure to solar radiation. They are found throughout the world ocean, in both open ocean and coastal regions where they are more likely to be exposed to larger and faster changes in environmental factors. A number of studies have investigated changes in coccolithophore morphology related to environmental changes over times ranging from tens of millions of years (Henderiks & Rickaby 2007, Henderiks 2008, Henderiks & Pagani 2008) to tens or hundreds of years (Iglesias-Rodriguez et al. 2008a, Rickaby et al. 2010a, Beaufort et al. 2011). There have also been modelling studies (e.g. Young 1994, Merico et al. 2006) and molecular clock investigations of changes in coccolithophore biochemistry (Young et al. 2012).

During the course of coccolithophore history, CO_2 (with implications for the rest of the inorganic carbon system and pH) and also Ca^{2+} and Mg^{2+} have varied dramatically (Orr 2011, Müller et al. 2011, Zeebe & Ridgwell 2011). The extent of stratification which controls solute transfer between deeper waters and the upper mixed layer, and therefore nitrogen and phosphorus availability, has also varied (Steinacher et al. 2010).

CO_2 concentrations over the last 220 million years have, with the exception of the last 10 to 20 million years, been higher than the present level. However, the calcite saturation index has probably varied little over the past 100 million years as documented in deep-sea sediments, due to the halving of the $[Ca^{2+}]$ and increase in $[CO_3^{2-}]$ (Tyrrell & Zeebe 2004). The decrease in $[Ca^{2+}]$ to around 10 mM in present-day seawater (Tyrrell & Zeebe 2004) and concomitant doubling of $[Mg^{2+}]$ to around 50 mM (Dickson & Goyet 1994), possibly modulated by changes in sulphate

concentration, resulted in the alternating 'aragonite ocean' and 'calcite ocean' (Bots et al. 2011, Müller et al. 2011, Orr 2011, Zeebe & Ridgwell 2011). Over shorter time scales, Rickaby et al. (2010a) found that the glacial Southern Ocean had a higher alkalinity than occurs today, with implications for calcification.

Past environments have also, with the exception of glacial episodes, been warmer than today, probably with a more stratified ocean, shoaling of the thermocline and the associated increase in the mean PAR and UVR incident on photosynthetic organisms, as well as decreased fluxes of phosphate and of combined nitrogen (i.e. nitrogen other than N_2) from the deep ocean to the surface (Rost & Riebesell 2004, Steinacher et al. 2010, Raven et al. 2011, 2012, Zeebe & Ridgwell 2011).

With the increase of CO_2 and temperature over the past 2 centuries since the start of the industrial revolution, and the predicted (inevitable) continuation of these trends, the coccolithophores will return to an approximation of what they have experienced over most of their existence, although the rate of change is probably higher than has generally occurred in the past. There will be some increases in dissolved inorganic carbon (DIC), as a result of dissolution of anthropogenic atmospheric CO_2 and of upwelling of waters in which sedimentary $CaCO_3$ has dissolved following interaction with the increased CO_2 in downwelled water. This latter process will also slightly increase surface water alkalinity and Ca^{2+} (Doney et al. 2009, Orr 2011). Anthropogenic combined nitrogen and sulphur inputs from the atmosphere reduce surface water alkalinity, but this accounts for only a few percent of the increase caused by CO_2 dissolution, although in localised coastal regions the effect may be 10 to 50% (Doney et al. 2007). The increased dissolved CO_2 and H^+, and reduced [CO_3^{2-}] in the oceans will result in the shoaling of the calcite saturation horizon over the coming centuries (Caldeira & Wickett 2003, Orr et al. 2005, Fabry et al. 2008). This may result in reduced calcification (see Merico et al. 2006) and increased dissolution of biogenic calcite. This would decrease the ballasting of organic particles and the carbonate transfer to deeper waters, resulting in a reduction of the CO_2 sink. Simultaneously, this outcome would decrease the CO_2 source in surface waters, owing to reduced calcification (Eq. 1) and increased dissolution (the reverse of Eq. 1).

Warming is increasing stratification with shoaling of the thermocline, so increasing mean fluxes of PAR and UVR, and decreasing fluxes of nutrients (phosphorus and combined nitrogen) to the upper mixed layer, resulting in decreased primary productivity (Steinacher et al. 2010, Boyd 2011, Joint et al. 2011, Raven et al. 2011, 2012). Further influences on nutrient availability come from anthropogenic inputs of atmospheric combined nitrogen (Doney et al. 2007), decreased nitrification (Beman et al. 2011) and iron availability (Shi et al. 2010) in an acidified surface ocean, as well as subsurface deoxygenation which increases denitrification (Oschlies et al. 2008, Keeling et al. 2010, Boyd 2011). Overall, the predicted future scenario is an increase in the low-productivity mid-ocean regions due to reduced nutrient fluxes caused by stratification (Behrenfeld et al. 2006, Cermeño et al. 2008, Doney et al. 2009, Steinacher et al. 2010, Tyrrell 2011). These highly stratified surface waters with high PAR and UVR are the very regions where coccolithophores are dominant due to their tolerance of strong light and high affinity for nutrients (Paasche 2001). However, the simultaneous increase in CO_2 concentrations and decrease in Ω_{cal} may have adverse effects on their ability to calcify and potentially increase dissolution of liths.

The experiments on coccolithophores were too short-term and were otherwise inappropriately designed to address evolutionary issues in the ways that were used in work on non-calcified microalgae (Collins & Bell 2004, Bell & Collins 2008, Collins & de Meaux 2009, Huertas et al. 2011) until the work of Lohbeck et al. (2012) on 500 generations of freshly isolated clones of *Emiliania huxleyi* which provided evidence of increased evolutionary fitness in higher CO_2 through genetic change in the cultures growing at high CO_2.

In order to compare the data presented in the literature, it is necessary to note that many different parameters pertaining to calcification are reported. In much of the recent literature examining the carbonate system, PIC production rates, or cellular PIC are reported. This is often accompanied by POC production rates and a PIC:POC ratio. This is informative as to the relative photosynthesis and calcification occurring within a cell. In studies with a different focus, coccolith mass, other dimensions or degree of malformation may be analysed. Although not directly comparable, the effect of environmental variables may still be extracted from the data sets.

CALCIFICATION AS A FUNCTION OF THE INORGANIC CARBON SYSTEM

Methodology

Much consideration has been given to the most appropriate methods to use in mimicking the contin-

uing increase in atmospheric, and hence surface ocean, CO_2 (e.g. Dickson & Goyet 1994, Hurd et al. 2009, Schulz et al. 2009, Shi et al. 2009, Riebesell et al. 2010, Gattuso & Hansson 2011, Hoppe et al. 2011, 2012). While the case for common methodology in future experiments is well made in the volume edited by Riebesell et al. (2010), earlier experiments can still be used to draw useful conclusions (see discussion by Shi et al. 2009). To briefly summarise, the most common methods of DIC manipulation are the addition of acid/base to the medium, or bubbling with either a CO_2/air combination or pure CO_2 to equilibrate the medium to the desired pCO_2. The addition of acid/base or bubbling alters the composite parameters of total alkalinity (TA) and DIC, respectively, whilst the other parameter remains constant. However, the effects of the 2 methods on the individual parameters of the carbonate system, i.e. pH, $[CO_2]$, $[CO_3^{2-}]$ and Ω_{cal}, are very similar (Schulz et al. 2009). Gas bubbling has generally been preferred because it more accurately reflects what will occur in the future; however, the mechanical effects of bubbling may adversely affect the study organisms (Shi et al. 2009, Hoppe et al. 2011). Alternative or complementary methods include the pre-equilibration of the medium and then growth of very dilute cultures in a closed system (Hoppe et al. 2011, 2012) or dilution with the CO_2-equilibrated medium (Riebesell et al. 2010). The use of $NaHCO_3$ or Na_2CO_3 followed by HCl is also possible (Schulz et al. 2009). The use of pH buffers has been found to introduce additional problems such as effects on growth (Blanchemain et al. 1994, Hurd et al. 2009) and trace metal speciation (Hurd et al. 2009, Shi et al. 2009). Due to apparently conflicting results of experiments on *Emiliania huxleyi* using different manipulative techniques (Riebesell et al. 2000a, Iglesias-Rodriguez et al. 2008a,b) several investigators set out to test the importance of the technique used on the outcomes for the same strain(s) (Shi et al. 2009, Bach et al. 2011, Hoppe et al. 2011), as well as comparing strains using a single technique (Langer et al. 2009). Hoppe et al. (2011) found no difference in the response of 2 strains of *E. huxleyi*, NZEH (as examined by Iglesias-Rodriguez et al. 2008a) and PLY M219, to closed-system TA or closed-system DIC manipulation. In these experiments, they did not see the large increase in PIC and POC seen by Iglesias-Rodriguez et al. (2008a). They also tested the more usual open system DIC manipulative technique of bubbling and found slightly different results. Shi et al. (2009) also compared the effects of closed TA and open DIC manipulations on strain NZEH, and they reported no significant differences between the treatments apart from a small decrease in growth rate in bubbled cultures which may be due to mechanical effects of bubbling or to real differences in the carbonate chemistry. Presumably all cultures were subject to similar mechanical stresses, so there is some additional reason for the apparent effect of increased pCO_2 when supplied by aeration.

Some measurements require quite a large biomass. As they grow, cells inevitably take up CO_2, so although the target gas may be added, this may not be what is seen in the experimental vessels. This leaves a philosophical question as to whether they are experiencing the pCO_2 that is added or the net pCO_2 that remains after carbon acquisition by the culture. The evidence suggests that manipulation by acid/base addition may mimic the future scenario sufficiently well to provide useful data. It is probably prudent to test this for individual species if dramatic results are seen. A thorough description of the carbonate system parameters is essential.

Strain differences

The alternative explanation proposed for the differences in response seen in *Emiliania huxleyi* cultures is that there are intra-specific responses. As indicated above, Langer et al. (2009) tested 4 different strains of *Emiliania huxleyi* and found different responses for all of them. Hoppe et al. (2011) tested the strain previously examined by Iglesias-Rodriguez et al. (2008a) and Shi et al. (2009) and, as indicated above, found somewhat different results. More experiments of this type are required.

Ω_{cal} and calcification

The intracellular calcification by coccolithophores (Mackinder et al. 2010, 2011) involves the supply of inorganic carbon to the coccolith-forming vesicle involving influx of HCO_3^- at the plasmalemma (Paasche 1964, Buitenhuis et al. 1999; cf. Maberly 1992). There is evidence that calcification can frequently still occur when the external Ca^{2+} and/or CO_3^{2-} are so low as to cause undersaturation of the medium with respect to calcite. This section will use evidence on the effects of Ω_{cal} on calcification from laboratory studies, field observations of modern day coccolithophores and from the sedimentary record.

In *Emiliania huxleyi* (as *Coccolithus huxleyi*), Paasche (1964) found that the calcification rate be-

came 0 at an external [HCO_3^-] of 0, with constant Ca^{2+}. Buitenhuis et al. (1999) found that calcification in *E. huxleyi* strain Ch 24-90 ceased when [HCO_3^-] was decreased to 0.5 mM or lower. Similar results were found for experiments at constant inorganic carbon with variable external Ca^{2+} concentration (Paasche 1964, Herfort et al. 2004, Trimborn et al. 2007, Leonardos et al. 2009, Xu et al. 2011). The intracellular precipitation of calcite by coccolithophores when the bulk medium is undersaturated has parallels in the intracellular deposition of celestite in acantharians (Raven & Knoll 2010) and of silica (opal) by diatoms (Raven & Waite 2004). However, in the case of celestite and silica, the present surface ocean is well below the saturation value for these 2 minerals, and for silica this has been the case since (at least) soon after the appearance of diatoms in the fossil record (Raven & Waite 2004, Raven & Knoll 2010). By contrast, the present surface ocean is supersaturated with respect to calcite, although this is forecast to change due to increases in CO_2 without compensatory parallel increases in ocean surface total inorganic carbon and alkalinity and/or Ca^{2+}, at least over timescales less than the ocean mixing time (Orr 2011, Tyrrell 2011, Zeebe & Ridgwell 2011).

A major recent preoccupation of those working on coccolithophores has, not unexpectedly, been the examination of the effects of increased CO_2 on coccolith formation. The problem of the use of different methodologies in comparing data sets has already been mentioned. Rather than deal in detail with the primary data, we refer mainly to review articles in presenting the main outcomes of the work. The analyses by Doney et al. (2009), Hurd et al. (2009), Ridgwell et al. (2009), Kroeker et al. (2010) and Moolna & Rickaby (2012) relate to work with coccolithophores grown with saturating concentrations of nutrients and at saturating fluxes of PAR. These show that the predominant response is a decreased rate of calcification when cells are grown at CO_2 levels higher than those found today (390 ppm) or at least a decrease in PIC:POC and a corresponding increase in calcification in low CO_2 concentrations such as the 190 ppm or so seen at the last glacial maximum 18 000 yr ago (e.g. Riebesell et al. 2000a,b, Zondervan et al. 2001, Casareto et al. 2009). However, different strains of *Emiliania huxleyi* and *Calcidiscus leptoporus* showed different responses, which are summarised in Table 2. The response patterns include no effect of changing CO_2 in the range examined (Langer et al. 2006, Rickaby et al. 2010b); a decreased calcification rate in both higher and lower CO_2 concentrations than the present values (Langer et al. 2006); an increased calcification rate, but not PIC:POC, with higher CO_2 concentrations (Iglesias-Rodriguez et al. 2008a,b, Riebesell et al. 2008); and both increased photosynthesis and calcification but usually greater photosynthesis leading to reduced PIC:POC (e.g. Rickaby et al. 2010b; see also Iglesias-Rodriguez et al. 2008a). However, a different response was seen by Hoppe et al. (2011) using the same strain of *E. huxleyi*. Reduced growth rates leading to increased PIC and POC were seen by Rickaby et al. (2010b) and Langer et al. (2009). Feng et al. (2009) incubated a natural population from the North Atlantic and saw much more abundant lightly calcified coccolithophores in their combined high temperature and CO_2 treatment.

A meta-analysis of the available laboratory studies by Findlay et al. (2011) suggests that for *Emiliania huxleyi*, the PIC:POC ratio can be predicted by the dissolved CO_2 concentration, TA and phosphate concentration. From a biogeochemical point of view, PIC production and growth rate must be examined together to determine whether there will be an overall increase or decrease in calcite production. This still cannot be directly related to calcite export without knowledge of the dissolution, aggregation and other variables affecting sinking rates of organic material. Bach et al. (2011) examined *E. huxleyi* grown either at constant alkalinity with CO_2 fugacity ranging from 2 to 600 Pa at sea level (20 to 6000 ppm), or at a constant pH (pH 8) with CO_2 fugacity of 4 to 370 Pa (40 to 3700 ppm). The constant alkalinity experiments showed optimal CO_2 fugacities for growth of ~20 Pa (200 ppm), for calcification of ~40 Pa (400 ppm) and for organic carbon production of ~80 Pa (800 ppm). Comparison with the constant-pH approach showed that the growth rates and organic carbon production were closely similar at the low and intermediate CO_2 values. However, at high CO_2, growth rates and organic carbon production were higher at constant pH than when pH decreased, suggesting an inhibitory effect of lower pH or allocation of resources to maintaining pH. pH dependence was also seen for calcification, though it was not clear which carbonate system parameter determined calcification at low CO_2 fugacities. These optima explain to some extent the general pattern of increased POC (optimum 80 Pa), decreased PIC (optimum 400 µatm) and sometimes decreased growth (optimum 20 Pa) seen in many of the studies performed. These optima can only be applied to this strain, and it would be interesting to test whether other strains, particularly CAWP-06, differ in these optimal values.

Table 2. Influence of species, strain and experimental methods on the outcome of experiments investigating the effects of ocean acidification on coccolithophore growth rate (μ), particulate inorganic carbon (PIC) production, particulate organic carbon (POC) production and PIC:POC ratio. ↓(↑): decrease (increase); ↔: no significant effect; ∩(∪): optima (minima). DIC: dissolved inorganic carbon; TA: total alkalinity; na: not applicable

Species/strain	Method	Light (μmol m^{-2} s^{-1})	PO_4^{3-} (μM)	NO_3^- (μM)	Temp. (°C)	pH_T range	μ	POC	PIC	PIC:POC	Mal-formation	CO_2sys	Source
Emiliania huxleyi													
RCC1256	Acid–base	400	6.25	100	17	8.33–7.69	↓	↑	↑	↔	–	DIC; TA	Langer et al. (2009)
RCC1256	CO_2	170	6	100	15	7.88–8.44	↓	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
RCC1256	Acid–base	170	6	100	15	7.72–8.32	↓	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
NZEH[b]	CO_2	150	6.25	100	19	8.15–7.79	↓	↑	↑	↔	N		Iglesias-Rodriguez et al. (2008a)
NZEH	CO_2	150	6.25	100	20	8.10–7.80	↔	–	–	↔	–	DIC; pH	Shi et al. (2009)
NZEH	Acid–base	150	6.25	100	20	8.10–7.80	↑	↑	↑	↓	–	pH; TA	Shi et al. (2009)
NZEH	CO_2 open	170	6	100	15	7.86–8.51	↔	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
NZEH	CO_2 closed	170	6	100	15	7.79–8.14	↔	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
NZEH	Acid–base	170	6	100	15	7.73–8.4	↔	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
PML B92/11A	Acid–base	150	6.25	100	15	8.45–7.80	↔	↑	↓	↓	Y	DIC; TA	Riebesell et al. (2000a)
PML B92/11A	Acid–base	15, 30, 80	6.25	100	15	8.39–7.81	↔	↑	↓	↓	–	DIC; TA	Zondervan et al. (2002)
Bergen	Acid–base	140	3.6	88	16	8.21–7.60	↓	↑	↓	↓	N	DIC; pH	Müller et al. (2010)
AC481	CO_2	150	1	32	13	8.3–7.60	↓	↔	↔	↔	Y + size↓	pH; TA	De Bodt et al. (2010)
AC481	CO_2	150	1	32	18	8.0–7.50	↔	↔	↔	↔	Y + size↓	pH; TA	De Bodt et al. (2010)
AC472 diploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↔	↑	↑	Size ↔	pH; TA	Fiorini et al. (2011a)
AC472 haploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↓	na	na	Size↓	pH; TA	Fiorini et al. (2011a)
RCC1212	Acid–base	400	6.25	100	20	8.33–7.69	↓	↔	↓	↓	–	DIC; TA	Langer et al. (2009)
RCC1238	Acid–base	400	6.25	100	20	8.33–7.69	↑	↓	↔	↔	–	DIC; TA	Langer et al. (2009)
Calcidiscus leptoporus													
AC370 diploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↔	↔	↔	Size ↔	pH; TA	Fiorini et al. (2011a)
AC370 haploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↑[a]	–	–	Size↑	pH; TA	Fiorini et al. (2011a)
AC365 diploid	Acid–base closed	350	6.25	100	20	7.86–8.74	↔	↔	∩	∩	Y	TA; DIC	Langer et al. (2006)
Syracosphaera pulchra													
AC418 diploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↓	↔	↔	Size↓	pH; TA	Fiorini et al. (2011a)
AC418 haploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↓	–	–	Size↓	pH; TA	Fiorini et al. (2011a)
Gephyrocapsa oceanica													
PC7/1	Acid–base	150	6.25	100	15	8.45–7.80		↑	↓	↓	Y	DIC; TA	Riebesell et al. (2000a)
Pz 3.1	Acid–base	200	6.25	100	18	8.13 DIC altered	↑	∩	↔	∪	N	DIC; TA; pH	Rickaby et al. (2010b)
***Coccolithus pelagicus* (spp. *braarudii*)**													
RCC 1200	Acid–base	140	3.6	88	16	7.6–8.21	↓	↔	↓	↓	Y	DIC; pH	Müller et al. (2010)
4762	Acid–base	200	6.25	100	18	8.13 DIC altered	↓	↑	↑	↔	Y + size↓	DIC; TA; pH	Rickaby et al. (2010b)
RCC 1200	Acid–base closed	350	6.25	100	17	7.81–8.56	↔	↔	↔	↔	N	TA; DIC	Langer et al. (2006)

[a]POC production but not POC content significant; [b]also known as PLY M219

Recent work has shown that experiments over much longer (~150 generations; Müller et al. 2010), or shorter (Barcelos e Ramos et al. 2010) than the normal several days of acclimation before measurements are made do not alter the response. The short-term experiments (Barcelos e Ramos et al. 2010) using net (rather than tracer) changes in calcite showed that changes occur over periods of hours, so all 3 experimental time scales permit changes to the proteome (acclimation). There is no evidence of the effects of carbonate system chemistry on holococcolith-bearing haploid cells. This was examined by Fiorini et al. (2011a) for *Calcidiscus leptoporus* and *Syracosphera pulchra*; however, the PIC of the haploid cells was below the detection limits. This may be of interest to pursue because holococcoliths persisted in the fossil record through variations in atmospheric and ocean chemistry (Medlin et al. 2008).

A recent study (Lohbeck et al. 2012) addressing the potential of coccolithophores to adapt to future CO_2 concentrations showed that the responses described above may be short term. Even within 1 yr, *Emiliania huxleyi* adapted to CO_2 partial pressure of 220 Pa so that PIC production and growth rate at this high level of CO_2 were significantly greater than that of cultures adapted to 40 Pa CO_2 when grown at 220 Pa CO_2. This adaptation also translated into increased PIC production when cells were returned from 220 Pa to 40 Pa CO_2. Cell diameter, growth rate, PIC production and PIC:POC were all reduced but POC cell^{-1} was increased in cells taken from 40 Pa directly into 220 Pa CO_2 conditions. Cells acclimated to 220 Pa were of the same size as those at 40 Pa with slightly reduced PIC cell^{-1}, PIC production and growth rate but increased POC cell^{-1}. This study also neatly demonstrated the emerging dominance of different genotypes selected from a mixed-genotype founding population at the different CO_2 concentrations.

In the modern ocean, Merico et al. (2006) found that *Emiliania huxleyi* blooms in the Bering Sea shelf over 7 yr correlated with high [CO_3^{2-}], with less calcification and production of malformed liths at lower [CO_3^{2-}]. However, Merico et al. (2006) did not claim that a high [CO_3^{2-}] was a critical factor in the success of *E. huxleyi.* Beaufort et al. (2011) found a clear pattern of distribution of differentially calcified species and morphotypes according to the carbonate chemistry. A decrease in coccolith mass, largely due to change in species composition, was strongly correlated with Ω_{cal} and [CO_3^{2-}], with other environmental variables only having a geographically localised influence. In this study, mixed-layer irradiance was not recorded, but was found by Charalampopoulou et al. (2011) to be a major determinant of species composition of the coccolith communities in the North Atlantic. pH or some related variable(s) of the inorganic carbon system was also a determining factor in the study by Charalampopoulou et al. (2011). Effects on community calcification were seen to vary with position on a transect between the North Sea and the Arctic Ocean, largely as a result of differences in calcification at the species level. Despite a generally consistent response of calcification to seawater inorganic carbon chemistry, Beaufort et al. (2011) found a heavily calcified R-like morphotype of *E. huxleyi* in the low-pH Patagonian shelf and Chilean upwelling waters. Another exception to the rule was found by Smith et al. (2012) during winter in the Bay of Biscay. Despite the low $CaCO_3$ saturation state, heavily calcified *E. huxleyi* type A predominated over the less calcified type A cells. However, the pH did not fall below 8.05, and cells were much reduced in number in winter. This seems to point to reduced growth rates, which may account for overcalcification. Hagino et al. (2011) also saw morphotypes associated with water masses with distinct temperature and nutrient conditions.

A recent analysis of calcite mass of dominant coccoliths in the sedimentary record over the last 40 000 yr (Beaufort et al. 2011; see Henderiks & Rickaby 2007, Henderiks & Pagani 2008) showed a clear pattern of decreasing calcification with increasing CO_2 and decreasing carbonate concentration in seawater. Both *Gephyrocapsa* and *Emiliania* showed a ~25% decrease in coccolith mass from the last glacial maximum to near-present in cores from the North Atlantic and South Indian Oceans. However, Iglesias-Rodriguez et al. (2008a) reported a 40% increase in coccolith mass over the past 220 yr, in the 0.65 to 10 µm fraction of a box core from a region of the subpolar North Atlantic with exceptional open-ocean sedimentation rates. This was not related to changes in species composition, although further analysis of the same core suggested that it was not consistent for all species (Halloran et al. 2008). The larger coccolithophores, including *Calcidiscus leptoporus* and *Coccolithus pelagicus*, showed increased lith sizes, but smaller species (*E. huxleyi*, *G. oceanica* and *G. mullerae*) showed more lightly calcified liths, or possibly dissolution of liths during or after sinking (Halloran et al. 2008). This increase in coccolith mass over the recent past was partly supported by Grelaud et al. (2009) in the Santa Barbara Basin (California, USA). They reported a 33% increase in coccolithophore shell carbonate mass for the order Isochrysi-

dales, comprising *E. huxleyi, G. oceanica* and *G. mullerae*, in response to increasing pCO_2 and sea surface temperature between 1917 and 2004. Obviously, these are the smaller species found to have the opposite response by Halloran et al. (2008). The coccolith mass enhancement was found only when the water mass was influenced by the Californian counter current flowing from the south, originating on the Chilean coast, and not when dominated by the south-flowing Californian current. Therefore, this morphotype may be the same as that seen by Beaufort et al. (2011) as an exception to the rule in their data set. The data sets of Beaufort et al. (2011) and Iglesias-Rodriguez et al. (2008a) do not span the same time period, so although they show an opposite response in coccolith mass to increased CO_2 concentrations, this may be due to other environmental variables. It appears that there are species- or strain-specific differences and influences of other variables so that broad generalisations cannot be made from individual studies.

Upwelling regions are characteristically low in pH and act as sources of CO_2 to the atmosphere, so species thriving here will be adapted to these conditions and may respond favourably whilst other species do not. At the level of the organism, modelling by Irie et al. (2010) suggested that an increase in coccolith mass (as reported by several but not all investigators: see above and Fukuda et al. 2011, Krug et al. 2011, Langer 2011, Richier et al. 2011) was, counter-intuitively, the optimal evolutionary response to decreasing ocean CO_3^{2-}. It must be emphasised that this conclusion rests on the untested assumption that increased mass decreases mortality. Where studies provide reports of malformations, or normally formed coccoliths, this is included in Table 2. Although it is normal for there to be some malformed coccoliths even under present-day conditions (Paasche 2001), there is certainly evidence that changes in carbonate chemistry can cause increasing malformation of coccoliths (Riebesell et al. 2000a, Langer et al. 2006, De Bodt et al. 2010, Müller et al. 2010, Rickaby et al. 2010b) but this is not always the case (Iglesias-Rodriguez et al. 2008a, Crawfurd 2010, Müller et al. 2010, Rickaby et al. 2010b). Rickaby et al. (2010b) suggested that there is a change in the interaction between the polysaccharide template and the calcite being laid down, due to a pH change within the coccolith-forming vesicle. Table 2 gives the outcome of experiments on the effect of Ω_{cal} on calcification, PIC, POC and PIC:POC ratio in coccolithophores as a function of strain and experimental method.

Ω_{cal} and dissolution

The calcified parts of coccoliths would be expected to dissolve in seawater undersaturated with calcite, with the rate increasing with the degree of undersaturation. This rate of dissolution might be slowed by the organic layer found around newly exocytosed coccoliths (Iglesias-Rodriguez et al. 2008a, Godoi et al. 2009, Hassenkam et al. 2011). Tyrrell et al. (2008) attributed the absence of coccolithophores from the brackish Baltic Sea to winter dissolution of the coccoliths in water undersaturated with calcite, coupled with low winter rates of metabolism and coccolithogenesis due to undersaturation. Dissolution of the externalised mineral skeleton in seawater undersaturated with respect to the mineral phase does not seem to be a major problem for acantharians (celestite) or diatoms (silica). At least for the diatoms, there is evidence that the organic layer surrounding the silicified frustules can decrease the dissolution rate of silica by 2 orders of magnitude (Natori et al. 2006) so that the first-order rate constant for dissolution (d^{-1}) falls from a value similar to the maximum specific growth rate (d^{-1}) to a value of 0.01× the maximum specific growth rate (Raven & Giordano 2009, Raven 2011a). Milligan et al. (2004) showed a small increase in the rate of dissolution of silica from diatom frustules with increasing CO_2 concentration in seawater; the mechanism of this effect is unknown. A further probable similarity between the dissolution of coccolith calcite and diatom silica is that, presumably, the organic layer restricting dissolution is gradually removed by the activity of heterotrophic microbes. A recent study by Hassenkam et al. (2011) showed that neither fossil nor modern coccoliths dissolved in Ca^{2+}-free artificial seawater at pH 8.2 despite $\Omega_{cal} = 0$, whereas inorganic $CaCO_3$ did dissolve. At pH 7.8 in Ca^{2+}-free artificial seawater, coccoliths dissolved completely. Biogenic calcite is more robust than inorganic calcite; it is thought that the organic coating protecting extant coccolithophores may also protect the liths during diagenesis, resulting in smaller crystals which are less prone to dissolution than inorganic crystals (Hassenkam et al. 2011). In comparing fossil and extant coccoliths, it must be acknowledged that any organic layer around fossil coccoliths might not reflect the original state but is a diagenetic effect. More work is needed on the extent to which organic coating on coccoliths restricts their dissolution.

EFFECTS OF PHOTOSYNTHETICALLY ACTIVE RADIATION ON CALCIFICATION

Calcification is an energy-dependent process (Raven 1980, 2011a, Brownlee et al. 1995a,b, Anning et al. 1996) and so is ultimately dependent on photosynthesis in the obligately photolithotrophic (Paasche 1965, 1966a,b, 2001) coccolithophores. Before considering the effect of PAR on calcification, we explore some aspects of the energetics of coccolith formation and photosynthesis, assuming that there is no close coupling of the carbon assimilation processes in calcification and in photosynthesis. From Falkowski & Raven (2007), the absolute minimum energy cost for the conversion of 1 mol CO_2 to carbohydrate is 8.43 mol of absorbed photons (400–700 nm), assuming that the additional ATP needed in addition to that produced in non-cyclic photophosphorylation comes from cyclic photophosphorylation; see also Tsuji et al. (2009) for the CO_2 assimilation mechanisms in coccolithophores. The implicit assumption is that the CO_2 concentration at the site of fixation by ribulose bisphosphate carboxylase-oxygenase (Rubisco) requires, in the present air-equilibrium surface ocean, a CO_2 concentrating mechanism (CCM). The minimum stoichiometry of a CCM is 1 mol ATP per mol CO_2 which, with cyclic photophosphorylation as the ATP source, with 1.14 mol photons needed to generate 1 mol ATP (Falkowki & Raven 2007), means a total of 8.43 + 1.14 = 9.57 mol photons per mol CO_2. Considerations of reductive assimilation of nitrate and sulphate, and ATP use in nutrient transport, biosynthesis and maintenance (Falkowski & Raven 2007) gives a total cost of photosynthetic growth of at least 15.5 mol photons per mol CO_2.

Turning to the energetics of coccolithogenesis, coccolithophores have the typical inside-negative electrical potential across the plasmalemma. The electrical potential of the cytosol relative to the medium has been estimated for *Emiliania* (as *Coccolithus*) *huxleyi* at −145 ± 8 mV (SD) (calcified strain) and −146 ± 18 mV (uncalcified strain), and for *Hymonomonas carterae* at −92 ± 11 mV, using the lipophilic singly-charged cationic dye 3,3′-di-propylthiocarbocyanine (Sikes & Wilbur 1982). Using the lipophilipic singly-charged cation tetra[^{3}H]phenylphosphonium, Nimer et al. (1992) found a value of −60 mV for *E. huxleyi.* Anning et al. (1996) found rather less negative values for *E. huxleyi* using the cationic fluorescent probe tetramethylrhodamine ethyl ester.

The process(es) maintaining the electrical potential difference across the plasmalemma in coccolithophores are unclear. An active electrogenic mechanism is needed to explain the electrical potential difference in the work of Sikes & Wilbur (1982) where the potential is more negative (–92.3 to –146 mV) than the K^+ diffusion potential (–65.5 to –86.5 mV). While there are reservations about the use of lipophilic cations to measure electrical potential differences across the plasmalemma (Ritchie 1984), it is very unlikely that the cytosol is not electrically negative by tens of mV relative to the medium.

The inside-negative electrical potential at the plasmalemma means that the entry of the Ca^{2+} used in calcite formation is only energized by maintaining the electrical potential difference in the face of positive charge entry which decreases the inside-negative value of the potential difference. However, the accumulation of HCO_3^- (as with the CCM involved in photosynthesis) requires direct or indirect energization (Raven, 1980, 1984, Berry et al. 2002). It is likely that 1 ATP is the minimum energy cost of moving 1 Ca^{2+} and 1 HCO_3^- in, and 1 H^+ out, at the plasmalemma. The argument for HCO_3^- is the same as for photosynthesis in the CCM considered above, although the H^+ flux is in the opposite direction.

Half as much ATP is probably needed for the transport of these 3 ions across the coccolith vesicle membrane. In this case, the directly energized process is likely to be Ca^{2+} entry using the Ca^{2+} ATPase (stoichiometry 2 Ca^{2+} influx and probably 2 H^+ efflux for 1 ATP converted to ADP + P_i using a P-ATPase: Evans et al. 1991, Anning et al. 1996, Araki & González 1998) from the very low free Ca^{2+} concentration in the cytosol. HCO_3^- entry could be driven with a relatively small, 10 to 20 mV electrical potential difference (lumen positive with respect to cytosol) produced by the active Ca^{2+}/H^+ antiport (but see below: Anning et al. 1996). However, with free Ca^{2+} in the cytosol of not more than 0.1 mmol m^{-3}, the 55 kJ mol^{-1} available from the conversion of 1 mol ATP to ADP and P_i could not give a concentration of free Ca^{2+} in the coccolith vesicle of more than about 1 mol m^{-3}, compared to 10.6 mol m^{-3} in seawater. To keep the product of free Ca^{2+} and CO_3^{2-} concentrations above the value equivalent to the saturation of calcite, this would require a relatively high pH and concentrations of inorganic C and of Ca^{2+}. Measurements of the pH in cytosol, coccolith vesicle and chloroplast of *Emiliania huxleyi* and *Coccolithus pelagicus* by Anning et al. (1996) showed that the values increase in that order, with the coccolith vesicle 0.2 units higher than the cytosol, but 0.6 to 0.8 units lower than the chloroplast, and 1.1 to 1.2 units lower than the seawater value of pH 8.3. The higher, even by only by a mean of 0.2 units, value of coccolith vesicle than of cytosol pH

is difficult to reconcile with the involvement of a V-type H^+ ATPase pumping H^+ into the coccolith vesicle (Araki & González 1998, Corstjens et al. 2001, Corstjens & González 2004). It must be borne in mind that the mean coccolith vesicle pH in *C. pelagicus* is relatively higher (7.6–8.3) when cytosol pH is higher than 7.2. It is lower (6.9–7.2), and not significantly different from cytosol pH, when the cytosol pH is lower than 7.2 (Anning et al. 1996). Another data set which does not favour the 'usual' direction of action of the H^+ V-ATPase, i.e. pumping H^+ from the cytosol to the endomembrane lumen, is that of the electrical potential of the coccolith vesicle lumen relative to the cytosol. The mean value is −6.2 mV, lumen negative relative to the cytosol (Anning et al. 1996), with a wide range from −30 mV for the highest coccolith-forming vesicle pH values and +13 mV for the lowest pH in the coccolith-forming vesicle. While a higher coccolith vesicle lumen pH than cytosol pH is expected if the dominant energization of the membrane is by the $2Ca^{2+}$:$2H^+$:1 ATP P-ATPase, this does not explain the inside-negative electrical potential of the coccolith vesicle relative to the cytosol (Anning et al. 1996).

A final twist is that, although the 'normal' direction of action of the H^+ V-ATPase does not agree with the mean values of pH and electrical potential differences across the coccolith vesicle membrane, the expression of the H^+ V-ATPase parallels that of calcification (Corstjens & González 2004). Although Ziegler et al. (2004) referred to 'polarity reversal' in a plasmalemma-located V-type H^+-ATPase in an epithelium in calcification–decalcification during moulting cycles in the terrestrial isopod *Porcellio scaber*, the 'polarity reversal' refers to the side of the epithelium in which the ATPase is expressed, not the direction of active H^+ flux relative to the side of the membrane which interacts with adenine nucleotides. Here deposition of $CaCO_3$ using soluble ions derived from the 'old' mineralised cuticle occurs on the side of the epithelium lacking the V-ATPase; when the deposited $CaCO_3$ is resorbed prior to deposition of the new, larger, mineralised cuticle, the V-ATPase relocates to the side from which $CaCO_3$ is resorbed.

Wieczorek (1992) and Wieczorek et al. (2000) showed how a plasmalemma V-ATPase in the luminal membrane in insect (*Manduca sexta*) midgut can account for alkalization to pH 11 of the gut lumen, with H^+ secretion from the goblet cells, and 2 H^+ taken up into these cells in exchange for 1 K^+, with a 200 mV potential difference (gut lumen positive) and a haemolymph pH of 6.8 (see also Raven 1994). This structurally complex arrangement is not readily envisaged with the coccolith-forming vesicle equivalent to the lumen of the goblet cell connected to the gut lumen, the coccolithophore cytosol equivalent to the goblet cell cytosol and the seawater medium equivalent to the haemolymph. The role, if any, of the endomembrane-located H^+-translocating pyrophosphatase in calcification is unclear.

Some energetic savings could be achieved by the movement of Ca^{2+} from a hypothesised relatively high free concentration just inside the plasmalemma to the coccolith vesicles via other components of the endomembrane system (Berry et al. 2002, Brownlee & Taylor 2004, cf. Corstjens et al. 1998). It seems doubtful that any such flux of Ca^{2+} to the endomembrane lumen is passive through Ca^{2+} channels yet would still allow the free Ca^{2+} in the coccolith-forming vesicle to be adequate to precipitate calcite. If there is still an involvement of the $1Ca^{2+}$:$2H^+$ ATPase, then any energy saving relative to uptake into the coccolith-forming vesicle from a cytosolic free Ca^{2+} of less than 100 µmol per m^3 would require variable stoichiometry of the ATPase. This suggestion could involve problems with HCO_3^- entry to provide a high enough concentration of CO_3^{2-} for calcite precipitation if HCO_3^- entry involves an electrical potential difference generated by the Ca^{2+} ATPase.

Such a mechanism was suggested in part (Berry et al. 2002) because of problems with maintaining a large flux of Ca^{2+} in the cytosol from the plasmalemma to the coccolith-forming vesicle, as a consequence of the low concentration of free and chelated Ca^{2+} in the cytosol (Raven 1980; see also Gussone et al. 2006). However, the endomembrane pathway presents a problem of charge balance if movement of only Ca^{2+} is considered. One solution would be for each Ca^{2+} destined for coccolith production that moves through the endomembrane system to move with a CO_3^{2-}. This would not necessarily involve CO_3^{2-} influx to the endoplasmic reticulum with Ca^{2+}; it could be achieved by entry of 1 Ca^{2+} and 1 HCO_3^- with the efflux of 1 H^+. If Ca^{2+} entry involves the $1Ca^{2+}$:$2H^+$ antiporter ATPase, then the required stoichiometry would involve the parallel entry of 1 H^+ and 1 HCO_3^-, or of 1 CO_2. If the endoplasmic reticulum lumen is, as usual, more acidic than the cytosol (by contrast with the coccolith vesicle, which is more alkaline: see Table 2 of Anning et al. 1996), then the CO_3^{2-} concentration in the lumen at passive equilibrium through a hypothetical CO_3^{2-} channel would be lower than that in the cytosol, although this would be counteracted by any inside-positive electrical potential difference. Another possible charge-balancing mechanism which does not involve the inorganic C fluxes (from just inside the plasmalemma to

the coccolith-forming vesicle) for coccolithogenesis occurring through the endomembrane system is for some other charge-compensating ion fluxes to occur upon entry of Ca^{2+} into the endoplasmic reticulum, with a corresponding reverse flux when the Ca^{2+} is consumed in coccolithogenesis. The circuit would be completed by cation flux through the cytosol from near the coccolith-forming vesicle to the plasmalemma, or anion flux in the opposite direction.

Despite the perceived problems with large Ca^{2+} fluxes through the cytosol, Allemand et al. (2004) suggested that diffusible Ca-binding proteins are involved in the calicoblastic layer outside the aboral endoderm adjacent to the aragonitic skeleton of scleractinian corals. Alternatives, such as the pinocytotic uptake of external Ca^{2+} (and other solutes), or in endomembrane vesicles loaded in the cytosol using a Ca^{2+}-ATPase, with movement across the epithelium in vesicles, have been ruled out by treatments with selective inhibitors (Tambutté et al. 1996). It is likely that Ca^{2+} entry for calcification in foraminifera involves fluid-phase endocytosis (pinocytosis) (Erez 2003, Bentov et al. 2009). Fluid-phase endocytosis could decrease the energy costs of Ca^{2+} transport associated with coccolithogenesis, and overcome problems with Ca^{2+} diffusion through the cytosol by endocytotic transport from the medium to the golgi and hence the endomembrane system (Berry et al. 2002). However, a search for the required fluid-phase endocytosis in coccolithophores did not yield positive results (Berry et al. 2002, Brownlee & Taylor 2004).

There seems to be a minimum energy cost of 1.5 mol ATP per mol $CaCO_3$ deposited. This involves a photon cost of 1.71 photons per $CaCO_3$ with cyclic electron flow generating ATP, i.e. 11% of the cost of photosynthetic growth. If it is assumed that the Mehler-peroxidase reaction is used to supply additional ATP in photosynthesis, and the ATP used in calcification, the photon costs are 24 mol photons and 4.6 mol photons, respectively, so the calcification cost is 19% that of photosynthetic growth. These values are rather lower than the 30% computed by Anning et al. (1996) which do not include all the cost of growth in the cost of photosynthesis.

This analysis suggests that calcification is unlikely to be a major energy sink for excess (to photosynthesis) excitation energy in photosynthesis. Coccolithophores are generally characterised as not being very susceptible to photoinhibition (with the haploid phase of *Emiliania huxleyi* being more sensitive to high PAR fluxes than the diploid phase: Houdan et al. 2005), but low-calcification cells are not generally more susceptible to photoinhibition (Nanninga & Tyrrell 1996, Harris et al. 2005; cf. Juneau & Harrison 2005, van de Poll et al. 2007). Nielsen (1995) had previously shown that high-calcification cells of *E. huxleyi* had higher values of α (the increase in rate of photosynthesis on a chlorophyll *a* basis per increment of incident PAR) than do low-calcification cells at all 3 concentrations of inorganic C tested. Another possible influence of the energy cost of calcification concerns the observed decrease in calcification in *E. huxleyi* as the seawater CO_3^{2-} concentration decreases (and external pH decreases) but HCO_3^- and, even more, CO_2 concentrations increase with increasing atmospheric CO_2 (Raven 2011a). The increased energy cost of calcification could, perhaps, explain the decreased calcification rate. However, as mentioned above, the changes in concentration of these solutes in seawater would not greatly alter the energetic cost unless leakage was increased as downhill energy gradients increase, or the increased energy requirement involved a doubling in the ratio of energy source consumed (e.g. ATP) to calcification substrate (or waste product) transported.

While the calcification rate increases with increasing irradiance from the very low dark rate, there is also an increase in the rate of photosynthesis, although calcification saturates at lower PAR values than does photosynthesis (Paasche 1964, Balch et al. 1992, Zondervan et al. 2002, Zondervan 2007). Even allowing for POC loss by respiration and loss of soluble organic compounds, it would be expected that PIC:POC would increase with decreasing irradiance below that required to saturate growth. As pointed out by Zondervan (2007), this was found in short-term experiments (Paasche 1964, Balch et al. 1992 Nimer & Merrett 1993, Müller et al. 2008). However, in more ecologically relevant growth experiments, this increase in PIC:POC with decreasing PAR only occurs from saturation down to below 30 µmol photons m^{-2} s^{-1} (Zondervan 2007). There is then a decrease in PIC:POC as a result of smaller liths with less calcite per lith and/or fewer coccoliths per unit POC (Zondervan 2007).

The available evidence (Paasche 1965, 1966a,b) is consistent with the light dependence of coccolithogenesis involving the photochemical reactions of photosynthesis more directly than use of the stored carbohydrate or lipid products of photosynthetic CO_2 assimilation. The action spectrum of calcification is similar to that of the action spectrum of photosynthesis and the absorption spectrum of the photosynthetic pigments apart from a greater activity of calcification in the blue region of the spectrum (Paasche 1966b). Calcification is less sensitive to the photosystem II (PSII) inhibitor 3-(p-chlorophenyl)-1,1-dimethyl urea

than is photosynthesis (Paasche 1965). Paasche (1965, 1966b) suggested an involvement of ATP from cyclic photophosphorylation which is energized by photosystem I (PSI) alone. Paasche (1965, 1966b) further suggested that part of the blue light stimulation of calcification is catalytic rather than energetic since light absorbed by carotenoids in the blue part of the PAR is not used significantly to energize PSI. This suggestion needs further investigation. If the higher rate of calcification than of photosynthesis in limiting irradiances of blue wavelengths, as compared to longer wavelengths, is confirmed, it could increase the PIC:POC ratio of coccolithophores living deep in the photic zone in clear oceanic waters where blue wavelengths predominate.

Low PAR may reduce both calcite content of the liths by around 35% (Paasche 1999) and the cell size (van Bleijswijk et al. 1994, Paasche 1999). This is seen especially when the light period is shorter (Paasche 1999), perhaps as a result of lower energy availability with a preferential allocation to processes other than calcification, or interaction between the photoperiod and the G1 phase of the cell cycle, as discussed below.

In conclusion, PAR indirectly affects calcification by regulating the energy supplied by photosynthesis. Protons released during calcification may be used to convert HCO_3^- to CO_2 to supply Rubisco with substrate. However, there is no obligate coupling of calcification and photosynthesis. There are indications that the energy for calcification may be supplied, at least partially, from cyclic phosphorylation involving PSI rather than PSII and/or that calcification may be stimulated by catalytic activity rather than energetic effects of light (Paasche 1966b).

A problem with discussing the relationship of calcification rate to PAR and to ocean acidification is uncertainty about the energy cost of calcification and the extent to which the energy cost increases with ocean acidification (Raven 2011a). The additional energy requirement for intracellular pH regulation is probably not more than 1% or so of respiratory energy output; intracellular pH regulation in an acidophilic *Chlamydomonas* species growing at an external pH of 2 uses less than 7% of the respiratory energy output (Messerli et al. 2005, Raven 2011a).

INTERACTIONS BETWEEN PAR AND OTHER ENVIRONMENTAL FACTORS

Zondervan et al. (2002) grew *Emiliania huxleyi* strain PML B92/11 at a range of CO_2 concentrations from 5 to 34 µmol l^{-1} (280 to 750 ppm) combined with a range of photon flux densities of 15, 30, 80 and 150 µmol m^{-2} s^{-1}. Their PAR values were based on a statement by Tyrrell et al. (1999) that the light attenuation by coccoliths in a bloom would reduce PAR to <35 µmol m^{-2} s^{-1} in the photic zone for >50% of the time, apart from in the top few meters. PIC and POC were both highly light dependent at subsaturating irradiance. An increase in photon flux density (PFD) from 15 to 80 µmol m^{-2} s^{-1} resulted in a 32% increase in PIC $cell^{-1}$ and 56% increase in POC $cell^{-1}$. The specific growth rate was doubled at 150 µmol m^{-2} s^{-1} compared to 15 µmol m^{-2} s^{-1}, but CO_2 concentration was found to have no effect. With increasing CO_2 concentration, PIC $cell^{-1}$ decreased only under saturating light intensity (80–150 µmol m^{-2} s^{-1}), whilst POC $cell^{-1}$ increased at intermediate light intensities (30–80 µmol m^{-2} s^{-1}). When adjusted for growth rate, POC l^{-1} increased at all irradiances with increasing CO_2 concentration, whilst PIC decreased only at the highest PFD giving an overall decrease in PIC:POC. It is important to realise that the decrease in PIC:POC is predominantly caused by increased photosynthesis here; however, when light is saturating, a decrease in calcification is seen as CO_2 concentration rises. For situations where Tyrrell et al.'s (1999) calculation is correct, this may mean that in a bloom situation, PIC is less likely to be affected by CO_2 concentration.

Feng et al. (2008) grew *Emiliania huxleyi* strain CCMP 371 at 2 levels of PAR (50 and 400 µmol m^{-2} s^{-1}) and 2 levels of CO_2 (376 and 750 ppm). PIC:POC at low light was independent of the CO_2 availability, while at high irradiances, PIC:POC was decreased, driven by decreased PIC, relative to low irradiances and was further decreased by high CO_2. This agrees with the findings of Zondervan et al. (2002). Further studies are needed of CO_2–PAR interactions in relation to the energetics of calcification, and the various mechanistic (Anning et al. 1996, Raven 2011a, Ries 2011) and evolutionary (Irie et al. 2010) implications.

INFLUENCE OF ULTRAVIOLET RADIATION ON CALCIFICATION

UVR can reduce phytoplankton productivity and growth by disturbing photosynthesis, nutrient uptake, amino acid synthesis and pigment production as well as damaging DNA (Buma et al. 2000). Due to the dominance of coccolithophores in stratified high-irradiance waters, it has been suggested that coccoliths play a protective role by scattering electromagnetic radiation, including UVR. Gao et al. (2009)

measured the transmission of radiation through naked and coccolith-covered cells and found a 20 to 25% decrease in UVR and 10 to 22% decrease in PAR due to coccoliths. While such experiments are technically very demanding, these findings suggest that coccolithophores may be at an advantage compared to other phytoplankton in this respect (Raven & Waite 2004). However, some results show that *Emiliania huxleyi* is in fact more sensitive to ultraviolet B radiation (UVBR; 280–320 nm) than other phytoplankton species, showing a 50% growth reduction at 150 J m^{-2} d^{-1} whilst the 5 other pelagic species tested, i.e. 3 diatoms and 2 (uncalcified) dinoflagellates, did not show 50% reduction in growth rates until at least 600 J m^{-2} d^{-1} (see Table 1 of Peletier et al. 1996). Incident doses of UVBR in excess of 1000 J m^{-2} d^{-1} are common in temperate waters (Buma et al. 2000 and references therein). With prolonged doses of UVBR, for 3 h d^{-1} for several days, *E. huxleyi* showed greatly reduced growth rates and increases in cell volume at 300 J m^{-2} d^{-1}. At 400 J m^{-2} d^{-1}, growth ceased, very high levels of cyclobutane pyrimidine dimers were evident, and the cell cycle was arrested in the G1 phase. It appeared that cells were unable to repair the DNA damage and so did not enter the S phase. This finding was corroborated by further field and laboratory studies showing increased cell size with UVBR exposure (Buma et al. 2000).

In some shorter-term experiments, Xu et al. (2011) examined the effect of UVR and temperatures of 20 and 25°C on calcification in an Australian *Emiliania huxleyi* strain CS-369, usually grown at 20°C. Cultures were grown for at least 148 generations (100 d) in normal present-day seawater calcium concentration (10 mM), and at low calcium concentration (0.1 mM) to restrict calcification. The treatment involved cultures which presumably had not been exposed to significant UVR since they were isolated from the ocean. The cells were exposed for 2 h to a range of wavelengths from >280 to >395 nm. This work showed that, for the cells in 10 mM calcium, UVBR, especially at 280 to 295 nm, inhibited photosynthesis by around 50% and calcification by around 65%. The resulting decrease in PIC:POC was not seen with ultraviolet A radiation (UVAR). PIC:POC of cells acclimated to 0.1 mM calcium was about a third that of the 10 mM calcium acclimated cells. UVBR inhibited photosynthesis by around 65% and calcification around 50%, but this was more variable so PIC:POC was not significantly different. There was no interactive effect of temperature and UVR. The decreased calcification of normal cells in the presence of UVBR does not support the hypothesis that cells are unable to divide and become more heavily calcified. It is also counter-intuitive, as cells become less calcified and potentially more susceptible to damage by UVBR, assuming the coccoliths play some protective role. As the lightly calcified cells show no further decrease in PIC:POC with UVBR, this may suggest that UVBR is attacking the energy supply for coccolithogenesis rather than the mechanism itself. Increased temperature would amplify this effect, and the combination of all these factors leads to an overall decrease in the calcification:photosynthesis ratio in a future scenario (Xu et al. 2011). Interesting though these results are, longer-term experiments using less extreme UVR fluxes would be more ecologically and evolutionarily significant.

Gao et al. (2009) found decreased rates of photosynthesis and calcification in response to UVAR and UVBR in combination with reduced pH in *Emiliania huxleyi* strain CS369. Cells cultured for 11 d at pH 7.6 were on average 7% smaller and the mean thickness of the coccolith layer was reduced by 31% compared to controls at pH 8.2; however, this was not significant. Exposure to UVBR almost totally inhibited calcification whilst reducing photosynthesis by ~10% in the lightly calcified cells at pH 7.6.

Although the experiments described above were conducted with the same strain and by the same research team, the controls showed much greater inhibition by both UVAR and UVBR, particularly of photosynthesis, in the study by Xu et al. (2011). This may be due to the PAR supplied being 150 µmol photons m^{-2} s^{-1} and then PAR of 290 µmol photons m^{-2} s^{-1} being used in the experiment (Xu et al. 2011) whilst cells of Gao et al. (2009) were acclimated to 425 µmol photons m^{-2} s^{-1}. With more available PAR, cells may be more able to repair damage to cellular machinery (Raven 2011b, 2012). However, the trend towards greater inhibition of photosynthesis than calcification in the low-calcium treatment and the opposite trend at low pH suggests that different mechanisms are at work. That energy is still being supplied to calcification at low calcium concentration in the presence of UVB but not at low pH may be to avoid the additional pH imbalance caused by calcification (Gao et al. 2009).

Guan & Gao (2010a,b) found that the shorter UVBR wavelengths damaged photosynthetic machinery and the longer UVAR damaged calcification machinery more in the same strain of *Emiliania huxleyi*. Damage was repaired and the overall specific growth rate was reduced by 25%, resulting in increased size and coccoliths per cell. After prolonged exposure to UVR, cells were more able to repair damage, had higher concentrations of UVR-absorbing compounds

and were increasingly calcified. These were interpreted as protective strategies in response to UVR. Gao et al. (2009) found reduced growth by 12% at pH 7.9 with UVBR. Guan & Gao (2010a) saw 52% and additional 10% reduction in POC fixation, with UVAR and UVA+BR respectively. PIC fixation was inhibited by 68 and 8% with UVA and UVA+BR respectively; these results were similar to those of Xu et al. (2011) but again much higher than those reported by Gao et al. (2009). In all of these studies, cells grown under normal conditions showed calcification to be more inhibited than photosynthesis by UVA and UVBR. Both UVA and UVB inhibited both photosynthesis and calcification, with UVA having had a greater effect than UVBR. Guan & Gao (2010b) suggested that the inhibition of photosynthesis may be caused by damage to the D1 protein of PSII. Growth rate reduction may be caused by damage or by allocation of resources to photoprotective compounds (Raven 1991, Garcia-Pichel 1994).

In summary, UVAR and UVBR reduce calcification and photosynthetic carbon fixation, increase photoprotective pigments but may reduce growth rates sufficiently to result in more heavily calcified cells. When combined with a stressor which reduces calcification, such as pH or low calcium concentration, different responses are seen. However, coccolithophores in nature are unlikely to experience low [Ca^{2+}], and pH as low as 7.6 is unlikely in the near future.

Holococcoliths have been much less studied; an exception is Quintero Torres et al. (2007), who modelled the probable scattering of radiation by their structure. They found that this would cause greater scattering of radiation in the 200 to 300 and 700 to 900 nm ranges. Thus holococcoliths could protect cells from UVR damage without attenuating light in the 400 to 700 nm range.

EFFECTS OF TEMPERATURE ON CALCIFICATION

Reduced temperature leads to increased dissolution of CO_2 in seawater, reduced carbonate and thus reduced Ω_{cal}. Temperature affects metabolic rates of both the phytoplankton and heterotrophic bacteria, giving optimal growth and dissolution rates, respectively.

Temperature alone

Early experiments on *Emiliania huxleyi* (as *Coccolithus huxleyi*) strain BT-6 (Watabe & Wilbur 1966) showed temperature effects on coccolith morphology, with increased malformations above and below the optimal temperature of 18°C. Growth rates were maximal at 18 to 24°C for this strain. Watabe & Wilbur (1966) found very low percentages of calcified cells when these cultures were grown at 7 or 27°C, the limits of their temperature range. Increases in width and decreases in length of coccolith elements were seen with increasing temperature between 12 and 27°C. The authors suggested that malformations were due to growth of crystals at different rates causing asymmetry and also suggested that local differences in calcium carbonate and inhibitory substances would also have the same effect. This work was followed up, again with *E. huxleyi*, by Paasche (1968) using a different technique. Maximum growth occurred at 17.5 to 26.5°C; at 12.5 and 26.5°C, growth was greatly decreased, but calcification (on a cell volume basis) was still 70 to 80% of the value at maximum growth rate. The cells had a complete covering of coccoliths at 12.5 to 23°C, but 30% of cells had an incomplete covering at 26.5°C. Paasche (1968) did not mention malformed coccoliths, although Langer et al. (2009) saw increased numbers of malformed coccoliths in *E. huxleyi* RCC1238 at 25°C compared to those grown at 10 to 20°C.

Interactions of temperature with other variables

The effect of the interaction of temperature and pCO_2 on calcification has been investigated by a number of authors (Feng et al. 2008, 2009, De Bodt et al. 2010, Borchard et al. 2011, Fiorini et al. 2011b, Xu et al. 2011). Xu et al. (2011) found that elevated temperature, within the range studied, increased photosynthesis and calcification of *Emiliania huxleyi* strain CS-369 at present-day seawater [Ca^{2+}], but reduced both if [Ca^{2+}] was reduced to 0.1 mM. Increased photosynthetic rate (but not POC) and growth rate of *E. huxleyi* strain CCMP 371 was also seen by Feng et al. (2008) under a higher temperature. Unlike Xu et al. (2011), Feng et al. (2008) found no effect of temperature (20 versus 24°C) on PIC, POC or PIC:POC at any of the light and CO_2 combinations used for their *E. huxleyi* CCMP 371 cultures. De Bodt et al. (2010) found a trend towards decreased PIC production with increasing CO_2 concentration (180, 375, 750 µatm) at both 13 and 18°C in *E. huxleyi* strain AC481. PIC:POC ratio decreased with increased growth temperature only at present-day CO_2 concentration; this was driven by greatly increased POC and slightly reduced PIC. At 18°C, both PIC and POC were

higher at present-day rather than future CO_2 concentrations. POC increased with increased CO_2 concentration only at 13°C between present and future treatments. There was also a reduction in cell size, but no difference in growth rate, with both increasing CO_2 concentration and increasing temperature. An increased number of malformed coccoliths was seen with increasing CO_2 concentration, but no effect of temperature.

Feng et al. (2009) examined the interactive effects of increased temperature and CO_2 concentration on a natural community from the North Atlantic, using ship-board continuous cultures. Addition of nitrate and phosphate but no silicic acid to the natural seawater caused a coccolithophore bloom. Increased temperature stimulated POC production rates per unit chlorophyll, with no difference caused by CO_2 concentration. Neither high temperature nor CO_2 concentration alone affected PIC, but despite a much higher abundance of coccolithophores in the high CO_2 concentration and temperature treatment, overall PIC was significantly reduced.

Fiorini et al. (2011b) grew *Syracosphaera pulchra* at 19 and 22°C and with 400 and 740 ppm CO_2; no significant differences in the PIC:POC ratio were found among the treatments. These experiments used realistic temperature and CO_2 values for today and later this century, and generally showed no effect of increased temperature on PIC:POC, or a decrease with increasing temperature in present, but not future, CO_2. This suggests that any alteration in [CO_3^{2-}] due to temperature does not alter net calcification.

Satoh et al. (2009) examined the interaction of HPO_4^{2-} limitation and low temperature on growth and calcification (as ^{45}Ca incorporation and microscopic observation) in batch cultures of *Emiliania huxleyi* NIES 837. They showed that temperature reduction from 20 to 12°C caused a much greater increase in calcification in HPO_4^{2-}-limited cultures than in HPO_4^{2-}-sufficient cultures. The different responses may be due to strain and species differences and/or differences in the sensitivities of calcification and photosynthesis to temperature (Xu. et al. 2011).

A possible mechanistic interpretation for the differential effects of temperature on the calcification and growth, at least below the optimal temperature for growth, is a lower activation energy for calcification than for growth. This 'explanation' could, of course, be regarded as a restatement of the observations in terms of physical chemistry. The relationship of calcification to the length of the G1 phase (Paasche 1998, Müller et al. 2008) of the cell cycle could relate to the temperature effects by the hypothesis that the fraction of the cell cycle time taken up by the G1 phase decreases up to the temperature optimum for growth, and decreases at higher temperatures (but see de Bodt et al. 2010). These suggestions could be followed up experimentally.

EFFECTS OF THE MACRONUTRIENTS NO_3^- AND PO_4^{3-} ON CALCIFICATION

Paasche & Bruback (1994) and Paasche (1998) were the first to investigate the effects of variations in nitrogen (as nitrate) and phosphorus (as phosphate) supply on calcification in a coccolithophore, in this case *Emiliania* (as *Coccolithus*) *huxleyi*; these, and later, experiments were reviewed by Zondervan (2007), while Langer et al. (2012) discussed more recent publications as well as providing original data. The general finding is an increasing PIC:POC ratio with decreasing NO_3^- and HPO_4^{2-} in the range which restricts growth rate (as POC increases). The increase in PIC:POC is often greater for decreasing HPO_4^{2-} than for decreasing NO_3^- (Zondervan 2007). Paasche (1998) noted that NO_3^- limitation reduces POC per cell, calcite per coccolith and coccolith size, but increases the number of coccoliths per cell, resulting in higher Ca:POC (on a cell basis) at reduced growth rates. However, Fritz (1999) found no change in coccolith size with a 3.3-fold change in growth rate of *E. huxleyi* 88E (CCMP 378) in NO_3^--limited chemostats at high irradiance. In that study, increased calcite per lith was seen as growth became more NO_3^--limited. Müller et al. (2008) found greatly decreased cell diameter with moderately increased calcite per cell when growth became NO_3^--limited. Coccolith calcite content was found to increase by 15% in PO_4^{3-}-limited chemostat cultures, whilst decreasing by 20% with a similar NO_3^--limitation (Paasche 1998).

Riegman et al. (2000) showed that *Emiliania huxleyi* has the highest affinity for PO_4^{3-} ever recorded in a phytoplankton species. At a specific growth rate of 0.14 d^{-1} (16% μ_{max}), the affinity of the PO_4^{3-} uptake system (defined as the initial slope of the plot of the rate of PO_4^{3-} uptake on a cell phosphorus basis against the external P concentration) was 19.8 l μmol^{-1} cell PO_4^{3-} h^{-1}. They found that NO_3^--limited cells were smaller and contained 50% less organic and inorganic carbon than PO_4^{3-}-limited cells. Both calcification and induction of the PO_4^{3-} uptake system were inversely correlated with growth rate in PO_4^{3-}-limited cultures. At the lowest growth rate (0.13 d^{-1}), the cells were 37% larger than in faster-growing cul-

tures and had more than 3 times greater PIC cell^{-1} due to increased lith coverage. Under NO_3^- limitation, no correlation of PIC or POC cell^{-1} was seen with variation in growth rate.

When PO_4^{3-}-limited, cells can continue to produce biomass and calcite but are unable to divide due to lack of PO_4^{3-} for nucleic acid synthesis. When NO_3^--limited, they cannot synthesise proteins, but calcification does continue and also results in higher calcite per cell. The cells are smaller due to reduced biomass, not calcite. Overall, nutrient limitation increases the PIC:POC ratio.

INTERACTIONS BETWEEN MACRONUTRIENTS AND OTHER VARIABLES

NO_3^--limited chemostat cultures of *Emiliania huxleyi* strain TW1 showed no change in PIC:POC ratio when grown at 700 rather than 400 ppm CO_2 (Sciandra et al. 2003); no data are given for (non-chemostat) NO_3^--replete cultures of the strain used. As indicated above when considering PAR, Müller et al. (2008) confirmed the speculation of Paasche (2001) that calcification is restricted to the G1 phase of the cell cycle, and showed in *E. huxleyi* that the length of the G1 phase increased under NO_3^- and PO_4^{3-} limitation and may be related to the increased calcite cell^{-1} in the nutrient-, particularly PO_4^{3-}-, limited cultures. Lefebvre et al. (2012) studied the interaction of CO_2 (166 to 194 ppm compared to 308 to 367 ppm) with nitrogen source (NH_4^+ plus NO_3^- compared to NO_3^- alone, both treatments with 200 µM nitrogen) in *E. huxleyi* strain CCMP371, and found that PIC:POC decreased with increasing CO_2 with NO_3^- as the nitrogen source while PIC:POC was lower and invariant with CO_2 when NH_4^+ plus NO_3^- was the nitrogen source. Lefebvre et al. (2012) pointed out that environmental change is increasing the availability of NH_4^+ relative to NO_3^-, with increasing cyanobacterial nitrogen fixation in the surface ocean and inhibition of nitrification by increased CO_2, so the results of their work have implications for future PIC:POC of coccolithophores. The nitrogen source for growth alters the Fe requirement: diazotrophy needs more Fe per unit nitrogen assimilation rate than NO_3^- or, particularly, NH_4^+ assimilation (Kustka et al. 2003). How the analysis of Lefebvre et al. (2012) is altered by consideration of the effects of increasing CO_2 on Fe availability awaits resolution of conflicting evidence as to the effects of ocean acidification on Fe availability (Millero et al. 2009, Breitbarth et al. 2010, Shi et al. 2010). Also, with high light and low PO_4^{3-} (i.e. usual bloom conditions for the diazotrophic cyanobacterium *Trichodesmium* and for many coccolithophores), *Trichodesmium* precipitates $CaCO_3$ as fibres of aragonite (Kranz et al. 2010). Marine pelagic cyanobacterial calcification is of relatively little quantitative importance in the present oceans. In the past, however, very large carbonate sediments have been produced by filamentous marine cyanobacteria on different occasions between 750 and about 50 million years ago (Riding 2006). Work on the coccolithophore *E. huxleyi* in phosphorus-limited chemostats investigated interactive effects of changes in CO_2, temperature and phosphorus: there were no significant trends with variation in the 3 factors (Borchard et al. 2011). The interaction between macronutrient supply and other factors is complex and needs further investigation.

EFFECTS OF MICRONUTRIENTS ON CALCIFICATION

Zondervan (2007) reviewed the limited information available up to 2007 on the effects of micronutrient availability on calcification of coccolithophores. Variations in Fe concentration in the medium which yielded a 6-fold range of growth rates had no significant effect on PIC:particulate organic nitrogen (PON), i.e. accumulation of PIC decreased in parallel with decreasing PON as Fe became more growth-limiting (Schulz et al. 2004, 2007). What will happen to Fe availability under increasing CO_2 is not clear. Millero et al. (2009) modelled Fe speciation under increased CO_2 and showed an increased fraction of Fe(II) and a slower oxidation of Fe(II) to Fe(III). Breitbarth et al. (2010) showed increased soluble Fe concentrations, Fe(II) concentration and Fe(II) half-life in a coastal mesocosm experiment with CO_2 enrichment. By contrast, Shi et al. (2010) found a decrease in the Fe uptake rate under increased CO_2 in the coccolithophore and 2 diatoms examined, although the cellular Fe requirement for growth is not changed with ocean acidification.

Limitation of growth rate by decreased Zn concentration led to a PIC:PON increase by over 2-fold (Schulz et al. 2004). There was no change in the rate of calcification, and cells with many layers of coccoliths were seen. Müller et al. (2008) pointed out that Zn is necessary for Zn finger proteins which play a central role in DNA replication and transcription, hence Zn deficiency may inhibit cell division. In the North Pacific, coccolithophore growth is limited by Zn (Crawford et al. 2003). On addition of Zn to natu-

ral samples, coccolithophore abundance increased 20-fold with a significant increase in total ^{14}C uptake into PIC. This suggests that although Zn limitation may cause increased cellular calcification, with the reduction in growth rate this does not translate to increased total PIC production. Zn concentrations similar to those in the study area of Crawford et al. (2003) are common in many oceanic regions (see Schulz et al. 2004). Increased cellular, but not total calcification, may also apply to phosphate limitation and UVB cell cycle arrest. Zn is also required for alkaline phosphatase needed to acquire phosphate from organic phosphate esters when phosphate is limiting, and for carbonic anhydrase required for carbon acquisition (Steele et al. 2009), noting that *Emiliania huxleyi* has low activity of extracellular carbonic anhydrase (Nimer et al. 1994). Buitenhuis et al. (2003) showed a co-limitation of growth of *E. huxleyi* by Zn and HCO_3^-, but the PIC:POC ratio was not addressed. Schulz et al. (2004) found that the effects of variation in the carbonate system parameters over a range of pH from 7.75 to 8.35 were not discernible due to the massive Zn response and variation amongst the CO_2 treatment results. These co-limitations will require further investigation.

EFFECTS OF CALCIUM, MAGNESIUM AND SULPHATE ON CALCIFICATION

Lower than present-day seawater [Ca^{2+}] (10 mM) have been used experimentally to decrease, or eliminate, calcification (Paasche 1964, Herfort et al. 2004, Trimborn et al. 2007, Leonardos et al. 2009, Xu et al. 2011). Xu et al. (2011) found that acclimation to 0.1 mM compared to 10 mM [Ca^{2+}] (present-day concentrations) reduced photosynthesis by 81.3% and calcification by 55.4% at 20°C. However, no effect on photosynthesis was suggested by the work of Herfort et al. (2002, 2004), Trimborn et al. (2007) and Leonardos et al. (2009). Trimborn et al. (2007) found only naked cells at 0.1 mM [Ca^{2+}].

Experiments with [Ca^{2+}] higher than present-day seawater concentrations (up to 50 mM) and varying Mg^{2+}, and hence Ca:Mg ratios (Herfort et al. 2004, Stanley et al. 2005, Katagiri et al. 2010, Müller et al. 2011), are relevant to understanding the effects on calcification of the changes in ocean chemistry over the last 220 million years of the fossil record of coccolithophores (Zeebe & Ridgwell 2011). Doubling the Ca^{2+} concentration from the present seawater concentration of 10 mM has no significant effects on the PIC:POC ratio, but 50 mM Ca^{2+} decreased the PIC:POC ratio and the rate of POC accumulation in *Emiliania huxleyi* (Herfort et al. 2004). High Mg^{2+} (87, 116 mM) and low Mg^{2+} (0, 14 mM) both caused malformation of coccoliths relative to Mg^{2+} at 29 and 58 mM (the present-day concentration); the extent of calcification was inhibited less by low than by high Mg^{2+} concentrations (Herfort et al. 2004). Stanley et al. (2005) examined the effect of Ca^{2+} (20–30 mM) and Mg^{2+} (≤20–30 mM) concentrations believed to have occurred in seawater on *Coccolithus neohelis*, *Ochrosphaera neopolitana* and *Pleurochrysis carterae*, with higher growth rates in the Cretaceous than in the modern seawater. In the only organism tested (*P. carterae*) calcite production was higher in Cretaceous than recent seawater, apparently giving more calcite per cell in the Cretaceous seawater (see Fig. 2 of Stanley et al. 2005). Calcification was not quantified, although Katagiri et al. (2010) examined the effects on calcification in *P. haptonemofera* of calcium in the concentration range of 0, 0.5, 5, 10 and 50 mM and Mg^{2+} at concentrations of 5, 50 and 140 mM. Calcification (measured as Ca^{2+} and Mg^{2+}) on a per cell basis was highest at 10 mM external Ca^{2+} when Mg^{2+} was constant at 50 mM, and at 50 mM Mg^{2+} when Ca^{2+} was varied (Katagiri et al. 2010). Müller et al. (2011) found no significant effect on PIC:POC in *E. huxleyi* of variations in Mg^{2+} from 5.5 to 92 mM with present Ca^{2+} (9.6 to 9.9 mM); for present or half the present Mg^{2+} and 2.6 to 51 mM Ca^{2+}, PIC:POC is essentially constant except for a decrease at the lowest Ca^{2+} concentrations. For *C. braarudii* with present or half the present concentrations of Mg^{2+} and 2.6 to 46.8 mM Ca^{2+}, PIC:POC decreases at the lowest Ca^{2+} concentration (Müller et al. 2011).

Herfort et al. (2004) and Katagiri et al. (2010) both showed that PIC:POC is greatest at [Ca^{2+}] and [Mg^{2+}] similar to present ocean concentrations at the present inorganic carbon concentration, which was the only one examined. Müller et al. (2011) found essentially constant PIC:POC with varying Ca^{2+} and Mg^{2+}, apart from a decrease at the lowest [Ca^{2+}]. With a rather different experimental design (constant divalent cation concentration with varying Ca:Mg ratio), Stanley et al. (2005) found that calcification was greatest with the Ca:Mg ratio of 1 found in the Cretaceous (see Stanley 2008). Further experimentation is needed to resolve these differences among the experiments. It is also desirable to examine the interaction between Ca:Mg and the absolute [Ca^{2+}] and increased CO_2. The high [Ca^{2+}] in the Cretaceous would partly offset the effect of the higher CO_2 (and lower carbonate) in decreasing the saturation state of calcite (see Fig. 1 of Stanley 2008, and Fig. 2.3 of Zeebe & Ridgwell 2011);

this could maintain the rate of calcification and prevent calcite dissolution.

A final aspect of the effect of $[Ca^{2+}]$ and $[Mg^{2+}]$ on calcification is the effect of $[SO_4^{2-}]$. Increased $[SO_4^{2-}]$ decreases the Mg:Ca ratio at which calcite is destabilised and aragonite becomes the commonest polymorph (Bots et al. 2011). Ocean $[SO_4^{2-}]$ has doubled over the last 65 million years (Kurtz et al. 2003), so variation in $[SO_4^{2-}]$ has been an important factor in marine calcification in the time for which coccolithophores have existed. However, the intracellular calcification by coccolithophores permits the organism to control the $[Ca^{2+}]$, $[Mg^{2+}]$ and $[SO_4^{2-}]$ in the coccolith-forming vesicle to at least some degree independently of the external concentrations. More widely, ocean $[SO_4^{2-}]$ changes could have been related to the evolutionary expansion of the alveolates and chromists, the latter containing the coccolithophores (Ratti et al. 2011).

The arguments of Ratti et al. (2011) are based on culture experiments in which 5 marine phytoplankton organisms, namely the cyanobacterium *Synechococcus* sp., a green alga (the prasinophycean *Tetraselmis suesica*), and 3 algae containing chlorophylls *a* + *c* derived from secondary endosymbiosis in which the plastids arose from a red algal endosymbiont, viz. the coccolithophorid *Emiliania huxleyi*, the dinoflagellate *Protoceratium reticulatom* and the diatom *Thalassiosira weissflogii*, were cultured with SO_4^{2-}, a nitrogen source and trace element concentrations reflecting modern seawater, Palaeozoic and Proterozoic seawater. In monospecific culture, all 5 species grew fastest in the Proterozoic-like seawater. In mixed cultures, *Thalassiosira weissflogii* outgrew the others in modern seawater, while *Tetraselmis suecica* outgrew the others in Palaeozoic seawater. These data are interpreted as suggesting that increases over time in the $[SO_4^{2-}]$ in seawater (Ratti et al. 2011, Halevy et al. 2012, Wortmann & Paytan 2012) could have been a factor in the rise of chlorophyll *a* + *c* phytoplankton relative to green algae and cyanobacteria in the Mesozoic (Ratti et al. 2011).

While the reported effects of changes in Ca^{2+}, Mg^{2+} and SO_4^{2-} on calcification and the PIC:POC ratio have relevance for the palaeoecology of coccolithophores, there will be no significant changes in these 3 ions in the ocean over the next few centuries.

EFFECTS OF SALINITY ON CALCIFICATION

Increased salinity increases Ω_{cal}, as does increased temperature (Green et al. 1998, Marion et al. 2009). These changes in Ω_{cal} are complicated, in terms of $CaCO_3$ precipitation, in the ocean by the general correlation of salinity with carbonate alkalinity, and the temperature and salinity effects on the speciation of inorganic carbon and the solubility of CO_2. These interactions lead, for example, to effects of low temperatures such as in winter (Tyrrell et al. 2008) and with ice melt input (Chierici & Fransson 2009). Coccolithophores as exemplified by *Emiliania huxleyi* are restricted to natural waters with salinity above 11 (Winter & Siesser 1994, Tyrrell et al. 2008). Beaufort et al. (2011) saw no strong correlation between calcification and salinity. After considering the various possible reasons for the absence of coccolithophores from the brackish Baltic Sea but abundance in the brackish Black Sea, Tyrrell et al. (2008) concluded that the most likely cause is not low salinity per se, but rather the decalcification of coccolithophores in the winter when calcite is close to being, or often is, undersaturated. This dissolution of coccoliths cannot be countered by replacement in a time of little or no growth. Chierici & Fransson (2009) are among those who have commented on the undersaturation with $CaCO_3$ of coastal arctic waters as a result of freshwater input from ice melt and temperature. Bollmann et al. (2009) noted that coccolith morphology changed with varied salinity.

INTERPRETING EARLIER CALCIFICATION BY COCCOLITHOPHORES

Over much of the 220 million years for which coccolithophores are known to have existed, the CO_2 concentration has been higher than at present, especially in the Pliocene and Pleistocene. At that time, the high CO_2 concentration with corresponding lower carbonate concentration was accompanied by a higher $[Ca^{2+}]$ and Ca:Mg ratio. The ocean surface calcite saturation value was apparently lower back to 220 million years ago than it was in the Pleistocene, but not so much lower as would have been the case had the $[Ca^{2+}]$ not been higher. With a warmer, more stratified ocean with shoaling of the thermocline there would have been a decreased input of nutrients (combined N, P, Zn) to the upper mixed layer; this may have increased the PIC:POC ratio, but probably reduced cell growth rates. Increased mean UVBR incident on the cells with less deep mixing may have had similar effects. However, the increased PAR incident on cells with less deep mixing would decrease the PIC:POC ratio. Overall, the sum of these effects could have helped explain the continuity of coccoliths in the fossil record up to the Miocene, with lower

CO_2 taking over in the Pleistocene and Pliocene outweighing the influence of a general increase in mixing depth.

PREDICTING CALCIFICATION BY COCCOLITHOPHORES IN THE NEXT CENTURY

Much emphasis has been placed on the effects of the continuing increase in CO_2 in decreasing the saturation state of calcite, and the related general decrease in PIC:POC in coccolithophores. As discussed above, increased stratification in a warmer ocean, with associated shoaling of the thermocline, will decrease the input of nutrients to the upper mixed layer, increasing the PIC:POC ratio in individual cells but reducing total PIC production if there is a more than compensating decrease in POC production rate per unit area. Increased mean UVBR incident on the cells with less deep mixing may also reduce growth rates but increase PIC:POC. However, the increased mean PAR incident on cells with less deep mixing would decrease the PIC:POC ratio, assuming that the initial mean PAR with deep mixing was high enough to give a decrease in PIC:POC with increased PAR. Overall, it is very likely that calcification will decrease in the future. However, with increased stratification, larger areas of the ocean may become dominated by coccolithophores. At increased temperature and irradiance, growth rates may increase, so, although PIC:POC per cell may be reduced, cell numbers may increase provided there are enough nutrients.

However, it is very likely that shoaling of the thermocline will mean decreased productivity as a result of nutrient limitation (Steinacher et al. 2010). If there is nutrient limitation, particularly if PO_4^{3-} limits cell growth, then these cells may become more heavily calcified, increasing PIC:POC but reducing growth rates. Increased UVR exposure in stratified waters could also potentially reduce growth rates and increase calcification. There are still many uncertainties and seemingly contradictory results despite the intense research effort targeting the responses of coccolithophores to environmental change. It is clear that changes in the carbonate chemistry, pH and PAR associated with environmental change will affect phytoplankton calcification. The balance between the effects on individual cells, population growth rates and species representation in the community will determine the global effects on PIC production from calcification by coccolithophores in the future, assuming no genetic changes (summarised in Table 3). Experimental evolution studies on coccolithophores are now in progress, with Lohbeck et al. (2012) having grown 500 generations of *Emiliania huxleyi* in high or ambient CO_2 concentrations and shown adaptive evolution to high CO_2. These data may alter predictions based solely on acclimatory changes in response to environmental changes.

MECHANISTIC UNDERSTANDING OF ENVIRONMENTAL EFFECTS ON CALCIFICATION

From the research presented it can be seen that very distinct mechanisms are at play (Table 3). There is evidence that factors affecting growth rates, and particularly those which halt cell division, seem to result in continued calcite production. Overall, this results in heavily calcified cells, both in terms of lith number and Ca^{2+} content. It has long been known that calcification is highly dependent on PAR, and to our knowledge, this is solely due to the energy supplied through photosynthesis. Direct effects on the calcification process are either related to Ω_{cal} or temperature. When these factors are outside the optimal range, they may cause malformation of liths. Coccolith production depends upon the physical process of crystal growth regulated by the cell and based on the organic template. Extreme changes in the physical environment are liable to disrupt crystal formation. The coccolith vesicle allows for a highly regulated environment, but temperature would be outside the control of the cell, and extreme ionic changes may be beyond its capabilities to control. Changes in cellular calcite production expressed as PIC $cell^{-1}$ may also reflect a range of different responses. Reduced PIC may occur if there are fewer liths, they are smaller or thinner, or incompletely formed (Zondervan et al. 2002).

CONCLUSIONS

Predicted future environmental changes are increased temperature, stratification leading to increased PAR, UVR and decreased nutrients in the photic zone accompanied by increased CO_2 concentrations resulting in decreased pH and $\Omega_{cal.}$ The interaction studies which have been reviewed here reveal some consistent trends (Table 3). At high light levels, increased CO_2 concentration either reduces calcification or does not affect it except in a few exceptional cases. Simultaneously, photosynthesis is often stimulated by increased CO_2 concentrations with high

Table 3. Summary of effects of environmental factors on the particulate inorganic carbon to particulate organic carbon (PIC:POC) ratio in coccolithophores. See discussions in the main text for more details and sources. PAR: photosynthetically active radiation; UVR: ultraviolet radiation; PON: particulate organic nitrogen

Environmental factor(s)	Effect on PIC:POC
CO_2	Usually a decrease with increasing CO_2 above the present level, but sometimes no effect. Usually an increase with decrease in CO_2.
PAR	Decreasing PAR below saturating level increases PIC:POC down to a low PAR below which PIC:POC decreases.
CO_2–PAR interaction	Relative to saturating PAR and present CO_2, decreased PIC:POC with increasing CO_2, limiting PAR increases PIC:POC with no effect of increased CO_2.
UVR	In short-term (2 h) experiments, no effect of UVAR added to PAR, decrease when UVBR is added to PAR and UVAR. In the longer term, UVAR had a greater relative effect on calcification but UVBR had a greater relative effect on photosynthesis, or continued calcification in UVBR after cell division had ceased.
Temperature	Generally no effect at saturating PAR and present CO_2.
Temperature–PAR–CO_2 interaction	Either no effect of any CO_2 and PAR, or temperature sensitive at higher but not at present CO_2.
NO_3^-, PO_4^{3-}	PIC:POC increases at limiting relative to saturating CO_2, especially for limiting PO_4^{3-}.
Temperature–PO_4^{3-} interactions	Larger temperature effect at limiting than at saturating PO_4^{3-}.
NO_3^-–CO_2 interactions	No effect of increased CO_2 on PIC:POC in nitrate-limited cultures.
Fe, Zn	Measured as PIC:PON; no effect of Fe deficiency, PIC:PON increased at limiting Zn.

light. For some species of coccolithophore, there is a general trend towards increased growth rates, and for others decreased rates; for *Emiliania huxleyi*, the 2 responses are found equally. Likewise in natural waters, *E. huxleyi* was found in greater numbers by Feng et al. (2009) and in lower numbers by Engel et al. (2005), but in both cases, PIC l^{-1} was reduced. With increased temperature and CO_2 concentration, PIC is also seen to decrease, while POC, abundance, size and malformation show varying responses. Nutrient limitation with both macro and micronutrients limits growth and so ultimately reduces PIC l^{-1}. In individual cells, PIC may continue to accumulate in extra liths, but these cells will ultimately be grazed or sink out and the population will decrease. These nutrient limitations are highly influential and mask any small effects of CO_2 concentration. UVR reduces photosynthesis and calcification and coupled with increased CO_2 concentration, but not due to the more lightly calcified liths, may almost completely halt calcification and/or cell division. This may also result in cells with extra liths but ultimately again PIC production per unit area or volume of culture (or habitat) will be reduced. The increased light available in a deeper mixed layer, with lower nutrients and thus less shading, may stimulate calcification and photosynthesis at depths where light is limiting if sufficient nutrients are available. Taken as a whole, the data and models suggest decreased oceanic calcification in the future, with possible exceptions in upwelling regions. The rate of change in the environment expected in the foreseeable future is greater than those commonly seen in the past. Migration is one possible solution, if appropriate habitat exists elsewhere. Adjustment to a changed environment at a given location poses problems when acclimation to the new environment using the existing genome is not possible or is too costly in resources, and genetic adaptation is too slow. However, the work of Lohbeck et al. (2012) on *E. huxleyi* suggests that such problems may not be insuperable.

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Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming

Kunshan Gao[1,*], E. Walter Helbling[2], Donat-P. Häder[3], David A. Hutchins[4]

[1]State Key Laboratory of Marine Environmental Science (B-606), Xiamen University, Daxue Rd 182, Xiamen, Fujian 361005, China
[2]Estación de Fotobiología Playa Unión and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Casilla de Correo 15, (U9103XAA) Rawson, Chubut, Argentina
[3]Neue Str. 9, 91096 Möhrendorf, Germany
[4]Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway, Los Angeles, California 90089, USA

ABSTRACT: Anthropogenic CO_2 is accumulating in the atmosphere and trapping reflected infrared radiation, resulting in warming of both terrestrial and ocean ecosystems. At the same time, the dissolution of CO_2 into seawater is increasing surface ocean acidity, a process known as ocean acidification. Effects of ocean acidification on marine primary producers have been documented to be stimulative, inhibitive, or neutral. Elevated CO_2 and reduced pH levels can interact with solar radiation, which fluctuates over different time scales from limiting to saturating or even stressful levels, to bring about synergistic, antagonistic, or balanced effects on marine primary producers at different depths or under changing weather conditions. However, shoaling of the upper mixed layer (enhanced stratification) due to ocean warming and freshening (rain, ice melting) can lead to additional photosynthetically active radiation (PAR) and ultraviolet (UV) exposure, which can have both benefits and costs to photosynthetic organisms. Elevated CO_2 concentrations under low or moderate levels of PAR have been shown to enhance photosynthesis or growth of both phytoplankton and macroalgae; excessive levels of PAR, however, can lead to additional inhibition of photosynthesis or growth under elevated CO_2, and addition of UV radiation (280 to 400 nm) can increase or down-regulate such inhibition, since solar UV-B (280 to 315 nm) radiation often harms algal cells, while UV-A (315 to 400 nm) at moderate levels stimulates photosynthetic carbon fixation in both phytoplankton and macroalgae. In view of warming effects, increased temperatures have been shown to enhance photorepair of UV-damaged molecules, though it simultaneously enhances respiratory carbon loss. The net effects of ocean acidification on marine primary producers are therefore largely dependent on the photobiological conditions (light limitation, light or UV stress), as well as interactions with rising temperature and other variables such as altered nutrient availability. Hence, feedbacks between changing carbonate chemistry and solar radiation across the entire spectrum present complications to interpret and understand ocean acidification effects based on single-factor experiments.

KEY WORDS: Algae · Carbon dioxide· Light · Phytoplankton · Photosynthesis · Ultraviolet radiation · Climate change

OCEAN ACIDIFICATION AND GLOBAL CHANGE

The oceans are currently absorbing over one million tons of CO_2 from the atmosphere each hour, and play an important role in mitigating global warming (Sabine et al. 2004). At the same time, enhanced dissolution of CO_2 from the air is also acidifying the oceans, a process known as ocean acidification (Doney et al. 2009). While standing biomass in the oceans accounts for only about 1% of that in terres-

*Email: ksgao@xmu.edu.cn

trial habitats, marine primary producers contribute about half of the global primary productivity due to their faster growth and turnover rates (Falkowski & Raven 1997). The ocean's biological pump effectively removes CO_2 from near-surface waters through photosynthetic carbon fixation and sequesters it at depth (Falkowski et al. 2000), thus promoting the absorption of CO_2 from the atmosphere (Sabine et al. 2004, Behrenfeld et al. 2006). This mechanism can both affect and be affected by the process of ocean acidification (Hutchins et al. 2009).

When CO_2 dissolves in seawater, it combines with water to form carbonic acid, which dissociates to bicarbonate, releasing protons (H^+) and so ultimately reaching a new equilibrium state. However, as the H^+ concentration rises with CO_2 dissolution, 'excess' H^+ releases can partially reverse the secondary dissociation reaction, resulting in a decrease in carbonate ions:

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (1)$$

$$H_2CO_3 \rightarrow H^+ + HCO_3^- \quad (2)$$

$$HCO_3^- \leftarrow H^+ + CO_3^{2-} \quad (3)$$

The carbonate chemistry of seawater is also strongly influenced by biological processes such as photosynthesis and respiration, which act to increase or decrease seawater pH, respectively. At the same time, assimilation of other nutrients such as nitrate or ammonia may also influence pH, as shown in the following assimilation equations (Stum & Morgan 1981):

$$106CO_2 + 16NO_3^- + HPO_3^- + 122H_2O + 18H^+ = [C_{106}H_{263}O_{110}N_{16}P_1] + 138O_2 \quad (4)$$

$$106CO_2 + 16NH_4^+ + HPO_3 + 108H_2O = [C_{106}H_{263}O_{110}N_{16}P_1] + 107O_2 + 14H^+ \quad (5)$$

However, considering the Redfield ratio (C:N:P stoichiometry), the hydrogen ion consumption or production due to nitrate or ammonia assimilation is small compared to changes in the seawater carbonate buffer system due to photosynthetic carbon removal.

Typical chemistry changes associated with ocean acidification are increased concentrations of CO_2, H^+, and HCO_3^- and decreased concentration of CO_3^{2-} and $CaCO_3$ saturation state (Gattuso et al. 2010). Since the beginning of the industrial revolution, the pH of oceanic surface seawater has been reduced by ~0.1 unit due to the increased atmospheric CO_2 concentration (Caldeira & Wickett 2003), corresponding to about a 30% increase in the H^+ concentration. With a further century-scale increase of CO_2 concentration in the atmosphere to 800–1000 ppmv (Intergovernmental Panel on Climate Change [IPCC] A1F1 scenario; Houghton et al. 2001), pH of the surface oceans will decrease by another 0.3 to 0.4 units (Feely et al. 2004, Sabine et al. 2004, Orr et al. 2005), thus increasing $[H^+]$ by 100 to 150%. The exchange of CO_2 between the sea and atmosphere depends on diffusive fluxes, the dynamics of physical mixing, and the marine biological CO_2 pump. About 50% of the CO_2 taken up by the oceans since the industrial revolution is still present in the upper ocean down to 400 m (Sabine et al. 2004). Consequently, organisms in the euphotic zone are exposed to a high-CO_2 environment, and their physiologies may respond to the increased CO_2 as well as to chemical changes, such as ionic speciation (Millero et al. 2009), related to the altered carbonate system or reduced pH, which may also alter their thermal windows (temperature range for species survival; Pörtner & Farrell 2008).

Although the ecological consequences of ocean acidification are likely profound, they are largely uncertain (Turley et al. 2010), especially when other concomitant global change variables are taken into consideration (Wu et al. 2008, Boyd et al. 2010). For instance, at the same time as the ocean is acidifying, global warming associated with increasing atmospheric CO_2 accumulation is leading to a 'greenhouse ocean', with increased sea surface temperature (SST) and a shoaling of the upper mixed layer (UML) (Hays et al. 2005, Doney 2006, Capotondi et al. 2011). Freshwater inputs from increased precipitation in temperate areas and ice melting in the polar regions, increasing temperature, and decreasing or increasing wind speeds, are all important in determining the UML depth. Enhanced stratification would expose plankton to increasing levels of photosynthetically active radiation (PAR, 400 to 700 nm) and UV radiation (280 to 400 nm), as well as to reduced availability of nutrients from underlying deeper waters due to enhanced strength in the pycnocline at the base of the UML (Steinacher et al. 2010). However, the penetration of solar radiation in the water column largely depends on the amount of particles and dissolved organic matter (DOM), which may increase in various inland waters and coastal ecosystems due to an increase in rainfall. In addition, a feedback mechanism is the photo-degradation of DOM that would partially counteract the decrease in nutrients due to enhanced stratification (Hörtnagl et al. 2011, Vähätalo et al. 2011).

Multiple stressors or factors such as these will influence the effects of ocean acidification on primary producers and the food web in both direct and indirect ways (Boyd et al. 2010, Boyd 2011). In addition to direct effects of the chemical changes in seawater on

the physiology of marine primary producers (see reviews by Wu et al. 2008 and Beardall et al. 2009 and references therein), ocean acidification can also alter chemical speciation, thereby affecting the availability of iron (Shi et al. 2010) and ammonium (Hutchins et al. 2009, Beman et al. 2011) to plankton assemblages. Increased solar UV-B radiation (280 to 315 nm) along with depletion of stratospheric ozone and global warming can interact to influence planktonic primary producers (Häder et al. 2011). Consequently, other anthropogenic factors will synergistically or antagonistically act with ocean acidification to influence the oceanic biological uptake of CO_2, complicating the understanding of ecological impacts of ocean acidification.

Our present understanding of the sensitivity of photosynthetic organisms to ocean acidification is based primarily on short-term experiments under either saturating or sub-saturating constant light levels, in which organisms are exposed to increased concentrations of CO_2. In contrast, phytoplankton cells in natural environments experience diurnal fluctuations of solar radiation, from light-limiting to light-saturating and potentially to stressful light levels in the UML of the euphotic zone in the presence of UV radiation. Along with the diurnal variation of solar radiation, surface water temperature and pCO_2 also often follow a diel pattern, especially in coastal waters of high biological production. Obviously, phytoplankton cells are exposed to dynamic environments under the sun. In this review, we focus on the combined effects of ocean acidification and solar radiation, by summarizing our increasing understanding of the physiological responses of primary producers and addressing the potential ecological implications of their interactive effects in an acidified, warmer, and more stratified ocean.

RESPONSES TO INCREASING CO_2 AND DECREASING pH

As more CO_2 accumulates in the atmosphere, seawater pCO_2 increasingly affects marine photosynthetic processes and energetics in direct and/or indirect ways. Low pH and/or increased pCO_2 can alter periplasmic electro-potential (periplasm negative relative to the medium or the oxidation-reduction potential) and affect proton or ion channels by altering the structure of periplasmic proteins (Beardall et al. 2009, Lü et al. 2011) or the activity of periplasmic extracellular carbonic anhydrase (Aizawa & Miyachi 1986, Sültemeyer 1998, Bozzo & Colman 2000). Changes in energetics, associated with down-regulation of CO_2-concentrating mechanisms (CCMs) with increasing pCO_2 or with up-regulated cost to cope with increased H^+ concentration, are likely to be responsible for the major acclimation processes of oceanic phytoplankton to rising CO_2 (Hopkinson et al. 2011). Acclimation to a rapid change in CO_2 concentration has been shown by a lessening of maximum photosynthetic efficiency and increase in cell membrane permeability in a diatom (Sobrino et al. 2005). Nevertheless, most of the studies to date have been restricted to short-term periods of time and the responses might have been obscured by the lack of proper acclimation (Beardall et al. 2009).

The concentration of dissolved inorganic carbon (DIC) in surface seawater is approximately 100 to 200 times that of CO_2 in the atmosphere at the present; however, its predominant form is HCO_3^-, with CO_2 usually accounting for <1% in pelagic waters (Gattuso et al. 2010). In addition, since CO_2 in seawater diffuses about 10 000 times slower than in air, its supply rate can limit photosynthetic carbon fixation (Raven 1993, Riebesell et al. 1993, Morel et al. 1994). These facts led to early laboratory and shipboard studies to assess whether increasing atmospheric CO_2 concentrations would enhance the primary production or growth of marine photosynthetic organisms (Gao et al. 1991, Riebesell et al.1993, Hein & Sand-Jensen 1997, Schippers et al. 2004) or reduce algal calcification (Gao et al. 1993, Riebesell et al. 2000). At the same time, the extent of enhanced primary productivity at elevated atmospheric CO_2 levels has been questioned (Beardall & Raven 2004), since most of the species investigated so far possess CCMs (Giordano et al. 2005, Raven et al. 2011). Laboratory as well as mesocosm studies have suggested that increased atmospheric CO_2 can sometimes stimulate photosynthesis and/or growth in both micro- and macroalgae (Gao et al. 1991, 1999, Riebesell et al. 1993, 2007, Riebesell 2004, Hutchins et al. 2007, Fu et al. 2007, 2008a, 2010; see also reviews by Wu et al. 2008, Beardall et al. 2009, Riebesell & Tortell 2011). However, since different physiological processes are involved, whether or not phytoplankton or macroalgae will benefit from increased CO_2 remains controversial (Wu et al. 2008). Although photosynthetic carbon fixation rate was enhanced in a diatom, mitochondrial respiratory (Wu et al. 2010) or photorespiratory (Gao et al. 2012) carbon losses were also stimulated. Additionally, decadal-scale global decreases (Behrenfeld et al. 2006, Boyce et al. 2010) and increases (Chavez et al. 2011) of phytoplankton productivity have been reported.

The literature includes reports of effects of simulated future CO_2-induced seawater acidification that range from positive (Hein & Sand-Jensen 1997, Fu et al. 2007, 2010, Hutchins et al. 2007, Riebesell et al. 2007, Wu et al. 2010), to neutral (Tortell et al. 2000, Tortell & Morel 2002, Chen & Gao 2003, Fu et al. 2007), to negative (Wu et al. 2010, Gao et al. 2012) (our Table 1; also see review by Riebesell & Tortell 2011 and references therein). Elevated CO_2 concentrations up to 10 000–50 000 µatm are known to down-regulate CCMs (Kaplan et al. 1980, Tsuzuki & Miyachi 1989, Raven 1991, Matsuda et al. 2001), and induction of

Table 1. Representative effects of elevated CO_2 concentration reported on diatoms, coccolithophores, cyanobacteria, phytoplankton assemblages, and macroalgae grown under different levels or qualities of light or solar radiation. Groups of cyanobacteria or calcifying algae are indicated by genus or species names. L-PAR: photosynthetically active radiation (PAR) levels <300 µmol photons m^{-2} s^{-1}; M-PAR: PAR levels ≥300 µmol photons m^{-2} s^{-1}; SL: solar visible radiation; +UV: presence of UV radiation; POC: particulate organic carbon. Additional references on different phytoplankton groups' response to ocean acidification can be found in Riebesell & Tortell (2011)

Source	Group	Study type	Light	Variables
Positive				
Riebesell et al. (1993)	Diatom	Lab	L-PAR	Growth
Schippers et al. (2004)	Diatom	Lab	L-PAR	Growth & photosynthesis
Chen & Gao (2004a)	Diatom	Lab	L-PAR	Photosynthesis
Wu et al. (2010)	Diatom	Lab	L-PAR	Growth & photosynthesis
Tortell et al. (2008)	Diatom	Ship-board	30% SL	Growth
Kim et al. (2006)	Diatom assemblage	Mesocosm	SL	Growth
Riebesell et al. (2007)	Diatom assemblage	Mesocosm	SL	POC production
Egge et al. (2009)	Phytoplankton assemblage	Mesocosm	SL	Photosynthesis
Hein & Sand-Jensen (1997)	Phytoplankton assemblage	Ship-board	L&M-PAR	Photosynthesis
Iglesias-Rodriguez et al. (2008a,b)	*Emiliania huxleyi*	Lab	L-PAR	Calcification
Feng et al. (2009)	Coccolithophore assemblage	Ship-board	SL	Calcification
Fu et al. (2007)	Pico-cyanobacteria	Lab	L-PAR	Abundance
Hutchins et al. (2007)	*Trichodesmium*	Lab	L-PAR	Growth
Fu et al. (2008a)	*Crocosphaera*	Lab	L-PAR	Growth & N_2 and CO_2 fixation
Fu et al. (2008b)	Raphidophyte	Lab	L-PAR	Growth & N_2 and CO_2 fixation
Gao et al. (1991, 1993, 1999)	Macroalgae	Lab	L-PAR	Growth
Chen & Gao (2003)	Diatoms	Lab	L-PAR	Photosynthesis
Kim et al. (2006)	Diatoms	Mesocosm		Growth
Gao et al. (2012)	Diatoms	Lab	Low SL	Growth
Neutral				
Tortell et al. (2000)	Phytoplankton assemblage	Ship-board	M-PAR	Growth
Tortell & Morel (2002)	Phytoplankton assemblage	Ship-board	30% SL	Growth
Feng et al. (2010)	Phytoplankton assemblage	Ship-board	7 and 33% SL	Photosynthesis
Fu et al. (2007)	Pico-cyanobacteria	Lab	L-PAR	Growth
Zou et al. (2011)	Macroalgae	Lab	L-PAR	Photosynthesis
Israel & Hophy (2002)	Macroalgae	Lab	SL	Growth
Fu et al. (2008b)	Dinoflagellate	Lab	L-PAR	Growth
Feng et al. (2008)	*Emiliania huxleyi*	Lab	L-PAR	Growth and photosynthesis
Gao et al. (2012)	Diatoms	Lab	~30% SL	Growth
Negative				
Wu et al. (2010)	Diatom	Lab	L-PAR	Respiration & photoinhibition
Gao et al. (2012)	Diatoms	Lab	>40% SL	Growth
Rokitta & Rost (2012)	*Emiliania huxleyi*	Lab	M-PAR	Growth
Levitan et al. (2010)	*Trichodesmium* sp.	Lab	L-PAR	Electron transport rate
Riebesell et al. (2000)	*Emiliania huxleyi*	Lab	L-PAR	Calcification & morphology
Feng et al. (2008)	*Emiliania huxleyi*	Lab	M-PAR	Calcification
Gao et al. (2009)	*Emiliania huxleyi*	Lab	PAR+UV	Calcification & photosynthesis
Gao & Zheng (2010)	Coralline algae	Outdoor	SL+UV	Calcification & photosynthesis & growth
Zou et al. (2011)	Macroalgae	Lab	L-PAR	Respiration
Chen & Gao (2011)	*Phaeocystis globasa*	Outdoor	SL+UV	Growth & photochemical yield
Russell et al. (2009)	Coralline algae	Lab	L-PAR	Abundance

CCMs is suggested to be closely related to the intracellular inorganic carbon (C_i) pool and be dependent on oxygen availability (Woodger et al. 2005). CO_2 levels (up to 1000 µatm) relevant to future CO_2 levels projected for 2100 have also been confirmed to down-regulate CCMs in marine diatoms (Chen & Gao 2003, 2004a, Rost et al. 2003, Wu et al. 2010), though CO_2 concentrations at which CCMs become completely switched off have not confirmed for different taxa. Down-regulation of CCMs can include decreased CO_2 affinity or increased CO_2 requirements for photosynthesis, inhibited carbonic anhydrase activity, and depressed HCO_3^- transport. Such down-regulation was found to be synchronized with diurnal photosynthetic performance in the diatom *Skeletonema costatum* (Chen & Gao 2004a,b). In a freshwater cyanobacterium (*Synechocystis* PCC6803), CO_2 concentration *in vitro* acts as a signal to control the activity of the NDH-1 complex (involved in cyclic electron flow around Photosystem I [PSI]), which in turn functions in the regulation of CO_2 uptake (Deng et al. 2003). Notably, doubling of ambient [CO_2] was recently suggested to save about 20% of the CCM-related energy expenditure in some diatoms, decreasing the total energy expended on carbon fixation by between 3 and 6% (Hopkinson et al. 2011). This estimate of saved energy expenditure parallels the 5% increase in growth rate observed in *Phaeodactylum tricornutum* when grown under 1000 µatm CO_2 (Wu et al. 2010). However, in the same diatom, CO_2-induced seawater acidification was shown to increase mitochondrial respiration and inhibit photosynthetic electron transport (Wu et al. 2010; our Fig. 1), which indicates an additional energy demand to re-equilibrate the perturbed acid–base balance during the night period under ocean acidification conditions. Although this additional energy demand should also happen during the daytime, this may be offset by the saved energy from down-regulation of CCM activity. On the other hand, cAMP metabolism was shown to be involved in controlling CCM in a diatom under elevated CO_2 levels (Harada et al. 2006). Recently, across a CO_2–pH gradient off the volcanic island of Vulcano (NE Sicily), periphyton communities altered significantly as CO_2 concentrations increased, with significant increases in chlorophyll *a* concentrations and in diatom abundance (Johnson et al. in press). This implies a possibility that the 'winners' could have increased their photosynthetic antenna to capture additional light energy to cope with the extra energy demand (such as enhanced respiration) due to increased seawater acidity.

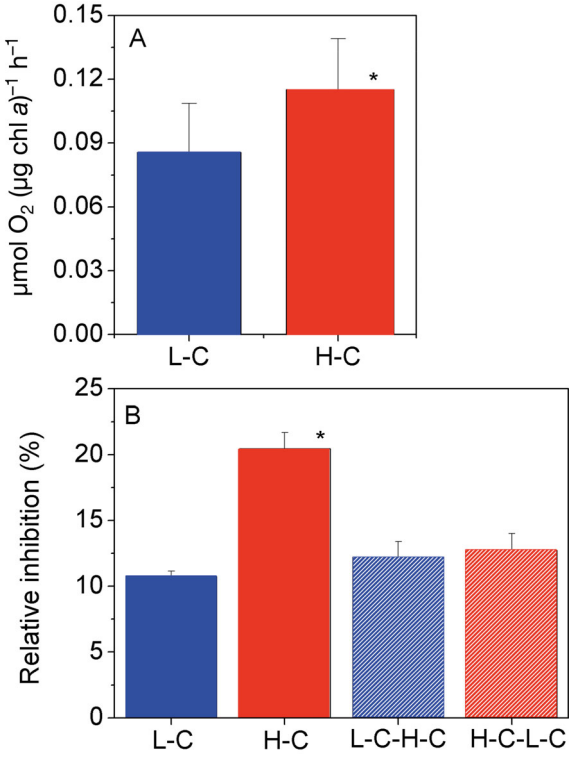

Fig. 1. *Phaeodactylum tricornutum*. (A) Dark respiration rate of low (L-C, 390 µatm) and high CO_2 (H-C, 1000 µatm) grown diatom cells, and (B) inhibition of electron transport measured under an actinic light level of 1200 µmol m^{-2} s^{-1} in L-C, H-C, or L-C grown cells measured under H-C condition (L-C-H-C) and vice versa (H-C-L-C); error bars represent SD (n = 3 to 12). *Significant ($p < 0.05$) differences. (Redrawn from Wu et al. 2010)

For calcifying coccolithophores, the efficiency of CCMs is still controversial. *Emiliania huxleyi* is able to concentrate inorganic carbon within its cells to a level about 10 times higher than the ambient (Sekino & Shiraiwa 1994); however, other studies show that the coccolithophorids *Pleurochrysis carterae* and *E. huxleyi* do not operate highly efficient CCMs (Nimer & Merrett 1992, Israel & Gonzalez 1996). On the other hand, it is still uncertain whether intracellular calcification in coccolithophores, which use bicarbonate and calcium ions to generate calcite and CO_2 ($2HCO_3^- + Ca^{2+} = CaCO_3 + CO_2 + H_2O$) (Hutchins 2011), provides a source of CO_2 for photosynthesis in these organisms (Zondervan 2007). A recent study demonstrated a photoprotective role of the calcification in *E. huxleyi* (Xu & Gao 2012). Calcification in some species of coccolithophores does not seem to be affected by elevated CO_2 and reduced pH (Langer et al. 2006). Calcification of one *E. huxleyi* strain was stimulated at low pH on a per-cell basis (Iglesias-Rodriguez et al. 2008a), a result that supported an earlier study on the same species (Fritz 1999). How-

ever, the Iglesias-Rodriguez et al. (2008a) results showed no increases in the cellular particulate inorganic carbon (PIC) to particulate organic carbon (POC) ratio (PIC:POC) at high pCO_2, and their methods and interpretations are still being debated (Riebesell et al. 2008, Iglesias-Rodriguez et al. 2008b). Notably, a recent global-scale water column and sediment core study found that coccolith mass declines nearly linearly with increasing pCO_2 or decreasing CO_3^{2-} ion levels (Beaufort et al. 2011). Other photosynthetic calcifiers may respond differently to ocean acidification; for instance, elevated DIC resulted in both enhanced photosynthesis and calcification in a coralline alga (Gao et al. 1993).

From a physiological point of view, increased CO_2 availability and the associated decline of pH should act differentially to affect primary producers, with the former saving energy required for active inorganic carbon acquisition and the latter potentially increasing energy demand to maintain cellular homeostasis relative to the increased acidity of seawater. Therefore, effects of ocean acidification on algal species or cyanobacteria would largely depend on their species-specific energetics and related physiological regulation, and subsequently their responses to ocean acidification would be altered by light as well as other environmental factors. Growth or photosynthetic responses to elevated pCO_2 are generally modulated by light energy availability, being typically most pronounced under low (Gao et al. 2012, Rokitta & Rost 2012) or stressful high (Gao et al. 2012) light levels. In a high-CO_2 ocean, increased pCO_2 and lowered pH cannot be considered separately as 2 independent factors, and double-edged effects of ocean acidification would be expected under different environmental conditions. Over long adaptation periods, ocean acidification may cause genetic alteration such as loss of CCM capabilities, and the evolved communities of the future are likely to be genetically different from contemporary communities (Collins et al. 2006). For instance, adaptation under ocean acidification condition for about 500 generations led to restored calcification in *Emiliania huxleyi* (Lohbeck et al. 2012).

PRIMARY PRODUCERS IN A WARMER AND ACIDIFIED OCEAN

The accumulation of CO_2 in the atmosphere traps heat reflected as infrared radiation (>700 nm), and both terrestrial and aquatic environments are thus being warmed. Although global mean SSTs are rising at only half the rate of those on land (0.13 vs. 0.27°C per decade since 1979), increasing temperature is one of the most pervasive of present-day influences on marine systems (Halpern et al. 2008). Warming trends are already believed to be changing the distributions and ecological niches of major phytoplankton groups like dinoflagellates (Peperzak 2003, Cloern et al. 2005, Hallegraeff 2010, Fu et al. 2012, this Theme Section), diatoms, and coccolithophores (Merico et al. 2004, Hare et al. 2007). A 50 yr (1960 to 2009) time series survey revealed a decline in dinoflagellate abundance in the northeast Atlantic and North Sea (Hinder et al. 2012) due to ocean warming and windy conditions. The combination of warming and higher light intensities during a mesocosm experiment resulted in significant acceleration of the spring phytoplankton bloom and changes in dominant species (Lewandowska & Sommer 2010).

Different habitats or latitudes have differences in annual and daily temperature means and ranges, and marine primary producers are subjected to substantial changes of temperature on various timescales. These include rapid shifts associated with tidal displacement of the thermocline or tidal immersion/emersion, diurnal fluctuations caused by clouds and changes in solar elevation, seasonal variations caused by changes in solar declination, and long-term inter-annual variability associated with natural climatic cycles and likely, human influence (Raven & Geider 1988, Davison 1991). These changes in temperature can influence the thermal windows of marine organisms by influencing their enzymatic efficiency and heat tolerance (Pörtner & Farrell 2008).

For primary producers, photosynthesis usually increases with increased temperature to reach an optimum and then declines with further warming, while respiration increases with increased temperatures, in a similar way to terrestrial plants (Zou et al. 2011). The respiration coefficient (Q_{10}, change in respiration rate over 10°C temperature change) increased with increased CO_2 concentrations in the brown macroalga *Hizikia fusiformis* (Zou et al. 2011). These authors documented a steeper slope of respiration versus temperature under increased levels of CO_2, reflecting a synergistic effect of elevated CO_2 and temperature on respiration. Respiration of phytoplankton may also increase under ocean acidification. In the diatom *Phaeodactylum tricornutum*, increase of pCO_2 from 390 to 1000 µatm (equivalent to 1000 ppmv in air) resulted in about a 30% increase in respiration (our Fig. 1; Wu et al. 2010), though combined effects of ocean acidification and warming on phytoplankton respiration, to the best of our know-

ledge, have not been documented. Since both photosynthesis and respiration will likely be influenced by ocean warming and ocean acidification, but probably to a different extent, the ratio of photosynthetic carbon fixation to respiratory carbon loss within the euphotic zone will vary in future warmer and acidified oceans.

Despite the obvious environmental relevance of these types of interactions between rising temperature and CO_2, there have been surprisingly few studies in which the effects of both factors have been considered together on marine primary producers. Recently, Connell & Russell (2010) found that simulated future CO_2 and temperature interacted synergistically to have a positive effect on the abundance of algal turfs. Species diversity and richness or ecological niche partitioning may be altered, since growth responses to the combined effects of ocean acidification and warming are likely to be species-specific. Two strains of the marine picocyanobacteria *Synechococcus* and *Prochlorococcus* responded differentially to warming as well as to elevated CO_2, with the growth rate of the former increasing and that of the latter not changing under the combined 'greenhouse' treatment (our Fig. 2) (Fu et al. 2007). Growth and nitrogen fixation rates of 2 isolates of the filamentous cyanobacterium *Trichodesmium* were strongly enhanced by either increasing CO_2 (750 µatm) or a 4°C temperature increase, but synergistic effects between the 2 variables were not observed (Hutchins et al. 2007). Levitan et al. (2010) reported similar findings for these 2 parameters in one of these same *Trichodesmium* isolates under the combination of warmer temperature (+6°C) and higher CO_2 (900 µatm).

In cultures of a Sargasso Sea isolate of the coccolithophore *Emiliania huxleyi*, growth and photosynthesis were stimulated by increases in both CO_2 and temperature, but no significant interactive effect was found between the 2 (Feng et al. 2008). That study found that although PIC production was affected by the combination of irradiance and acidification changes (see 'Light limitation and stress interactions with ocean acidification'), these calcification processes were independent of a 4°C temperature rise. In cultures of another *E. huxleyi* strain, De Bodt et al. (2010) found that elevated CO_2 stimulated photosynthetic carbon fixation, while both high CO_2 and temperature independently decreased calcification, but with no apparent interactions between the 2 factors. Another recent study found that temperature and CO_2 did have significant interactive effects on POC quotas and production rates in the coccolithophore

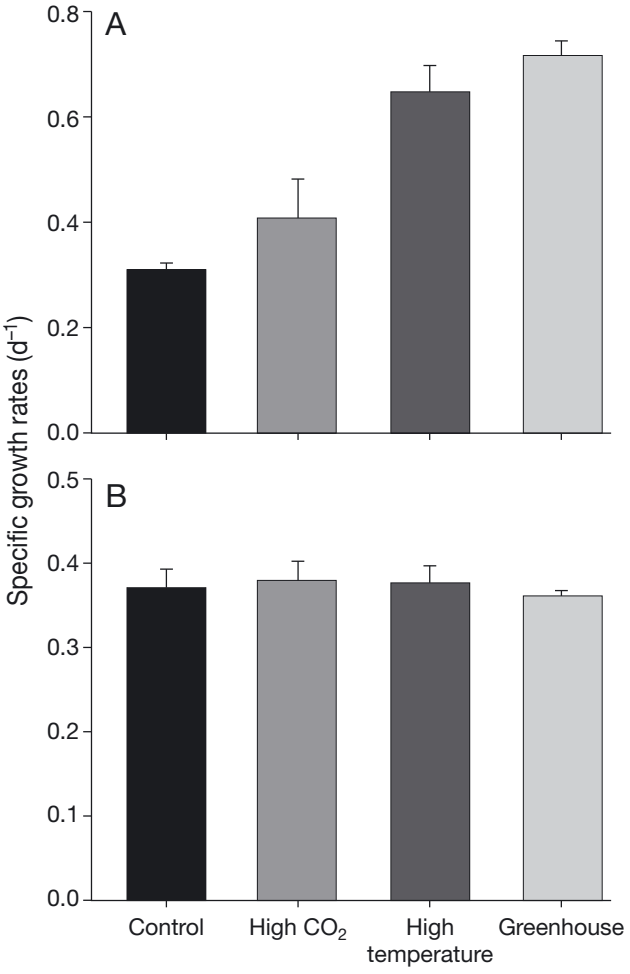

Fig. 2. Cell-specific growth rates of cultured (A) *Synechococcus* strain CCMP 1334 and (B) *Prochlorococcus* strain CCMP 1986 in a temperature and CO_2 matrix experiment. Treatments used were 20°C and 380 µatm CO_2 (control), 20°C and 750 µatm CO_2 (high CO_2), 24°C and 380 µatm CO_2 (high temperature), and 24°C and 750 µatm CO_2 (greenhouse). In (A), the right 2 bars are significantly different from the left 2, but no other differences; in (B), no significant differences. Error bars are SD of triplicate cultures for each treatment. (From Fu et al. 2007)

Syracosphaera pulchra, but these interactions were life-stage specific, with much greater impacts in haploid than in diploid cells (Fiorini et al. 2011). A 5°C temperature rise increased photosynthesis and calcification of *E. huxleyi* only at the ambient Ca^{2+} concentration (10 mM), whereas it decreased both processes in cells grown at 1% of ambient Ca^{2+} concentration (Xu et al. 2011). In contrast, in an experiment using a mixed natural North Atlantic bloom community, Feng et al. (2009) found that coccolithophore cell abundance was greatly increased under the 'greenhouse' combination of increased pCO_2 and temperature, but not when either one was increased

alone (our Fig. 3A). Paradoxically, they also found that calcite production was significantly lower in this greenhouse treatment (our Fig. 3B), even though it had by far the highest numbers of coccolithophores. They suggested that future trends in the North Atlantic bloom could include larger and denser blooms of coccolithophores, but that at the same time these cells might be much less calcified than under present-day conditions. Recent culture work, however, suggests large variability in the ability of different phytoplankton taxa to adapt to sequentially increasing temperatures, and 2 strains of *E. huxleyi* were among the species that were found to be unable to adapt to pronounced warming (e.g. a growth temperature increase from 22 to 30°C; Huertas et al. 2011). Further studies looking at the potential for long-term adaptation of algae to higher temperatures is needed, especially in combination with acidification and other global change variables.

While ocean acidification and warming lower coral-reef resilience (Anthony et al. 2011), algal reef builders also respond to these factors. Macroalgal calcifiers, which deposit $CaCO_3$ in their intercellular spaces or thallus surface, respond negatively to elevated pCO_2. Ocean acidification reduces the calcification of the red coralline algae (Gao et al. 1993, Gao & Zheng 2010), green algae *Halimeda* spp. (Sinutok et al. 2011), and brown algae *Padina* spp. (Johnson et al. 2012). The combination of ocean acidification and warming decreased photochemical yield, chlorophyll content, and calcification in *Halimeda* spp., indicating that 32°C and 1000 µatm CO_2 are the upper limits for survival of these organisms on the reef at Heron Island, Australia (Sinutok et al. 2011).

The combined effects of warming and acidification are likewise quite variable in other eukaryotic algae. In a comparative study of 2 co-occurring estuarine harmful bloom flagellate species, the maximum light-saturated carbon fixation rate (P^B_{max}) of the raphidophyte *Heterosigma akashiwo* was increased only with simultaneous increases in both CO_2 and temperature, whereas P^B_{max} of the dinoflagellate *Prorocentrum minimum* responded to CO_2 enrichment with or without increased temperature (Fu et al. 2008b). A shipboard incubation study that examined rising temperature and CO_2 in 2 natural Bering Sea assemblages found large community shifts away from diatoms and towards nanoflagellates in the 'greenhouse' treatment, although these appeared to be due more to changes in temperature than in CO_2 (Hare et al. 2007). Likewise, higher temperatures reduced the abundance of diatoms in the Feng et al. (2009) North Atlantic bloom incubation study, while

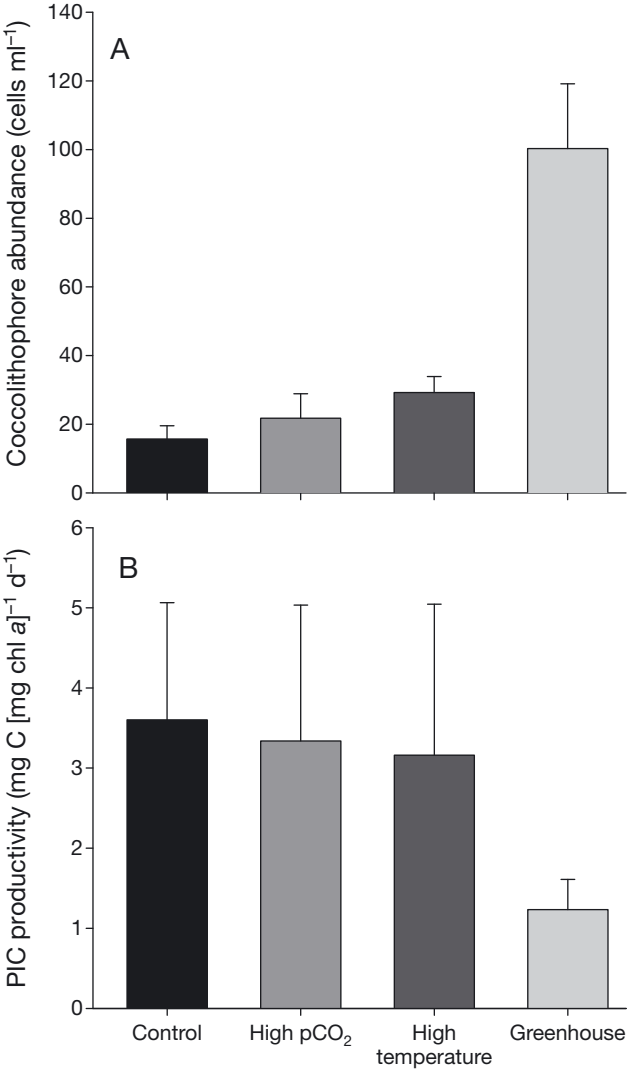

Fig. 3. (A) Final coccolithophore cell abundance and (B) particulate inorganic carbon (calcite or PIC) production rates in a temperature and CO_2 matrix experiment using a natural North Atlantic spring bloom phytoplankton community. Treatments used were 12°C and 390 µatm CO_2 (control), 12°C and 690 µatm CO_2 (high CO_2), 16°C and 390 µatm CO_2 (high temperature), and 16°C and 690 µatm CO_2 (greenhouse). In (A), right bar is different from the other 3 ($p < 0.05$), but none of the other 3 differ from each other ($p > 0.05$); in (B), same pattern as (A). Error bars are SD of triplicate treatments. (From Feng et al. 2009)

CO_2 changes had no apparent effect on diatom dominance. A similar finding was reported in a coastal regime, Daya Bay in China, where long-term inputs of thermal effluent from a power plant shifted the community from diatoms to dinoflagellates, although CO_2 was not considered in that study (T. Li et al. 2011). However, a 50 yr (1960 to 2009) survey in the northeast Atlantic and North Sea did not observe any decline in the abundance of diatoms (Hinder et al.

2012). On the other hand, there may be a strong shift in the biogeochemical cycling of organic matter in the upper ocean in response to increased temperature that will affect pelagic food web structure and the biological sequestration of organic matter (Wohlers-Zöllner et al. 2011), though interactive effects of ocean warming and acidification on important biogeochemical cycles are still uncertain.

LIGHT LIMITATION AND STRESS INTERACTIONS WITH OCEAN ACIDIFICATION

Visible light or PAR (400 to 700 nm) drives photosynthesis in the marine environment in a way that is more dynamic than in the terrestrial environment, since light quantity and quality changes rapidly with depth due to differential attenuation of different wavelengths. Light levels under which phytoplankton cells must photosynthesize can range from limiting to stressful depending on their mixing regime within the UML, as well as the season and time of day.

Under low light or reduced levels of solar PAR, growth or photosynthesis of many phytoplankton groups, including diatoms (Riebesell et al. 1993, Kim et al. 2006, Wu et al. 2010, Sun et al. 2011), coccolithophores (Riebesell et al. 2000, Leonardos & Geider 2005), dinoflagellates and raphidophytes (Fu et al. 2008b), and cyanobacteria (Hutchins et al. 2007, Fu et al. 2008a, Kranz et al. 2010, Levitan et al. 2010, Garcia et al. 2011), as well as some macroalgae (Gao et al. 1991, 1993, 1999, Zou et al. 2011), is often stimulated under elevated CO_2 conditions (our Table 1). For intertidal algae, increasing atmospheric CO_2 concentration enhances their photosynthetic CO_2 fixation rate during emersion at low tide (Gao et al. 1999, Zou & Gao 2002). However, non-responsiveness of photosynthetic carbon fixation to increased CO_2 or ocean acidification has also been shown in both phytoplankton (Tortell & Morel 2002) and macroalgae (Israel & Hophy 2002) (our Table 1).

Some of the best documented interactive effects between ocean acidification and PAR have been observed in *Trichodesmium*. Kranz et al. (2010), Levitan et al. (2010), and Garcia et al. (2011) all showed that CO_2-driven enhancement of N_2 fixation and growth rates is greatest at low, limiting light levels, while this effect is considerably muted at higher levels of PAR, possibly due to down-regulation of nitrogenase enzyme iron protein (NifH) synthesis (our Fig. 4). Under high light, elevated CO_2 concentration significantly depressed NifH levels compared to low light (our Fig. 4). Garcia et al. (2011) also found that gross:net N_2 fixation (gross being total N fixed, while net is N retained by the cell, as in gross or net photosynthetic carbon fixation) ratios were highest at low light and high pCO_2, and declined with successive increases in light or decreases in pCO_2. Trends in trichome (cell chain) length in the cultures were inversely related to those of the gross:net N_2 fixation ratios, although whether these 2 effects are connected, and why, is uncertain. These gross:net ratio effects imply that *Trichodesmium* cells exude or lose much more of their total fixed N at high CO_2. This effect is likely to be greatest deep in the euphotic zone, but may be reduced in future shallow mixed layers at high light intensities. In general, the overall

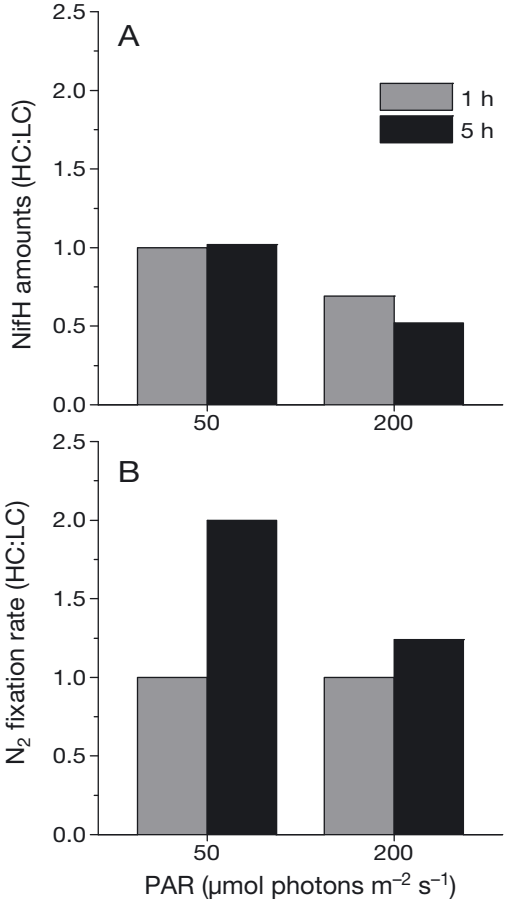

Fig. 4. *Trichodesmium* strain IMS101. (A) Changes in the amount of the nitrogenase iron protein, NifH (pmol mg protein^{-1}) and (B) nitrogen fixation rate in response to different light (50 and 200 µmol photons m^{-2} s^{-1}) and pCO_2 (150 and 900 µatm) levels. The values are indicated as the ratios of high (HC, 900 µatm) to low CO_2 (LC, 150 µatm), measured at 1 and 5 h after the onset of light. PAR: photosynthetically active radiation. (Data re-drawn from Kranz et al. 2010 and Levitan et al. 2010)

weakening of the well-documented positive effect of high pCO_2 on N_2 fixation in this biogeochemically critical cyanobacterium at higher irradiances also means that shoaled UML depth may need to be taken into account for accurate predictions of ocean acidification impacts on future N_2-supported new production. In addition, changes in PAR quality due to differential attenuation of different light wavelengths with changed UML depth might also interact with ocean acidification or warming to affect cyanobacterial physiology and N_2 fixation as well as that of other phytoplankton groups. Nevertheless, the high flexibility in resource and energy allocation by *Trichodesmium* should enable this important organism to flourish in future surface oceans characterized by elevated pCO_2, higher temperatures, and increased light exposures (Levitan et al. 2010), although little has been documented about its physiological performance under solar radiation.

Ocean acidification and PAR interactions can often be identified and quantified by examining photosynthesis versus light (*P*–*E*) relationships under varying pCO_2. For instance, in the toxic harmful bloom diatom *Pseudo-nitzschia multiseries*, Sun et al. (2011) found significant sequential increases in P^B_{max} (maximum biomass-normalized carbon fixation rates) across 3 pCO_2 levels. However, for this diatom, α values (the slope of the light-limited portion of the curve) and E_K (the light saturation point) did not change with acidification. Frequently, these types of experiments demonstrate non-linear effects on *P*–*E* parameters. For instance, Feng et al. (2008) found that in *Emiliania huxleyi* cultures grown under low light, P^B_{max} was much higher in combined high-pCO_2, high-temperature 'greenhouse' treatments than when either CO_2 or temperature were increased alone. Similar additive or non-linear 'greenhouse' enhancements of P^B_{max} have been seen in the raphidophyte *Heterosigma* sp. (Fu et al. 2008b), the cyanobacteria *Synechococcus* sp. and *Trichodesmium* sp. (Fu et al. 2007, Hutchins et al. 2007), and in a natural assemblage from the Bering Sea (Hare et al. 2007). In contrast, Feng et al. (2010) found no physiological interactive effects of light and CO_2 on community P^B_{max} in an experiment using a Ross Sea diatom–*Phaeocystis* assemblage. Instead, they observed a diatom community structure shift away from small pennate diatoms towards much larger centric diatoms when high CO_2 was combined with high light. This might be related to the difference in photo-physiological performance between large and small diatoms (Wu et al. 2011). Susceptibility to photo-inactivation under high light is less in larger than in smaller diatom species, and is higher under ocean acidification conditions compared to ambient CO_2 level (our Fig. 5; Key et al. 2010, McCarthy et al. 2012). Phytoplankton cell size controls the efficiency of elements or energy transfer, and thus the assembly of higher trophic levels in marine food chains (Raven 1998, Finkel et al. 2010). Light absorption (Fujiki & Taguchi 2002) and photosynthesis (Raven & Kübler 2002) are also known to differ among differently sized algal cells. The pigment-specific light absorption increases with decreasing cell size (Fujiki & Taguchi 2002), leading to higher light-use efficiency as well as solar radiation exposures per unit pigment or per cell volume (Jeffrey et al. 1996). On the other hand, even when experiencing the same pH reduction with the ongoing ocean acidification, smaller cells suffer differentially from the acidity increase due to differences in thickness of the diffusion layer surrounding cells (Flynn et al. 2012).

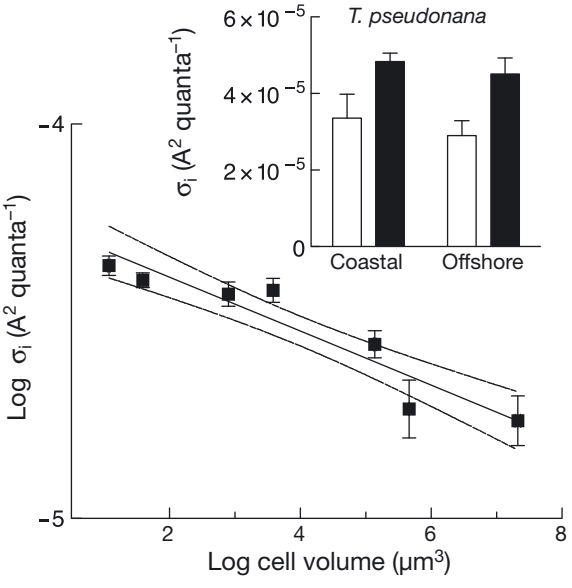

Fig. 5. *Thalassiosira* and *Coscinodiscus* spp. Smaller diatoms show higher susceptibility to photo-inactivation than large diatoms. Susceptibility to photo-inactivation of Photosystem II (PSII) (log σ_i) estimated under moderately high light for a size range of marine centric diatoms from the genera *Thalassiosira* and *Coscinodiscus*, plotted versus log cell volume (μm^3). Error bars are SD (n = 4 to 6 independent culture replicates for each strain), and dotted lines plot the 95% confidence interval for the regression. (Data re-drawn from Key et al. 2010 and Wu et al. 2011.) Inset: Susceptibility to photo-inactivation of PSII (σ_i) under moderately high light increases under increased pCO_2 for small diatoms from coastal and offshore habitats. Open bars: cells grown under 390 µatm CO_2; closed bars: cells grown under 750 µatm CO_2. Error bars are SD (n = 3 to 4). (Data re-drawn from McCarthy et al. 2012)

This implies that the combination of light and ocean acidification influences competition among species or differently sized groups, and is likely to change phytoplankton community structure in the future shoaled UML. Under laboratory constant-light levels, growth of larger diatoms is stimulated to higher extents than smaller ones, with differentially affected elemental stoichiometry (C:N:Si ratios) (Y. Wu, Z. Finkel, D. Campbell unpubl. data). Hoogstraten et al. (2012) found that growth rates of the colonial prymnesiophyte *Phaeocystis globosa* decreased with increasing pCO_2 in saturating light treatments, and suggested that this harmful bloom species could be less competitive in the future ocean as a consequence. Chen & Gao (2011) also showed depressed quantum yield and growth rate under high levels of sunlight, but they also found stimulated rates under heavy cloud cover when this species was grown under ocean acidification conditions under solar radiation.

While many laboratory or indoor studies under constant low or high PAR levels have demonstrated differential responses of different taxa to ocean acidification, few experiments have been performed under natural sunlight with changing light levels (Chen & Gao 2011). Notably, phytoplankton assemblages or diatoms showed different responses when grown under different levels of solar radiation and elevated pCO_2 (Gao et al. 2012). When exposed to CO_2 concentrations projected for the end of this century in combination with high light intensities representative of the UML, natural phytoplankton assemblages from the oligotrophic South China Sea responded with decreased primary production and increases in photo-physiological indicators of light stress (non-photochemical quenching, NPQ). At the same time, community dominance shifted away from diatoms and towards prymnesiophytes. However, under reduced sunlight intensities representing a deeper mixed layer, simulated ocean acidification did not cause such reduction in primary production (K. Gao et al. unpubl. data). To model this interactive acidification and light effect in the laboratory, Gao et al. (2012) grew representative diatoms at CO_2 concentrations of 390 and 1000 µatm across a range of solar radiation typically experienced by phytoplankton at different depths of the euphotic zone (5 to 100% of incident surface irradiance). Growth and photochemical quantum yield of the high-pCO_2 cultures were positively related to light intensity at lower light levels (5 to 36% of incident surface irradiance), but at higher light levels these parameters were negatively related to an increase in light intensity and significantly decreased compared to the cells grown at ambient CO_2 levels. This reversal of the effects of ocean acidification with increasing light levels suggests that under low levels of sunlight or at deeper depths, ocean acidification could stimulate diatom growth, but would be inhibitory under high solar exposures at shallower depths or at midday. Down-regulated CCMs due to increased availability of CO_2 and up-regulated energy demands due to acid–base perturbation appear to act synergistically to induce additional light stress to the cells, as evidenced by the increased NPQ in both the diatom cultures and the natural phytoplankton assemblages from the South China Sea and decreased PAR threshold in the diatoms grown at the elevated pCO_2 (Gao et al. 2012). Such enhanced light stress under ocean acidification and stimulated mitochondrial and photo-respiration could be mechanistically responsible for the decline of primary production or growth rate (our Fig. 6, based on the results presented in Gao et al. 2012). Hypothetically, with increasing availability of CO_2 and progressive acidification of seawater, phytoplankton cells at deeper depths receiving low light levels would increase their growth, while those near the surface receiving higher levels of sunlight would decrease their growth, with the PAR threshold at which growth becomes saturated declining (Fig. 7). Whether such a synergistic effect of high levels of CO_2 and light are unique for diatoms or are widespread among phytoplankton taxa remains to be investigated. However, considering the key role of diatoms in the ocean, both as the predominant source of food for higher trophic levels and as the main driver of export production, the impacts this effect may have on ocean productivity would be severe. Since future shoaling of UML depths is expected to expose phytoplankton to increased mean light intensities, reduced marine primary production along with community structure changes seem likely in future high-CO_2 oceans.

Photoautotrophs experience subsaturating, saturating, and stressful light levels even during a single daily solar movement. Under supra-optimal light-saturated conditions, algal cells need to dissipate additional energy they receive, and any other environmental stresses could affect the threshold at which PAR becomes excessive (Fig. 7). Therefore, increased acidity or a disturbed acid–base balance could affect light use efficiency or lower the PAR levels above which light becomes excessive (Gao et al. 2012). Photoinhibition of electron transport was exacerbated under ocean acidification when the diatom *Phaeodactylum triconutum* was exposed to high light levels

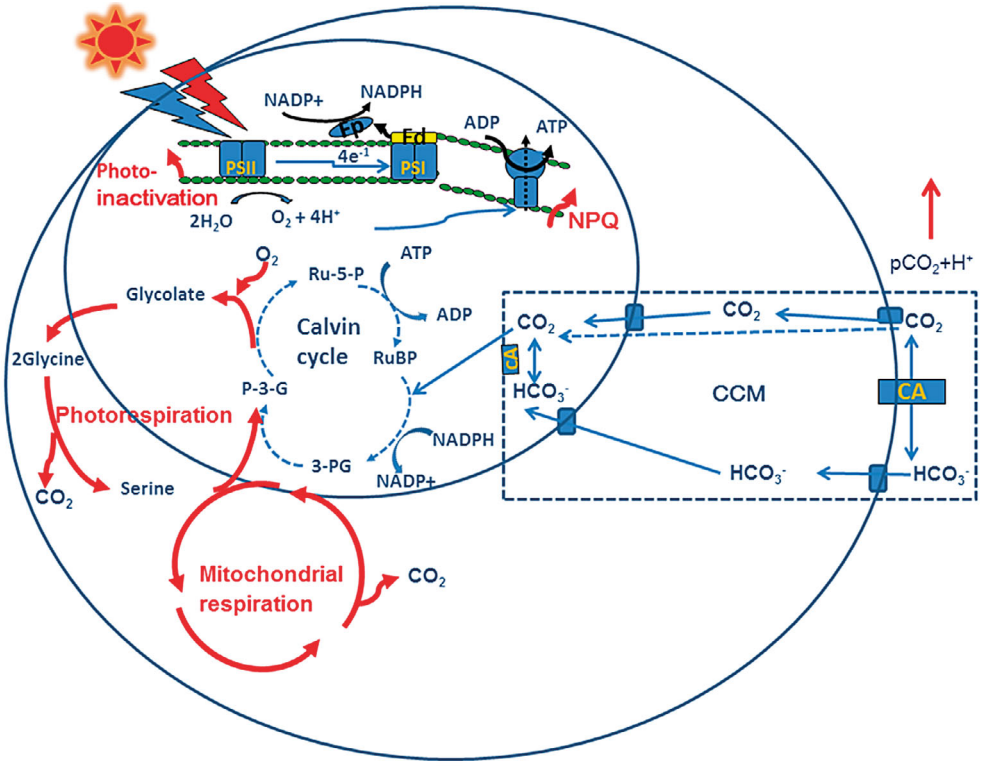

Fig. 6. Metabolic pathways up-regulated (red and solid) and down-regulated (blue and dotted) under ocean acidification. Phytoplankton down-regulate their CO_2-concentrating mechanisms (CCMs) under increased CO_2 levels and save energy expenditure on carbon acquisition, but this may trigger additional light stress when cells are exposed to high light intensity at the same time. Meanwhile, cellular defenses are enhanced, including increased non-photochemical quenching (NPQ), enhanced mitochondrial respiration, and photorespiration. However, these protective activities are not enough to fully compensate for photodamage, thus carbon fixation (the Calvin cycle) and growth rate are ultimately reduced under sunlight. PSI: Photosystem I; PSII: Photosystem II; CA: carbonic anhydrase; Fd: ferredoxin (Generated from the concepts in Gao et al. 2012)

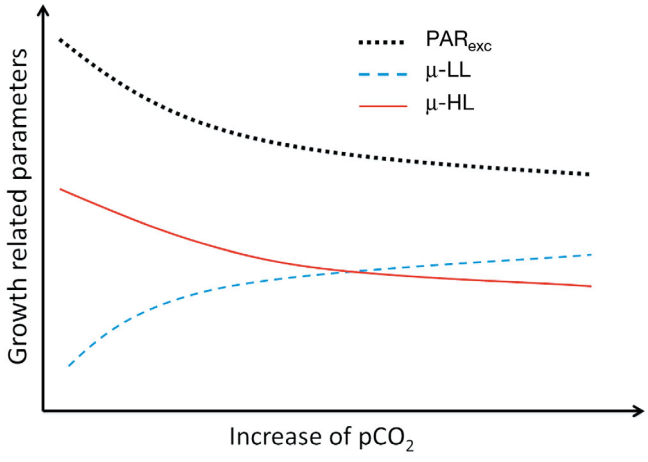

Fig. 7. Hypothetical relationship between growth-related parameters and increasing pCO_2, based on recent findings in some diatoms (Gao et al. 2012). PAR_{exc}: photosynthetically active radiation (PAR) threshold beyond which growth rate of a phytoplankton species declines with increasing light levels; μ-LL: specific growth rate under sub-saturating light levels; μ-HL: specific growth rate under super-saturating light levels

(our Fig. 1; Wu et al. 2010). In another diatom, *Thalassiosira pseudonana*, however, such an inhibition was not observed under the same CO_2 level (Yang & Gao 2012). In *Skeletonema costatum*, growth was not affected by elevation of CO_2 concentration (up to 1000 μatm) under either 30 or 210 μmol photons m^{-2} s^{-1} of PAR; however, at 30 μmol photons m^{-2} s^{-1} PAR and reduced CO_2 levels of 100 μatm, the growth of this diatom was significantly decreased (Chen & Gao 2003), likely reflecting that light energy availability affects the operation of CCMs that provide the carbon source for carboxylation. In contrast, in a mesocosm study under reduced levels of natural solar radiation, only the diatom *S. costatum* showed an increased growth rate with increased pCO_2 (Kim et al. 2006). In cultures of the diatom *Chaetoceros muelleri*, low-light treatments showed lower growth rates in elevated CO_2 or reduced pH conditions, but no CO_2 or pH effect was recorded under high light exposure, reflecting that light supply does alter the cell's response to ocean acidification (Ihnken et al. 2011). In *T. pseudonana*, since photosynthetic carbon fixation and respiratory

carbon loss were equally stimulated under the ocean acidification, growth rate was not affected (Yang & Gao 2012). Although indoor laboratory studies have provided useful information about the relationship of ocean acidification and light levels, it should be noted that fluctuating light levels in the mixed layer might modulate C_i acquisition efficiencies in different ways compared to the constant light levels under which cultures are usually maintained in the laboratory. Further interactive ocean acidification–light experiments are needed that use more realistic variable-irradiance regimes.

Coccolithophores, as key photosynthetic $CaCO_3$ producers, form extensive blooms in many regimes, and are sensitive to both acidification and PAR (Boyd et al. 2010). A recent field survey in the North Sea–Arctic Ocean identified pH and light as the 2 key environmental controls on coccolithophore community composition throughout this region (Charalampopoulou et al. 2011). When grown under indoor conditions (either light-limiting or -saturating), they usually demonstrate a decline of calcification rates under high pCO_2 and low pH conditions (Riebesell et al. 2000, Sciandra et al. 2003, Delille et al. 2005, Gao et al. 2009). In *Emiliania huxleyi,* different light:dark cycles (16:8 or 24:0 h) did not result in significant growth rate differences, and both PIC and POC production per cell increased with increased PAR within a range of 20 to 150 µmol photons m^{-2} s^{-1}, leading to insignificant changes in the PIC:POC ratio at the lowest irradiance, at which it was much lower. Increased CO_2 up to about 1050 µatm did not affect the specific growth rate as much as increased light levels, which increased POC content per cell (Zondervan et al. 2001). In the same species, the PIC:POC ratio was decreased when the cells were grown at 400 µmol photons m^{-2} s^{-1} compared to those at 50 µmol photons m^{-2} s^{-1}, and were further decreased by increased CO_2 and lowered pH, but only at the elevated PAR intensity (Feng et al. 2008). On the other hand, seawater acidification associated with elevated pCO_2 enhanced photosynthesis in coralline algae under indoor low PAR (Gao et al. 1993), but reduced it under incident high solar PAR (Gao & Zheng 2010). Photosynthetic carbon fixation or POC production often shows a positive relationship with calcification or PIC generation in coccolithophores (Paasche 2001, Zondervan et al. 2001, Feng et al. 2009, Xu & Gao 2012) and coralline algae (Borowitzka 1981, Gao et al. 1993) with increased light. Light limitation would result in less availability of ATP to drive algal calcification. However, under ocean acidification conditions, even photosynthesis-saturating levels led to decreased calcification in *E. huxleyi* (Feng et al. 2008, Gao et al. 2009). When grown at a PAR level of 300 µmol photons m^{-2} s^{-1}, the growth rate of both diploid and haploid *E. huxleyi* decreased at the elevated pCO_2 compared to those grown at the ambient level (Rokitta & Rost 2012). These findings indicate that, even when there is sufficient light energy, calcification is still suppressed by altered seawater chemistry under ocean acidification, which contrasts to the finding that calcification in some bivalves (Melzner et al. 2011) or corals (Holcomb et al. 2010) is not affected by ocean acidification when sufficient energy (food) is available.

COMBINED EFFECTS OF OCEAN ACIDIFICATION AND UV RADIATION

Solar UV radiation (280 to 400 nm) harms both primary producers and their consumers in aquatic ecosystems, decreasing productivity and disturbing reproduction and development (Häder et al. 2011). Although enforcement of the Montreal Protocol has slowed down ozone depletion, recent studies indicate a 10% increase in UV-B irradiance (280 to 315 nm) reaching northern temperate regions between 1983 and 2003 (Josefsson 2006). Most recently, a massive ozone hole was discovered in the Arctic, reflecting an interactive impact of climate change on ozone depletion (Manney et al. 2011). Global warming leads to less heat release to the stratosphere, and thereby enhances ozone depletion reactions, which occur faster when the stratosphere becomes further cooled. UV irradiances can penetrate as deep as 80 m in pelagic oceans (Tedetti et al. 2007), depending on the transparency of seawater. Open-ocean phytoplankton cells are generally more sensitive to UV inhibition than coastal assemblages (Fritz et al. 2008, G. Li et al. 2011). This may be because positive UV-A effects on photosynthetic carbon fixation were only found in coastal water phytoplankton assemblages, and because pelagic phytoplankton species are often smaller and more light-acclimated due to less mixing, and must deal with less availability of nutrients compared to those in coastal waters (G. Li et al. 2011).

Solar UV-B irradiance is known to decrease photosynthetic carbon fixation (Helbling et al. 2003, Gao et al. 2007, Häder et al. 2011), damage DNA (Buma et al. 2001, 2006, Gao et al. 2008) and proteins (Bouchard et al. 2005, Wu & Gao 2009), and even alter morphology (Wu et al. 2005) of photosynthetic organisms. It is also suggested to reduce genome stability in terrestrial plants (Ries et al. 2000). On the other hand, mod-

erate levels of UV-A (315 to 400 nm) are recognized to stimulate photosynthetic carbon fixation of phytoplankton assemblages (Barbieri et al. 2002, Helbling et al. 2003, Gao et al. 2007) and macroalgae (Gao & Xu 2008, Xu & Gao 2010), and enhance the activity of carbonic anhydrase, which facilitates bicarbonate utilization in a diatom (Wu & Gao 2009). Low levels of UV-A irradiances are known to stimulate repair of UV-B-induced DNA damage (Buma et al. 2001). DNA repair is also enhanced at higher temperatures (Gao et al. 2008). Therefore, contrasting effects of UV radiation (280 to 400 nm) on photosynthetic organisms can be observed between cloudy periods, when light is limiting and UV-A stimulative, and sunny days, when PAR becomes excessive and UV radiation more harmful (Gao et al. 2007). When the negative and positive effects are in balance, UV impacts can be neutral and disappear entirely (Wahl et al. 2004, Molis & Wahl 2009). Since UV-A and UV-B can affect photosynthetic CO_2 fixation in various and even opposing ways, it may interact with ocean acidification to affect marine primary producers differently under varying climate conditions (Chen & Gao 2011).

For the coccolithophore *Emiliania huxleyi* (Gao et al. 2009) and a coralline alga *Corallina sessilis* (Gao & Zheng 2010), reduced levels of calcification due to ocean acidification are further decreased by the addition of UV radiation. This reflects that the calcified layer for both the micro- and macro-algal calcifiers plays a protective role against UV. The coccoliths of *E. huxleyi* reduce the transmission of harmful UV radiation by about 26% (Gao et al. 2009), and reduced coccolith thickness leads to decreased electron transport rate as well as a lower PIC:POC ratio (our Fig. 8).

While ocean acidification increases the sensitivity of photosynthesis and calcification of some algal calcifiers to UV radiation (Gao et al. 2009, Gao & Zheng 2010), the presence of UV stimulates the calcification of *Emiliania huxleyi* when grown at ambient CO_2 levels under natural solar radiation, but at the cost of reduced growth rates (Guan & Gao 2010). Although increased accumulation of UV-screening compounds, such as mycosporine-like amino acids (MAAs), is a defensive strategy employed under ocean acidification conditions by coralline algae (Gao & Zheng 2010), it is usually not sufficient for the plant to cope with the combined impacts of both stressors at once, reflecting a likely future decrease of vegetation cover by these reef-building organisms.

Elevated CO_2 levels have been shown to increase the sensitivity of some marine and freshwater phytoplankton to UV-B (Sobrino et al. 2008). In contrast, ocean acidification and moderate levels of UV-A stimulated photochemical yield and specific growth rate of the harmful bloom prymnesiophyte *Phaeocystis globosa* (Chen & Gao 2011). Intense UV-B exposure on sunny days, however, caused significant reductions of the photochemical yield and growth of this alga, and these were further decreased under ocean acidification conditions in acclimated cultures of *P. globosa* grown under natural solar radiation (Chen & Gao 2011).

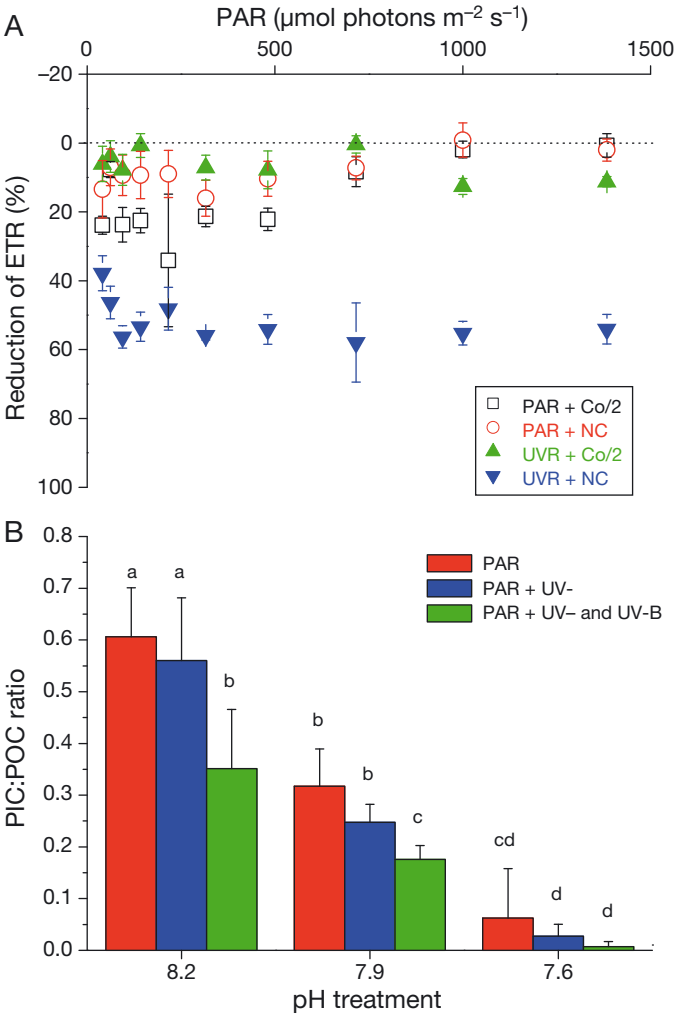

Fig. 8. *Emiliania huxleyi*. (A) Coccoliths play an important role in shielding cells from UV radiation (UVR). Removal of the coccoliths barely affects the electron transport rate (ETR) in the absence of UV (red, NC), but leads to about a 60% reduction in the presence of UV (blue, NC). PAR: photosynthetically active radiation. (Z. X. Ruan & K. Gao unpubl. data.) (B) UV-A and UV-B act synergistically with CO_2-induced seawater acidification to reduce the production ratio of particulate inorganic to organic carbon (PIC:POC ratio). Different letters indicate significant differences among the treatments. (Gao et al. 2009, with copyright permission from the Association for the Sciences of Limnology and Oceanography)

Interactive effects of UV radiation and CO_2-induced seawater acidification on phytoplankton are likely to differ between algal taxa due to species-specific differences in tolerance of UV radiation or UV-defensive strategies. For instance, coastal and pelagic phytoplankton species and assemblages can exhibit different responses to the effects of UV-A and UV-B (G. Li et al. 2011), and the extent of diel variations in seawater carbonate chemistry also differs between these 2 ecosystems. For many major functional groups of primary producers, like harmful algal species, N_2-fixing cyanobacteria, and picocyanobacteria, however, virtually nothing is presently known about their responses to combined ocean acidification and UV exposure. It is clear though that the net or balanced effects of ocean acidification on primary producers in general will depend on mixing depths, mixing rates, and fluctuations of solar radiation, which together mediate the balance between UV-induced damage and repair. Photosynthetic performance in phytoplankton assemblages (Helbling et al. 2003) or in diatoms (Guan & Gao 2008) is inhibited by UV to a lesser extent under fluctuating solar radiation or during the mixing path as compared to static (non-mixed) conditions. Therefore, it is likely that effects of ocean acidification on phytoplankton would differ when they are exposed to fluctuating light or solar radiation, as when they receive changing levels of solar radiation during vertical mixing. In general, how an organism responds to UV radiation under ocean acidification conditions may give insight as to which organisms may operate with better physiological performance in future lower-pH oceans. Li et al. (2012) showed that ocean acidification appears to counteract UV-B-induced harm to the diatom *Phaeodactylum tricornutum*. In addition, beneficial effects of increased temperature on photosynthesis under UV radiation stress have been previously documented (Sobrino & Neale 2007, Gao et al. 2008, Halac et al. 2010, Helbling et al. 2011), showing lower UV-induced inhibition or damages at higher temperatures. Differential sensitivities to UV radiation have been reported in marine picoplankters when grown under elevated CO_2 concentrations, with *Nannochloropsis gaditana* having lower sensitivity and *Nannochloris atomus* showing a neutral response (Sobrino et al. 2005). The diatom *Thalassiosira pseudonana*, when grown at elevated CO_2 concentration, became more sensitive to UV radiation (Sobrino et al. 2008). These issues require further studies to look into the physiological energetic costs and benefits in changing oceans.

CONCLUSIONS AND FUTURE WORK

Heat from solar radiation trapped in oceanic surface waters together with global warming is responsible for ocean warming. Surface ocean warming enhances stratification and reduces nutrient availability due to lower diapycnal transport of nutrients from deeper layers, as well as increases in UV exposure to phytoplankton cells circulating in a shallower mixed layer (our Fig. 9; Steinacher et al. 2010). In the tropics and at mid-latitudes, phytoplankton are typically nutrient-limited, and stratification with upper-ocean warming could lead to reduced nutrient supply and decreased growth (Fig. 9); at higher latitudes, phytoplankton cells are often light-limited, so stratification would keep them close to the surface where light levels are higher and may stimulate growth (Doney 2006). Nitrogen, phosphorus, and iron are key elements that limit marine primary production. The concentrations of these elements vary in different oceanic waters, and therefore may affect the physiological responses of phytoplankton to ocean acidification, probably differentially under different quality or intensity of solar radiation. However, only a few studies have addressed ocean acidification and nutrient interactions (Lefebvre et al. 2012), although like CO_2 and solar radiation, changes in nutrient supply rate and ratios could have large implications for biogeochemical cycles and food web interactions (e.g. the food quality of phytoplankton). Iron-limited phytoplankton in the Gulf of Alaska increase their growth rates and biomass when incubated at high CO_2 levels, probably due to increases in photosynthetic efficiency through reduced energetic demands for CCMs (Hopkinson et al. 2010). Irradiance and temperature both have documented interactions with phytoplankton iron requirements (Rose et al. 2009, Sunda & Huntsman 2011), but how these multi-variate feedbacks may change in a high-CO_2 ocean remains speculative. Even phytoplankton vitamin B_{12} requirements can be affected by acidification, and B_{12} and CO_2 levels together can help determine cellular quotas of iron and other trace metals (King et al. 2011).

Reduced thickness of surface mixed layer or UML with stratification can increase UV exposure to phytoplankton cells (Fig. 9). Increased PAR or UV dose exposure can decrease the ratio of chlorophyll to organic carbon in phytoplankton, and reduced availability of nutrients and iron can further alter this ratio, since both nutrient limitation and light stress decrease the pigmentation of photosynthetic organisms. Ocean acidification can lead to increased C:N

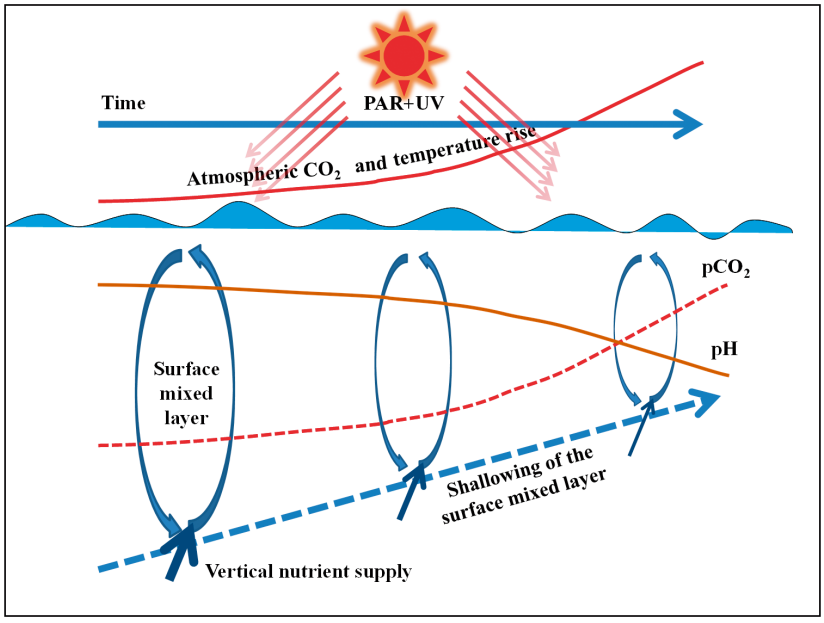

Fig. 9. Conceptual illustration of ocean changes associated with climate change. Atmospheric CO_2 and global temperature increases (red lines) lead to more CO_2 (dashed red line) dissolving in surface oceans, decreased pH (solid yellow line), and ocean warming. Increases in stratification and decreases in the thickness of the surface mixed layer due to ocean warming and freshening lead to less vertical transport of nutrients (decreasing size of upward arrows) and increasing exposure of cells to solar photosynthetically active radiation (PAR) and UV radiation. These multiple driving factors may synergistically or antagonistically interact to influence the physiology of marine plankton

and/or C:P ratios (Riebesell et al. 2007, Hutchins et al. 2009), consistent with trends in many terrestrial plants, whose food value can consequently decrease under elevated CO_2 (Ziska & Bunce 2006). P:N:C ratios changed from 1:6.3:121 to 1:7.1:144 to 1:8.25:168 in a pelagic phytoplankton assemblage when pCO_2 was raised from 350 to 700 to 1050 µatm (Bellerby et al. 2008), with enhanced organic carbon transport to the deeper layer (Schulz et al. 2008). Ocean acidification effects on the elemental stoichiometry of phytoplankton can also alter the ocean's large-scale biogeochemical cycles (Hutchins et al. 2009, Finkel et al. 2010, Tagliabue et al. 2011). Since reduced mineralization of algal mineralizers may affect their UV sensitivity (Gao et al. 2009; our Fig. 8), ocean acidification and UV may act synergistically to alter the PIC:POC rain ratio (Hutchins 2011).

Despite an apparent need to consider all of these many environmental changes in a holistic context, most of the ocean acidification studies so far have been conducted under laboratory conditions without considering multiple factors (Boyd 2011). This is one of the main limitations on our knowledge about the ecosystem-level impacts of ocean acidification and other global change factors in the real ocean. The relevance of changes in PAR and temperature to photoautotrophic organisms in the ocean is obvious, but surprisingly few phytoplankton studies have addressed their physiological and ecological interactions with high CO_2 and lower pH. Future studies may also need to focus on the impacts of elevated CO_2 levels under different concentrations or ratios of nutrients, such as N, P, and Fe. Additionally, effects of solar UV radiation have not been considered in indoor laboratory experiments due to the common use of UV-free light sources or UV-opaque vessels. Solar UV radiation can be either harmful or beneficial for photosynthetic carbon fixation by phytoplankton assemblages, depending on the incident levels of solar radiation, and its presence definitely raises daily production in coastal water columns (Gao et al. 2007). Experimental tests of the ecological effects of ocean acidification under real sunlight would allow more realistic predictions of future biological processes in a high-CO_2 ocean.

Equally important is the need to assess the potential for algae to adapt in response to selection by the combination of changing pCO_2 and solar radiation exposure. Micro-evolutionary processes have the potential to result in responses to global change factors that differ considerably from those observed in short-term (typically weeks) experiments using phytoplankton that are physiologically acclimated, but not genetically adapted through extended natural selection. Pioneering studies on evolutionary responses of the freshwater green alga *Chlamydomonas* to long-term growth at high CO_2 demonstrated that some adapted cell lines lost CCM capabilities, although evolution of novel traits was not observed (Collins & Bell 2004, Collins et al. 2006). Beaufort et al. (2011) discovered a very heavily calcified coccolithophore morphotype growing in high-CO_2 coastal upwelling waters, suggesting the possibility of local adaptation to low pH. This field study was supported by experimental evolutionary culture studies by Lohbeck et al. (2012), who found that 500 generations of selection at high CO_2 resulted in recovery of coccolithophore growth rates and calcification. However, 100 generations of selection by high CO_2 resulted in no apparent

adaptation or clade selection in the diatom *Thalassiosira pseudonana* (Crawfurd et al. 2011). Obviously, more studies on genetic change in response to ocean acidification are needed, and flexible and reversible epigenetic changes beyond the DNA sequence have not been evaluated at all.

The evolutionary experimental studies cited in the previous paragraph have all addressed the adaptive responses of phytoplankton to increased pCO_2, and at least one study has taken a similar approach to understanding evolution driven by warming (Huertas et al. 2011). It seems reasonable to suppose that larger cell types might be favored if ocean acidification helps relieve diffusion limitation by CO_2, while at the same time warmer temperatures and lower nutrient supplies might exert selection for smaller cell sizes. To date though, no published data exist on evolutionary responses of phytoplankton to multiple simultaneous selection factors, such as elevated CO_2, light, and warming together, and complex adaptive responses to multiple variables are likely to be extremely difficult to rigorously attribute and interpret. Nevertheless, phytoplankton communities of the future will clearly be forced to adapt to many concurrent environmental changes, and so more realistic multi-variate experimental evolution studies are necessary if marine scientists are to accurately predict the trajectory of potential adaptive changes in the ocean biota.

In light of the studies summarized here, we suggest that priorities for future research in this field should include:

(1) Comparative studies of coastal water with open-ocean species or phytoplankton assemblages. Coastal water species might respond to ocean acidification differently from open-ocean species due to their acclimation to fluctuating and diel pH regimes, respectively. In coastal waters, high primary productivity results in day–night reversals of pH changes, that is, pH increases with increasing solar radiation due to photosynthetic CO_2 fixation and decreases with time after sunset due to respiration. Typically, light exposures of coastal phytoplankton are often lower due to near-shore turbidity, while incident visible and UV light penetrate much deeper in clearer offshore waters. Thus, these 2 types of communities may offer valuable lessons about the net effects of changes in both pCO_2 and solar radiation together.

(2) Community-level studies. Community structure changes or dominant species may differ under future lower pH, warmer, and higher light conditions. It is important to explore the involved mechanisms that are responsible for the establishment of 'winners', the species that are superior competitors under future ocean conditions. Studies on the responses of different taxa or different life stages to ocean acidification and altered light and temperature levels are needed that specifically assess their relative competitive abilities under global change scenarios.

(3) Combined effects of multiple stressors or factors, including light, UV, temperature, hypoxia, and eutrophication or nutrient limitation. While more interactive studies of this nature are appearing in the literature, capturing the net responses of marine algae and cyanobacteria to the full spectrum of expected environmental changes remains a daunting logistical challenge for experimentalists and modelers.

(4) Species living at different layers within the euphotic zone experience different light qualities and quantities, and therefore, their physiological responses to ocean acidification should be distinguished. As an example, work comparing the effects of elevated pCO_2 on high-light- and low-light-adapted ecotypes of the cyanobacterium *Prochlorococcus* (Moore et al. 1998) may yield insights into the physiological mechanisms governing interactions between light and ocean acidification. Notably, recent work looking into the effects of elevated pCO_2 under different levels of sunlight on diatoms revealed that different metabolic pathways are involved for the algae to adapt to ocean acidification and increased light exposures (Gao et al. 2012).

(5) For field studies, phytoplankton physiological responses to pH changes in upwelling areas or low pH waters should be studied as a natural analog to ocean acidification. However, upwelling waters are usually also colder and nutrient-rich, whereas much of the future ocean is expected to be warmer and more oligotrophic. Consequently, it is important to keep in mind when interpreting such studies that present-day upwelling regions are not perfect analogs for the future greenhouse ocean. Nevertheless, pH, temperature, and nutrient gradients and mixing depths in upwelling areas are the driving forces for abundance or primary production of phytoplankton, and the interaction of these variables have been little explored.

(6) Long-term adaptation studies. Key phytoplankton species should be grown for thousands of generations, under solar radiation and pCO_2 levels projected in their future natural environments, and their physiological, genetic, and epigenetic responses should be followed. Comparison of adaptation in cells grown under sunlight and constant indoor light would be of interest to evaluate the tremendous number of studies based on indoor experiments.

(7) Molecular methods should be used to examine gene regulation in model organisms and natural phytoplankton communities during acclimation or adaptation to altered pCO_2 and solar radiation, and these transcriptomic studies should be linked to simultaneous studies of adaptive genetic changes and physiological performance.

(8) For macroalgae, effects of fluctuating pH during the diel cycle on their physiological behavior should be investigated, which would enhance understanding of their mechanistic strategies to cope with coastal ocean acidification. At the same time, different life stages of a macroalga's life cycle may respond differently to ocean acidification, so long-term experiments either indoor or in the field should be launched. Some species may experience difficulties in completing their life cycles due to the impact of ocean acidification, such as the red algal genus *Porphyra*, which depends on calcified shells for the conchocelis stage.

(9) Growth as a function of different levels of CO_2 should be examined for different taxa acclimated or adapted to the CO_2 levels. Such a relationship should be examined under sub-, optimal, and supra-saturating light conditions as well as different levels of temperature and/or nutrients.

In conclusion, it is clear that combinations of ocean warming, PAR, UV radiation, and ocean acidification are all likely to interact to influence species competition, community structure, and biogeochemical cycles in the oceans. Biogeography and ecological niches of marine phytoplankton will also shift due to the combined effects of these factors. A full understanding of the ecological and physiological impacts of global change on marine ecosystems will require that future studies do a much better job of addressing the challenge of understanding the individual and combined effects of ocean acidification and solar irradiance across the entire electromagnetic spectrum.

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Influence of ocean warming and acidification on trace metal biogeochemistry

Linn J. Hoffmann[1,2,*], Eike Breitbarth[1,2], Philip W. Boyd[3], Keith A. Hunter[1]

[1]Department of Chemistry, University of Otago, PO Box 56, Dunedin 9054, New Zealand
[2]GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany
[3]National Institute of Water and Atmosphere Centre of Chemical and Physical Oceanography, Department of Chemistry, University of Otago, PO Box 56, Dunedin 9054, New Zealand

ABSTRACT: Rising atmospheric CO_2 concentrations will have profound effects on atmospheric and hydrographic processes, which will ultimately modify the supple and chemistry of trace metals in the ocean. In addition to an increase in sea surface temperatures, higher CO_2 results in a decrease in seawater pH, known as ocean acidification, with implications for inorganic trace metal chemistry. Furthermore, direct or indirect effects of ocean acidification and ocean warming on marine biota will affect trace metal biogeochemistry via alteration of biological trace metal uptake rates and metal binding to organic ligands. We still lack a holistic understanding of the impacts of decreasing seawater pH and rising temperatures on different trace metals and marine biota, which complicates projections into the future. Here, we outline how ocean acidification and ocean warming will influence the inputs and cycling of Fe and other biologically relevant trace metals globally and regionally in high and low latitudes of the future ocean; we discuss uncertainties and highlight essential future research fields.

KEY WORDS: Ocean acidification · Ocean warming · Trace metals

INTRODUCTION

Rising atmospheric CO_2 concentrations affect the marine environment directly via the uptake of CO_2 that acidifies the oceans (Caldeira & Wickett 2003, Doney et al. 2009b) and indirectly via global temperature increase that also results in sea-surface warming. Ocean warming is particularly evident in the Arctic (Wassmann et al. 2011) and in some Antarctic regions (Schofield et al. 2010). Modeling simulations reveal further effects of a changing climate, such as increasing stratification that results in reduced upwelling and wind-driven mixing (Doney 2006), changing wind patterns (Tokinaga et al. 2012), expansion of the areal extent of oxygen minimum zones (Gruber 2011), and changes in the thermohaline circulation (Rahmstorf & Ganopolski 1999, Boyd & Doney 2003).

Although there is a wealth of published information on the importance of trace metals and their inputs and cycling in marine systems (Morel & Price 2003, Boyd & Ellwood 2010), the impacts of rising atmospheric CO_2 on trace metal biogeochemistry are presently difficult to foresee. The role of trace metals for oceanic carbon sequestration, and thus their climate relevance, has received much attention since the seminal work of Martin (1990) and was recently highlighted again by Smetacek et al. (2012). However, investigations into the influence of rising atmospheric CO_2 on marine processes and trace metal biogeochemistry still appear as isolated disciplines. This is puzzling, as pH and temperature are 2 master variables in all chemical and biological processes and therefore intimately link the disciplines of trace metal biogeochemistry, ocean acidification, and sea surface warming.

Dissolved metals in seawater are usually present at low concentrations due to their low solubility (as in the case of Fe) and/or because of adsorption onto particles. Seawater pH and temperature will affect both

*Email: lhoffmann@geomar.de

solubility and adsorption of metals, and thus likely change the dissolved concentrations of metals in the future ocean. The inorganic solubility of Fe is greater in colder waters (Liu & Millero 2002) but also largely depends on the organic content of seawater. Furthermore, increasing temperatures will increase the rates of all chemical reactions, which should increase ferric oxide and hydroxide precipitation as well as oxide aging with negative effects for dissolved Fe concentrations. A clear dependency of metal solubility upon temperature has not been established for many elements, and therefore quantifications of the effect of rising sea surface temperature (SST) on metal solubility in the future ocean are difficult. On the other hand, the expected decrease in seawater pH from pre-industrial 8.25 to 7.85 within this century, and of up to 0.7 units by the year 2300 (Caldeira & Wickett 2003, Jacobson 2005), will significantly affect the inorganic solubility of several trace metals, particularly those forming strong complexes with hydroxide and carbonate ions (Millero et al. 2009). The case of Fe is the most multifaceted and is discussed here briefly. When seawater pH falls below 8, changes in the inorganic speciation result in an increase in the thermodynamic solubility of Fe(III) hydroxide. Based on measurements of Kuma et al. (1996) and Millero (2001), the overall Fe(III) solubility may increase by approximately 460 pmol kg^{-1} as a result of the expected drop in seawater pH from currently 8.10 to 7.85 by the year 2100. In parallel, Fe(II) is much more soluble than Fe(III) but is unstable at current seawater pH due to rapid oxidation. Ocean acidification will slow Fe(II) oxidation rates significantly and thus increase the residence time of Fe(II) (Kuma et al. 1996, Millero 2001, Breitbarth et al. 2010b).

Compared to the well-studied Fe redox reactions in seawater, only a few studies have addressed Cu redox reactions in the field (Moffett & Zika 1987, 1988). As for Fe, a shift in Cu speciation can be expected (Millero et al. 2009). Cu forms strong carbonate complexes, and the decrease in CO_3^{2-} ions due to ocean acidification will result in an increase in the free Cu(II) ion concentration (Millero et al. 2009). In parallel, Cu(II) reduction will increase, while Cu(I) complexation by chloride and reoxidation kinetics by H_2O_2 remain largely unchanged (Millero et al. 1991). It is unclear today whether these changes in the Cu redox cycle will be of biological relevance. In contrast, Cd and Zn are not subjected to a dynamic redox cycle in the surface ocean, and their solubility in seawater is much higher than for Fe(III). The effect of ocean acidification on the inorganic Cd and Zn solubility may thus be negligible.

However, the influence of changing ocean acidity and temperature on trace metal biogeochemistry is more complex than a direct pH/temperature relationship with solubility. Metal solubility is controlled by the interrelationship of inorganic solubility, organic complexation, redox chemistry, and the phytoplankton–trace metal feedback mechanisms (Fig. 1). The majority of the total concentration of bio-active metals such as Fe, Co, Cd, Cu, Ni, Zn, and Pb are not in their inorganic form, but bound to organic complexes. The ligand-bound fraction of metals can be up to 100% for Co (Saito & Moffett 2001, Saito et al. 2005), >99% for Fe and Cu (Sunda & Hanson 1987, Coale & Bruland 1988, Sunda & Huntsman 1991, Rue & Bruland 1995), from 50 to 90 and >98% for Zn (Bruland 1989, Baars & Croot 2011), and >70% for Cd (Bruland 1992). Possible effects of rising oceanic CO_2 concentrations on organic trace metal ligands will therefore play a major role in overall trace metal bioavailability in the future ocean, but only very few studies have addressed this topic so far. Assessments of ocean acidification effects on marine trace metal chemistry are still largely based on theoretical considerations of inorganic and organic metal speciation and therefore tell us little about potential effects on metal uptake, requirements, toxicity thresholds, and possible biological feedback mechanisms (Millero et al. 2009, Breitbarth et al. 2010a).

In the last decade, an enormous amount of published work on the influence of ocean acidification on marine biota has been assembled (Gattuso & Hansson 2011). However, publications concerning the interactions of multiple factors such as seawater pH, temperature, and trace metals are scarce but point towards the significance and complexity thereof (Boyd et al. 2010). For example, it has been demon-

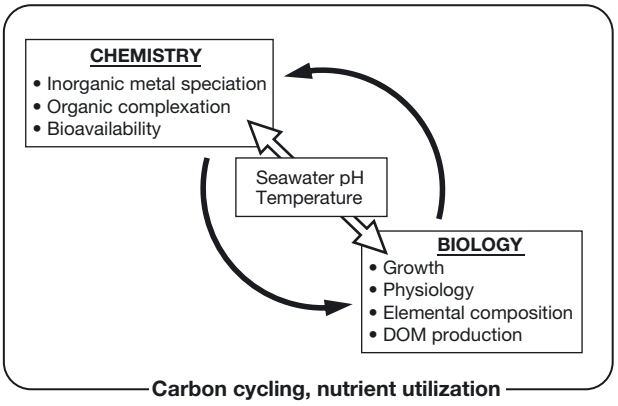

Fig. 1. Predicted relationship between the effects of ocean acidification and warming on trace metal chemistry and phytoplankton biology in seawater

strated that N_2 and CO_2 fixation rates as well as growth of the marine unicellular diazotrophic cyanobacterium *Crocosphaera* only increased with increasing pCO_2 if Fe concentrations were not limiting (Fu et al. 2008).

Temperature and Fe have synergistic effects on phytoplankton growth rates in the Ross Sea (Rose et al. 2009). Moreover, King et al. (2011) state that complex feedback mechanisms between vitamin B_{12} and pCO_2 interactions affect the uptake and metal net use efficiency of Fe, Co, Zn, and Cd by *Atthyea* sp., a subarctic diatom. Under B_{12}-replete conditions and high pCO_2, this diatom grew faster, had lower Fe, Zn, and Cd quotas, and used the metals more efficiently compared to low pCO_2 conditions. Under B_{12} limitation, however, diatom growth rate was much lower and did not change with changing pCO_2. Also, the net use efficiency of Fe, Zn, Co, and Cd decreased with increasing pCO_2 under B_{12} limitation (King et al. 2011). At a higher trophic level, work by Lacoue-Labarthe et al. (2009, 2011, 2012) suggests potential ecotoxicological impacts of trace metals with seawater warming and decreasing seawater pH due to altered permeability of egg shells and the embryonic metabolism in the cephalopod species *Loligo vulgaris* and *Sepia officinalis*. All of these examples demonstrate the need to consider multiple factors when trying to assess the implications of ocean acidification and warming on the marine ecosystem.

The importance of a multivariate approach especially when investigating essential trace elements is founded in Liebig's law of the minimum (Salisbury & Ross 1992). For example, changes in Fe bioavailability may not result in increased phytoplankton growth if another nutrient is ultimately limiting. Likewise, ocean acidification effects on phytoplankton growth and nutrient uptake may be masked if nutrient limitation, which in the open ocean is often governed by Fe, is elevated due to contamination artifacts associated with the experimental set-up. Acknowledging this, some groups now have developed 'trace-metal clean' experimental protocols for ocean acidification work (Hoffmann et al. in press).

It seems that decreasing seawater pH and warming will both influence trace metal–phytoplankton interactions in various ways; however, robust predictions of these interactions are not yet possible. In order to assess the effects of ocean acidification and SST warming on trace metal biogeochemistry in the future ocean, we need to look at how trace metals are supplied, cycled, and lost from the upper ocean. Table 1 summarizes how global change may affect metal input in the future ocean on a global scale, as well as in high- and low-latitude regions. In the following sections, we will discuss the primary factors that control the interplay of temperature, ocean acidification, and trace metals in the ocean and how they might affect marine phytoplankton both globally and regionally.

Table 1. Overview of potential global change effects on trace metal chemistry. TM: trace metal; DOM: dissolved organic matter

	Global				High latitudes			Low latitudes		
Parameter	SST ↑	pH ↓	Precipitation ↕	Wind ↕	Sea ice cover ↓	Land ice Glaciers ↓	Permafrost ↓	Desertification ↑	Riverine input ↓	Coastal upwelling ↑
Metal input			↕ Dry/wet deposition	↕ Aerosol input; vertical mixing	Geographic shift of seasonal deposits from ice	↑ Erosion, riverine TM input (coastal)	↑ Direct and riverine TM input (coastal)	↕ TM input via dust (open ocean)	↓ Riverine TM input (coastal)	↑
Inorganic chemistry	↓ Fe solubility ? solubility of other metals ↑ metal oxidation	↑ Fe solubility ↓ Fe, Cu inorganic complexation ↑ metal reduction	? Possible cloud chemistry effects on metal solubility		Effects on photoreductive metals					
Organic complexation	?	?			?	↑ Erosion, riverine DOM input (coastal)	↑ Direct and riverine DOM input (coastal)		↓ Riverine DOM input (coastal)	

Legend: ↑ increase, ↓ decrease, ↕ regionally dependent, ? unknown effect

GLOBAL EFFECTS OF CLIMATE CHANGE AND OCEAN ACIDIFICATION ON MARINE TRACE METAL SUPPLY AND CYCLING

Metal sources

Climate change is likely to influence global trace metal biogeochemistry by affecting both metal sources and cycling in the future ocean. For surface waters, the main global trace metal inputs are aerosol particles from desert dust (Jickells et al. 2005), anthropogenic sources (Sedwick et al. 2007, Sholkovitz et al. 2009), and volcanic eruptions (Olgun et al. 2011). In coastal regions, riverine inputs and upwelling of trace metal-rich deep water play an additional important role (Boyd 2009). Sediments are a major source of trace metals in shelf waters (e.g. Laës et al. 2007, Ussher et al. 2007), and the 'island wake' effect is of local importance in remote ocean systems (Blain et al. 2007). It has been suggested recently that trace metals from deep-sea hydrothermal vents can potentially reach surface waters via ocean circulation allied to an excess of organic compounds that bind to and stabilize the metals in hydrothermal fluids (Sander & Koschinsky 2011).

Climate change will potentially influence global dust and riverine trace metal inputs into the ocean, but accurate predictions are not yet possible (Boyd & Ellwood 2010). Global dust models predict scenarios that vary from a 60% decrease to a 3.8% increase in dust emissions during the 21st century (Mahowald & Luo 2003, Tegen et al. 2004, Woodward et al. 2005, Stier et al. 2006), making robust projections about future changes in dust as a trace metal source a major challenge. Model predictions suggest an increase in precipitation for the monsoon regimes, over the tropical Pacific, as well as at high latitudes, while a decrease in precipitation is predicted in the subtropics (Meehl et al. 2007). Therefore, riverine trace metal inputs are likely to change significantly on a global scale with large regional differences depending on a range of factors, such as complex interactions of their terrestrial sources, redox processes, and colloid chemistry in estuaries.

The supply of trace metals, in particular from aerosols, will further be influenced by the projected changes in CO_2 and other anthropogenically-influenced gases. Increasing atmospheric concentrations of carbon dioxide, sulfur dioxide, and nitrogen oxides decrease the pH of cloud water and result in acid rain (Badr & Probert 1993, Bogan et al. 2009, Vahedpour & Zolfaghari 2011). The direct contribution of sulfur and nitrogen gases to ocean acidification will be negligible (Hunter et al. 2011), but the effect on atmospheric trace metal inputs could be significant. As in seawater, a lower pH of cloud water results in increased solubility of trace metals from aerosol particles such as desert dust or volcanic ash with potentially significant effects for the net flux of trace metals from these sources to the future ocean (Desboeufs et al. 2001). Mahowald et al. (2009) estimated that the higher acidity in the atmosphere caused by human activities may double the bioavailable Fe in the ocean via increased solubility from desert dust.

On a global scale, model predictions suggest a future increase in precipitation as well as water vapor and evaporation, while regional variations are pronounced (Meehl et al. 2007). Together, altered patterns in wind and precipitation, as well as riverine transport, will ultimately modify the supply of trace metals to the open ocean. The effects of these physicochemical changes on marine productivity and biogeochemical cycling may be profound (Boyd & Doney 2003, Feely et al. 2004, Jickells et al. 2005, Orr et al. 2005).

Inorganic metal speciation

As mentioned above, the inorganic speciation of metals will be altered by ocean warming and ocean acidification. One example thereof is the observed increase in Fe(II) concentrations and Fe(II) half-life times under lower pH in a coastal mesocosm experiment (Breitbarth et al. 2010b). In addition, changes in surface ocean stratification will likely have effects on particle residence times in the euphotic zone. When stratification increases, there is less mixing with deeper waters, which means that particles in the surface mixed layer may reside for longer in this stratum (Doney 2006). This would increase the mean light intensity to which particles in the surface mixed layer are exposed, with possible implications for photochemical metal redox dynamics. For example, increased light and temperature increase Mn oxide dissolution rates (Sunda & Huntsman 1994). Furthermore, the photoreduction of Fe(III) complexes increases under higher light intensities, resulting in higher Fe(II) concentrations (Kuma et al. 1995). In addition, the photosynthetic apparatus of marine phytoplankton is downregulated under higher light intensities, resulting in lower Fe requirements (Sunda & Huntsman 1997, Maldonado et al. 1999, Feng et al. 2010). However, stronger stratification will also lead to increased warming of the surface waters, which may decrease Fe(II) concentrations due to higher

reoxidation rates, as observed in the Gulf of Aquaba (Shaked 2008). Lower Fe requirements for marine phytoplankton, as a result of elevated light intensities, are thus mainly to be expected in colder oceanic regions (Sunda & Huntsman 2011). Likewise, ocean acidification will affect the redox speciation of Cu, yielding a larger Cu(I) fraction (Millero et al. 2009). The potential biological implications thereof are unknown today.

In general, even slight changes in the bioavailability of Fe and Cu may have profound effects for marine ecosystems, as these metals are known to interact with each other. Cu is needed by some marine phytoplankton for sufficient Fe acquisition (Peers et al. 2005, Wells et al. 2005, Maldonado et al. 2006, Annett et al. 2008), and Cu requirements of natural phytoplankton communities increase under Fe limitation (Semeniuk et al. 2009). On the other hand, Cu is a potentially toxic metal whose toxicity to marine phytoplankton is strongly regulated by organic ligand complexation.

Organic metal speciation

Organic trace metal complexation in seawater is controlled by the functional groups within dissolved organic matter, including phytoplankton exudates, siderophores produced by heterotrophic bacteria, and bioremineralization products (Boyd & Ellwood 2010, Breitbarth et al. 2010a). To date, research on trace metal bioavailability has focused on measurements of ligand concentrations and ligand binding strength in natural waters, and metal uptake in controlled laboratory experiments using metal chelators. Studies addressing ocean acidification effects on organic ligand binding are rare. In a first study, Shi et al. (2010) investigated the effect of pH on Fe uptake from 3 Fe-ligand complexes with different functional groups. They found reduced Fe uptake by marine phytoplankton under lower pH in EDTA- and DFOB-buffered media but no pH effect on Fe uptake from the siderophore Fe-Azotochelin. This was expected, as the catechol groups of Azotochelin are protonated in seawater and thus the free Fe concentration should not be affected by changes in seawater pH (Shi et al. 2010). In the case of EDTA, cellular Fe acquisition decreases with decreasing seawater pH as the dissociation of Fe-EDTA (and any chelator with acidic binding groups that are not protonated in seawater) becomes less favored at low pH due to reduced competition with OH^- for Fe chelation, and thus the Fe′ concentration decreases (Stumm & Morgan 1996). However, as acknowledged by Shi et al. (2010), Fe′ uptake from Fe-EDTA is a simplistic scenario in which Fe′ is the only bioavailable form of Fe. Such an approach has limited applications to the open ocean, as natural organic ligands possess a variety of metal binding sites (Barbeau et al. 2003) with different H^+ stoichiometries (Sillén & Martell 1971, Breitbarth et al. 2010a). The observed decrease in Fe uptake from Fe-DFOB under lower pH was not caused by a pH effect on the dissociation of the Fe-DFOB complex, as this is not affected by seawater pH (Shi et al. 2010). Rather, this was most likely due to pH effects on the enzymatic cell surface reduction of the Fe-DFOB complex (Shi et al. 2010). In addition, a substantial body of work points towards the multiplicity of Fe uptake mechanisms in marine phytoplankton, which include models of ligand-bound Fe (FeL) uptake, cell surface reduction of the FeL complex, and direct uptake of Fe(II) (Hutchins et al. 1999, Maldonado & Price 1999, Shaked et al. 2005, Salmon et al. 2006, Morel et al. 2008). Therefore, the diverse range of chemical and biological processes in marine trace metal biogeochemistry, and how they each will be altered by ocean acidification, is likely to be multifaceted and complex.

Short-term (1 to 2 h) Fe uptake experiments with the coastal diatom *Thalassiosira weissflogii* in natural seawater did not show significant differences between different pH/pCO_2 treatments within the same water mass (Shi et al. 2010). However, a body of literature is evolving on physiological effects of ocean acidification on marine phytoplankton (Engel et al. 2004, Doney et al. 2009a, Hutchins et al. 2009, Boyd et al. 2010). Therefore, experiments over periods long enough for phytoplankton to grow could result in significant impacts on organic Fe complexation. Results from a coastal mesocosm CO_2 enrichment experiment show 2 to 3 nM higher dissolved Fe concentrations under lower pH compared to the mid- and high pH treatments (Breitbarth et al. 2010b). This large difference cannot be solely explained by an increased inorganic Fe solubility under lower pH (Millero et al. 2009), and thus pH effects on the organic Fe complexation are one likely explanation next to pH effects on colloid formation, Fe chelation, and Fe hydroxide precipitation (Breitbarth et al. 2010b).

While the effect of ocean acidification on heterotrophic bacterial growth seems to be small (Liu et al. 2010, Weinbauer et al. 2011), bacterial enzymatic activity and polysaccharide degradation increases under lower seawater pH in coastal waters (Grossart et al. 2006, Piontek et al. 2010). Increasing tempera-

tures have been shown to increase bacterial growth, production, and respiration (Vaqué et al. 2009, Kritzberg et al. 2010). The direct effects thereof upon trace metal bioavailability have not been assessed at present, but it is possible that these mechanisms will also affect the siderophore production by heterotrophic bacteria with consequent implications for Fe bioavailability in the future ocean. Moreover, changes in organic complexation, either via shifts in ligand: H^+/OH^- stoichiometry or as an effect of ligand concentration changes, should also affect Fe retention rates in surface waters (Sunda 2010).

The Fe(III)-binding groups of marine siderophores can be hydroxamate, catecholate, or α-hydroxy carboxylate moieties (Barbeau et al. 2003). While hydroxamate and catecholate groups are photochemically resistant when bound to Fe(III), α-hydroxy carboxylate groups undergo light-induced ligand oxidation and reduction of Fe(III) to Fe(II) (Barbeau et al. 2001, 2003). Increased mean light intensities in the predicted future shallower surface mixed layers (Doney 2006) would thus enhance the photolysis of some organic Fe(III) complexes and increase reactive Fe(II) in surface seawater. Cu-binding ligands differ from Fe-binding ligands in that they can be directly produced by some marine phytoplankton species when exposed to higher concentrations of Cu (and other toxic metals such as Cd and Zn) (Ahner & Morel 1995, Moffett & Brand 1996, Croot et al. 2000, Ahner et al. 2002, Dupont & Ahner 2005). Thereby, the ligand can be released into the water and bind Cu (or other toxic metals such as Cd and Zn) extracellularly (Moffett & Brand 1996, Croot et al. 2000, Dupont & Ahner 2005), or the metals are taken up into the cell and detoxified by intracellular binding to the ligand (Ahner et al. 2002, Dupont et al. 2004). These thiols are low molecular weight sulfhydryl-containing compounds such as glutathione and phytochelatin. Both glutathione and phytochelatin bind Cd, Cu, and Pb via sulfhydroxyl coordination (Rabenstein 1989, Strasdeit et al. 1991). Louis et al. (2009) described a decrease in the interaction between organic ligands and Cu(II) when seawater pH fell below 8. As a result, the inorganic Cu fraction increased with decreasing pH, similar to what was described earlier for fresh waters (Averyt et al. 2004). It is unknown so far whether Cu ligand production will be affected by ocean acidification and warming and what the effects for Cu toxicity would be. Intracellular Cu binding is unlikely to be directly affected by surrounding seawater pH but could possibly be indirectly affected via ocean acidification and warming effects on phytoplankton physiology.

A recent study described the effect of pH on the uptake of Zn and Cd in marine phytoplankton (Xu et al. 2012). Here, short-term (3 to 4 h) Zn and Cd uptake in natural phytoplankton assemblages decreased with decreasing pH in contrast to experiments with single metal chelators in the laboratory. The authors concluded that in natural systems, Zn and Cd bioavailability is lower under lower pH, which they explained by the potential interaction of strong and weak ligands in natural waters. These results illustrate that care must be taken when extrapolating results from laboratory experiments using artificial metal chelators to the field.

For future research, the interplay between SST, pH, and dissolved organic matter (DOM) content of seawater and their effects on metal bioavailability needs to be established in order to better understand the effect for metal uptake by marine phytoplankton at a global level. In the context of ocean acidification and ocean warming effects on trace metal biogeochemistry, it is important to establish a holistic view of the many facets of trace metal chemistry. Future predictions of the nature of trace metal–phytoplankton interactions are difficult, as most studies have so far focused on Fe and neglected possible impacts of other metals. Because of growing evidence that the interplay of a particular metal with other metals, as opposed to the bioavailable concentration of one metal alone, will determine its biological impact (Sunda & Huntsman 1983, 1996, 2000, Peers et al. 2005, Maldonado et al. 2006), we have to move away from the dominant focus on Fe as a sole controlling trace metal in marine biogeochemistry. In addition to the importance of Cu for Fe uptake, the interplay between Cd, Fe, and Zn might have important ramifications with regard to the effects of ocean acidification. Zn is needed in the enzyme carbonic anhydrase in marine phytoplankton and can be substituted by Cd and/or Co under Zn limitation (Price & Morel 1990). Low Zn concentrations have been shown to decrease HCO_3^- uptake and thus limit phytoplankton growth (Morel et al. 1994, Buitenhuis et al. 2003). Higher pCO_2 in a more acidic ocean should lower the Zn requirements of marine phytoplankton and would thus also lower the need for substitution by Cd (Cullen et al. 1999).

The 'kink' in the Cd:PO_4 relationship at intermediate PO_4 concentrations (~1.3 µmol kg^{-1}) and deviations thereof are also associated with Fe chemistry (Cullen 2006). Experiments have shown that Cd interferes with the uptake of Fe(II) and that the Cd:C and Cd:P ratios increase under Fe limitation and decrease with increasing Fe (Cullen et al. 2003, Lane et

al. 2008, 2009). It is not clear, though, how important phylogenetic differences in the Cd uptake are in this context (Quigg et al. 2003), or what the interrelationship of Cd, Fe, and Zn in a warmer, more acidic ocean may be. Since changes in the biogeochemistry of Fe and Zn may likewise affect Cd chemistry in seawater, this also complicates the use of Cd as a tracer for past seawater nutrient concentrations (Cullen 2006). Furthermore, Cd and Zn appear to compete for the same ligands in fresh water (Sander et al. 2007); however, investigations on this topic are lacking for seawater.

In summary, ocean acidification and ocean warming will ultimately alter metal biogeochemistry on multiple levels, from affecting trace metal sources, via primary chemical effects on inorganic trace metal speciation, through to physiological effects on microbial cellular metal acquisition, and finally as a product of potential biological feedback mechanisms of heterotrophic bacteria and phytoplankton ecophysiology and altered community structure.

INTERPLAY OF TRACE METAL SUPPLY, OCEAN ACIDIFICATION, AND TEMPERATURE AT HIGH LATITUDES

The variability in the projections from recent global dust and precipitation model simulations points to the need for a more regional view to better identify future changes in trace metal supply. In polar regions, the main sources of trace metals to the ocean are aerosol deposition and ice melting, as well as riverine input in the Arctic (Wagener et al. 2008, Shaw et al. 2011, Boyd et al. 2012, Klunder et al. 2012; Table 1). Asian dust fluxes that can reach the North Pacific are expected to decrease markedly in the future (Tsunematsu et al. 2011), but as mentioned earlier, these fluxes are difficult to predict on a basin scale because of regional differences in soil moisture and vegetation (Ravi et al. 2011).

Ocean acidification and warming have already resulted in distinct changes of the polar marine ecosystem. Sea ice cover and the areal extent of land ice glaciers have decreased significantly in recent years both in the Arctic and Antarctic (Anisimov et al. 2007, Perovich & Richter-Menge 2009, Comiso 2012), and this trend is expected to increase in the near future. Further, in the Arctic Ocean, increasing pCO_2 concentrations and sea ice melting have already resulted in aragonite undersaturation (Yamamoto-Kawai et al. 2009).

The result of rapid ice melting in both regions is an increased input of fresh water, which, together with an increase in SST, will enhance stratification at high latitudes and reduce the exchange with nutrient- and trace metal-rich deeper waters with impacts on deep-water formation and the thermohaline circulation (Marsland et al. 2007, Wassmann & Reigstad 2011). An additional consequence of melting ice in both polar regions is an effect on the light climate of the mixed layer. A reduction in Arctic sea ice cover strongly increases the light penetration into surface waters and thus will influence the photochemical redox-processing of trace metals. In Antarctic waters, where light limitation is mainly caused by deep mixing, the effects will probably not be as pronounced as in the Arctic. However, Boyd et al. (2008) predicted a shoaling of mixed layer depths in the Southern Ocean, which will also increase the mean light intensity in surface waters. In the case of Fe, increased photochemical Fe(III) reduction will lead to a larger pool of Fe(II) supported by a higher residence time due to slower oxidation rates at lower pH and cold temperatures (Kuma et al. 1995, Sunda & Huntsman 2003).

A further consequence of increased melting of drifting Arctic sea ice, which is formed in the shallow coastal zones and contains entrained sediments, may be a major input of Fe, other trace metals, and terrestrial organic matter to offshore waters of the Arctic Ocean. It is suggested that Fe incorporated in sea ice and subsequently released with meltwaters may also contribute to observed intense ice edge blooms (Hölemann et al. 1999, Measures 1999, Fitzwater et al. 2000). Similar processes have been described for the Antarctic (van der Merwe et al. 2011), and it has been suggested that particularly the role of icebergs for metal supply to the open ocean may increase with the decline of Antarctic ice shelves (Lin et al. 2011). However, Lannuzel et al. (2011) suggested that seawater is the main source for metal accumulation in Antarctic sea ice and that seasonal melt mainly affects Fe input into seawater. Hendry et al. (2008) showed increased Cd in coastal Antarctic waters from terrestrial and continental shelf sediments with implications for the local $Cd:PO_4$ ratio. Their results suggest that sea ice cover influences the metal content of Antarctic deep water.

An important factor that will most likely influence the organic trace metal complexation in the Arctic Ocean is permafrost melting. A significant reduction of the permafrost regions around the Arctic Ocean is expected by the end of this century (Delisle 2007, Lawrence et al. 2008). In its 2007 report, the Intergovernmental Panel on Climate Change emphasized that '[t]he most sensitive regions of permafrost degrada-

tion are coasts with ice-bearing permafrost that are exposed to the Arctic Ocean' (Lemke et al. 2007, p. 372). However, no information about potential effects of meltwater runoff on marine ecosystems is given. Permafrost melting will introduce nutrients, organic substances, and trace metals from land into the sea in a manner that is comparable to spring snowmelt (Rember & Trefry 2004). As the Arctic Ocean is N limited as a result of denitrification in the Pacific Ocean (Yamamoto-Kawai et al. 2006), the input of nitrate will likely have a significant effect on Arctic primary productivity. Further, an increased input of organic substances could increase or decrease the bioavailability of trace metals in the Arctic Ocean via changes in their organic complexation. This could further affect the productivity of coastal ecosystems and thus may have serious consequences for the arctic marine biogeochemistry. Arrigo & van Dijken (2011) reported that the total annual net primary production (NPP) in the Arctic increased by 20% between 1998 and 2009, and the authors concluded that this was mainly caused by a reduction in sea ice cover and a subsequent increase in light intensity. The possibility that at least part of this increase in NPP could also be influenced by an increase in metal supply was not discussed, but the authors stated that: '[n]utrient fluxes into Arctic surface waters need to be better understood to determine if these projected increases are sustainable' (Arrigo & van Dijken 2011, p. 1).

Trace metal cycling is further strongly affected by the metal uptake and downward export of biota. Climate-change driven alterations in polar phytoplankton and bacterial species composition as reported by Tortell et al. (2008) and productivity might therefore have significant implications for marine trace metal cycling in the future. Phytoplankton growth is limited by Fe supply (and light) in the Southern Ocean and the subarctic Pacific. When Fe is not limiting, temperature can have a significant additional effect on diatom growth (Rose et al. 2009). Climate-related changes in the bioavailability of Fe (and other trace metals) as discussed above might therefore have strong implications for the phytoplankton community at high latitudes, especially in combination with increasing SST.

INTERPLAY OF TRACE METAL SUPPLY, OCEAN ACIDIFICATION, AND TEMPERATURE AT LOW LATITUDES

At low latitudes, coastal upwelling, aerosol deposition, and riverine input are the major sources of trace metals to the surface oceans (Landry et al. 1997, Mackey et al. 2002, Mahowald et al. 2005, Tovar-Sanchez et al. 2006). Riverine trace metal inputs are likely to change significantly as a result of climate change-driven effects on rainfall in these areas. In general, models predict a decrease in rainfall in subtropical regions and an increase in some equatorial regions of east Africa and Asia (Meehl et al. 2007).

Coastal upwelling brings nutrient- and trace metal-rich deep water to the surface ocean at low latitudes. Due to climate change, upwelling events are expected to become less frequent but stronger and longer in duration (Bakun 1990, Iles et al. 2012). Temperature and seawater pH shifts are expected to be less extreme at low latitudes versus high latitudes (Gruber 2011). Therefore, ocean acidification and ocean warming effects on the inorganic metal solubility are likely to be less important at low latitudes. On the other hand, the areal extent of oxygen minimum zones at high and low latitudes has already expanded (Whitney et al. 2007, Stramma et al. 2008). Future modeling predictions indicate that this process will be more pronounced in the low-oxygen regions at low latitudes (Gruber 2011), which will play an important role for nutrient and metal cycling in these regions. Oxygen minimum zones harbor a large amount of Fe in its reduced form Fe(II) that would otherwise rapidly precipitate as Fe(III) and thus get lost from the upper ocean Fe cycle (Fig. 2). Oxygen minimum zones could be a source of Fe(II) for surface waters as discussed for the Baltic Sea (Breitbarth et al. 2009). Paralleled by their role for P and Mn cycling (Turnewitsch & Pohl 2010), the regional importance of oceanic oxygen minimum zones for Fe cycling could therefore increase in the future. Similar to Fe, Cu reduction is higher under low oxygen conditions, and therefore Cu(I) concentrations could be higher here.

The reduced freshwater input into the future oceans in subtropical regions will ultimately also reduce the amount of DOM that is transported to the oceans. Therefore, organic trace metal complexation may decrease here. Whether phytoplankton/bacterial feedback mechanisms could counteract this, e.g. by an increased production of metal-binding ligands, remains speculative, but these mechanisms could play a role if the bioavailable fraction of essential metals becomes limiting.

A reduced input of DOM could also reduce the substrate availability for marine bacteria and subsequently their trace metal ligand production. On the other hand, ocean acidification has been shown to increase the activity of some microbial enzymes, which might result in enhanced polysaccharide pro-

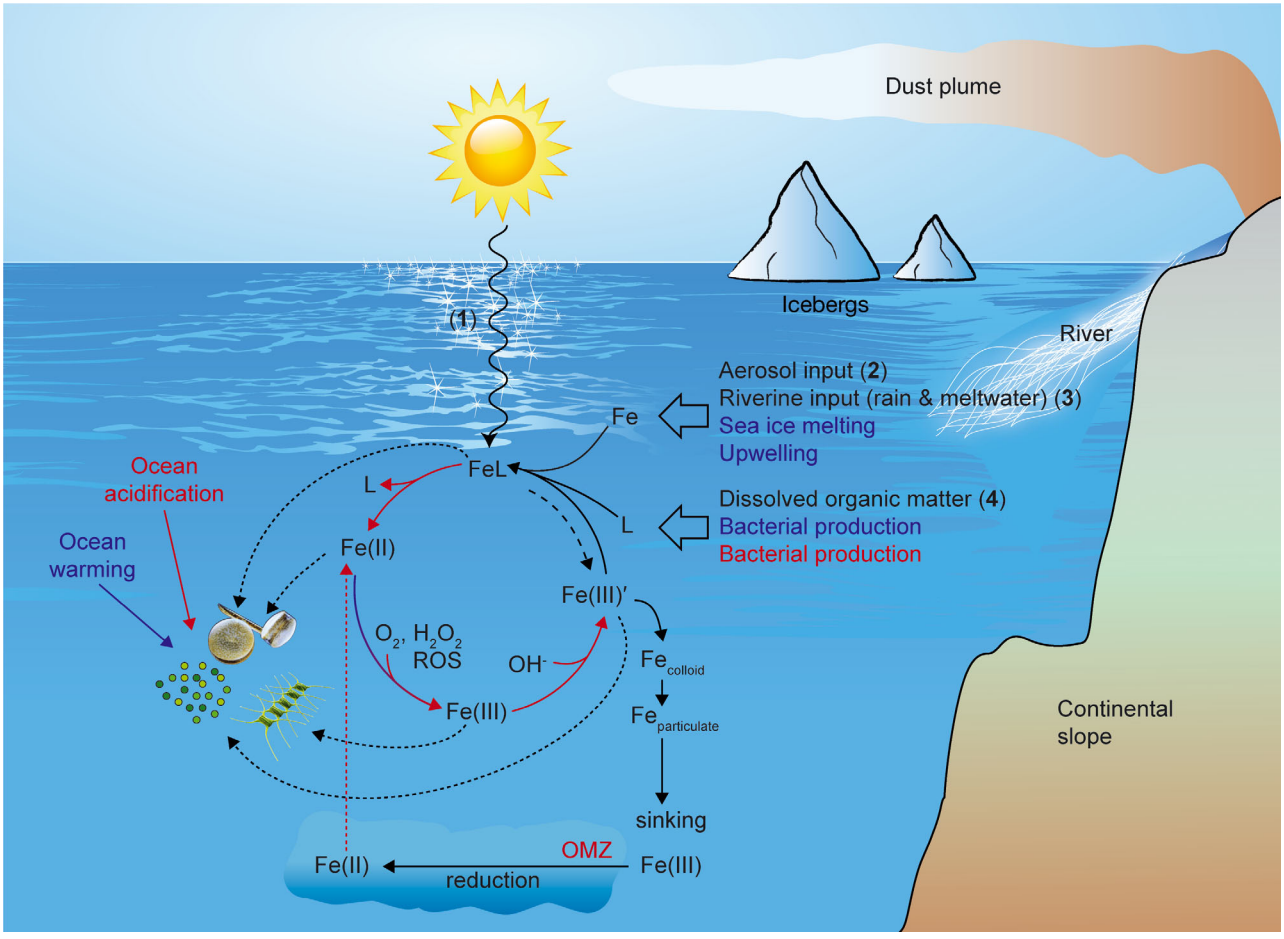

Fig. 2. Direct effects of ocean acidification (red) and ocean warming (blue) on Fe chemistry in seawater. Ocean acidification and ocean warming both influence phytoplankton and bacterial physiology with possible effects for biological Fe uptake and ligand production. Further climate change factors that may influence the marine Fe cycle are (1) changes in stratification and the effects thereof upon light climate, (2) changes in the input of dissolved organic matter, (3) changes in the input of Fe from aerosol particles, and (4) riverine inputs. FeL: ligand-bound iron; L: ligand; Fe(III)′: sum of all inorganic Fe(III) species; OMZ: oxygen minimum zone; ROS: reactive oxygen species. Iron cycle redrawn after Sunda (2001), Croot et al. (2005), and Breitbarth et al. (2009)

cessing in the future surface ocean (Piontek et al. 2010). However, effects of ocean acidification for marine microorganisms are often contradictory (Weinbauer et al. 2011), and implications for future trace metal cycling are thus difficult to predict.

At low latitudes, diazotrophic phytoplankton are a major group within the phytoplankton community (Boyd et al. 2010). Both carbon and nitrogen fixation rates of the dominant nitrogen-fixing cyanobacterium *Trichodesmium* are higher under lower seawater pH (Barcelos e Ramos et al. 2007, Hutchins et al. 2007, 2009, Levitan et al. 2007). Increasing growth rates will further increase the cellular Fe demands of nitrogen fixers, which are already higher compared to other phytoplankton groups (Kustka et al. 2003). This could result in a further increase in Fe limitation of N_2 fixation in the future ocean. This is supported by observations that nitrogen fixation rates do not increase under high pCO_2 when Fe concentrations are depleted (Fu et al. 2008, Law et al. 2012).

Overall, increased upwelling, changes in dust deposition, and reduced riverine input will expose marine ecosystems at low latitudes to a multitude of environmental stressors in the future. Today, it is impossible to predict how these will influence the trace metal budget of these waters, but their implications for marine productivity are highly likely.

CONCLUSIONS

Understanding the potential effects of global change on trace metal biogeochemistry requires an integrated, multidisciplinary approach combining tra-

ditionally segregated fields of geochemistry, physical oceanography, biology, and marine trace metal chemistry. The abiotic effects of ocean acidification and warming cascade into interwoven basic processes of marine biogeochemistry which need to be addressed individually as well as on a system level. These are as follows: (1) seawater pH and temperature effects on inorganic trace metal complexation, redox reactions, precipitation, and oxide aging; (2) seawater pH and temperature effects on organic metal complexation and redox cycling; (3) seawater pH and temperature effects on microbial community composition, growth, organic ligand production, phytoplankton metal uptake, requirements, and toxicity thresholds; (4) changing bioavailability of trace metals to marine primary producers as a result of (1), (2), and (3); and (5) potential biological feedback mechanisms on all of the above (Fig. 1).

We have illustrated how several environmental factors at high versus low latitudes may affect trace metal biogeochemistry in the future and how large the uncertainties of these estimations still are. However, studying these aspects results in significant methodological challenges. While recent advances in multi-element trace metal analytical protocols allow for a much improved sample throughput with excellent accuracy and precision (Milne et al. 2010, Biller & Bruland 2012), detailed metal speciation studies still require manual titrations of the metal of interest versus a competing ligand added to the seawater sample. Some working groups have developed automated protocols (S. Sander pers. comm.), which represent a great advance for sample processing of the essential organic speciation measurements. However, trace metal concentration and speciation methods still have in common the approach that the analytical solution is buffered to a standard measurement pH and measurements are performed at room temperature, making their application for ocean acidification research difficult. Refined protocols are thus needed to determine metal–organic interactions at different seawater pH and temperatures.

During its emerging phase, the ocean acidification research field was hindered by a lack of consensus on methodological issues, which has now been overcome by a community agreement in the form of a 'Guide to best practices for ocean acidification research and data reporting' (Riebesell et al. 2010). It would be unfortunate if the evolution of the interdisciplinary ocean acidification field (or in a broader context, multiple ocean change effects) and trace metal biogeochemistry, have to undergo a similarly redundant process. As an example, while performing constant pH monitoring during a trace-metal-clean phytoplankton incubation experiment, we observed that the acid cleaning treatment of the incubation bottles had lowered the seawater pH by ~0.2 units in the control treatment (Hoffmann et al. in press). A bottle pre-conditioning step with seawater was required, even though the material (LDPE) was rigorously rinsed with purified water after the last acid cleaning step. This effect likely went unnoticed and provided an accidental ocean acidification treatment in many previous trace metal clean seawater incubations. A full characterization of the seawater carbonate system throughout experiments is advised in the aforementioned guide (Riebesell et al. 2010). A community-based agreement for interdisciplinary ocean acidification–trace metal biogeochemical research that adapts this guide is needed to define suitable standards for future studies.

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Global change and the future of harmful algal blooms in the ocean

Fei Xue Fu*, Avery O. Tatters, David A. Hutchins

The University of Southern California, Department of Biological Sciences, 3616 Trousdale Parkway, Los Angeles, California 90089, USA

ABSTRACT: The frequency and intensity of harmful algal blooms (HABs) and phytoplankton community shifts toward toxic species have increased worldwide. Although most research has focused on eutrophication as the cause of this trend, many other global- and regional-scale anthropogenic influences may also play a role. Ocean acidification (high pCO_2/low pH), greenhouse warming, shifts in nutrient availability, ratios, and speciation, changing exposure to solar irradiance, and altered salinity all have the potential to profoundly affect the growth and toxicity of these phytoplankton. Except for ocean acidification, the effects of these individual factors on harmful algae have been studied extensively. In this review, we summarize our understanding of the influence of each of these single factors on the physiological properties of important marine HAB groups. We then examine the much more limited literature on how rising CO_2 together with these other concurrent environmental changes may affect these organisms, including what is possibly the most critical property of many species: toxin production. New work with several diatom and dinoflagellate species suggests that ocean acidification combined with nutrient limitation or temperature changes may dramatically increase the toxicity of some harmful groups. This observation underscores the need for more in-depth consideration of poorly understood interactions between multiple global change variables on HAB physiology and ecology. A key limitation of global change experiments is that they typically span only a few algal generations, making it difficult to predict whether they reflect likely future decadal- or century-scale trends. We conclude by calling for thoughtfully designed experiments and observations that include adequate consideration of complex multivariate interactive effects on the long-term responses of HABs to a rapidly changing future marine environment.

KEY WORDS: Climate change · CO_2 · Ocean acidification · Temperature · Stratification · Nutrient limitation · HAB · Algal toxins · Phycotoxins

INTRODUCTION

Anthropogenic eutrophication has been linked to worldwide increases in harmful algal bloom (HAB) frequency and intensity in recent decades (Honjo 1993, Anderson et al. 2002, Glibert et al. 2005, Hallegraeff 2010). In response to these observations, a great deal of research has focused on the influence of changing nutrient availability on algal bloom establishment and growth. Eutrophication, however, is only one of multiple global anthropogenic biogeochemical impacts.

In addition to disturbance of natural nutrient cycles, humans are also causing a massive perturbation of the global carbon cycle. The atmospheric partial pressure of CO_2 (pCO_2) has risen by >30% due to the burning of fossil fuels, deforestation, industrialization, and cement production (IPCC 2007). These already elevated current CO_2 levels will approximately double from ~385 to 750–800 ppm by 2100, and ocean pH will consequently decrease by as much as 0.77 units over the next several hundred years (Caldeira & Wickett 2003), with unknown consequences for many pH-sensitive marine organisms

*Email: ffu@usc.edu

(Royal Society 2005, Orr et al. 2005, Hoegh-Guldberg & Bruno 2010). We know that ocean acidification can have potentially far-reaching consequences for the physiology of many algal groups, including altered growth and carbon fixation rates, shifts in nutrient uptake, changes in elemental ratios, and increased sensitivity to ultraviolet radiation (Riebesell 2004, Fu et al. 2007, 2008a,b, 2010, Feng et al. 2008, 2009, 2010, Hutchins et al. 2007, 2009, Riebesell et al. 2007, 2008, Rost et al. 2008, Beardall et al. 2009a, Gao et al. 2012a, this Theme Section). These physiological responses may be reflected at the ecosystem level through changes in algal competitive interactions, ecological dominance, and overall community structure (Tortell et al. 2002, Riebesell 2004, Hare et al. 2007, Feng et al. 2009, 2010).

Primary producers including HAB species must adjust not only to altered seawater carbonate chemistry, but also to numerous other concurrent environmental changes. Over the next 50 to 100 yr, greenhouse warming will increase average sea surface temperatures by as much as 5°C, and increased precipitation, runoff, and ice melting will lower surface salinities in many parts of the ocean (Bopp et al. 2001, Sarmiento et al. 2002). The combined influence of warming and freshening on the density of seawater will cause much of the surface ocean to become more stratified, driving fundamental shifts in key biological variables such as nutrient supplies and light exposure regimes (Boyd & Doney 2003, Boyd et al. 2008, 2010, Cermeño et al. 2008, Hutchins et al. 2009). As a consequence of these environmental changes, marine ecosystems all over the world are currently changing at an alarming rate. Long-term data sets from around the world suggest that ongoing changes in coastal and estuarine phytoplankton communities are likely due to the combination of climate shifts and other anthropogenic influences (Edwards et al. 2006, Smetacek & Cloern 2008).

Only a few studies to date have directly addressed the implications of ocean global change for HAB species, and most of these have considered the effects of warming (Peperzak 2003, 2005, Cloern et al. 2005, Moore et al. 2008, 2009, Paerl & Huisman 2008). However, it is virtually certain that harmful blooms of the future will also be simultaneously affected by interactions with the complex network of other changing environmental variables discussed above. Recent evidence demonstrates that some coastal ecosystems and estuaries are already experiencing significant levels of anthropogenic acidification (Feely et al. 2008, Cai et al. 2011). Since HABs often occur in these types of ecosystems, there is an urgent need to investigate how they will respond to changing CO_2 and/or pH both alone and in combination with other variables. Accurately predicting the responses of HABs to these many interacting anthropogenic changes is a top priority for everyone who must deal with the negative impacts of toxic algal blooms, including marine resource managers, policy makers, governmental management agencies, and marine resource users such as the seafood harvesting and aquaculture industries. The goal of this review is to summarize the effects of rising pCO_2 in concert with other global change factors on the physiological and ecological responses these organisms.

Our review is intended to expand on the excellent recent review of HABs and global change by Hallegraeff (2010), by focusing on how we may apply the results of a large body of prior work on environmental perturbation effects on HABs to understand their responses to a rapidly changing ocean. Equally importantly, we also emphasize the results of new experiments specifically targeting global change effects on HABs in this rapidly expanding field. First, we briefly review the relevant literature on the effects of individual global change-relevant variables on growth and toxicity, including nutrients, temperature, solar radiation, and salinity. In the final sections of our review, we cover the limited but particularly important body of HAB-related research examining multivariate interactions between these environmental factors and CO_2-driven ocean acidification.

EFFECTS OF INDIVIDUAL GLOBAL CHANGE FACTORS ON HARMFUL ALGAE

pCO_2/pH

Despite the extensive recent research effort that has been directed toward understanding ocean acidification effects on diverse marine organisms, only a handful of studies have so far addressed how CO_2 or pH changes affect HAB physiology and toxicity. Some bloom-forming dinoflagellates may especially benefit from higher pCO_2, due in part to their CO_2-fixing enzyme, a type II Rubisco (ribulose-1,5-bisphosphate carboxylase-oxygenase; Tortell 2000, Rost et al. 2003). Type II Rubisco has a low affinity for carboxylation and is therefore extremely inefficient at processing CO_2 at present-day atmospheric concentrations, compared to the type I Rubisco found in most other algae. Dinoflagellates overcome this limitation, in part, by compartmentalizing Rubisco within the chloroplast to avoid photorespiration (Jenks &

Gibbs 2000, Nassoury et al. 2001). This low-affinity CO_2-fixing system may also be compensated for by efficient carbon-concentrating mechanisms (CCMs) such as various forms of carbonic anhydrase (CA), which allow algae to access the much more abundant pool of HCO_3^- in seawater. These adaptations allow some dinoflagellates to grow rapidly at present day pCO_2 levels, and it is unknown for most species whether elevated CO_2 will enhance growth further by offsetting the physiological constraints of their type II Rubisco.

Dason et al. (2004) showed that the marine dinoflagellates *Amphidinium carterae* and *Heterocapsa oceanica* do not possess an external CA, and thus their photosynthesis is dependent on free CO_2 alone. Consequently, the growth of these 2 species is suggested to be CO_2-limited (Colman et al. 2002, Dason et al. 2004), and their growth could potentially be stimulated by increasing CO_2 concentrations in the future ocean. In contrast, the growth rates of 3 other marine dinoflagellates (*Prorocentrum minimum*, *H. triquetra*, and *Ceratium lineatum*) are most likely not limited by dissolved inorganic carbon, since they preferentially take up HCO_3^- instead of CO_2 to support photosynthesis (Rost et al. 2006). This observation is supported by the finding that increasing CO_2 does not significantly affect the growth rate of another isolate of *P. minimum* (Fu et al. 2008a).

Because phycotoxin biosynthesis is directly linked to the autotrophic metabolism of most HAB species, it is perhaps not entirely surprising to find that changing CO_2 availability can also affect cellular toxicity. Photosynthesis is not only the essential process in primary metabolism, but is also required for toxin production (Pan et al. 1996a). For example, the yield of saxitoxin per cell in the dinoflagellate *Alexandrium catenella* is proportional to hours of daylight (Proctor et al. 1975). Also, *A. minutum* is not capable of producing saxitoxin after a 22 d incubation period in the dark, while parallel light-grown cultures produced 1.17 µg per 10 000 algal cells (Maas & Brooks 2010).

pCO_2 and/or pH changes affect toxicity of the diatom genus *Pseudo-nitzschia*, which causes notoriously damaging blooms along the Pacific coast of North America and elsewhere around the world (Scholin et al. 2000, Trainer et al. 2000, 2009, Schnetzer et al. 2007). Two recent studies have examined the influence of seawater pH on the toxicity of cultures of *Pseudo-nitzschia* spp.: Sun et al. (2011) and Tatters et al. (2012) found that domoic acid concentrations increase dramatically in treatments combining high pCO_2/low pH (adjusted by bubbling the seawater with CO_2-enriched air) with nutrient limitation. The authors speculated that pCO_2-induced domoic acid production is perhaps a consequence of an excess in carbon supply when elevated CO_2 occurs together with nutrient-limited growth conditions. Interestingly though, 2 previous studies found results that differ from these 2 recent studies, in that domoic acid levels increased instead at higher pHs (e.g. lower pCO_2; Lundholm et al. 2004, Trimborn et al. 2008). It is worth noting that unlike the 2 more recent studies, in these earlier experiments pH was adjusted by HCl and NaOH addition rather than by CO_2 bubbling. The 2 recent multivariate studies, and possible reasons for these apparently contradictory results, are considered further in the 'Interactive effects of CO_2 and nutrients' section below. Despite the differences in their findings, all 4 of these studies support the suggestion that pCO_2/pH can strongly influence the production of domoic acid by this globally distributed diatom genus.

The impact of elevated CO_2 on the growth of CCM-utilizing diatoms versus algal species without a CCM (Riebesell 2004) suggests that those species which lack CA will likely benefit most from rising CO_2 levels. Notably, the raphidophyte *Heterosigma akashiwo* does not appear to use CA (Nimer et al. 1997), suggesting that it may be especially favored by rising CO_2 levels. In fact, the growth of *H. akashiwo* is significantly stimulated by increasing CO_2, again achieved by bubbling the seawater with air/CO_2 mixtures (Fu et al. 2008a). However, this finding may not apply to all raphidophytes. For instance, the growth of *Chattonella marina* is not affected by pH over a range from 7.5 to 8.5 (adjusted by acid and base additions), although growth greatly decreases at pH values over 9.0 (Liu et al. 2007). Coupled with these reduced growth rates, rates of ichthyotoxic reactive oxygen species (ROS) production by *C. marina* also increase at this elevated pH, but remain stable within the pH range of 7.5 to 8.5. Liu et al. (2007) suggested that high pH may enhance the activities of enzymes that regulate ROS production, and/or that high pH may reduce iron bioavailability to the algae.

The prymnesiophyte *Phaeocystis globosa* can form massive harmful blooms in temperate areas such as the North Sea. Recent evidence suggests that its physiological responses to changing pCO_2 may be dependent on its polymorphic life history, which alternates between solitary flagellated cells and colonies composed of numerous cells embedded in a gelatinous matrix. Wang et al. (2010) demonstrated that bubbling *P. globosa* cultures with elevated CO_2

stimulates the formation and growth rates of colonies, but the growth rates of solitary cells are unchanged. Based on the observed increases in colony formation, these authors suggest that future rising CO_2 may affect carbon and sulfur cycles as well as marine trophic structure both locally and regionally. However, a natural assemblage of the closely related polar species *P. antarctica* is relatively unaffected by extended incubation at elevated pCO_2 (Feng et al. 2010).

This short list summarizes the published studies on HAB species responses to ocean acidification in isolation; these experiments and a few others examining rising pCO_2 in combination with other variables are summarized in Table 1 and are reviewed below. This surprising paucity of information on high pCO_2/low pH effects needs to be remedied by further studies using a variety of environmentally relevant species, thus there is likely to be new information available on this subject within the next few years.

Temperature

Temperature is probably the most widely recognized component of climate change and also plays a crucial role in determining potential algal growth rates. Consequently, temperature can influence community dynamics of harmful bloom species relative to their competitors and grazers. In diatoms, for example, nitrate uptake and reduction decline rapidly at elevated temperatures (Lomas & Glibert 1999), potentially favoring competing algae. Likewise, temperature can differentially impact the growth rate, pigment content, light-harvesting capacity, and photosynthetic carbon fixation of many microalgae (Sosik & Mitchell 1994, Coles & Jones 2000, Anning et al. 2001, Stramski et al. 2002).

Increasing sea surface temperatures are already leading to prolonged and more intense temperatures during bloom seasons (Peperzak 2003, 2005, Edwards et al. 2006, Hallegraeff 2010, Paerl & Scott 2010), and this trend is likely to continue with the potential for establishment of temporally and spatially expanded bloom windows (Fig. 1; Moore et al. 2008). Many HABs have a window of temperature that is reached and often exceeded within a given year (Gobler et al. 2005, Moore et al. 2008). Therefore, in some cases increasing temperature may not intensify HABs throughout the growing season, but perhaps instead change the timing of their initiation and termination during the annual seasonal cycle. Warm water temperatures, calm conditions, and accompanying stratification seem to promote the proliferation of many microalgae, including several harmful species (Paerl & Scott 2010). Cellular toxicity can also be sensitive to rising temperature. For instance, cultures and field samples of *Karlodinium veneficum* exhibit increased cellular toxicity at temperatures >25°C (Kempton et al. 2002, Adolf et al. 2009).

The relationship between HABs and warming is not always straightforward. As toxic diatoms of the genus *Pseudo-nitzschia* typically respond to seasonal patterns, temperature is likely a critical driver in their bloom development. Depending on geographical region, seasonal blooms have been correlated with pulses of cool, nutrient-rich upwelled water (Horner et. al 1997, Trainer et al. 2002, Kudela et al. 2010), and also with warmer, stratified conditions (Bird & Wright 1989, Buck et al. 1992, Horner et al. 1997, Scholin et al. 2000). In the laboratory, growth rates of a temperate isolate of *P. pseudodelicatissima* increase up to 25°C (Lundholm et al. 1997). Temperature could also play a role in regulation of enzymatic pathways involved in domoic acid biosynthesis by *Pseudo-nitzschia*. Although there have been laboratory studies of *Pseudo-nitzschia* spp. growth rates as a function of temperature (Lundholm et al. 1997, Thessen et al. 2009), effects on domoic acid production have been examined surprisingly seldom. In 2 culture studies, warmer temperatures were not demonstrated to accelerate domoic acid production (Lundholm et al. 1994, Bates et al. 1998). Similarly, little or no correlation was observed between cellular domoic acid and temperature during field observations in Chesapeake Bay and the northern Gulf of Mexico (Thessen & Stoecker 2008, MacIntyre et al. 2011).

Temperature shifts may affect the spread of *Pseudo-nitzschia* to new habitats. The persistent seasonal nature of these blooms once seed populations become established in supportive areas can be quite remarkable. For instance, recent data from Barron et al. (2010) suggest that cooling waters of the North Pacific influenced by the negative Pacific Decadal Oscillation have coincided with the sudden 1999 appearance of *P. australis* and *P. multiseries* in the sedimentary record of California's Santa Barbara basin. These HAB diatoms remained significantly more abundant relative to other diatoms such as *Chaetoceros* and *Rhizosolenia* spp. as of 2003. Although blooms of toxic *Pseudo-nitzschia* spp. are now a common annual feature of this region, this study could also be taken to suggest that long-term warming trends might contract the ranges of these organisms.

Table 1. Published studies on harmful algal bloom (HAB) responses to ocean acidification in isolation. pCO_2: atmospheric partial pressure of CO_2; UV: ultraviolet; UVR: ultraviolet radiation; PAR: photosynthetically active radiation

HAB taxon	Variables	Variable ranges	Response	Source
Heterosigma akashiwo and *Prorocentrum minimum* (rhaphidophyte and dinoflagellate)	CO_2 and temperature	380–750 ppm 20–24°C	Increased growth at high pCO_2 in *Heterosigma* but not *Prorocentrum*; increased growth with warming in both species	Fu et al. (2007)
Chattonella subsalsa (rhaphidophyte)	CO_2 and temperature	380–750 ppm 20–24°C	Increased growth at high pCO_2 and temperature	Present work (Fig. 4)
Chattonella marina (rhaphidophyte)	pH	7.5–9.5	Growth reduced and reactive oxygen species production at pH >9.0	Liu et al. (2007)
Karlodinium veneficum (dinoflagellate)	CO_2 and phosphate	190–750 ppm 0.5 and 20 µM P	Increased growth and toxicity at high pCO_2; karlotoxins induced under P limitation	Fu et al. (2010)
Alexandrium catenella (dinoflagellate)	CO_2 and temperature	380–800 ppm 15–19°C	Increased saxitoxin at high pCO_2 and low temperature	Present work (Fig. 5)
Alexandrium minutum (dinoflagellate)	pH	pH 5.5, 7.5 and 8.5	Growth and cellular gonyautoxin-1 and -4 quota highest at pH 7.5	Hwang & Lu (2000)
Alexandrium minutum (dinoflagellate)	CO_2 and temperature	pH 7.5 and 8.0; 20 and 25°C	Growth increase under combined increasing temperature and low pH, but toxicity trends unpredictable	Flores-Moya et al. (2012)
Pseudo-nitzschia multiseries (diatom)	CO_2 and phosphate	190–750 ppm; 0.5–20 µM P	Increased domoic acid under P limitation and under high pCO_2	Sun et al. (2011)
Pseudo-nitzschia fraudulenta (diatom)	CO_2 and SiO_4	200–765 ppm; 10.6–106.1 µM Si	Increased domoic acid under Si limitation and under high pCO_2	Tatters et al. (2012)
Pseudo-nitzschia seriata, *P. multiseries*, *Nitzschia navis-varingica* (diatoms)	pH	7.9–8.9	Domoic acid levels increased at high pH in *Pseudo-nitzschia* spp. but unchanged in *Nitzschia*	Lundholm et al. (2004), Trimborn et al. (2008)
Phaeocystis globosa (prymnesiophyte)	CO_2 and UV	393 ppm and 1013 ppm; varying PAR, UVA, and UVB	Under high PAR, high CO_2 inhibited growth without UVA and UVB, but either type of UVR further inhibited growth	Chen & Gao (2011)
Phaeocystis globosa (prymnesiophyte)	CO_2 and light	190–750 ppm 80 and 240 µmol photons m^{-2} s^{-1}	Under high light, growth increased with decreasing CO_2 but was unaffected by CO_2 under low light	Hoogstraten et al. (2012)
Phaeocystis globosa (prymnesiophyte)	CO_2	380 and 750 ppm	Elevated CO_2 stimulated the formation and growth of colonies, but growth rates of solitary cells were unchanged	Wang et al. (2010)

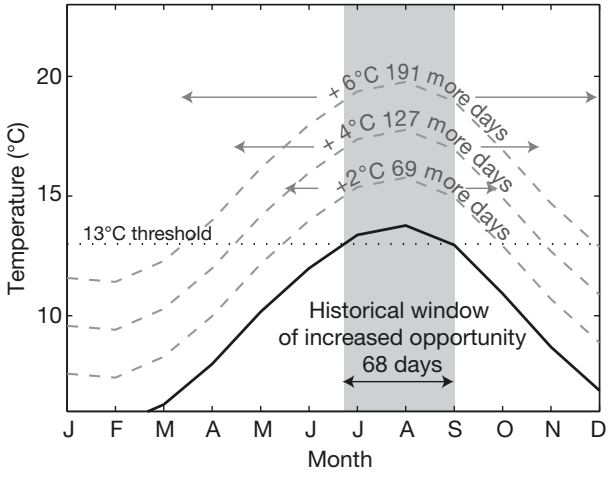

Fig. 1. *Alexandrium catenella.* Expansion of the annual temporal window for blooms of the saxitoxin-producing dinoflagellate in Puget Sound, Washington, USA, at projected sea surface warming levels of +2, +4, and +6°C. The species is limited to blooming at temperatures over 13°C; with a 6°C average increase, the annual bloom window could expand from the historical value of 68 d to as long as 259 d (from Moore et al. 2008)

Proliferation of paralytic shellfish poisoning (PSP)-producing dinoflagellates of the genus *Alexandrium* also tends to be seasonally and regionally specific. As described by Anderson et al. (2012, p. 29)

> Overall, the *Alexandrium* species that have been studied in detail have proven to be remarkably resilient and capable of colonizing a wide spectrum of habitats and hydrographic regimes. It is thus of no surprise that the biogeographic range of these species has expanded in recent times and that associated PSP outbreaks remain a significant global problem.

Once cyst beds become established in a given locality, temperature may determine periods of excystment and vegetative growth (Anderson et al. 2005). Annual variability in PSP-contaminated shellfish could result from either changing seasonal incidence of toxic *Alexandrium* blooms, variations in toxicity by resident dinoflagellates, or a combination of both (Siu et al. 1997). Arguably, each of these scenarios could be temperature-related. Correlations between cooler temperature and enhanced *Alexandrium* toxicity have been reported by numerous culture and field investigations (e.g. Hall et al. 1982, Ogata et al. 1987, Cembella et al. 1988, Anderson et al. 1990). In contrast, enhanced toxicity at median or increased temperature is less common but has also been documented (Siu et al. 1997, Etheridge & Roesler 2005, Lim et al. 2006).

Pyrodinium bahamense var. *bahamense* and var. *compressum*, 2 other PSP-producing dinoflagellates, are commonly found in tropical/sub-tropical waters (Usup et al. 2012). The western Atlantic form of *P. bahamense* was formerly thought not to produce the neurotoxins associated with PSP, but recently, accumulated saxitoxins have been found in puffer fish in the Indian River Lagoon in Florida (Landsberg et al. 2006). These results show that *P. bahamense* is a putative saxitoxin source. As with other dinoflagellates, laboratory studies have demonstrated that they have broad temperature windows, e.g. 22 to 35°C (Usup et al. 1995) and 23 to 37°C (Gedaria et al. 2007). The distribution of *P. bahamense* var. *bahamense* in coastal waters of Florida suggests that the minimum temperature that limits its occurrence is 20°C (Phlips et al. 2006). These temperature tolerances support potential climate-related range expansion (Usup et al. 2012). Although the majority of environmental variables examined influence the PSP toxin profile, not total toxin content, one study demonstrated increased toxicity at low temperature (Usup et al. 1994).

The increased abundance, geographical range expansion, and growing severity of ciguatera fish poisoning occurrences are likely indicators that several members of the benthic/epiphytic dinoflagellate genus *Gambierdiscus* are responding to warming sea surface temperatures and habitat transformation by concurrent spreading of the marine macroalgae with which they are associated (Morton et al. 1992, Hales et al. 1999, Chateau-Degat et al. 2005, Parsons et al. 2012). One culture study examining effects of temperature on ciguatoxins of *G. toxicus* demonstrated a positive correlation (Bomber et al. 1988). The range of *Gambierdiscus* is rapidly expanding along with another toxic dinoflagellate genus, *Ostreopsis,* which is not closely related to *Gambierdiscus* and produces quite different toxins, but also shares a benthic/epiphytic lifestyle (Tindall & Morton 1998, Rhodes 2011, Parsons et al. 2012). The majority of laboratory experiments examining temperature suggest that *Ostreopsis* grow more efficiently at high temperatures, but are more toxic at lower temperatures (Shears & Ross 2009, Granéli et al. 2011, Rhodes 2011).

Growth and toxicity of other HAB dinoflagellates can also be positively or negatively related to seawater warming. Temperature affects toxicity in some diarrhetic shellfish poisoning (DSP)-producing *Prorocentrum* spp. (Morton et al. 1994) and *Dinophysis* spp. (Kamiyama et al. 2010, Tong et al. 2011). In a study by Peperzak (2003), *P. micans* and *P. minimum* doubled their growth rates in simulated warm stratified conditions. The majority of studies on yessotoxin and analogues produced by *Protoceratium reti-*

culatum suggest that toxicity increases with temperature (Guerrini et al. 2007, Paz et al. 2007). The brevetoxin-producing dinoflagellate *Karenia brevis,* which causes mass mortality of marine life in the Gulf of Mexico, has been observed in the field between 7 and 34°C (Brand et al. 2012). However, optimal growth in laboratory cultures is between 22 and 29°C (Magana & Villareal 2006, Vargo 2009). The closely related *K. mikimotoi* has also been found over a wide range of temperatures (4 to 32°C; Gentien 1998, Brand et al. 2012). Toxin production in *K. brevis* demonstrates a trend of slightly higher toxicity at low temperatures that impair growth (Lamberto et al. 2004), suggesting the possibility of reduced brevetoxin impacts in a future warming ocean.

A recent 50 yr time series study in the northeast Atlantic and North Sea shows that phytoplankton community structure has shifted away from dinoflagellates, including harmful species such as some *Prorocentrum* spp. and non-harmful taxa such as *Ceratixum fuca,* and towards diatoms such as the potentially toxic *Pseudo-nitzschia* spp. and non-HABs such as *Thalassiosira* spp. (Hinder et al. 2012). The combined effects of increasing sea surface temperature and increasingly windy conditions in summer were suggested to be the main reasons for this observation. However, Hinder et al.'s (2012) results do not necessarily apply to many HAB species, since the survey focused on an open ocean phytoplankton community, and most HABs occur in estuaries or coastal waters. Local physical dynamics in these 2 regions are completely different. Nutrients are generally much more enriched in estuaries than in the open ocean, and estuaries and bays are usually less affected by wind-driven physics. Some harmful taxa are warm-water species and hence slightly increasing temperature may favor their growth, in particular many dinoflagellates. Calm winds and warmer temperatures will stratify the water column and suppress mixing long enough for motile dinoflagellates to grow and accumulate in surface waters, and hence allow them to bloom.

Recent data link harmful dinoflagellate blooms to warmer temperatures. For instance, increasing temperature stimulates blooms of the toxic dinoflagellate *Alexandrium* in Puget Sound in Washington state (Moore et al. 2009). A large unprecedented dinoflagellate bloom was observed in San Francisco Bay in September 2004, and one of the conditions that was thought to have caused this bloom was high air temperatures (Cloern et al. 2005). Although the study by Hinder et al. (2012) convincingly demonstrated multi-decadal changes in oceanic plankton communities due to altered ocean temperature and mixing, whether climate change will similarly affect the abundance or distribution of nearshore and estuarine HABs is far from clear.

Nutrients

Future climate variations such as changing storm frequencies and wind patterns will affect coastal water column dynamics, including frequency and intensity of upwelling events, tidal mixing, and mixed layer depths (Doney et al. 2009, Hallegraeff 2010). Both warming and freshening of the surface ocean from increased precipitation will promote increased seasonal water column stratification in coastal waters (Hallegraeff 2010, Paerl & Scott 2010), as well as increases in permanent stratification in the open ocean gyres (Gentien et al. 2005, Polovina et al. 2008). The implications of this increased stratification for HABs are likely profound, since many coastal and offshore blooms depend on vertical mixing to supply nutrients from below (Cermeño et al. 2008, Boyd et al. 2010). More rapid depletion of surface nutrients and concurrent decreases in replenishment from deeper water will likely favor pico- and nano-size species (Hallegraeff 2010). Reductions in bioavailable silicate (Goffart et al. 2002) may also lead to decreased diatom abundance (Hallegraeff 2010), which could inhibit harmful blooms in some cases (e.g. those of the toxic diatom genus *Pseudo-nitzschia*), and promote them in others (e.g. when non-toxic diatom species are important competitors with toxic dinoflagellates).

In a classic aquatic ecology paper, Margalef (1978) suggested that diatoms are best adapted to nutrient-enriched, well-mixed water columns, while dinoflagellates dominate in stratified, more oligotrophic environments. More recently, it has been repeatedly suggested that motile species such as many harmful dinoflagellates and raphidophytes have a distinct advantage in obtaining nutrients by vertical migration (Smayda 1997, Handy et al. 2005, Hallegraeff 2010, Paerl & Scott 2010); thus enhanced stratification could offer these groups a competitive advantage. For instance, *Alexandrium tamarense* cells living in N-limited waters are likely able to sustain growth and moderate toxicity if they are able to perform diel vertical migration to N-rich depths (MacIntyre et al. 1997). Along with many other genera (Gentien et al. 2005), blooms of *Alexandrium* are usually found subsurface under stratified conditions (Cembella & Therriault 1989).

The mixotrophic capabilities of many dinoflagellates (Stoecker 1999, Glibert & Burkholder 2011) may afford these organisms even more flexibility under future stratified, low-nutrient conditions (Caron & Hutchins in press). Growth rates of some facultatively mixotrophic harmful species increase when they are supplemented with prey (Adolf et al. 2006, Glibert et al. 2009). This alternative metabolic strategy would offer a potential for broader niches and alternative resource exploitation under both nutrient-poor and eutrophic conditions.

Of course, in many coastal and estuarine regimes, cultural eutrophication may be more important than increased stratification in determining future nutrient availability. In particular, in future climate regimes, some regions of North America are predicted to experience either more precipitation, or the same amount of precipitation delivered in fewer and thus larger pulses (IPCC 2007). This could result in heavier and more intense nutrient loading to coastal and estuarine ecosystems, and this perhaps stimulation of HAB events.

In addition to generally increased nutrient loading, coastal ecosystems can also experience unbalanced nutrient ratios from anthropogenic inputs, potentially leading to limitation by either phosphorus (P) (high N:P ratios) or nitrogen (N) (low N:P ratios) (Smayda 1997). These skewed nutrient ratios can have significant but genera-specific effects on physiological characteristics, in particular their cellular toxicity. For instance, Guerrini et al. (2007) found that yessotoxin production by the dinoflagellate *Protoceratium reticulatum* is stimulated by P limitation, but not by N limitation. Likewise, saxitoxin-producing members of the dinoflagellate genus *Alexandrium* demonstrate increased toxicity only under P limitation (Boyer et al. 1987, Anderson et al. 1990, Siu et al. 1997). In contrast, for the dinoflagellate *Karlodinium veneficum,* karlotoxin concentrations increase significantly under conditions of either N or P limitation (Adolf et al. 2009). In the dinoflagellate *Prorocentrum lima*, N and P limitation both increase cellular concentrations of the toxin okadaic acid (Vanucci et al. 2010), but in *Dinophysis acuminata*, okadaic acid levels increased only under N limitation (Johansson et al. 1996). Intracellular concentrations of domoic acid in some toxic *Pseudo-nitzschia* species are enhanced by Si and P limitation, but not by N limitation (Bates et al. 1991, Pan et al. 1996b,c). Often, the synthesis of N-rich toxins such as domoic acid and PSPs is reduced with N limitation (Boyer et al. 1987, Bates et al. 1991), while that of toxins containing no N, such as yessotoxins and karlotoxins, are less dependent on the availability of this nutrient (Adolf et al. 2009).

In addition to various limiting nutrient scenarios, the chemical form or speciation of nutrients can also affect algal toxicity. The bloom-forming dinoflagellate *Karenia brevis* shows little response of brevetoxin production to nutrient limitation (Lekan & Tomas 2010), but is enhanced when grown on urea versus nitrate (Shimizu et al. 1995). N speciation can also have implications for toxicity in *Alexandrium* spp., since saxitoxin production is enhanced when cultures are grown on ammonium as opposed to either nitrate or urea (Levasseur et al. 1995, John & Flynn 2000, Hamasaki et al. 2001). In natural blooms of the diatom *Pseudo-nitzschia*, domoic acid levels increase with N source in the order urea > nitrate > ammonium (Armstrong Howard et al. 2007). However, a recent *Pseudo-nitzschia* spp. laboratory study showed that N sources may affect cellular domoic acid content in species- or strain-specific ways (Thessen et al. 2009). That work (op.cit.) suggests that there is no general trend regarding effects of N source on cellular domoic acid levels, and that it is consequently important to consider intra- and interspecies variability in ecophysiology and toxicity.

Ocean acidification may be relevant to this dependency of HAB toxicity on specific N sources, since low pH has been shown to inhibit nitrification and so could ultimately shift the speciation of the overall ocean N inventory away from nitrate and towards reduced species such as ammonium and organic nitrogen (Hutchins et al. 2009, Beman et al. 2011). A model of the North Sea at 1000 ppm CO_2 suggests that ammonia oxidation rates could be inhibited by as much as 20%, resulting in a decrease of the nitrate to total dissolved inorganic ratio by up to 10% (Blackford & Gilbert 2007). Such a substantial shift in the chemical form of N supplied to phytoplankton communities under acidified conditions could potentially favor smaller organisms that are more competitive for ammonium, such as picoeukaryotes and cyanobacteria, as well as some HAB species such as dinoflagellates and raphidophytes.

Solar irradiance

Light is obviously a key factor affecting the physiological responses of all photoautotrophs, including HAB species. Irradiance regimes will change for primary producers in many areas of the future ocean due

to the increased stratification and mixed layer shoaling (discussed in the 'Nutrients' section above). Phytoplankton circulating in a shallower mixed layer will necessarily be exposed to higher mean daily doses of photosynthetically active radiation (PAR; Boyd et al. 2010), as well as to more potentially deleterious ultraviolet (UV) radiation (Gao et al. 2012a; UV is considered further in the 'Interactive effects' section below).

In general, it should not be surprising that in the absence of other limiting factors, HAB growth increases with PAR, within physiologically tolerable limits. For instance, light-dependent growth kinetics occur in many species of *Alexandrium* (Anderson et al. 1984, Maranda et al. 1985, Ogata et al. 1987, 1989, Parkhill & Cembella 1999, Lim et al. 2006). Laabir et al. (2011) found a positive relationship between light intensity and growth rates and biomass of a Mediterranean *Alexandrium catenella* isolate up to 90 µmol photons m^{-2} s^{-1}; photoinhibition was not observed until a light intensity of 260 µmol photons m^{-2} s^{-1} was reached. Another *A. catenella* culture showed no sign of photoinhibition up to 800 µmol photons m^{-2} s^{-1} (Carignan et al. 2002). These results support the suggestion that the genus *Alexandrium* is adapted to high light (Smayda 2008), which could provide it with a competitive advantage in future shallower mixed layers.

Baek et al. (2008) showed that optimal growth rates of the bloom-forming dinoflagellates *Ceratium furca* and *C. fusus* occur at irradiances ranging from 216 to 796 µmol photons m^{-2} s^{-1}. Like *Alexandrium*, these results indicate that *Ceratium* is well-adapted to intense light levels and hence has an advantage in highly transparent or shallow mixed layers (Baek et al. 2008).

Similarly, increasing light intensity stimulates the growth of the estuarine raphidophytes *Heterosigma akashiwo* and *Chattonella subsalsa,* which exhibit maximum growth rates over a light range of 100 to 600 µmol photons m^{-2} s^{-1}; no sign of photoinhibition was observed for either species even at the highest light intensity tested, >600 µmol m^{-2} s^{-1} (Zhang et al. 2006). These results support the suggestion that raphidophycean flagellates generally can tolerate and even prefer very high light intensities (Kahn et al. 1998). Another recent study demonstrated that light effects on the growth of *H. akashiwo* are temperature-dependent (Martinez et al. 2010). They also found differences in growth responses to light between *H. akashiwo* strains, suggesting that light could play a role in intraspecific dominance shifts and that generalizations for the whole genus may need to be made cautiously.

In contrast to these HAB species, there is evidence that the dinoflagellate *Karenia brevis* appears to be relatively low-light adapted. This species has a low light saturation point of around 65 µmol m^{-2} s^{-1} (Shanley & Vargo 1993, Magana & Villareal 2006), and its light compensation point is around 20 to 30 µmol m^{-2} s^{-1} (Wilson & Collier 1955, Aldrich 1962, Eng-Wilmot et al. 1977). Brown tides (*Aureococcus* and *Aureoumbra*) are another group of HABs that benefit from low light, as both genera commonly bloom in severely light-attenuated environments (Gobler & Sunda 2012). These 2 genera can attain nearly maximum growth rates under a light intensity of 50 µmol m^{-2} s^{-1} at 20°C (MacIntyre et al. 2004). Consistent with their low light adaption, genetic evidence for adaptation to low light was obtained from the *Aureococcus* genome (Gobler et al. 2011). These results suggest that both *K. brevis* and brown tides have an advantage when growing at depth, and also may have a competitive advantage during dense self-shaded blooms. This trait, however, means that they may not benefit from future increases in mean light exposures as much as many other non-HAB taxa.

Light is required for production of many algal toxins, including PSPs, domoic acid, and DSP toxins (Proctor et al. 1975, Bates et al. 1991, Pan et al. 1996a, Carneiro et al. 2009, Tong et al. 2011). Parkhill & Cembella (1999) and Etheridge & Roesler (2005) revealed that the highest cellular toxin levels in *Alexandrium tamarense* and *A. fundyense* were observed at light intensities between 100 and 150 µmol photons m^{-2} s^{-1}. Analysis of PSP composition in *Alexandrium* demonstrated that toxin composition did not vary with increasing light (Boyer et al. 1987, Cembella et al. 1987, Ogata et al. 1987, Oshima et al. 1990, Cembella & Destombe 1996, Parkhill & Cembella 1999, Lim et al. 2006), suggesting that light regulated the total toxin concentrations rather than changing the toxin profiles. In contrast to these observations of toxin stimulation by increasing light, an inverse relationship between cellular toxicity and light-dependent growth was also documented (Ogata et al. 1987, Hamasaki et al. 2001, Cembella 1998). There appears to be no general trend that applies to the effect of light on the production of PSPs in all *Alexandrium* species and strains, and in general the effect of light variation on toxicity is less remarkable compared to other factors such as temperature, salinity, and nutrients (Ogata et al. 1987, Lim et al. 2006). Thus, the responses of these dinoflagellates to any future increases in irradiance doses due to mixed layer shoaling are difficult to predict.

The responses of toxicity to light are also complex in HAB raphidophytes. In *Heterosigma*, there is an inverse relationship between light-limited growth rates and toxicity (Ono et al. 2000). Conversely, hemolytic activity of *Fibrocapsa japonica* is positively affected by light (de Boer et al. 2009). Other studies with *Chattonella marina* have related light intensity positively to its ichthyotoxicity (Ishimatsu et al. 1996, Marshall et al. 2001). Similar results were also observed by Oda et al. (1997) and Marshall et al. (2001, 2005), who reported that light is also involved in the production of ROS by raphidophytes, including *F. japonica*.

The influence of light on the growth and toxin production of dinoflagellates has been more extensively investigated compared to toxic diatoms. Several studies have documented physiological responses of *Pseudo-nitzschia* spp. to light intensity (Bates et al. 1991, Whyte et al. 1995, Pan et al. 1996a, Fehling et al. 2005, Thessen et al. 2009), but only one of these described light as a basic requirement for domoic acid production (Bates et al. 1991). Domoic acid production by *Pseudo-nitzschia* is inhibited in darkness, but resumes soon after cultures are shifted into the light (Bates et al. 1991). Although cultures of *P. seriata* exposed to a long photoperiod (18 h light: 6 h dark) compared to a short photoperiod (9 h light: 15 h dark) have higher growth rates, biomass, and total domoic acid production, their cellular domoic acid content is reduced (Fehling et al. 2005).

The majority of published studies showing light-regulated growth and toxin production in HAB species have been done with laboratory cultures. Recently, however, field observations assessing the environmental factors regulating *Pseudo-nitzschia* blooms in the northern Gulf of Mexico have found that the mean cell toxin quotas and abundance of *Pseudo-nitzschia* species were strongly correlated with several factors, including high irradiance (Fig. 2C,D; MacIntyre et al. 2011).

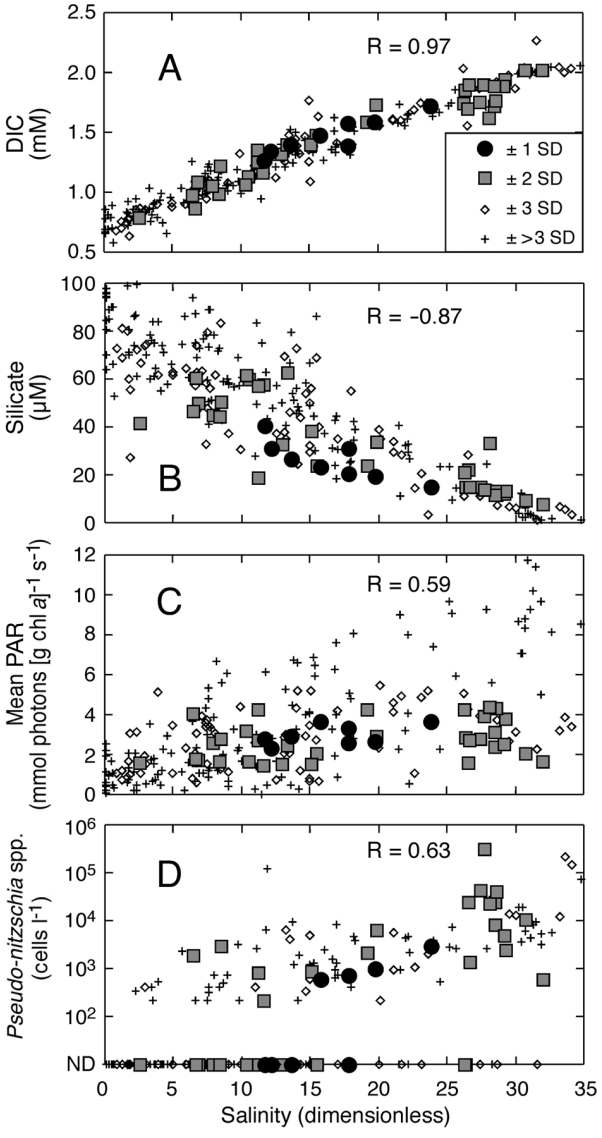

Fig 2. *Pseudo-nitzschia* spp. Environmental correlations with abundance of domoic acid-producing diatoms in samples in which domoic acid was detected across an estuarine salinity gradient in the Gulf of Mexico. Plotted against salinity are (A) total dissolved inorganic carbon (DIC), (B) silicate concentrations, (C) mean down-welling photosynthetically active radiation (PAR) in the water column, and (D) *Pseudo-nitzschia* spp. abundance. ND: counts that were below the limit of detection; R-values represent correlation coefficients for each entire data set (from MacIntyre et al. 2011, used by permission)

Salinity

Altered future rainfall and climate patterns could significantly increase salinity variability in coastal areas, and especially in estuaries (Hallegraeff 2010, Paerl & Scott 2010). Such salinity fluctuations may favor halotolerant and euryhaline organisms such as many HAB dinoflagellates and raphidophytes. For instance, many species of the dinoflagellate *Prorocentrum* are euryhaline in culture and in nature (Grzebyk & Berland 1996). In a clonal culture of *P. lima*, growth rate and toxicity were inversely correlated with salinity (Morton et al. 1994). In the dinoflagellate *Karlodinium veneficum*, reduced growth rates due to low salinity significantly enhance cellular toxin quotas (Adolf et al. 2009). However, the diatom *Pseudo-nitzschia multiseries* demonstrates reduced growth rates and cell-normalized toxicity at

lower salinities; at full seawater salinity, toxicity increases several fold (Doucette et al. 2008). Species-specific salinity-related growth tolerances have been reported by several investigators for this diatom genus (Jackson et al. 1992, Lundholm et al. 1994, 1997, Thessen et al. 2005).

Dense proliferations of *Alexandrium* cells normally occur in coastal zones. Consistent observations within estuaries, and the generally wide salinity tolerance of this genus, suggest that salinity is an influential growth factor (Franks & Anderson 1992, Parkhill & Cembella 1999, Lim & Ogata 2005). Reported relationships between toxicity and salinity range from inverse (Grzebyk et al. 2003, Lim & Ogata 2005) to no difference (Anderson et al. 1990) to positive (White 1978, Lim & Ogata 2005). For instance, Grzebyk et al. (2003) reported that a clone of *A. minutum* grew most favorably at salinity 20 to 37, but toxicity was highest at salinity 15.

A range of responses of growth and toxicity to changing salinity has also been reported in other dinoflagellates. One study on *Pyrodinium bahamense* revealed a high tolerance to salinity changes, but natural blooms are usually encountered only at salinities of 20 or more (Usup et al. 2012). Guerrini et al. (2007) reported that *Protoceratium reticulatum* grows over a salinity range of 22 to 42, with the highest yessotoxin concentration at salinity 32. Paz et al. (2007) also reported that yessotoxin production decreased with increasing salinity in this species. Toxicity in this species was demonstrated to be relatively unchanged above salinity 24, but was enhanced 3-fold at salinity 20 (Usup et al. 1994). *Karenia brevis* is thought to tolerate a range of salinities (18–45) but seems to grow best in full-salinity seawater (Magana & Villareal 2006, Brand et al. 2012). However, a recent laboratory study by Errera & Campbell (2011) demonstrated a close connection between salinity and brevetoxin production by *K. brevis*. Three of 4 clones of *K. brevis* responded to hypoosmotic shock dramatically: cellular brevetoxin quota increased by 14-fold, while cell volume remained unchanged. This study implies that brevetoxin production by *K. brevis* may be affected by variations in salinity due to semi-daily tidal rhythms, and that this species could become more toxic if future precipitation increases result in lower salinities in the coastal regions where it blooms. *K mikimotoi* exhibits a similar salinity tolerance of 9 to 35 (Gentien 1998, Vargo 2009, Brand et al. 2012).

Benthic and epiphytic HABs can also be affected by salinity. For instance, in *Gambierdiscus*, both growth and toxicity respond to salinity changes. In general, members of this genus grow optimally at or near full-strength seawater, but can tolerate mild fluctuations (Parsons et al. 2012), and toxicity is partially determined by salinity (Bomber et al. 1988, Roeder et al. 2010). The effects of salinity on the ecologically similar genus *Ostreopsis* are quite variable, ranging from negative to positive correlations with growth and toxicity (Pistocchi et al. 2011). A Mediterranean *O. ovata* isolate displays optimal growth rates at high salinity (36–40), but toxicity is highest at salinity 32 (Pezzolesi et al. 2012). While there is an evident lack of consistency in the responses of these various HAB species to changing salinity, species-specific increases or decreases in toxicity and growth rate are commonly reported and may become important to consider under future changing precipitation and evaporation regimes in coastal areas and estuaries.

INTERACTIVE EFFECTS OF CO_2 AND OTHER ENVIRONMENTAL VARIABLES ON HABs

CO_2 and temperature

As discussed above, rising temperature may increase the bloom frequencies of cyanobacteria and dinoflagellates relative to other algae such as diatoms (reviewed by Beardall et al. 2009b). Although a number of studies have determined how global warming affects various harmful species, very little is known about the effects of rising temperature in concert with rising CO_2 on their physiology or ecology. To our knowledge, only 1 published study has focused on interactive effects of CO_2 and temperature increases in HAB species. Fu et al. (2007) compared the combined effects of CO_2 and temperature on cultures of the raphidophyte *Heterosigma akashiwo* and the dinoflagellate *Prorocentrum minimum* isolated from the same Delaware Inland Bays (USA) estuary. Maximum light-saturated photosynthetic rates (P^B_{max}) increase in *H. akashiwo* only with simultaneous CO_2 and temperature increases (Fig. 3A), whereas P^B_{max} in *P. minimum* responds significantly to CO_2 enrichment, with or without increased temperature (Fig. 3B; Fu et al. 2007). CO_2 availability and temperature also have pronounced effects on cellular C and N content in *H. akashiwo*, but not in *P. minimum*. Evidently, there can be major differences in responses to combined warming and acidification even between 2 HAB species that commonly bloom together in the same body of water.

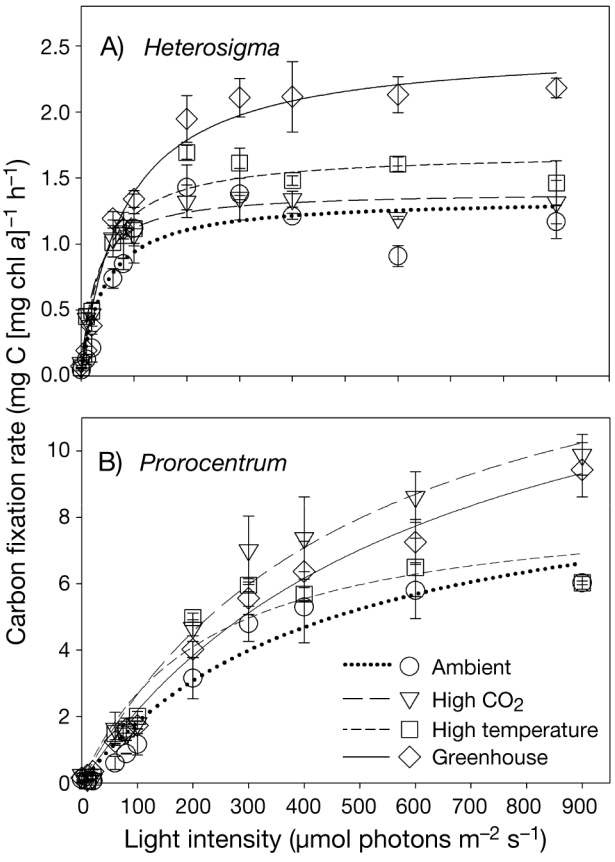

Fig. 3. *Heterosigma akashiwo* and *Prorocentrum minimum.* Photosynthesis versus irradiance curves for (A) the raphidophyte *H. akashiwo* and (B) the dinoflagellate *P. minimum* in a CO_2/temperature interaction experiment. Treatments included ambient (20°C and 375 ppm CO_2), high CO_2 (20°C and 750 ppm CO_2), high temperature (24°C and 375 ppm CO_2), and greenhouse (24°C and 750 ppm CO_2). Maximum photosynthetic rates increased only in the greenhouse treatment for *Heterosigma,* but in both high CO_2 treatments for *Prorocentrum,* regardless of temperature (from Fu et al. 2008a, used by permission)

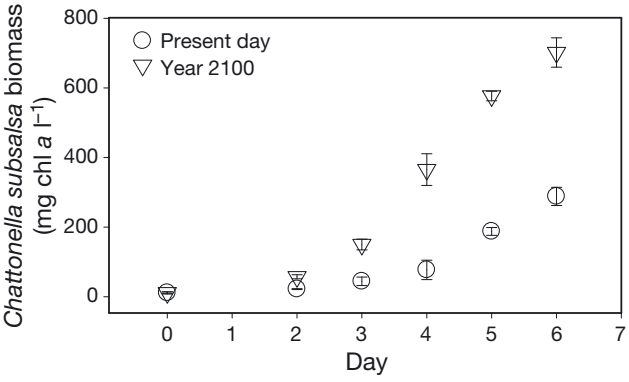

Fig. 4. *Chattonella subsalsa.* Growth of an estuarine harmful algal bloom raphidophyte (mean ± SD, n = 3) at present-day temperature and pCO_2 (20°C and 375 ppm CO_2), and at levels projected for the year 2100 (24°C and 750 ppm CO_2) (F. X. Fu & D. A. Hutchins unpubl. data)

Heterosigma akashiwo is not the only HAB species likely to benefit from a greenhouse climate regime. Our preliminary experiments with *Chattonella subsalsa,* another bloom-forming raphidophyte from the same Delaware Inland Bays estuary, also demonstrated enhanced growth under CO_2 and temperature conditions predicted for the end of this century (Fig. 4). These 2 studies suggest that some raphidophytes may be favored by the combination of future rising CO_2 and temperature, possibly more than other co-occurring HAB groups such as dinoflagellates.

In preliminary work, we have observed interactions between temperature and CO_2 that strongly influenced PSP production by the marine dinoflagellate *Alexandrium catenella*, in a strain isolated from coastal southern California. Within 15 and 19°C temperature treatments, triplicate bottles were equilibrated at 2 different CO_2 concentrations (380 ppm pCO_2: present day; 800 ppm pCO_2: future). pCO_2 levels were obtained by gentle bubbling with filtered commercial gas (Tatters et al. 2012). Four treatments were used in this study: control (15°C, 380 ppm CO_2); high CO_2 (15°C, 800 ppm); high temperature (19°C, 380 ppm CO_2); and 'greenhouse' (19°C, 800 ppm CO_2). Experiments used identical semicontinuous culturing methods with each species to measure temperature and CO_2 effects during acclimated steady-state growth (Fu et al. 2010); saxitoxin measurements were made using HPLC (Abbott et al. 2009) with calibration standards obtained from the National Research Council of Canada (Halifax, Nova Scotia). At current 380 ppm pCO_2 levels, cellular toxin contents did not differ between temperatures, but in the high pCO_2 treatment (800 ppm), the slowly growing cultures maintained at 15°C had cellular toxin contents that were ~50% higher than those in faster-growing cultures maintained at 19°C (Fig. 5). Regardless of temperature conditions, an enrichment of pCO_2 significantly stimulated cellular saxitoxin equivalent contents, with an increase of ~1.5 to 2.3 times in the 800 ppm pCO_2 treatments relative to those grown at 380 ppm pCO_2. These results suggest that already damaging *Alexandrium* blooms could potentially become much more toxic under acidified conditions, but that these toxicity increases could be partially offset by simultaneously rising temperature.

Another perspective on this issue is offered by a recent study with 2 strains of *Alexandrium minutum* isolated from NW Spain (Flores-Moya et al. 2012). These cultures were grown for 2 yr under simulated global change conditions, including increased tem-

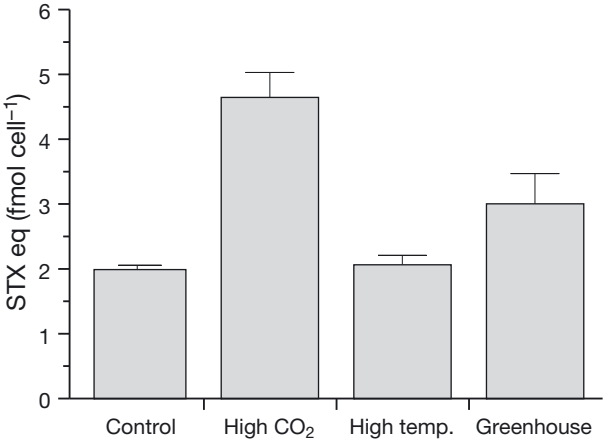

Fig. 5. *Alexandrium catenella.* Preliminary experiment showing cellular paralytic shellfish toxin contents (fmol cell^{-1} saxitoxin equivalents, STX eq) in a southern California isolate of the dinoflagellate grown at control (15°C and present-day CO_2 at 380 ppm), high CO_2 (15°C, 800 ppm CO_2), high temperature (19°C, 380 ppm CO_2), and greenhouse (19°C, 800 ppm CO_2) (A. O. Tatters et al. unpubl. data)

perature (from 20 to 25°C) and decreased pH (from 8.0 to 7.5). The results suggest that long-term adaptation and acclimation to future global change may stimulate their growth rates, but effects on cellular toxicity were unpredictable due to high variance in cellular toxin contents between different cell lines (Flores-Moya et al. 2012).

Rising CO_2 and temperature will not only affect the responses of phytoplankton among different major taxonomic groups, but may also elicit different responses within closely related taxa. For instance, cyanobacteria are generally thought to possess efficient CCMs, and hence they may not be especially vulnerable to carbon limitation (Badger et al. 1998). However, a culture isolate of the picocyanobacterium *Synechococcus* has a greater response to CO_2 (controlled by bubbling) and temperature increases for many physiological parameters, compared to a closely related *Prochlorococcus* culture (Fu et al. 2007). However, at this time, no published studies have tested how combined CO_2 and temperature increases may influence the growth and toxicity of marine and estuarine harmful cyanobacteria such as *Lyngbya.*

The interactive effects of CO_2 and temperature on phytoplankton physiology can be species- or even strain-specific. Such differences between closely related groups can have large consequences for whole natural community changes. Currently no information is available to document the interactive effects of CO_2 and temperature on natural community shifts involving HABs, but experimental results using other types of algal communities are available. For instance, in phytoplankton communities examined during the North Atlantic spring bloom, increased temperature alone promotes whole-community photosynthesis, while phytoplankton community composition is affected by both elevated CO_2 and temperature (Feng et al. 2009). Similarly, the effect of rising CO_2 alone on photosynthesis of phytoplankton communities was minor in the tropical North Pacific (Tortell et al. 2002) and the Bering Sea (Hare et al. 2007). However, elevated CO_2 and increased temperature (the 'greenhouse' treatment) stimulates whole-community carbon fixation by 2.6- to 3.5-fold, and also resultes in community structure shifts from diatoms toward nanophytoplankton (Hare et al. 2007). Similar studies in estuaries and the coastal ocean are needed for HAB-dominated communities.

CO_2 and nutrients

A large number of studies have investigated the influence of nutrient availability on growth and toxicity in HAB species, examining compounds such as PSPs, karlotoxins, brevetoxins, and domoic acid (see 'Nutrients' section above). However, to date, the interactive effects of changing CO_2 with nutrient availability on HAB cell potency have been investigated only for domoic acid and karlotoxins in laboratory culture experiments (Fu et al. 2010, Sun et al. 2011, Tatters et al. 2012).

Domoic acid production increases dramatically in nutrient-limited laboratory cultures of the toxic diatoms *Pseudo-nitzschia* spp. as the CO_2 concentration at which the cells are grown increases from 190 ppm (glacial era levels) to 380 ppm (approximately present-day atmospheric concentration) to 750 ppm (projected levels for the year 2100). These trends have been documented in a *P. multiseries* isolate from eastern Canada under P limitation (Fig. 6; Sun et al. 2011), and a *P. fraudulenta* clone from southern California under Si limitation (Fig. 7; Tatters et al. 2012). These CO_2-mediated toxin increases are far greater in nutrient-limited cultures than in nutrient-replete treatments, but occur under both regimes (see panel insets in Fig. 7). These studies demonstrate not only that outbreaks of amnesic shellfish poisoning events could worsen in the future high-CO_2 world, but also that CO_2 effects on toxicity can be influenced by changing nutrient availability.

The interactions between CO_2 and nutrient availability in natural *Pseudo-nitzschia* communities have

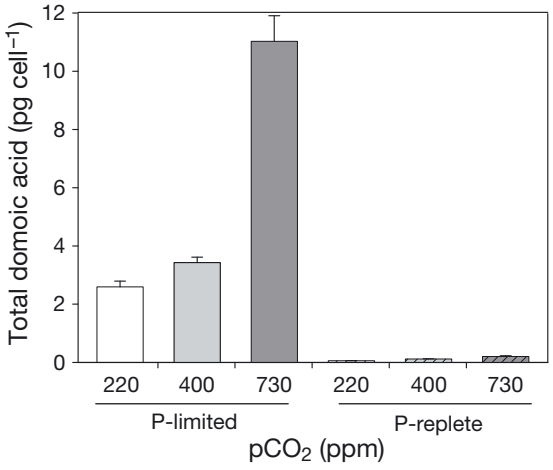

Fig. 6. *Pseudo-nitzschia multiseries.* Domoic acid production (mean + SD, n = 3) by the Canadian east coast isolate CCMP 2708 grown at 3 pCO_2 levels (220, 400, and 730 ppm) under both P-limited (open bars, 0.5 µmol l^{-1}) and P-replete (cross-hatched bars, 20 µmol l^{-1}) conditions (data from Sun et al. 2011)

not been experimentally investigated. However, in *Pseudo-nitzschia* blooms from the Gulf of Mexico, both cell densities (Fig. 2D) and domoic acid levels (not shown) are negatively correlated with silicate concentrations (Fig. 2B) and positively correlated with dissolved inorganic carbon or CO_2 (Fig. 2A; MacIntyre et al. 2011). This *in situ* observational study supports the culture results of Tatters et al. (2012), suggesting that domoic acid levels are enhanced in Si-limited *Pseudo-nitzschia* cells under high CO_2 conditions.

As noted in the pCO_2/pH section above, 2 prior studies examined the influence of seawater pH on the toxicity of *Pseudo-nitzschia* cultures and found that domoic acid concentrations increased instead at higher pHs (Lundholm et al. 2004, Trimborn et al. 2008). It is not clear whether these previous results and those of Sun et al. (2011) and Tatters et al. (2012) are actually conflicting, since the earlier and recent studies used different methodologies. Lundholm et al. (2004) and Trimborn et al. (2008) used nutrient-replete batch cultures grown to stationary phase, while Sun et al. (2011) and Tatters et al. (2012) used semi-continuous cultures in steady-state, nutrient-limited growth. pH values were adjusted by additions of HCl and NaOH in the 2 earlier studies, while CO_2 or pH levels were obtained through bubbling the cultures with different pCO_2 concentrations in the 2 newer studies. The 2 sets of studies also used different *Pseudo-nitzschia* species or strains, differing experimental conditions, and analytical methods.

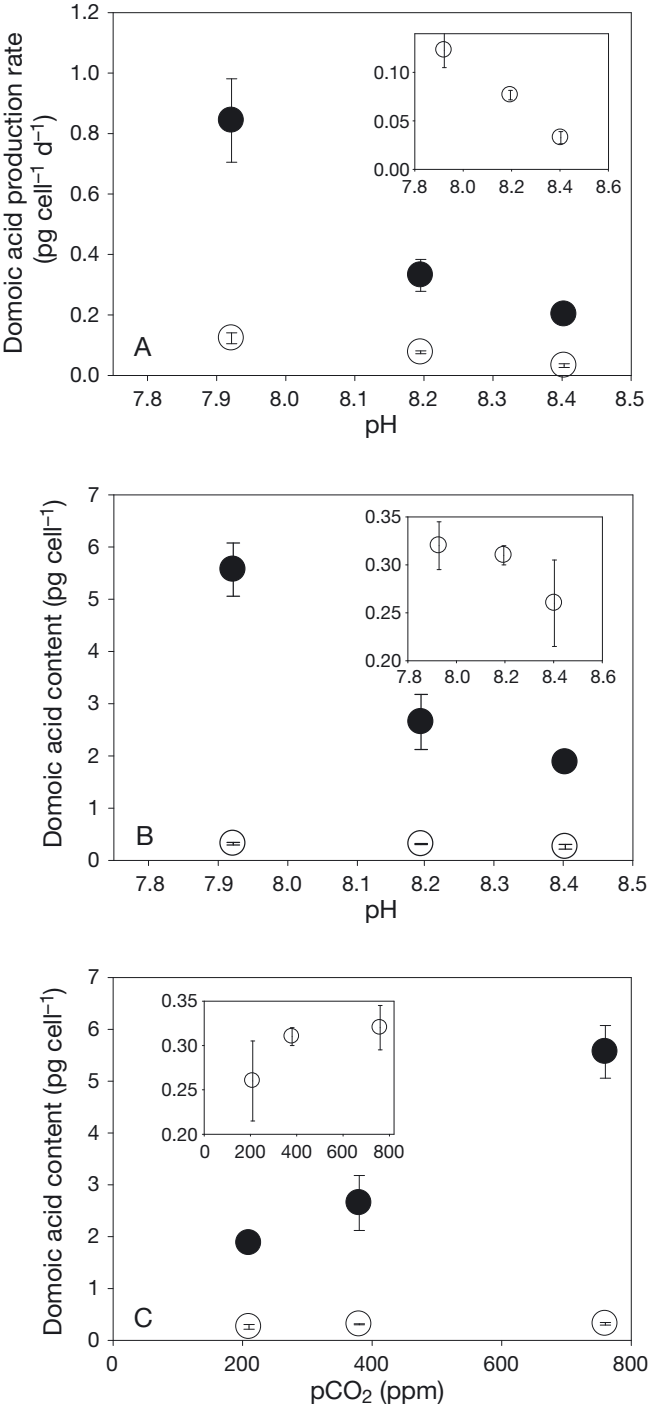

Fig. 7. *Pseudo-nitzschia fraudulenta.* Domoic acid production (mean ± SD, n = 3) by a Southern California isolate grown under $Si(OH)_4$-limited (●) and nutrient-replete (○) conditions at seawater CO_2 concentrations of 210 ppm (preindustrial atmospheric levels), 380 ppm (modern levels), and 760 ppm (projected year 2100 levels). (A) Cellular domoic acid production rates (pg cell^{-1} d^{-1}) versus pH, (B) domoic acid cell contents (pg cell^{-1}) versus pH, and (C) domoic acid contents (pg cell^{-1}) versus pCO_2. Panel insets: nutrient-replete data with an expanded *y*-axis scale for clarity (from Tatters et al. 2012)

Lundholm et al. (2004) also did not examine the low pH levels that are relevant to future ocean acidification trends. These contrasting results emphasize the need for an in-depth examination of CO_2/toxicity interactions with nutrient availability in other *Pseudo-nitzschia* species using standardized experimental protocols.

Fu et al. (2010) showed that the toxicity of the toxic estuarine dinoflagellate *Karlodinium veneficum* is also strongly affected by changing CO_2 under nutrient-limited growth conditions. Enhanced toxin levels under N or P limitation have been reported previously in this species (Adolf et al. 2009). However, Fu et al. (2010) found that the highest levels of overall cellular toxicity by far were observed in P-limited, high-pCO_2 cultures, suggesting a synergistic effect between the 2 variables (Fig. 8C). This potency effect was due to a large shift in the biochemical composition of the cellular toxin pool, with cells grown under high-CO_2 conditions producing much higher levels of a more toxic karlotoxin congener (Karlotoxin 1, Fig. 8A) and lower amounts of a less potent congener (Karlotoxin 2, Fig. 8B). As a result, overall cellular toxicity increased by up to 300% between 230 and 745 ppm CO_2 in the P-limited cultures (Fig. 8C). This work demonstrates that it is important to consider the effects of future increases in atmospheric CO_2 not only on levels of total cellular toxin production, but also on the entire suite of chemical congeners produced by many harmful dinoflagellate species.

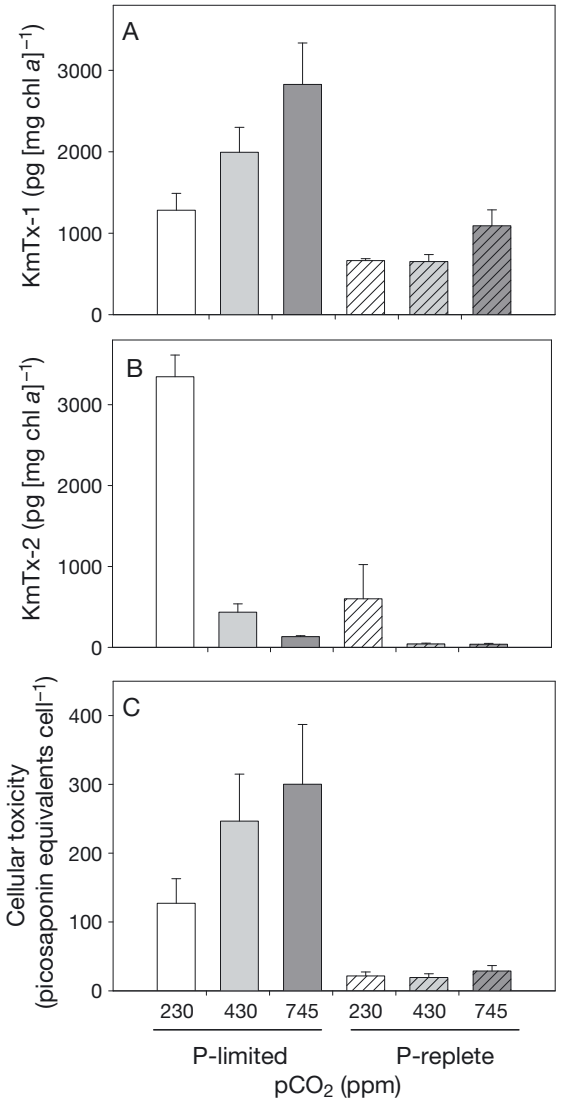

Fig. 8. *Karlodinium veneficum.* Chlorophyll *a*-normalized karlotoxin production (mean + SD, n = 3) by estuarine harmful algal bloom dinoflagellate (isolate CCMP 2936) grown at 3 pCO_2 levels (230, 430, and 745 ppm) under both P-limited (open bars, 0.5 µmol l^{-1}) and P-replete (cross-hatched bars, 20 µmol l^{-1}) conditions. (A) Levels of the highly potent congener karlotoxin 1 (KmTx-1); (B) levels of the less potent congener karlotoxin 2 (KmTx-2); (C) resulting relative toxicity levels of the cultures (in picosaponin equivalents cell^{-1}) (from Fu et al. 2010)

CO_2 and solar irradiance

To date, no published studies have shown how the interactions between increasing CO_2 and PAR may affect the growth and toxin production of HABs. Gao et al. (2012b), however, demonstrate that ocean acidification reduces the ability of diatoms to cope with super-saturating PAR levels (see also Gao et al. 2012a). A few studies have examined effects of elevated CO_2 in combination with UV radiation (UVR) on various phytoplankton species. UVR reaching the sea surface increased dramatically during the late 20th century due to the thinning of the stratospheric ozone layer, especially at high latitudes (Kerr & McElroy 1993). Although the upper atmosphere concentrations of anthropogenic ozone-reactive chlorinated compounds have been reduced since the implementation of the Montreal protocol, it is still unclear when the ozone layer will fully recover (Weatherhead & Anderson 2006). The expected enhancement of stratification in the open ocean water column will increase the exposure of phytoplankton in the upper mixed layer to all wavelengths of solar radiation, making deleterious exposures to UVR more likely (Gao et al. 2012a).

Mengelt & Prézelin (2005) reported that the presence of UVA (320 to 400 nm) stimulates carbon fixation by *Pseudo-nitzschia*-dominated communities in the Santa Barbara Channel, although UVB (280 to 320 nm) photoinhibition is also observed. Their re-

sults suggest that *Pseudo-nitzschia* may not be photoinhibited even in shallower portions of the water column. Because their sampling occurred during a bloom event, CO_2 concentrations were likely decreasing rapidly. They suggested that exposure to UVA may increase extracellular CA activity to help maintain higher intracellular CO_2 levels, despite reduced external CO_2.

Sobrino et al. (2005) demonstrated that UVR sensitivity showed different responses to increased CO_2 levels in 2 marine picoplanktonic eukaryotes with similar morphology but different CCMs. The 2 marine picoplankters *Nannochloropsis gaditana* and *Nannochloris atomus* were grown with constant aeration in air containing 0.03 and 1% CO_2. The former species, which relies on bicarbonate uptake for photosynthesis, shows decreased sensitivity to UVR after growing for 4 d under elevated CO_2 conditions. In contrast, *N. atomus*, a species with active CO_2 transport, shows similar sensitivity to UVR with and without supplemental CO_2. These studies do not verify the potential effect of UVR on CCMs, but suggest that differences in UVR sensitivity related to external CO_2 concentrations can affect taxonomic composition in open ocean algal communities. Whether this might apply to estuarine and coastal HAB assemblages has not yet been determined.

Studies with the diatom *Thalassiosira pseudonana* grown at 2 pCO_2 levels (380 versus 1000 ppm, obtained by bubbling) also have shown that the presence of UVR may affect CO_2 uptake more than that of HCO_3^- (Sobrino et al. 2008). With another diatom, *Skeletonema costatum*, Wu & Gao (2009) found that the presence of UV promotes external CA activity as a result of enhanced CO_2 supply. They suggested that this helps cells avoid UV-induced photoinhibition of photosynthesis. Rising CO_2 stimulates the growth of *T. pseudonana* regardless of exposure to UVR, but the presence of UVR does not affect its growth (Sobrino et al. 2008). Future work is necessary to determine whether the observations of these diatom studies can be applied to harmful diatoms such as toxic *Pseudo-nitzschia* spp., or to other HAB groups such as dinoflagellates and raphidophytes.

Chen & Gao (2012) focused on the interactive effects of UV and ocean acidification on the photosynthetic performance of the HAB species *Phaeocystis globosa*. The major finding of this study was that the effect of CO_2 on physiological responses, including growth rates and photochemical efficiency, is dependent on light levels (Chen & Gao 2011). Under high light levels, enrichment with CO_2 inhibits the growth of *P. globosa* with or without UVA and UVB, but the presence of either type of UVR further inhibits its growth (Fig. 9). When CO_2 and UVA are combined, the effects are synergistically magnified. In contrast to its responses to UVA, UVB exposure always inhibits *P. globosa* growth regardless of the light levels and CO_2 conditions. Enrichment with CO_2 imposes a significant but minor negative effect of UVB and UVA on growth.

Another recent study investigated the combined effects of CO_2 and PAR on *Phaeocystis globosa*, isolated from the North Sea (Hoogstraten et al. 2012). Their study showed that the physiological effect of CO_2 is dependent on light conditions. The growth rates of high-light cultures decrease with increasing CO_2 levels, while photosynthetic efficiency increases with increasing CO_2. However, no CO_2 effect is observed in light-limited cultures. Together with the

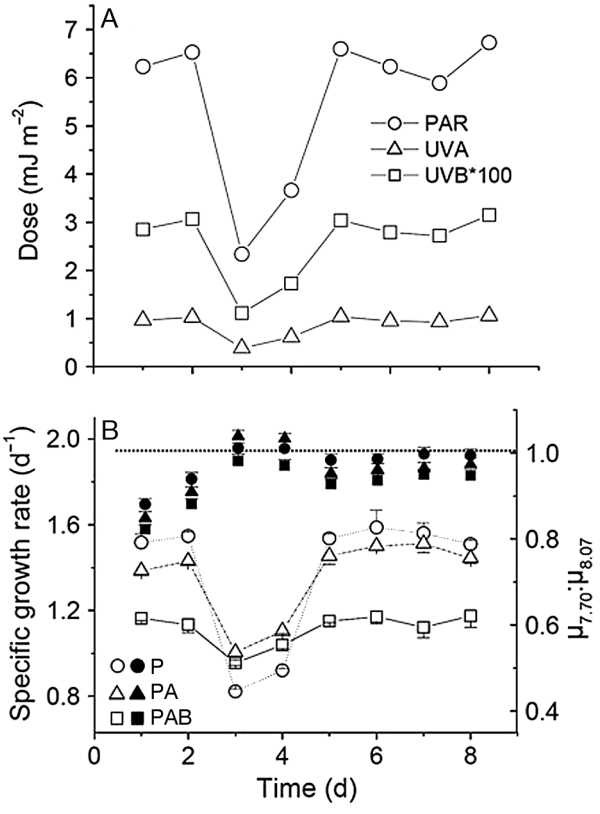

Fig. 9. *Phaeocystis globosa.* Effects of solar irradiance on growth of harmful algal bloom prymnesiophyte. (A) Daily doses of solar photosynthetically active radiation (PAR), ultraviolet radiation A (UVA, 315–400 nm), or ultraviolet radiation B (UVB, 280–315 nm) during the experimental period of 1 to 8 May 2009. (B) Specific growth rates of cultures grown at pH 8.07 (open symbols) and the ratios (mean +/– SD, n = 3) of the specific growth rates at pH 7.70 to those at pH 8.07 (7.70:8.07; solid symbols), under solar PAR (P), PAR + UVA (PA), or PAR + UVA + UVB (PAB), respectively (from Chen & Gao 2011, used by permission)

observations of Chen & Gao (2011), this study indicates that *P. globosa* may not be well suited to grow under expected future ocean conditions of higher PAR and UV.

No information is currently available to make a clear statement about how interactions between UVR and CO_2 may affect possibly the most important physiological trait of many HAB species: toxin production. If the presence of UVB inhibits growth and photosynthesis, toxin production may be stimulated since it is often thought that toxicity is stimulated by growth-limiting conditions. If the results of Chen & Gao (2011) and Sobrino et al. (2008), suggesting that phytoplankton are more sensitive to UVR under high CO_2 conditions, are representative of HAB groups in general, toxicity may increase in the future ocean. However, the presence of moderate doses of UVA will often stimulate algal growth and carbon fixation, thus potentially counteracting this phenomenon. Algae will likely experience stronger doses of UVR as well as PAR in the future ocean, due to shallower mixed layers. Thus 3-way interactions among PAR, UVR, and CO_2 will exert combined effects on the physiological responses of HABs. These alternate scenarios for toxin effects from CO_2 and UVR should be examined in future work.

FUTURE DIRECTIONS

The interactive global change effects on HABs may differ significantly between marine regimes. For instance, CO_2 sources, levels, and trends are likely to be quite different for estuarine, coastal, and offshore bloom events. Many freshwater-influenced estuarine systems lack robust carbonate buffer systems, and hence tend to have relatively low alkalinity and bicarbonate concentrations. In contrast, marine systems have significant pools of bicarbonate accessible to phytoplankton. Due to these chemical differences, the possible role of CO_2 availability in influencing estuarine versus oceanic HAB ecology remains to be extensively investigated. Recently, Nielsen et al. (2012) investigated the effect of pH manipulated by additions of acid and base on an estuarine plankton community in the Derwent River estuary, Tasmania, Australia. pH effects on community structure (which included diatoms and dinoflagellates) were not observed across a pH range between 8.0 and 7.7. They pointed out that large fluctuations in seasonal and diurnal pH (ranging from 7.5 to 9.6) and in salinity levels in estuaries have selected phytoplankton assemblages in these regimes to tolerate a broad range of water chemistries, and hence they may not be affected by changes in pH within the range expected for the next 100 yr.

Coastal upwelling regions where organisms such as *Pseudo-nitzschia* bloom are especially vulnerable to ocean acidification, since anthropogenic CO_2 can augment already naturally elevated pCO_2 in upwelled water (Feely et al. 2008). In addition, coastal eutrophication and resulting suboxia or hypoxia can also strongly elevate regional pCO_2 (Hales et al. 2005, Cai et al. 2011). Nutrient and light trends are also likely to be regime-specific. For instance, the commonly assumed trends of higher light exposures and reduced nutrient supplies for phytoplankton due to enhanced stratification may apply largely to the open ocean. In contrast, expected continuing future increases in anthropogenic eutrophication of estuaries are likely to result in opposite trends, with simultaneous increases in nutrient concentrations and decreases in available light due to higher turbidity. Thus, rising pCO_2 and acidification will interact with other crucial environmental variables in varying ways for future HAB events in different types of marine ecosystems.

Many of these ocean acidification and climate change effects will manifest themselves through competition-related changes in algal community structure. Rising CO_2 and temperature in concert with irradiance changes and increased eutrophication are likely to affect the ecological dominance of groups such as raphidophytes and dinoflagellates relative to competing non-harmful species. These global change effects may also favor particular HAB species over others. We tested whether CO_2 availability could affect interspecific competition between 2 HAB dinoflagellates, *Karlodinium veneficum* and *Prorocentrum minimum,* isolated from the same estuary (Delaware Inland Bays). For this, we carried out a simple batch culture competition experiment in which the 2 species were inoculated at a 1:1 ratio (cell:cell) into nutrient-replete medium and grown for 10 d at 3 pCO_2 levels (190, 380, and 750 ppm). *K. veneficum* growth rates (Fig. 10A) and biomass (Fig. 10B) responded positively to increasing pCO_2, whereas those of *P. minimum* did not. At the end of the experiment, ratios of *K. veneficum* to *P. minimum* cells were still ~1:1 in the low pCO_2 treatment, but had increased incrementally to ~3:1 in the high pCO_2 replicates (Fig. 10C). These differing responses may reflect their different CCM efficiencies, but this hypothesis will require further work to verify. Regardless of the reason for this outcome, our artificial HAB community experiment suggests that pCO_2 may be 1 factor

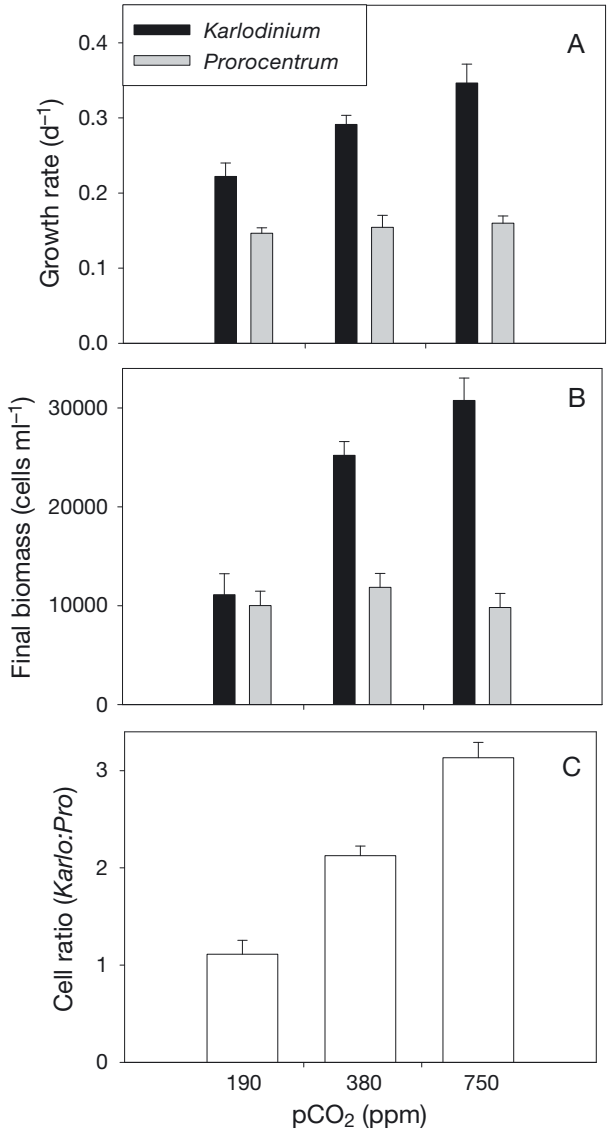

Fig. 10. *Karlodinium veneficum* and *Prorocentrum minimum*. Outcome of an ocean acidification interspecific competition experiment using mixed cultures of the dinoflagellates *K. veneficum* (black bars) and *P. minimum* (grey bars), isolated from the same estuary in Delaware Inland Bays, USA, at various CO_2 concentrations. Mean (+SD, n = 3) (A) growth rates (d^{-1}) during the last 10 d of the experiment, (B) final biomass (cells ml^{-1}), and (C) final abundance ratios (cell:cell) (F. X. Fu & D. A. Hutchins unpubl. data)

(among many) determining the outcome of interspecific competition in mixed natural assemblages. The dominance of toxic *K. veneficum* over non-toxic *P. minimum* at high pCO_2 could be due to pCO_2-induced karlotoxin (Fu et al. 2010) inhibiting the growth of *P. minimum*. In a similar vein but between more closely related taxa, Davis et al. (2009) showed that toxic populations of the freshwater harmful cyanobacterium *Microcystis* can outcompete non-toxic ones at higher temperatures. Consequently, the effects of global climate change on toxin production may be important to understand in order to explain inter- and intraspecific competitive interactions within HAB communities in a range of aquatic ecosystems.

An emerging ocean acidification issue with potentially serious environmental implications is the new evidence that elevated pCO_2 along with nutrient limitation greatly increases the cell-specific toxicity of some HAB species, including the dinoflagellate *Karlodinium veneficum* (Fig. 8; Fu et al. 2010) and some *Pseudo-nitzschia* spp. diatoms (Figs. 6 & 7; Sun et al. 2011, Tatters et al. 2012). Our preliminary work with the dinoflagellate *Alexandrium catenella* shows that saxitoxin levels are greatly stimulated by ocean acidification as well (Fig. 5). These early results suggesting that toxin production can be regulated by CO_2 or pH raise an alarming question: Will harmful algal blooms become more toxic in the future ocean? New studies are needed that encompass a broad range of harmful taxonomic groups, and that include physiological measurements along with examinations of gene and protein expression patterns with an eye towards identifying CO_2- and toxin-responsive genes for future follow-up work.

These new studies with multiple diatom and dinoflagellate species suggest a possible general principle of toxic HAB responses to ocean acidification: that the combination of low pH and growth limitation strongly stimulates their cellular toxin levels. This common physiological response may be shared by species from divergent taxonomic groups, and occurs when growth is limited by a variety of different nutrients, or by other environmental factors such as lower temperatures. The literature on algal toxins already highlights a generalized 'growth rate hypothesis,' in that limitation by nutrients or other factors often promotes higher toxin levels in many HAB species (Bates et al. 1991, Cembella 1998, Pan et al. 1998, Maldonado et al. 2002, Granéli & Flynn 2006, Lim et al. 2006, Mitra & Flynn 2006, Sunda et al. 2006, Adolf et al. 2009, Fu et al. 2010), as long as the limiting resource is not directly required for toxin synthesis (e.g. light energy or nitrogen in some cases). Thus, toxins may increase in slowly dividing cells at least partly due to the availability of a supply of 'excess' fixed carbon and photosynthetically-derived energy that can be directed into toxin precursors. Of course, any roles that toxins may play in reducing grazing mortality (Waggett et al. 2008, 2012), facilitating supplemental prey capture (Adolf et al. 2007, Sheng et al. 2010), or obtaining limiting nutrients such as iron (Maldonado et al. 2002, Wells et al. 2005), also

become increasingly important to the HAB species when growth rates are severely limited. Our new work suggests that when elevated pCO_2 is also incorporated, this growth limitation effect on toxin production is tremendously exacerbated.

It is instructive in this respect to consider the large body of previous work on the effects of changing pCO_2 on secondary metabolism in terrestrial plants. The major consensus is that plant secondary metabolites increase with higher CO_2 levels (Schonhof et al. 2007, Ziska et al. 2008, Ghasemzadeh & Jaafar 2011, Ibrahim & Jaafar 2011). Since most known algal toxins, including domoic acid, saxitoxins, brevitoxins, and karlotoxins, are classified as secondary metabolites, it should not be entirely surprising that increased CO_2 levels can result in higher toxin cell quotas. The specific mechanism for this increase would be likely dependent on the class of toxins. For lipophilic toxins such as karlotoxins and yessotoxins, this could result from increased glycolate levels (the starter unit for toxin synthesis; Yamazaki et al. 2011), allowing more fixed carbon to be diverted to the toxin machinery. A major cellular source of glycolate is photorespiration (Spencer & Togasaki 1981); however, it might be expected that this source would be reduced when ambient CO_2:O_2 ratios are high, and so this does not appear to be consistent with the observed trends in lipophilic toxin synthesis under acidified conditions. However, another study with a freshwater green alga showed that glycolate production rates were 15 to 20 times higher in elevated CO_2-grown cells relative to air-grown cells (de Veau & Burris 1989).

A similar 'high CO_2 subsidy' argument can be made for hydrophilic toxins such as domoic acid (Ramsey et al. 1998) and saxitoxin (Srivastava et al. 2011) with regard to their biosynthetic precursors (e.g. acetate and arginine). Of course, an alternate hypothesis is that lowered seawater pH directly affects the activity of crucial enzymes in toxin biosynthetic pathways and hence synthesis; external pH decreases can indeed affect intracellular pH in several phytoplankton species (Dason et al. 2004, Suffrian et al. 2011). In order to mechanistically understand the future impacts of toxic algal blooms in a high CO_2 ocean, more studies should be focused on distinguishing between these 2 possible causes: direct pH effects on biosynthetic pathways, and indirect stimulation of toxin synthesis by a high CO_2 subsidy.

The potential impacts on the growth, toxin production, and community structure of harmful algae from increasing CO_2, either alone or in conjunction with other variables, are currently poorly understood. So far, however, interactive global change effects have been examined in very few organisms. Physiological responses of dinoflagellates to even pCO_2/pH shifts alone have been examined only for a handful of species (Rost et al. 2006, Fu et al. 2008a). Our ability to predict the long-term synergistic effects of climate change and ocean acidification on HAB and competing non-harmful species depends to a large extent on understanding their basic physiological and growth responses under changing conditions of CO_2, temperature, irradiance, salinity, and nutrients. Some modeling work based on laboratory and field studies has attempted to predict how HABs may respond to future rising temperature (Peperzak 2003, 2005, Moore et al. 2008, 2011). For truly predictive capabilities, additional modeling studies will be necessary, which incorporate rising CO_2 along with all of the other relevant global change factors as they affect growth and toxicity of harmful species.

Even less is known about the effects on HAB species of changing 'bottom-up' variables such as pCO_2 and temperature when combined with altered 'top-down' controls such as microzooplankton herbivory. Aside from direct grazing effects on HAB mortality, indirect interactions between prey availability and a changing physical and chemical environment could be especially significant for mixotrophic species, such as many dinoflagellates (Caron & Hutchins in press). Rising CO_2 or interactions between CO_2 and temperature have a significant effect on microzooplankton communities, abundances, and grazing rates in the North Atlantic spring bloom (Rose et al. 2009). These findings emphasize the importance of determining how CO_2 and other climate variables affect trophic interactions in marine regimes where HABs are a major environmental issue.

The validity of extrapolating short-term experiments with natural HAB communities or laboratory cultures to long-term ecosystem trends needs to be explored. Incubation experiments examining responses to CO_2 alone and in concert with other global change variables typically cover only a few generations, so it is hard to predict whether they accurately reflect likely future decadal- or century-scale trends in nature. Most of the published high-CO_2 experiments have placed present-day phytoplankton immediately into a high-CO_2 environment, and then analyzed how they respond after a given acclimatization time, which is usually shorter for field and longer for culture experiments. An exception to this is the recent study of Flores-Moya et al. (2012), which examined acclimation and adaptation to warming

and acidification in dinoflagellates over a 2 yr period; more studies of this sort are needed. Marine phytoplankton have large population sizes and relatively fast generation times, which means that they have a high probability of being able to adapt to ongoing environmental changes. Such potential long-term evolutionary changes in response to gradually changing pCO_2 and temperature may be quite likely, complicating the interpretation of short-term studies using abruptly modified environments. For instance, the rate of environmental change affects the physiological and possibly adaptive responses of the model alga *Chlamydomonas* to increasing CO_2 (Collins & de Meaux 2009). The rate of change of environmental variables will likely affect responses of all phytoplankton, including HAB species, to future anthropogenic ocean acidification and climate change. The challenge for marine scientists will be to understand and accurately predict the responses of harmful algal groups to all of the many interacting factors to which they must adapt in a rapidly changing ocean.

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Phytoplankton niches, traits and eco-evolutionary responses to global environmental change

Elena Litchman[1,2,*], Kyle F. Edwards[1], Christopher A. Klausmeier[1,2], Mridul K. Thomas[1]

[1]Michigan State University, Kellogg Biological Station, Hickory Corners, Michigan 49060, USA
[2]Centre for Ocean Life and Section of Ocean Ecology and Climate, DTU Aqua, 2920 Charlottenlund, Denmark

ABSTRACT: Phytoplankton are major primary producers in aquatic ecosystems and are sensitive to various aspects of global environmental change. They can respond through phenotypic plasticity, species sorting, genetic adaptation, or a combination of these processes. Here we present conceptual, experimental and theoretical ways to predict different phytoplankton responses to global change. Using phytoplankton ecological niches to predict their responses to multiple environmental stressors is a promising new approach. Functional traits of phytoplankton, such as resource utilization traits and tolerance curves for various environmental factors like temperature, can be used to define niches along major axes. Characterization of pairwise and higher dimension trade-offs among traits should help predict possible niche changes along multiple dimensions simultaneously. The potential for evolutionary responses to global change can be assessed using evolution experiments with individual strains, as well as in communities, because the responses may depend on the presence of competitors, grazers, and parasites. The evolutionary pressures induced by multiple stressors may have interactive effects and, thus, should be investigated simultaneously. Novel models of trait evolution in a community context should provide additional insights into potential adaptation trajectories under diverse global change scenarios.

KEY WORDS: Ecological niche · Climate change · Trait-based approach · Trade-off · Experimental evolution · Community · Adaptive dynamics

INTRODUCTION

Phytoplankton are responsible for about half the global primary productivity, play a major role in most biogeochemical cycles, and form the basis of many aquatic food webs (Field et al. 1998, Falkowski et al. 2008). The contribution to major elemental cycles differs greatly between the major functional groups of phytoplankton. Processes such as nitrogen fixation, calcium uptake, and silicon uptake occur almost solely in single groups, in these cases the cyanobacteria, coccolithophorids, and diatoms. Some taxa, such as the cyanobacterium *Trichodesmium* sp. and the dimethyl sulfide-producing coccolithophorid *Emiliania huxleyi*, are believed to exert regional and possibly global effects (Brown & Yoder 1994, Staal et al. 2003). Phytoplankton are extremely sensitive to global environmental change, responding not only through total biomass but community composition as well (Li et al. 2009). In order to understand global elemental cycles and predict the climate change-driven alterations to them, it would be valuable to understand patterns in community structure and productivity of major phytoplankton taxa.

Predicting how phytoplankton communities will reorganize in the future in response to changes in climate and other global change stressors is, therefore, a major challenge facing oceanographers, aquatic ecologists, and environmental scientists. Will the biomass of phytoplankton decline or increase in the

*Email: litchman@msu.edu

future? What taxonomic groups and individual species could benefit or be hit especially hard by changing environmental conditions? Answers to these questions will be required if we want to understand how oceanic ecosystems will function in the future.

Organisms can respond to changing environmental conditions in 3 distinct ways that we briefly outline here (Fig. 1). First, a species' phenotypic plasticity (Table 1) can allow it to persist as the environment changes (Charmantier et al. 2008), essentially widening its ecological niche (Polechova & Storch 2008). Species, as well as traits, differ in their plasticity ranges, and higher plasticity is often (but not always) beneficial for persisting under global change (Nicotra et al. 2010). Second, if the limits of phenotypic plasticity of individual species are reached, species sorting (replacement) may occur as a response to changing environmental conditions (Ackerly 2003). Species that are better adapted to novel conditions will increase in abundance, competitively displacing more poorly adapted species. Such species may already be present in a community or may arrive via immigration (Urban et al. 2011). Finally, species may also genetically adapt to changing conditions, via clonal selection or selection on new genotypes arising through mutation, horizontal gene transfer, or recombination.

Therefore, phenotypic plasticity, species sorting, and genetic adaptation can all contribute to species responses to global environmental change and can act simultaneously or sequentially. It is, however, extremely difficult to predict or determine in retrospect the relative importance of individual processes. Further complications arise because environmental changes are multidimensional and responses may be both nonlinear and nonadditive. A promising approach to dealing with this complexity and increasing our mechanistic understanding of the effects of multiple stressors is to quantitatively define multidimensional ecological niches of phytoplankton and determine the influences of environmental conditions on niches of individual species and functional groups. As we describe below, mechanistic approaches to the niche have the potential to unify our understanding of how communities respond to global change via phenotypic plasticity (e.g. thermal tolerance curves), genetic change (e.g. selection on intraspecific variation in thermal tolerance curves), and species sorting (e.g. sorting due to interspecific variation in thermal tolerance curves). Furthermore, characterizing multidimensional niche space for individual species or functional groups could help predict phytoplankton responses to diverse global change stressors acting simultaneously. By constructing trait-based eco-evolutionary models informed by these data it will be possible to make short-term and long-term predictions as to how communities will respond via multiple mechanisms to multiple stressors.

Here we describe the concept of the niche and how it can be made more mechanistic using functional traits of organisms, we discuss the role of traits in explaining present and future community structure and dynamics, and we suggest theoretical and experimental ways to predict species eco-evolutionary responses to multiple environmental stressors.

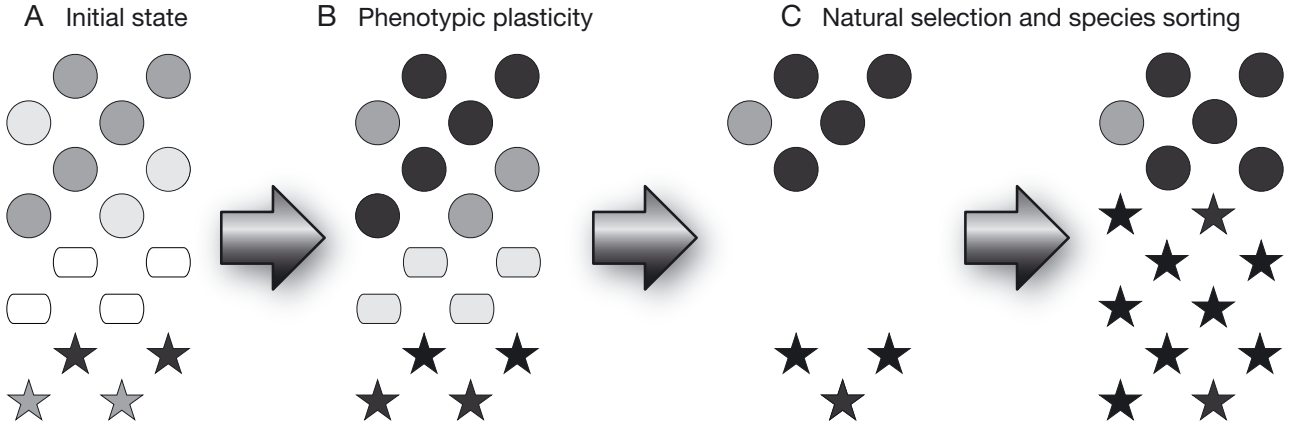

Fig. 1. Diagram of possible responses of a community to changing environment. (A) A community consists of 3 species (stars, circles, and rectangles), different shades represent different phenotypes (and potentially genotypes). (B) All species respond to changing environment by altering their phenotypic traits (phenotypic plasticity). (C) One species (rectangles) is maladapted to novel conditions and cannot persist in the community, while the best-adapted species (stars) increases in abundance more than the other persisting species (circles) (species sorting). Certain phenotypes and, potentially, genotypes (dark ones) in both species are better adapted to novel conditions and increase in frequency (natural selection)

THE NICHE CONCEPT

The concept of the niche is one of the fundamental ideas in ecology. It was introduced by J. Grinnell and C. Elton early in the 20th century, and further developed by G. E. Hutchinson and R. MacArthur in the 1950s and 1960s, after which it fell out of favor for some time (Chase & Leibold 2003). Since the early 2000s, the concept of the niche has been experiencing a renaissance, with multiple papers and books reinvigorating the idea (Peterson et al. 1999, Chase & Leibold 2003), including the applications of the niche concept to predicting the effects of global change on various organisms (Wiens et al. 2009). There are several views of the niche, placing emphasis on different aspects. The niche can be considered a part of environmental space that a species can occupy, defined primarily by abiotic factors, which is sometimes called the Grinnellian niche (Wiens et al. 2009). Another view puts more emphasis on the functional role that a species or a group of species plays in an ecosystem and is referred to as the Eltonian niche (Polechova & Storch 2008, Wiens et al. 2009). Hutchinson's idea of a niche as an *n*-dimensional hypervolume combines the 2 views (Polechova & Storch 2008) and also defines and distinguishes the fundamental and realized niche. A fundamental niche describes the potential space along the multiple resource and environmental factor dimensions where a species can live in the absence of biotic interactions (Hutchinson 1957). A realized niche is a subset of a fundamental niche that allows species persistence in the presence of competition (original Hutchinson's definition) and other biotic interactions such as predation (Colwell & Rangel 2009, Wiens et al. 2009).

LINKING NICHES TO TRAITS

Although the concept of the niche is very attractive, applying it to real-world questions is challenging because of the difficulty in identifying all the relevant axes and determining the ranges on all those axes that lead to species persistence. These challenges may be somewhat lessened in phytoplankton, because the main niche dimensions are probably fewer and easier to identify than in other, more complex, organisms. These dimensions include resources, such as nutrients and light, as well as temperature, pH, grazers, and parasites (Litchman et al. 2010). Once potential niche axes are known, the most commonly used way to characterize species niches is to analyze species abundances (or presence-absence) along environmental gradients and use statistical techniques to delineate the niche (Guisan & Thuiller 2005). These approaches are often called environmental niche modeling or species distribution models (SDMs) and are now widely used in terrestrial ecology, including applications to predicting species responses to global change (Pearman et al. 2008, Thuiller et al. 2008). However, much niche modeling suffers from a lack of a clear mechanistic basis and unrealistic assumptions, such as niche conservatism and absence of biotic interactions (Pearman et al. 2008, Kearney & Porter 2009, Wiens et al. 2009; reviewed further in the 'Niche models' section).

A possible solution to these issues is to use a species' traits to define its ecological niche (Colwell & Rangel 2009, Wiens et al. 2009, Kearney et al. 2010). Trait-based approaches have gained popularity, especially in terrestrial plant ecology, and can help increase our mechanistic understanding of community structure and dynamics (McGill et al. 2006, Bruggeman & Kooijman 2007, Litchman et al. 2007). Using functional traits to mechanistically define ecological niches has been proposed recently (Chase & Leibold 2003) and applied to terrestrial ectotherms (Kearney & Porter 2009, Kearney et al. 2010) but has not been developed for phytoplankton or other microbes. Kearney and colleagues propose 3 major frameworks to connect functional traits to ecological niches: biophysical ecology, the geometric frame-

Table 1. Main terms used in the text and their definitions

Term	Brief definition
Niche	Conditions where a species' growth rate is positive
Genotype	Genetic makeup of an organism
Phenotype	Observed characteristics of an organism
Phenotypic plasticity	Changes in an organism's phenotype in response to changes in the environment
Genetic adaptation	Changes in a population's distribution of genotypes in response to changes in the environment
Trait	An element of an organism's phenotype
Functional trait	A trait that determines fitness
Species sorting	Changes in community composition due to interspecific interactions leading to exclusion

work for nutrition, and dynamic energy budget (DEB) models (Kearney et al. 2010). Similarly, Leibold and Chase (Leibold 1995, Chase & Leibold 2003) developed mechanistic niche models by adapting the resource competition framework (Tilman 1982) to other processes that affect an organism's fitness. This approach is well suited for phytoplankton, as the resource competition theory itself was first developed for phytoplankton (Tilman 1982, Tilman et al. 1982). Furthermore, a trait-based approach based on resource competition theory mechanistically unites the Grinellian and Eltonian perspectives, because species' traits determine the conditions under which they can persist in isolation, as well as their impacts on other species in the community. This combination of requirements and impacts translates, in Hutchinson's terms, from fundamental to realized niches (Chase & Leibold 2003). Therefore, this approach to the niche can connect traits, niches, and the biogeochemical effects of phytoplankton.

For example, if a species has certain trait values for nutrient-dependent growth and mortality, it is then possible to define a range of a given resource where the net growth rate would be positive. This range would, then, delineate the fundamental niche of the species along the axis of that resource (Fig. 2A). If resource levels are affected by competitors, this approach can define the realized niche as well, e.g. if under particular environmental conditions competitors reduce nutrient concentrations below that required for the focal species to persist (R^*; Fig. 2A), then the current environmental conditions are not within that species' realized niche. Identifying traits for multiple resource- or environmental factor–related growth would then allow us to define the ecological niche of a species in all these dimensions (Fig. 2B). Therefore, defining species trait ranges along the multiple environmental axes should help us to quantitatively describe the multidimensional niche space. As species differ in their trait values (Litchman et al. 2007, Litchman & Klausmeier 2008), the quantitative knowledge of species traits will help determine niche differences across species, as well as the consequences of such differences for species coexistence and diversity (Chase & Leibold 2003).

The niche perspective on species coexistence and diversity contrasts with neutral theory that assumes no meaningful differences in traits of species and, thus, in their fitness (Hubbell 2001). According to neutral theory, changes in communities may only occur due to stochastic processes (demographic stochasticity, stochastic dispersal, and random speciation) but are not driven by selection on trait differ-

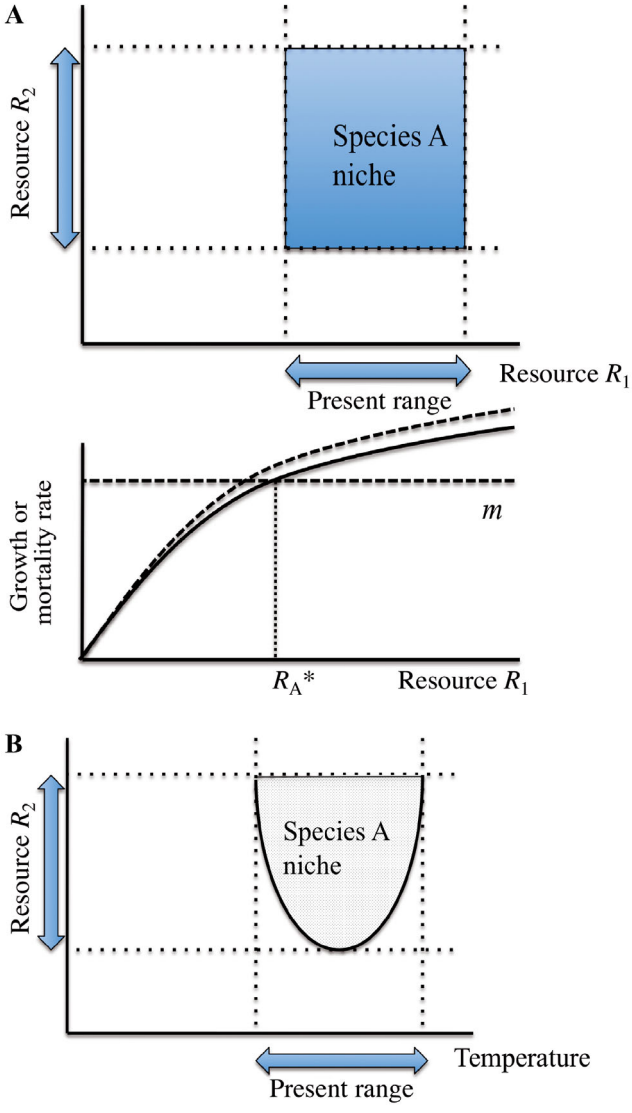

Fig. 2. Mechanistic representation of a niche using functional traits. (A) The growth rate of Species A is described as a function of species traits and resource concentration (Monod function, $\mu = \mu_{max}[R_1/(R_1 + k)] - m$, where μ is the growth rate, μ_{max} is the maximum growth rate, R_1 is the resource concentration in the environment, k is a half-saturation constant for growth, and m is mortality). A range of that resource occurring in a given environment and leading to positive growth corresponds to the niche range along that resource dimension (upper panel). A similar function can be used for another resource, R_2 to define the niche range along that resource dimension. The resource concentration at which growth rate equals mortality is called R^* and is a measure of species competitive ability for a given resource. For the Monod model, $R^* = [mk/(\mu_{max} - m)]$. R_A^* is the R^* of Species A. The dashed line is a growth curve for a different genotype that is a better nutrient competitor (lower R^*). (B) Ecological niche in 2 dimensions, along resource (nutrient) and environmental factor (temperature) axes. Shaded area represents space where species' growth rate is positive, e.g. the niche). The R^* (thick lower border of the shaded area) depends on the temperature (Tilman 1982)

ences (Hubbell 2001). It is clear that most phytoplankton species differ in many aspects of their ecological niches, therefore, not following the assumptions of neutral theory. However, it is intriguing to think that, in some instances, phytoplankton species may appear effectively functionally identical, at least for periods of time. For example, cryptic species documented in phytoplankton (Amato et al. 2007) may not differ noticeably in their traits that affect fitness, thereby behaving neutrally. The differences between the neutral and niche perspectives have been discussed extensively in ecological literature and will not be detailed here.

INTER- AND INTRASPECIFIC TRAIT VARIATION

A quantitative characterization of species traits and comparisons among species or higher taxa (Edwards et al. 2012) should help predict what species and groups are likely to dominate under changed conditions. For example, species that are good nutrient competitors should increase in their abundance under more severe nutrient limitation that may result from weaker mixing in a warmer ocean (Doney 2006). Functional trait compilations averaged over major functional groups can be used to parameterize phytoplankton community models to predict responses of different groups to various global change stressors (Litchman et al. 2006).

Assessing the range of trait variation under different conditions within a species is needed to characterize the limits to phenotypic plasticity. Most physiological and ecological traits in phytoplankton are inherently plastic, but the ranges differ for different traits. Phytoplankton cell size is highly dependent on the levels of nutrients, light, and temperature (Doucette & Harrison 1990, Riegman et al. 2000, Montagnes & Franklin 2001). The growth affinity for nitrate (μ_{max}/k; variables in Fig. 2) was shown to increase with increasing temperature, while the affinity for ammonium had little temperature dependence (Reay et al. 1999). A comprehensive meta-analysis of published physiological experiments measuring species traits under different conditions has not been done, but could provide much needed information on phenotypic plasticity ranges. It is also unknown how species differ in their degree of plasticity for the same traits.

Laboratory experiments measuring physiological traits in phytoplankton are usually carried out on individual strains. In addition to intraspecific trait variation due to different environmental conditions (phenotypic plasticity), different strains grown under identical conditions also exhibit variation in trait values due to genotypic differences (Rynearson & Armbrust 2004, Kardinaal et al. 2007), providing a basis for selection. Strains better adapted to novel conditions will outcompete less adapted strains, thus changing genotypic frequencies in the population.

NICHE MODELS

Statistical niche models

Most current SDMs use observational data on species distributions and relate them statistically to various environmental variables, thereby attempting to define species-realized niches (Kearney et al. 2010). Several major statistical approaches are commonly used; these include generalized linear models (GLMs), generalized additive models (GAMs), genetic (genetic algorithm for rule set production, GARP), and machine-learning algorithms such as Maxent (Wiens et al. 2009).

Due to the prohibitive amount of sampling required to characterize phytoplankton abundance at finer scales, we lack data on the global distributions of most phytoplankton species. However, we can place constraints on their geographic ranges using SDMs. As SDMs are typically correlative, estimating optimal abiotic conditions based on current abundances in the environment, they are not perfect for phytoplankton because of the difficulties estimating species abundances. SDMs have several other shortcomings, among them the inability to clearly separate biotic and anthropogenic influences from underlying abiotic forces and the absence of mechanistic explanations for the observed patterns (Wiens et al. 2009, Kearney et al. 2010).

Mechanistic niche models

Mechanistic SDMs overcome these issues by using physiological parameters to construct a niche model that is then related to current environmental conditions to derive a maximal species range (Kearney & Porter 2009). The species' range by this model is therefore the geographic limit of its fundamental niche. This approach has not been widely applied to marine taxa, despite being well suited to them by virtue of having stable, highly predictable, and well-connected environments (Robinson et al. 2011).

Phytoplankton meet many of the assumptions of this method, including being strongly influenced by abi-

otic factors, being less dispersal-limited than most taxa due to ocean circulation, and lacking in behaviors that can modify environmental influences. In addition, the physiological traits needed to parameterize niche models, such as the effects of changes in nutrient concentration, light, temperature, and salinity on fitness have been measured in a number of phytoplankton species (Litchman & Klausmeier 2008, Schwaderer et al. 2011, Edwards et al. 2012). The reactions norms that phytoplankton exhibit in response to these environmental drivers are strongly non-linear, which is difficult to capture in correlative SDMs. For example, species responses to temperature are highly skewed, with lethal temperatures often a few degrees above the optimum temperature for growth (Kingsolver 2009).

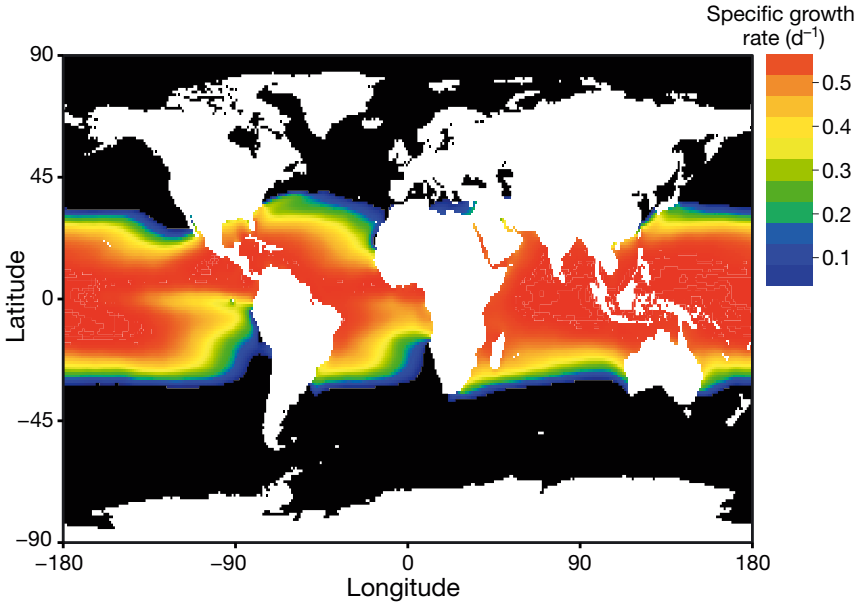

Fig. 3. Predicted species range of cyanobacterium *Trichodesmium erythraeum* Strain GBRTRLI101 based on its thermal tolerance curve (Chappell & Webb 2010) Colour variation indicates predicted variation in positive growth rates (which could imply variation in abundance), while growth rates at or below zero are shown as black. This species is generally found in tropical and subtropical waters (Karl et al. 2002)

An example of using mechanistic SDMs is shown in Fig. 3. The geographic range of the tropical cyanobacterium *Trichodesmium erythraeum* was estimated using its thermal tolerance curve (Chappell & Webb 2010), fitted to the function of Norberg (2004), and the monthly mean ocean temperature data from the NOAA World Ocean Atlas 2009 (Locarnini et al. 2010). Growth rates were calculated based on monthly temperatures to account for seasonal temperature changes, and the range depicted with color variation covers areas where the mean annual growth rate is estimated to be positive. Though our ability to validate these model predictions is currently limited, they do agree with our understanding of *Trichodesmium erythraeum* as a tropical and subtropical species (Karl et al. 2002). The predicted range corresponds to the fundamental thermal niche of this species, and it is likely that temperature also controls its abundance and growth indirectly, by regulating stratification and nutrient supply (Monteiro et al. 2011). Disentangling the effects of different environmental factors on species niches is complicated, and caution should be exercised when interpreting the resulting species ranges. We may extend this approach by incorporating multiple environmental parameters simultaneously, but we are currently limited by our understanding of how major environmental drivers interact to affect population growth rates. Characterizing the nature of the interactions between temperature, nutrients, and light in a group of species would provide a useful base to extend this into multiple trait and environmental dimensions. Additionally, this approach would greatly benefit from the incorporation of intraspecific variation in traits, as local adaptation would tend to bias estimates based on single cultures. Other factors, such as competition, grazing, and dispersal will limit this range further, though source–sink dynamics may extend the boundaries as well.

Range predictions may similarly be made based on environmental predictions from global climate models. A comparison of existing and future ranges would enable us to better predict changes in elemental cycling in different parts of the ocean, such as potential poleward shifts in populations of nitrogen-fixing cyanobacteria.

USING TRAITS TO REVEAL MECHANISMS BEHIND COMMUNITY STRUCTURE

A fundamental challenge in basic and applied ecology is the prediction of community composition from environmental conditions. A common approach to this problem is to use time series data or spatially extensive surveys to analyze how different taxonomic groups respond to environmental variation. A limitation of this approach is that the resulting patterns can be difficult to interpret in terms of underly-

ing mechanisms. For example, in the western English Channel there is a somewhat regular procession of diatom species/genera as the environment shifts from presumed light limitation during the winter, through spring bloom conditions, to presumed nutrient limitation during the summer (Widdicombe et al. 2010). What mechanism(s) cause this seasonal shift in diatom composition? Because multiple environmental variables change in a partially correlated way (light, temperature, nitrate, phosphate, silicate, grazer abundance, and composition), and because different species or genera vary in multiple ways, it is difficult to dissect the causes of seasonal variation in composition. However, in order to predict how communities will respond to environmental change, including potentially new combinations of environmental conditions, it will be necessary to have a strong mechanistic foundation for understanding community composition and dynamics.

A focus on functional traits has the potential to provide a more mechanistic basis for understanding the causes of shifts in community composition. Much work in this vein has already been performed in terrestrial plant communities. For example, Cornwell & Ackerly (2009) measured plant community composition across the varied topography of coastal California, and at the same time measured 14 leaf and stem traits on 54 species that occurred in these communities. These data allowed them to test whether community-averaged trait values changed along environmental gradients. Among other patterns, they found that community-average specific leaf area (leaf area divided by leaf dry mass) increased with increasing soil water content. This pattern is consistent with a trade-off, whereby higher specific leaf area increases potential productivity at the expense of resource use efficiency. Therefore, their results suggest that changing soil water content alters community composition by selecting for species with the locally optimal specific leaf area.

There are distinct advantages and disadvantages of applying such a trait-based approach to community variation in marine phytoplankton. A primary advantage is the strong link between commonly measured phytoplankton traits and the processes of resource acquisition and usage (Litchman & Klausmeier 2008). For example, much of the ecophysiology of nutrient acquisition and nutrient-limited growth can be captured by measuring the Michaelis-Menten uptake curve and the Droop curve for growth rate as a function of nutrient quota (Grover 1991). Compared to common terrestrial plant traits such as wood density and seed mass (e.g. Cornwell & Ackerly 2009), the parameters of these curves are more clearly linked to ecophysiology and population dynamics. The disadvantage of applying these traits to community analyses is that they are relatively labor intensive to measure and hence have been measured for relatively few species. Therefore, quantifying the community average of a trait, such as the minimum subsistence quota for nitrogen, will be a significant undertaking.

Although quantifying trait values for an entire community is ideal, it is possible to use a subset of species to test for the role of functional traits in determining community response to multiple environmental factors. For example, we may predict that, in the western English Channel, better nitrogen competitors should have a relative advantage during summer periods, when nitrate is often reduced below the detection limit (Smyth et al. 2010). Using laboratory-measured parameters of Michaelis-Menten uptake and Droop growth curves, we can make *a priori* predictions of the relative competitive ability of nitrate for those species for which we have such data (Edwards et al. 2011). We can then test whether species-specific responses to nitrate concentration are consistent with interspecific trait differences. In particular, we can predict that good nitrogen competitors will experience a relative advantage as nitrate becomes rare. In a regression framework, this will be

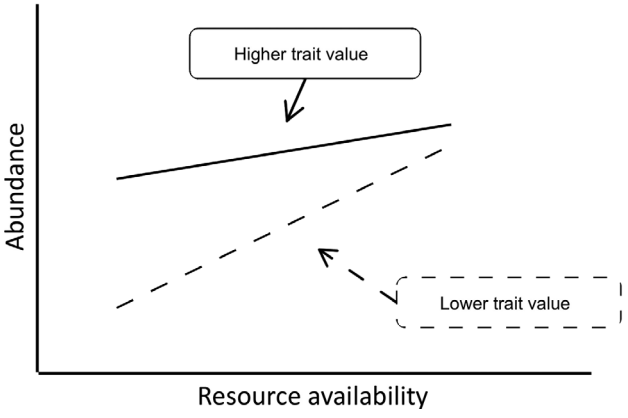

Fig. 4. Example of how functional trait differences will result in predictable differences between species in response to environmental variation. Assume a trait is measured that approximates competitive ability for a resource. Across a gradient of resource availability, species' abundances should change in such a way that better competitors (higher trait) increase in relative abundance as the resource decreases and becomes more limiting. In a linear statistical model, the effect of the trait can be quantified as an interaction between the trait value and the slope of abundance versus resource availability. The particular slopes and intercepts shown here are merely one example, as the prediction concerns the relative values of the slopes, i.e. higher trait

evident as a difference in slopes of abundance versus nitrate concentration, which can be tested as an interaction between a species' trait and its slope versus nitrate (Webb et al. 2010, Schwaderer et al. 2011; Fig. 4).

Preliminary analyses indicate that this approach can detect strong effects of functional traits on interspecific differences in response to environmental change (Edwards et al. in press). By using multiple regression, this approach can be applied to multiple trait–environment interactions simultaneously, which allows one to test for particular trait–environment linkages while controlling for the effects of other environmental factors that vary simultaneously. In summary, we can establish a mechanistic foundation for the relation between environmental change and community composition, by formulating *a priori* predictions of trait–environment interactions and testing those with appropriate statistical models.

This approach also integrates naturally with a mechanistic approach to the niche and the geographic distribution of species. The same traits that are used in a mechanistic niche model can be used to make predictions about the relative performance of species under particular environmental conditions. For example, in a nitrogen-limited region of the ocean, a mechanistic niche model will predict that some set of species can each individually persist under those particular nitrogen supply conditions; in Hutchinsonian terms, this region is therefore part of the fundamental niche of these species. However, within this set of species, some species will be relatively better nitrogen competitors and will therefore be expected to attain higher abundance, and perhaps exclude other species entirely. In this way, it is possible to translate from models of the fundamental niche to the observed realized niche, using trait-based predictions. Furthermore, by using functional trait information to parameterize population dynamic models (see section 'Models of trait evolution and community assembly'), it is possible to make explicit predictions about what species or trait values can coexist under particular environmental conditions.

TRADE-OFFS AND RESPONSES TO MULTIPLE STRESSORS

The majority of species traits are not independent but positively or negatively correlated; these correlations often represent trade-offs and result in contrasting ecological strategies (Margalef 1978, Litchman et al. 2007, Schwaderer et al. 2011). Trade-offs are essential for explaining species coexistence and diversity (Tilman 1990) and niche differences (Chase & Leibold 2003).

Trade-offs are especially important when multiple stressors act simultaneously. Because of the trade-offs, a species that performs well under one environmental stressor, such as an elevated temperature, may not fare well when another stressor, such as increased CO_2, is acting simultaneously. The trade-offs reflect energy and material constraints on the investment into major functions: for example, investing into cellular machinery for growth (ribosomes) may limit investment into light-harvesting machinery (chloroplasts) (Klausmeier et al. 2004). If higher CO_2 increases sensitivity to photoinhibition (Wu et al. 2010), a simultaneous production of heat shock proteins in response to rising temperatures may limit the investment into photoprotective compounds. However, heat shock proteins may also be useful in photoprotection (Schroda et al. 1999). Determining the nature of trade-offs in phytoplankton is, therefore, crucial for predicting species and community responses to diverse global change factors. This is an active area of research and many trade-offs have been documented for phytoplankton, including a 3-way trade-off between nitrogen and phosphorus competitive abilities and cell size (proxy for grazer resistance) (Litchman et al. 2007, Edwards et al. 2011, Schwaderer et al. 2011). However, many more trade-offs remain to be discovered and their mechanistic basis understood. It is also important to determine how rigid the observed trade-offs are and whether species can deviate from them.

MULTIVARIATE TRAIT STRUCTURE

We may expect that different environmental factors have distinct effects on community composition and ecosystem processes. However, whether this is true will depend on patterns of interspecific covariation of multiple performance functions. For example, nitrogen pollution could shift production in coastal ecosystems from nitrogen limitation to phosphorus limitation. Furthermore, if there is a strong trade-off across species in competitive ability for N versus P, we would expect a large shift in composition, from better N competitors to better P competitors. However, the regional species pool may not exhibit such trait variation, and instead, multiple performance criteria may be positively correlated. For example, if smaller cells tend to be better competitors for all nutrients (Edwards et al. 2011), but species of similar

size do not vary in N versus P ability, then a shift from N to P limitation may not lead to a strong shift in composition. Therefore, it is important to distinguish between the existence of functional trade-offs and the degree to which interspecific variation is organized along those trade-off axes: past selection may not have led to significant phenotypic variation along all possible trait axes.

Some evidence for the importance of this perspective comes from an analysis of the covariation among the competitive ability for nitrogen, the competitive ability for phosphorus, and cell size in freshwater and marine phytoplankton (Edwards et al. 2011). When combining freshwater and marine species, there is evidence for a general 3-way trade-off among the competition for N, the competition for P, and cell size. However, the dominant axes of trait variation differ between freshwater and marine systems: freshwater algae vary primarily along an N versus P competition axis, while marine species vary primarily along a size axis, where increased size is correlated with decreased competitive ability for both nutrients. Based on these results, it is plausible that a large change in the N:P supply ratio would result in a large change in the composition in freshwater systems, but a smaller change in the composition in marine systems. More generally, the response of a community to environmental change will depend on the structure of trait covariation created by past environments and evolution. By quantifying the multivariate patterns of interspecific trait variation, it is possible to test for trade-offs and to quantify what performance functions tend to covary positively and which vary independently. Using these patterns, it is then possible to project what kinds of communities can possibly occur in response to rapid environmental change. If climate change leads to novel combinations of environmental conditions, the resulting community may be poorly adapted to those conditions until evolution is able to explore new regions of trait space.

TRAIT AND NICHE EVOLUTION

Changing environmental conditions, including anthropogenic global change, may alter not only the levels of resources and environmental factors, thereby affecting species-realized niches, but may also lead to evolutionary changes in trait values. Traits characterizing resource utilization or responses to environmental factors (e.g. thermal niche) may evolve, leading to a better adaptation to novel selection regimes. Changes in trait values will thereby alter a species' fundamental niche. Niche evolution may be widespread in phytoplankton because large population numbers, fast generation times, high genotypic diversity, and various selective pressures likely lead to trait evolution. Understanding trait evolution is, therefore, necessary for characterizing niche dynamics at present and in the future. In the following section we outline ways to address the potential for trait evolution, both experimentally and theoretically.

EVOLUTION EXPERIMENTS

Experiments with individual species

Experimental investigation of how key traits may evolve in response to global change is a promising and much needed approach for marine phytoplankton and other planktonic organisms. There is a rich field of experimental evolution that uses bacteria, insects, terrestrial plants, and other organisms (Elena & Lenski 2003, Garland & Rose 2009, De Meester et al. 2011), but experimental studies on phytoplankton evolution are just beginning. The most relevant studies to what we propose here are the pioneering experiments on the evolutionary responses of the freshwater algae *Chlamydomonas* to a global change stressor, increased CO_2, carried out by G. Bell and S. Collins (Collins & Bell 2004, 2006). Evolution experiments are often done with single clones, and evolutionary changes arise as a result of mutations or genetic recombination (Garland & Rose 2009). However, in nature, most populations, including marine phytoplankton species, are comprised of many different genotypes and, as a result, exhibit intraspecific variation in trait values (Rynearson & Armbrust 2000, Rynearson et al. 2006). This intraspecific trait variation provides a rich basis for selection of the best adapted genotypes, often leading to a faster evolutionary response compared to monoclonal responses (Yoshida et al. 2003, Barrett & Schluter 2008). However, it is also possible that in large populations of asexual organisms, clonal interference, where clones with beneficial mutations compete with each other, may slow down adaptation (Kao & Sherlock 2008). We suggest that evolution experiments with marine (and freshwater) phytoplankton and other organisms should be carried out both with single clones, to address the role of mutation in evolutionary response, and with multiple clones, to better reflect selection scenarios in nature. A just published study on the bloom-forming coccolithophorid *Emiliania huxleyi* experimentally

evolved both individual clones and clone mixtures under increased CO_2 concentrations (Lohbeck et al. 2012). Experiments with monocultures combined with genetic analyses allowed determination of what genes are involved in a particular phenotypic/trait change, as, even for the same selection regimes, there may be distinct adaptation paths involving different genes and resulting in different states of fitness (Elena & Lenski 2003). Using ecologically and biogeochemically important species and species whose genomes were or are being sequenced and annotated would maximize the efficiency and usefulness of the experimental evolution studies.

The initial assessment of the genetic and phenotypic (trait variation) diversity will allow us to make predictions on the adaptation potential, both in experimental and natural populations. If the trait variation present in different genotypes is high, the population is more likely to respond adaptively to changing conditions and the rate of such response may be faster (Barrett & Schluter 2008). In marine phytoplankton, standing genetic variation and, possibly, trait variation may be high not only due to frequent mutations and changing selection pressures, but also due to gene flow from populations experiencing different environmental conditions via long-distance transport by currents and other types of water movement (Rynearson & Armbrust 2005).

To determine the pattern and rates of trait evolution, the trait distribution is assessed at the beginning of and throughout the experiment; the trait's mean values and variance are usually of interest. Adaptation can lead to changes either in means or variance or both, and will arise due to mutations, recombination, or clonal selection.

Most evolution experiments consider a single selective factor, and, to the best of our knowledge, investigations of the effects of multiple environmental stressors simultaneously on trait evolution have not been carried out. This is clearly a research direction that needs to be pursued urgently in the face of multidimensional global environmental change. For example, determining how rising temperatures and CO_2 concentration would affect evolution of phytoplankton functional traits would be very informative, as it is unknown if the selection pressures from the 2 stressors have an opposing or a synergistic effect (or no interaction) on traits and the niche.

A possible approach would be to conduct complete or incomplete factorial experiments with factors being major global change stressors, such as temperature, high CO_2, or high or low nutrient concentrations. Such experiments can be carried out both with single strains and strain mixtures to reflect the genetic diversity occurring in nature.

Community experiments

Although evolution experiments with individual species may show the potential of different species to adapt to various stressors, even acting simultaneously, the evolutionary responses in natural conditions will most likely also depend on the community composition in which the focal species is embedded (Van Doorslaer et al. 2010). Experiments with *Daphnia* showed that its microevolutionary responses to high temperature had the opposite pattern in a community compared to that a in monoculture (Van Doorslaer et al. 2010). Collins (2011) found that the evolutionary response of *Chlamydomonas* to increased CO_2 depended on whether the strains were grown in isolation or in mixtures.

It has been shown theoretically that, in communities, evolutionary responses of individual species to changing conditions may be hindered by the presence of other species that are already better adapted to the new conditions (de Mazancourt et al. 2008). With increasing diversity, the probability that such well-adapted species are present increases, and consequently, the evolutionary response may not be as strong in diverse communities as in monocultures or low-diversity communities (de Mazancourt et al. 2008). As a result, instead of evolutionary changes, species sorting (replacement) may be the dominant community (or metacommunity) response to novel environmental conditions.

These and other theoretical findings make it necessary to investigate evolution of species traits in the context of other species. The presence of predators or parasites may also alter the trajectory and the end result of adaptation. Therefore, we propose that evolution experiments investigating the effects of competitors, predators, and parasites on species evolutionary responses are a much-needed direction for experimental evolution studies. Of course, community composition changes frequently, and it would be impossible to run evolution experiments with all the species combinations that a species in question encounters. However, as a first step, it would be informative to contrast species trait evolution in the absence and presence of a competitor and/or predator. A more thorough investigation of trait evolution in the community context could be done using modeling approaches that we briefly outline in the following section.

MODELS OF TRAIT EVOLUTION AND COMMUNITY ASSEMBLY

A range of theoretical frameworks exists for the trait-based modeling of marine communities. These include (1) extensions of traditional community ecology, where a large number of species are allowed to sort out (randomly chosen species, Follows et al. 2007; a near-continuous range of species, Bruggeman & Kooijman 2007); (2) moment methods that approximate the community by total biomass, mean trait, and trait variance (Wirtz & Eckhardt 1996, Norberg et al. 2001, Savage et al. 2007, Merico et al. 2009); (3) multispecies models that combine population and trait dynamics as in quantitative genetics (Abrams & Matsuda 1997, Norberg et al. 2012); (4) mutation-limited evolution in adaptive dynamics (Geritz et al. 1997), and (5) evolutionary game theory, which focuses on uninvadable sets of species (Brown & Vincent 1992, Klausmeier et al. 2007, Litchman et al. 2009). What these approaches have in common is that they explicitly define species by their ecologically relevant traits. Moreover, they often make the same prediction about the endpoint of community assembly/evolution (Abrams 2001). They differ primarily in their assumptions about the sources of phenotypic diversity (rare mutation, genetic variation maintained by mutation-selection balance, immigration, 'everything is everywhere').

Few of these approaches have been used to predict how species and communities will respond to global change, but it is likely that they will differ in their predictions, because adaptation to a changing environment relies on phenotypic diversity. For example, in 3 disparate modeling frameworks (quantitative genetics [QG], adaptive dynamics [AD], and moment methods [MM]), the response of a trait to selection is proportional to the product of the trait variance and the selection gradient (QG: Lande 1979; AD: Dieckmann & Law 1996; MM: Norberg et al. 2001, Savage et al. 2007), but the source of variance differs (common or rare mutation or immigration). Therefore, accurately parameterizing the mechanisms that maintain or bring new phenotypes into the community is a key challenge in using trait-based models to predict response to global change.

A related issue particularly relevant in the response to multiple stressors is the covariance among multiple traits. This sets the ability for short-term adaptation (Lande 1979, Arnold 1992, Savage et al. 2007). When there are negative correlations between traits that are both being selected for, adaptation is hampered. This trait covariance is set by both the past selective regimes, as well as the input of new phenotypes due to mutation, which is subject to functional constraints (Arnold 1992), or immigration, which is determined by the traits of species in the regional species pool (Savage et al. 2007). Elucidating these functional constraints and determinants of metacommunity diversity (Norberg et al. 2012) is another important task.

CONCLUSIONS

Species and communities will undoubtedly respond to multifaceted global environmental change. Several mechanisms with overlapping time scales, such as phenotypic plasticity, species sorting, and genetic adaptation, will likely be involved. It is, however, unknown what the rate and the relative importance of the potential response mechanisms would be. Given the pressing need to assess how species and communities will respond to multiple stressors, ecologists and evolutionary biologists can approach the problem from multiple angles, using diverse experimental and modeling approaches. We propose several research directions to help advance the field and increase its predictive power. First, we can collect experimental data on major functional traits (and their plasticity) and combine it with the data on species distributions along major environmental gradients to map ecological niches. A combination of statistical and mechanistic niche descriptions may result in the most precise niche characterizations. Second, we can conduct evolution experiments, both with single strains and diverse populations to determine the adaptation potential and the exact mechanisms of adaptation. A simultaneous consideration of several key environmental stressors, such as temperature and CO_2, is necessary to increase the realism and to understand multiple stressor interactions. Third, the development of novel models of (phyto)plankton community organization and evolution should allow a detailed exploration of various global change scenarios and the role of community and food web composition in adaptation to global change.

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The biological pump in a high CO_2 world

Uta Passow[1,*], Craig A. Carlson[1,2]

[1]Marine Science Institute, University of California Santa Barbara, California 93106, USA
[2]Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California 93106, USA

ABSTRACT: The vertical separation of organic matter formation from respiration can lead to net carbon sequestration within the ocean's interior, making the biological pump an important component of the global carbon cycle. Understanding the response of the biological pump to the changing environment is a prerequisite to predicting future atmospheric carbon dioxide concentrations. Will the biological pump weaken or strengthen? Currently the ocean science community is unable to confidently answer this question. Carbon flux at approximately 1000 m depth, the sequestration flux, determines the removal of carbon from the atmosphere on time scales ≥100 yr. The sequestration flux depends upon: (1) input rates of nutrients allochthonous to the ocean, (2) the export flux at the base of the euphotic zone, (3) the deviation of carbon fixation and remineralization from Redfield stoichiometry, and (4) the flux attenuation in the upper 1000 m. The biological response to increasing temperature, ocean stratification, nutrient availability and ocean acidification is frequently taxa- and ecosystem-specific and the results of synergistic effects are challenging to predict. Consequently, the use of global averages and steady state assumptions (e.g. Redfield stoichiometry, mesopelagic nutrient inventory) for predictive models is often insufficient. Our ability to predict sequestration flux additionally suffers from a lack of understanding of mesopelagic food web functioning and flux attenuation. However, regional specific investigations show great promise, suggesting that in the near future predictions of changes to the biological pump will have to be regionally and ecosystem specific, with the ultimate goal of integrating to global scales.

KEY WORDS: Biological pump · Climate change · Ocean acidification · Rising temperature · Marine carbon cycle · Carbon sequestration · Export flux · Sequestration flux

INTRODUCTION

Concerns regarding the increase of atmospheric partial pressure of carbon dioxide (pCO_2) have focused attention on the biological carbon pump, because the ocean sequesters one-fourth to one-third of the carbon released by human activities (e.g. fossil fuel oxidation, deforestation and cement manufacturing) each year (Sabine et al. 2004, Sabine & Tanhua 2010). The term biological carbon pump refers to the suite of biologically mediated processes responsible for transporting carbon against a concentration gradient from the upper ocean to the deep ocean. According to our best estimates, approximately two-thirds of the vertical gradient in carbon in the ocean is attributed to the biological pump with the rest due to the solubility pump, but estimates of the relative activity of the different pumps are poorly constrained (Gruber & Sarmiento 2002, Reid et al. 2009). The questions of (1) whether the ocean will continue to take up carbon, (2) at which rate, (3) for how long the exported carbon will remain removed from the atmosphere, and (4) how the biological pump will respond to the consequences of increased carbon input combined with warming have become central both for scientists and politicians. In this

*Email: uta.passow@lifesci.ucsb.edu

review article we will explore potential impacts of predicted changes in the global temperature and carbonate system on the ability of the biological pump to sequester carbon and highlight exciting new research directions attempting to address these questions.

THE BIOLOGICAL CARBON PUMP AND ITS CONTROLS

Components of the biological pump

The term biological carbon pump describes the combination of biologically driven processes that spatially separate particulate and dissolved organic matter production from remineralization (organic carbon pump), and the biologically mediated carbon incorporation in calcium carbonate shells from their dissolution (calcium carbonate pump). The organic carbon pump exports about 10 times more carbon than the calcium carbonate pump (Sarmiento et al. 2002).

Photosynthetic production of organic matter is confined to the surface waters, whereas respiration occurs throughout the water column. Photosynthesis converts CO_2, thereby drawing down dissolved inorganic carbon (DIC), to organic matter. The subsequent export of particulate organic matter (POM) via gravitational flux, of dissolved organic matter (DOM) via mixing and advection, and the active transport of carbon via vertically migrating zooplankton removes the organic matter to deeper regions where it accumulates or is respired (Fig. 1). The effect of the biological pump on carbon storage in the ocean interior can be partitioned into short-term (months to decades) and long-term (centuries to millennia) storage. The depth of remineralization or the remineralization length scale (RLS) of the exported carbon determines the extent to which carbon can be effectively removed through exchange with the atmosphere.

Here we define the vertical export of organic matter from the base of the euphotic zone (~100 m; 1% light level) or from the mixed layer depth as export flux. Photosynthesis sets the upper limit for export flux, however, a greater portion of organic matter produced via photosynthesis is recycled within the euphotic zone of the ocean (regenerated production), thereby escaping export (Buesseler 1998). New production sensu Dugdale & Goering (1967) is defined as production that is supported by input of new nitrogen (nitrate) into the euphotic zone via convective mixing, upwelling, atmospheric deposition, nitrogen fixation or horizontal advection. On an annual average, new production is often considered to be equivalent to the export flux at the base of the surface ocean (Jenkins & Goldman 1985).

Long-term sequestration of carbon, as stipulated by the International Panel of Climate Change (IPCC 2007), requires removal from the atmosphere for over 100 yr. This criterion is met after carbon is transported below 1000 m on average (Primeau 2005) and coincides with the nitrate maximum, the base of the mesopelagic zone, and the greatest extent of vertically migrating zooplankton. In this review we consider removal of carbon by the biological pump to

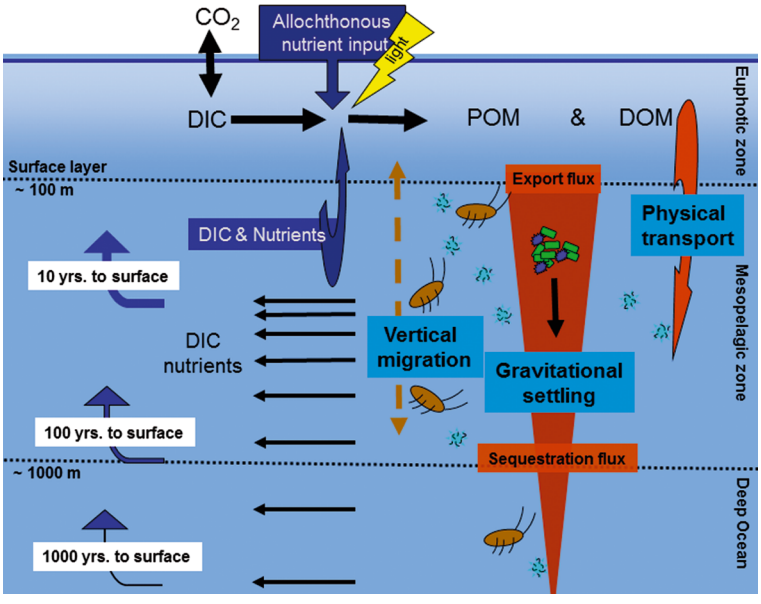

Fig. 1. Schematic of the biological carbon pump. Organic matter formed within the euphotic zone is transported to depth by (1) vertical migrating zooplankton (brown ovals with antennas), (2) gravitational settling of marine snow (green clusters with black bacteria) or (3) physical transport of dissolved organic matter (DOM). The sinking flux of aggregates (symbolized by green cells and blue transparent exopolymer particles) decreases with depth as organic matter is remineralized (turned into its inorganic form). The average time until the dissolved inorganic carbon (DIC) and nutrients are returned back to the surface depends on the depth of remineralization, the remineralization length scale (RLS). Sedimentation out of the surface layer (euphotic zone or mixed layer depth, whichever is deeper) is termed export flux, whereas the sedimentation out of the mesopelagic is called sequestration flux. The carbon sequestration flux determines the storage of carbon within the ocean for 100 yr or more

≥1000 m as the sequestration flux sensu Lampitt et al. (2008) (Fig. 1), but caution that the actual depth of the sequestration flux varies regionally depending on ventilation depth. The magnitude and change to the sequestration flux is a critical variable to constrain in order to assess long-term carbon storage and efficiency of the biological pump.

The sequestration flux of carbon at the base of the mesopelagic zone is smaller than export flux and constitutes between 6 and 25% of new production as estimated from deep-moored trap fluxes (Berelson 2001a, Francois et al. 2002). The reduction in flux between the base of the euphotic and mesopelagic zone is referred to as flux attenuation and is caused by intense biological remineralization (conversion of organic carbon to inorganic carbon) and solubilization (conversion of particulate matter to dissolved matter) within the mesopelagic (100 to 1000 m) during downward transport (Martin et al. 1987, Karl et al. 1988, Steinberg et al. 2008a). These biological processes in the mesopelagic zone reduce export flux by ~90% within the upper 1000 m (Nelson et al. 2002, Fasham 2003).

Two schools of thought exist when referring to the efficiency of the biological pump (De La Rocha & Passow 2012). The efficiency of nutrient utilization in the euphotic zone, e.g. the ratio between export flux and primary production, may be meant. In this review we use the alternative, defining the efficiency of the biological pump as the ratio between sequestration flux and export flux.

How the biological pump varies in the face of increased CO_2 and temperature and decreased pH is of critical importance for assessing the role the ocean plays in the oceanic carbon cycle. Here, we provide a summary of several potential scenarios that describe how small changes in the nutrient stoichiometry during production or remineralization of organic matter coupled with changes in allochthonous nutrient inputs and shifts in flux attenuation may affect carbon sequestration via the biological pump.

POM flux

A variety of drivers, such as adherence to or departure from Redfield stoichiometry of organic matter and an increase or decrease in flux attenuation, can result in various scenarios that affect both sequestration flux and nutrient inventories in the mesopelagic zone (Fig. 2).

- Scenario 1: Assuming Redfield stoichiometry and that new production is driven solely by the entrainment of nutrients from depth, equivalent amounts of CO_2 to support the consumption of those inorganic nutrients (i.e. nitrate, phosphate) are also brought to the surface. In such a scenario the export flux of POM returns the C, N and P to depth in amounts equal to the original vertical supply with no net flux of carbon to depth over an annual cycle (Michaels et al. 2001, Hopkinson & Vallino 2005; Fig. 2a).
- Scenario 2: Under assumptions of Redfield stoichiometry and steady state of mesopelagic nutrient inventories, decreased delivery of nutrients into the euphotic zone from below, due to increased stratification, leads to decreased new production and export flux but has no impact on sequestration flux, which remains negligible as in the first scenario (Fig. 2b)
- Scenario 3: Allochthonous input of nutrients (nitrogen, iron, phosphorous) from aeolian or fluvial inputs or due to nitrogen fixation to the ocean can result in an increased sequestration flux, independent of the export flux, if steady states of mesopelagic nutrient inventories and Redfield stoichiometry are maintained (Fig. 2c). Data on sedimentation rates of POM below 1000 m (from traps or ^{234}Th determinations) indicate that in today's ocean some fraction of organic matter is indeed transported to great depths supporting the idea of increased allochthonous nutrient inputs (Gruber & Sarmiento 1997, Gruber 2005).

The above 3 scenarios are constrained by Redfield stoichiometry and based on the tight coupling between essential nutrients (nitrogen, phosphorus) and carbon during photosynthesis and respiration.

- Scenario 4: Any mechanism that relaxes the C: N: P stoichiometry and results in the production or accumulation of C-rich organic matter has the potential to change the amount of carbon export- and sequestration flux (Fig. 2d). A longer RLS for carbon compared to nitrogen results in the preferential transport of organic carbon to depths (Michaels et al. 2001). This could increase the sequestration flux of carbon without necessarily impacting nutrient inventories within the mesopelagic zone. Experimental and field data support the preferential remineralization of nutrients over carbon (Anderson & Sarmiento 1994, Michaels et al. 2001, Boyd & Trull 2007); however, the effect is small and spatially and temporally variable, making it difficult to quantify on a global scale using current techniques
- Scenarios 5 and 6: Alternatively a change in flux attenuation has the potential to impact the sequestration flux, without deviating from the Redfield stoichiometry and without changes in allochtho-

nous nutrient input, if the steady state of mesopelagic nutrient inventories is relaxed (Fig. 2e,f). The impact of climate change on sequestration flux and nutrient inventories in the mesopelagic is ultimately dependent upon the RLS. A shallow RLS would increase nutrient inventories within the mesopelagic zone and reduce sequestration flux (Fig. 2f). Modeling experiments indicate that if the RLS was to increase by 24 m globally, carbon sequestration flux would increase enough to reduce atmospheric CO_2 by 1 to 27 ppm (Kwon et al. 2009). But, is such a decrease in flux attenuation and the associated increase of the sequestration flux potentially possible on time scales of 100s of years?

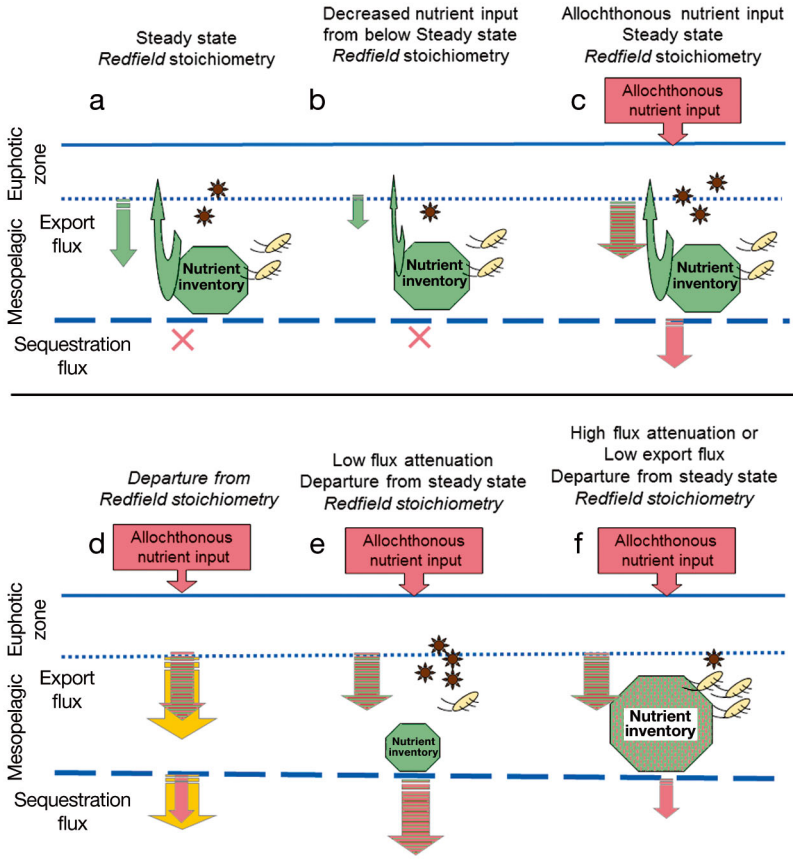

Fig. 2. Six scenarios highlighting processes that impact sequestration flux (see 'POM flux'). Under steady state conditions (nutrient inventory of the upper 1000 m of the ocean (symbolized in green) remains constant on an annual basis), and assuming Redfield stoichiometry, (a–c) sequestration flux (flux through 1000 m) equals allochthonous (red) nutrient input. Under the same assumptions, the export flux at the base of the euphotic zone depends on mixing of nutrients into the euphotic zone from depths and allochthonous nutrient input. (d) A deviation from Redfield stoichiometry during production or remineralization of organic matter allows for the preferential sedimentation of carbon over limiting nutrients (carbon in excess to that associated with nutrients in yellow), potentially having an impact on both export and sequestration flux. (e,f) The relaxation of the steady state condition allows for a change in the nutrient inventory of the upper 1000 m, thus decoupling sequestration flux from allochthonous nutrient input. (e) Low flux attenuation results in increased sequestration flux, supported by organic matter generated from nutrients from the upper 1000 m. (f) Inefficient nutrient utilization within the euphotic zone, or high flux attenuation, both result in a sequestration flux smaller than the allochthonous nutrient input and an increase in the nutrient inventory of the upper 1000 m. Brown stars symbolize sinking marine snow, ovals with antenna heterotrophs and the relative abundance of both denotes the food supply vs. respiration, e.g. flux attenuation. The size and color of green polygons symbolizes the relative size and origin of the limiting nutrient within the upper 1000 m. Red denotes allochthonous nutrients, green stands for nutrients from the upper 1000 m of the ocean. Down arrows denote flux out of the upper layer (export flux) or out of the mesopelagic (sequestration flux). Color of arrows denotes the origin of the exported limiting nutrient as specified above

The upper 1000 m of the ocean contain about 8.06×10^{15} mol of nitrate + nitrite (World Ocean Atlas 2005) whereas annual POC flux through 1000 m (sequestration flux) is estimated as 0.07 Pmol yr^{-1} (0.86 Pg C yr^{-1}; Jahnke 1996, Dunne et al. 2007). Under Redfield conditions this mesopelagic nitrogen inventory could support the annual sequestration flux of POC for almost 750 yr assuming the sequestration flux remained constant. A doubling of the annual sequestration flux based on the mesopelagic nutrient inventory would reduce the mesopelagic nutrient reservoir by only 23% over the next 100 yr. This decrease in the global mesopelagic nutrient inventories would be difficult to resolve with current methods on times scales of a few decades. Either, a decrease in flux attenuation and the resulting increase in sequestration flux (Fig. 2e), or an increase in flux attenuation resulting in a concomitant decrease in sequestration flux (Fig. 2f), may result from changing ecosystem structure and function.

These theoretical considerations illustrate that sequestration flux may be primarily dependent on flux attenuation of organic matter in the mesopelagic. Flux attenuation of sinking organic particles and their RLS are determined by the sinking velocity of particles and their loss rates (De La Rocha & Passow 2007).

DOM flux

Research over the past 2 decades has demonstrated significant contribution of dissolved or suspended organic matter to export flux in some regions of the global ocean (Copin-Montégut & Avril 1993, Carlson et al. 1994, Hansell et al. 2009). Vertical transport of DOM can contribute up to 20% to the bio-

logical pump (Hansell 2002), but as a dissolved phase it can only be exported to depth during isopycnal exchange or convective overturn of the host water mass (Carlson et al. 1994, Hansell & Carlson 2001, Kähler & Koeve 2001, Hansell et al. 2002, Hopkinson & Vallino 2005, Carlson et al. 2010) or trapped within aggregates (Noji et al. 1999, Antia 2005). The abiotic formation of transparent exopolymer particles (TEP) from DOM is another potential pathway for the export of dissolved organic matter, because TEP contribute significantly to sinking aggregates. Export of DOM via mixing processes requires that it resists degradation in the surface waters until it can be entrained to depth. Only the forms of DOM that survive in surface waters for weeks to months can be effectively exported (Carlson 2002).

These differences in the transport mechanisms of POM and DOM mean that potential changes in the partitioning of organic carbon between the dissolved and the particulate pools are of particular interest. Any increase in the fraction of photosynthate that is DOM or an increase in heterotrophic activity compared to autotrophy would shift the balance between the DOM and POM pumps.

Active zooplankton pump

Zooplankton that feed in the ocean's surface layer migrate to depths of 300 to 800 m where they respire and defecate, actively transporting carbon to depths (active zooplankton pump). Active vertical transport by migrating zooplankton to below the euphotic zone is estimated as 4 to 34% on average of POC flux at those depths (Ducklow et al. 2001, Steinberg et al. 2002), but the transport to 1000 m has, to our knowledge, not been estimated. Changes in the populations of vertically migrating zooplankton due to environmental change could affect the contribution of this component of the organic carbon pump.

ENVIRONMENTAL CHANGE AND RESPONSE OF THE BIOLOGICAL PUMP

Environmental changes

Rising atmospheric pCO_2 is reflected in the global mean surface ocean with oceanic pCO_2 rising at nearly the same rate as atmospheric pCO_2. The increased anthropogenic CO_2 induces change in the carbonate system, whereby DIC increases and the subsequent shift in speciation between bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions and CO_2 (including CO_2 plus H_2CO_3) results in the release of protons. The increase in proton concentration, while total alkalinity (TA) remains roughly constant, results in a decrease in pH (i.e. ocean acidification; Caldeira & Wickett 2003, Orr et al. 2005).

The global increase in the average air temperature (Kerr 1995a, Vinnikov & Grody 2003) due to the trapping of infrared radiation (i.e. greenhouse effect; Harries et al. 2001) leads to rising temperatures in the surface ocean (Levitus et al. 2000). This increase is observed despite the partial off-set by the inadvertent release of additional aerosols (Kerr 1995b, Kaufmann et al. 2011). The increase in water temperatures of the surface ocean can directly affect the stratification of the ocean, physiological rate processes, and planktonic community structure. Stratification of the surface ocean can locally be intensified by predicted changes in precipitation patterns and the melting of sea ice (Reid et al. 2009). The resulting changes in mixing regimes as well as possible changes in allochthonous nutrient inputs affect nutrient and light availability and nutrient stoichiometry.

In the following sections we will explore how the direct and indirect effects of changes in the carbonate chemistry, temperature, water column stratification and nutrient regime may impact the different components of the biological pump focusing on the sequestration flux.

Factors that potentially affect export flux

Nutrient availability

Changes in weather and wind patterns are anticipated to increase dust deposition to the ocean (Mahowald et al. 1999) and ocean acidification is predicted to increase biological availability of iron (Millero et al. 2009, Hoffmann et al. 2012 this issue). Both these factors may partially relieve iron limitation of primary production especially in the HNLC (high nutrient, low chlorophyll) areas of the ocean (Breitbarth et al. 2010). Increased iron availability will likely alter export flux, but the magnitude and duration of this effect and how it impacts sequestration flux remain unknown. As iron limitation is relieved, primary production will subsequently be limited by another resource.

Globally, anthropogenic nitrogen inputs into the biosphere have increased by a factor of 3 to 4 since 1860, increasing oceanic nitrogen supply. This allochthonous input can potentially induce an imbal-

ance between nitrogen and phosphorus (Peñuelas et al. 2012). The anthropogenic inputs of nitrogen to the ocean via enhanced riverine effluent (Smith et al. 2003) and as atmospheric fixed nitrogen have already contributed to measurably higher algal production in some coastal areas (Duce et al. 2008). Additionally several laboratory studies have demonstrated increased nitrogen fixation under ocean acidification simulations leading to speculation that some of the nitrogen-limited regions of the ocean could experience increases in new production in the future (Barcelos e Ramos et al. 2007, Hutchins et al. 2009, Kranz et al. 2010, Liu et al. 2010). Increased temperature can further promote the growth of nitrogen fixers, if nutrients, especially phosphorus and iron, are available (Langlois et al. 2008, Stal 2009), suggesting the possibility of a geographic expansion of nitrogen fixation. Nitrogen fixation supplies up to 50% of nitrogen for export flux at station ALOHA (near Hawaii) and has increased through the turn of the century (Michaels et al. 2001). However, for elevated nitrogen fixation to be sustained, demand for phosphate must be met and that source remains unclear in the face of increased ocean stratification.

The balance between nitrogen fixation and denitrification must be considered as this balance constrains the net input of nitrogen into the system. Increased stratification, for example, is predicted to reduce ventilation of the surface ocean, thereby leading to a decrease in oxygen concentrations in the ocean interior (Bopp et al. 2002). Expansion of the suboxic habitats of denitrifying bacteria may enhance global denitrification rates (Deutsch & Weber 2012). However, ecosystem responses to such changes in nitrogen sinks and sources may result in further feedbacks to the marine nitrogen cycle and the biological pump, which are only beginning to reveal themselves (Weber & Deutsch 2010, Deutsch & Weber 2012). Nitrification rates are also sensitive to ocean acidification with rates decreasing at several open ocean sites (Beman et al. 2011) but increasing at an estuarine site (Fulweiler et al. 2011).

Increased stratification of the surface ocean restricts nutrient delivery into the euphotic zone from below. For example, reduced availability of nitrate for primary production due to a shoaling of the thermocline between 1909 and 2002 has been inferred for large parts of the ocean from a temperature–nitrate relationship (Kamykowski & Zentara 2005). A significant decrease in global marine photosynthesis attributed to rising temperatures and increased stratification has also been postulated from global data sets combining Secchi depths, transmissometer and satellite information (Boyce et al. 2010), indicating a negative relationship between primary production and surface temperature observed *in situ* during the last decade (Behrenfeld et al. 2006). These latter studies suggest that export flux is decreasing globally because nutrients from the mesopelagic are not made available for primary production due to their reduced entrainment into the euphotic zone. However, these findings remain controversial (Mackas 2011, Rykaczewski & Dunne 2011) with contrasting results showing increases in phytoplankton biomass in several oceanic biomes for the past several decades (McQuatters-Gollop et al. 2011). Lomas et al. (2010) demonstrated that primary production and carbon export flux in the oligotrophic Sargasso Sea has increased over 50% since 1996 despite increased stratification. However, they found that the attenuation of export flux in the upper mesopelagic zone had also doubled, partially due to increased zooplankton biomass, so that flux at 300 m remained largely unchanged. This study reiterates the potential importance of mesopelagic food web structure and its link to export flux out of the euphotic zone (Michaels & Silver 1988, Steinberg et al. 2008a). Changes in export flux may not necessarily translate to a change in sequestration flux (Fig. 2).

Light field

Stratification may lead to photoinhibition in the surface layer of some regions (oligotrophic gyres), but the deeper penetration of light due to a nutrient- and phytoplankton-poor surface layer may compensate for the lack of primary production at the surface. In other regions, for example in parts of the Southern Ocean where deep mixing prevails, light limitation may be relieved. Relief of light limitation would allow for more efficient use of the nutrients available within the euphotic zone and initially could lead to increased export flux. However, this increased flux could only be sustained until the system became limited by another factor such as nutrient availability. Regionally specific information on the timescales of depth penetration of increased temperatures is required for a more accurate evaluation of the consequences of a changed light field for export flux.

Temperature

Rising temperatures generally increase metabolic processes including phytoplankton growth and microbial respiration (Sarmento et al. 2010). Several

mesocosm simulation experiments have examined the potential impacts of rising temperatures alone (Sommer & Lengfellner 2008, Lassen et al. 2010, Taucher et al. 2012), or in combination with perturbations of pCO_2 (Kim et al. 2011), light (Lewandowska & Sommer 2010), nutrients (Wohlers-Zöllner et al. 2011) or grazing (Sommer & Lewandowska 2011), on phytoplankton blooms. All of these studies observed an early onset and peak of the spring phytoplankton blooms, which has also been observed *in situ* (Edwards & Richardson 2004). Peak biomass was generally (Wohlers et al. 2009, Lassen et al. 2010, Lewandowska & Sommer 2010, Kim et al. 2011, Sommer & Lewandowska 2011), although not always (Taucher et al. 2012), smaller at elevated temperatures. Shifts in phytoplankton community composition at elevated temperatures especially towards smaller cells have been documented in several studies (Lassen et al. 2010, Lewandowska & Sommer 2010, Sommer & Lewandowska 2011). Diminished bloom biomass and smaller average cell sizes at elevated temperatures have also been observed *in situ* (Richardson & Schoeman 2004, Behrenfeld et al. 2006, Morán et al. 2010). Paleoproxy analysis of phytoplankton cell size supports the idea that body size decreases as temperature increases (Falkowski & Oliver 2007). The reduction in plankton cell size could arguably result in decreased export flux; however, other studies have challenged this argument suggesting that small cells are also incorporated into sinking aggregates in proportion to their abundance (Richardson & Jackson 2007).

The relative effect of temperature increase on microbial respiration compared to that of phytoplankton production may have a larger impact on export production (Harris et al. 2006, López-Urrutia et al. 2006, Sarmento et al. 2010) compared to absolute changes in primary production. One anticipated effect of global warming deduced from experiments, ecological theory and long term *in situ* observations is the expansion of oligotrophic areas of the ocean and the strengthening of the relative contribution of heterotrophy associated with a reduced export flux, although the evidence of this outcome is equivocal (Sarmento et al. 2010).

Inorganic carbon speciation and availability

Culture experiments have revealed that many phytoplankton species grow well over a large range of pH (Hinga 2002, Berge et al. 2010, Liu et al. 2010, Joint et al. 2011) especially when the pH change was caused by TA perturbations. However, truly oceanic species growing *in situ* are not exposed to large fluctuations in pH and may be more sensitive to such changes. The effect of increased surface water CO_2 concentrations due to ocean acidification (DIC perturbation) on phytoplankton taxonomic groups is highly variable (Rost et al. 2008). The CO_2 specificity of the carboxylating enzyme ribulose biphosphate carboxylase/oxygenase (Rubisco) is relatively high in diatoms compared to coccolithophores or dinoflagellates (Raven 1991). Kinetic carbon uptake studies suggest that at present day pCO_2 levels the photosynthetic carbon fixation rates of diatoms are close to their maximal rates (CO_2 saturation at present day levels), whereas coccolithophores have a low affinity for inorganic carbon and appear carbon limited under present day conditions (Rost & Riebesell 2004). Such physiological differences between taxa or species may lead to shifts in phytoplankton composition (Boyd et al. 2010). Shifts in phytoplankton composition are important to consider when assessing how increased DIC content affects new production and aggregation, and these are likely one of the main factors explaining ambiguous or contrasting results from mesocosm experiments (i.e. Kim et al. 2006, Egge et al. 2009, Riebesell et al. 2008).

Role of phytoplankton

The efficiency with which phytoplankton are exported is thought to be dependent on species composition, and shifts in dominant phytoplankton taxa will have consequences for export because of their different life strategies. The export of carbon fixed by small cells like picoplankton, which compared to diatoms have a higher surface area to volume ratio and potentially release a greater percentage of fixed carbon as DOM (Karl et al. 1998), will depend more on transport via the DOM-pump. Diatoms, in contrast, are very efficient in transporting carbon to depths by forming large, rapidly sinking aggregates (Smetacek 1985, 1998) and a decrease in diatom abundance due to increased stratification is expected to lead to a decrease in carbon flux (Bopp et al. 2005). *Phaeocystis* spp. provide non-sinking, carbon-rich mucus that requires attachment to heavy particles or physical processes prior to export (Passow & Wassman 1994, Wassman et al. 1995), whereas dinoflagellates rarely aggregate directly (Alldredge et al. 1998). Coccolithophores play a prominent role in the hard tissue pump and their coccoliths may be central for aggregation and ballasting (see 'The calcium

carbonate pump' below). However, the argument has been made that all phytoplankton, including picoplankton cells, contribute equally to flux (Richardson & Jackson 2007) and increased export flux concomitant with higher phytoplankton stocks dominated by picoplankton support this suggestion (Lomas et al. 2010).

Shifts in phytoplankton composition due to ocean acidification (Tortell et al. 2002, Rochelle-Newall et al. 2004, Low-Décarie et al. 2011, Meakin & Wyman 2011) or increased temperatures (Hare et al. 2007) are commonly observed experimentally. The impact of ocean acidification on the calcifying coccolithophores has received special attention. Ocean acidification makes calcification more difficult, but increased pCO_2 appears to facilitate primary production for this group with variable outcome for the growth success of the species (Zondervan 2007, Iglesias-Rodriguez et al. 2008, Bach et al. 2011). Inclusion of other factors for an evaluation of the competitiveness of coccolithophores in the future ocean (Irie et al. 2010, Xu et al. 2011) makes predictions even less robust at present. The degree of water column stability exerts an additional strong selective pressure on phytoplankton composition (Falkowski & Oliver 2007). A shift towards a phytoplankton community dominated by smaller cells (picoplankton) adapted to be more competitive at low nutrient concentrations and input may be expected (Bopp et al. 2005, Lomas et al. 2010, Morán et al. 2010). Phytoplankton species that utilize organic nitrogen or phosphorus (Benner & Passow 2010) may gain a competitive advantage under increase stratification. Modified speciation of trace elements (Millero et al. 2009) promoting or inhibiting (e.g. iron versus copper) growth of phytoplankton may cause further shifts in phytoplankton composition.

The interactive and synergistic effects of these different environmental stressors and the results for the competitive ability of individual species are extremely complex and difficult to predict (Feng et al. 2009, Boyd et al. 2010, Low-Décarie et al. 2011). However, shifts in phytoplankton composition appear likely and are becoming apparent: Coccolithophores appear to have expanded their geographic range (Merico et al. 2003, Cubillos et al. 2007) and a shift towards *Prochlorococcus* and *Trichodesmium* due to increased stratification has become visible in the North Atlantic (Lomas et al. 2010) and Pacific Subtropical Gyre (Karl et al. 2001). A regime shift in phytoplankton abundance, composition and seasonal cycle due to changed river discharge has been documented in the Adriatic (Mari et al. 2012).

Role of aggregation

The importance of marine snow for carbon sequestration lies in its rapid sinking velocities. Marine snow is formed from feeding structures or via aggregation of small component particles (Alldredge & Silver 1988). Aggregation, especially of diatoms, plays a critical role for the rapid transfer of algal carbon to depth (Alldredge & Jackson 1995, Boyd & Newton 1995, Buesseler 1998, Boyd & Newton 1999). However, on varying regional or temporal scales the contribution of sinking feces (Wilson et al. 2008) or feeding structures (Noji et al. 1997, Passow et al. 2001, Robison et al. 2005) may be large. Fecal pellet flux may vary between <5 and up to 100% of sinking flux, and zooplankton species may dramatically affect the amount of primary production consumed, the composition and sedimentation rate of sinking particles and the flux of organic carbon to the deep ocean (Ducklow et al. 2001, Steinberg et al. 2008a).

Aggregation rates are a function of particle numbers and sizes (Jackson & Burd 1998, Burd & Jackson 2009), implying that the marine snow sized aggregates that sink rapidly only form during times of high particle concentrations, especially during large blooms (Jackson 2005). The suggested reduction of large phytoplankton blooms (see 'Role of phytoplankton' above) could potentially result in a decrease of aggregation events. Few studies have investigated the dependence of aggregation rate on temperature or ocean acidification directly. Increased aggregation rate at elevated temperatures was detected during one experimental study, possibly due to increased abundance of transparent exopolymer particles (TEP), but degradation of aggregates was also enhanced, with the net result of increased or decreased sedimentation of aggregates dependent upon temperature (Piontek et al. 2009).

A reduced availability of bio-minerals due to decreased production may negatively impact sinking velocity of aggregates. Calcifying coccolithophores aggregated more rapidly than their non-calcifying counterparts (Engel et al. 2009b), in agreement with the concept that the presence of minerals promotes aggregation (Passow & De La Rocha 2006, De La Rocha et al. 2008). Experimental evidence suggests that the sinking velocities of coccolithophore aggregates decreased under ocean acidification conditions (Biermann & Engel 2010).

TEP are an essential component of aggregates, having impacts on both their formation (Alldredge et al. 1993, Alldredge & Jackson 1995) and sinking velocity (Engel & Schartau 1999, Azetsu-Scott & Passow 2004). Ocean acidification and increased tempera-

tures are hypothesized to increase TEP production (Engel et al. 2004), which in turn is thought to result in increased aggregation and sedimentation rates (Arrigo 2007, Riebesell et al. 2007). However, stickiness of TEP may decrease due to ocean acidification (Mari 2008), challenging this assumption. To date the impact of ocean acidification and temperature on TEP production and thus aggregation and flux remain equivocal. Increased TEP production by phytoplankton under ocean acidification conditions has been demonstrated in one mesocosm experiment (Engel et al. 2004), but results were contradictory in another two (Schulz et al. 2008, Egge et al. 2009). These contrasting results indicate that additional factors, such as total alkalinity (Mari 2008, Passow 2012) or nutrient stoichiometry (Corzo et al. 2000, Staats et al. 2000, Passow 2002, Beauvais et al. 2006), must be carefully considered in future experiments that test the role of ocean acidification and TEP in aggregation.

Based on the correlation between sinking POC and minerals (calcium carbonate, opal, lithogenic minerals) in deep sediment traps (Armstrong et al. 2001, Francois et al. 2002, Klaas & Archer 2002), mineral ballasting has been proposed as an important mechanism controlling POM flux. Model results based on the 'Ballast Hypothesis' suggest a weakening of the biological pump in the future (Bopp et al. 2005, Gehlen et al. 2006) due to the expected decrease in ballasting by coccoliths (Zondervan et al. 2001, Beaufort et al. 2007). Depending on the relative contribution of minerals, some ballasting does occur, and increased aggregation and sinking velocities may be expected in some coastal systems that experience increased dust input or river run off. Laboratory-made aggregates or feces ballasted with diatom frustules or coccoliths effectively increased settling velocities of aggregates in laboratory experiments (Ploug et al. 2008a,b), but *in situ* data from the Mediterranean suggest that mineral ballasting did not drive sinking velocity (Lee et al. 2009b). Furthermore, controlled laboratory experiments show that as a result of decreasing aggregate size coincident with increasing density the effect of scavenged minerals on aggregate sinking velocity is more complex than originally conceived (Hamm 2002, Passow & De La Rocha 2006).

Altering elemental stoichiometry of organic matter

Considering stoichiometry of POM

Organic matter production and remineralization operate with approximately Redfield stoichiometry of 106C:16N:1P. Although the bulk particulate organic matter measured in the upper water column over vast geographical ranges does not deviate significantly from the canonical Redfield molar ratio, laboratory studies have demonstrated that elemental ratios in phytoplankton can be highly plastic, with elemental allocation varying as a function of growth rates and nutrient stress (Goldman et al. 1979, Geider & La Roche 2002). Whereas the ratios of recycled nutrients suggest remineralization according to Redfield (e.g. Anderson & Sarmiento 1994), the POC:PON ratios of sinking particles increase with depths, indicating preferential remineralization of nitrogen over carbon (Schneider et al. 2003). Deutsch & Weber (2012) suggest that the variability of nutrient stoichiometry is not random and that it may depart systematically from Redfield on regional scales, but that circulation patterns obfuscate the regional signatures leading to a globally homogenous (Redfield) nutrient ratio.

Assuming that remineralization of carbon and the limiting nutrient is vertically decoupled so that remineralization of carbon occurs deeper than that of nitrogen, a net export of carbon in the absence of an input of allochthonous nutrients is possible (Michaels et al. 2001, Schneider et al. 2004; Fig 2d). Thus, if conditions prevail that allow organic matter to be fixed into carbon-rich exportable POM (C:N > 6.6), or if there is preferential loss of N and P relative to C as sinking particles are remineralized, than net sequestration of C into the interior can occur (Michaels et al. 2001).

Enhanced photosynthesis in the absence of increased nutrient supply may be expected under ocean acidification conditions (Doney et al. 2009) and the production of C-rich POM (Burkhardt & Riebesell 1997, Wolf-Gladrow et al. 1999) under high CO_2 conditions has been postulated based on experimental evidence. Variable stoichiometry based on uptake ratios was observed in a mesocosm experiment simulating different ocean acidification scenarios (Riebesell et al. 2007, Bellerby et al. 2008) but was not reflected in the respective pools of particulate or dissolved organic matter (Riebesell et al. 2008, Schulz et al. 2008). Additionally, there is a paucity of experimental evidence supporting a systematic increase of the C:N ratio in POM due to ocean acidification or temperature in mesocosom (Kim et al. 2011, Wohlers-Zöllner et al. 2011) or laboratory culture experiments (Burkhardt et al. 1999, Hutchins et al. 2009). This issue may be critically dependent on taxa (Riebesell & Tortell 2011) or on the nutrient availability and temperature conditions associated with the ocean acidification perturbation.

Deviations from the Redfield ratio during production or remineralization are difficult to determine *in situ* because of the high spatial and temporal variability in the C:N ratio of organic matter, the homogenizing effect of circulation patterns, and the synergistic effects of different biological processes. Sedimentary denitrification, for example, masks the signature of organic matter remineralization during sedimentation (Deutsch & Weber 2012). Nevertheless, indications of preferential remineralization of nitrogen during sedimentation are observed (see overview in Boyd & Trull 2007) and oceanic nutrient ratios are found to be dynamic on decadal to geological scales (Finkel et al. 2010, Deutsch & Weber 2012).

Considering stoichiometry of DOM

In contrast to POM, the production of DOM can more easily be decoupled from nutrient supply (Hopkinson & Vallino 2005). When phytoplankton deplete ambient nutrient stores (including upwelled CO_2), some continue to fix C as an energy dissipation mechanism and release it as C-rich DOM in vast excess of Redfield stoichiometry (Williams 1995, Hopkinson & Vallino 2005, Conan et al. 2007, Wetz & Wheeler 2007). This surplus fixation of carbon is called carbon overconsumption (Sambrotto et al. 1993, Williams 1995, Carlson 2002, Conan et al. 2007) and in the short term produces 'new' organic carbon in the sense that it allows organic matter production by phytoplankton to exceed the CO_2 supplied to the surface waters by vertical exchange. Accumulation of nitrogen-rich but phosphorus-poor DOM in the surface layer of the North Pacific Subtropical Gyre between 1993 and 1999, possibly due to increased nitrogen fixation and as a result of a shift in autotrophic community structure, gives evidence for the multi-year storage of carbon as dissolved organic matter (Church et al. 2002).

DOM released by phytoplankton varies with species, growth stage and environmental conditions (Nagata 2000, Carlson 2002), but if under ocean acidification conditions or increased temperature exuded DOM is on average more carbon rich and less bioavailable to heterotrophs, this may represent a highly efficient C sequestration mechanism with global implications for the operation of the biological pump assuming convective overturn or isopycnal exchange is deep enough (Hopkinson & Vallino 2005). Experimental evidence on increased C:N ratios of DOM due to temperature or ocean acidification effects is scarce and often equivocal (Wohlers-Zöllner et al. 2011, Taucher et al. 2012). Nonetheless accumulation of C-rich DOM represents a potentially important shift in carbon storage away from particle export to sequestration in a suspended/dissolved form as proposed in the microbial carbon pump (Jiao et al. 2010, see below).

The role of DOM in carbon storage or export is ultimately dependent upon its bioavailability to heterotrophic bacterioplankton. If DOM becomes resistant to extant microbial remineralization then it is potentially available for export (Carlson 2002). Initial studies of the effects of ocean acidification have demonstrated shifts in chemical composition (i.e. variability in the mol fraction of neutral sugars and amino acids) and changes in some exoenzyme activities (Grossart et al. 2006, Arnosti et al. 2011, Engel et al. 2011, Wohlers-Zöllner et al. 2011), but the overall impact on DOM bioavailability is currently under investigation. Changes in the C:N ratio of organic matter production or utilization are supported by experimental and geological observations. However an understanding of remineralization and physical transport processes are required to estimate their potential global impact on sequestration flux.

Recently the production of refractory DOM (rDOM) via heterotrophic microbial processes has been proposed to result in the production of C-rich DOM that can persists for hundreds to thousands of years (microbial carbon pump, MCP; Jiao et al. 2010). Several studies have shown that heterotrophic bacterial biomarkers are observed in this rDOM pool lending support to the MCP (Benner 2002, Benner & Kaiser 2003). However, the bioavailability of DOM to marine microbes is controlled by a number of factors such as the inorganic nutrient availability, molecular composition of DOM and the extant microbial community (Carlson et al. 2004). Little is known regarding the impact of ocean acidification, warming or increased stratification on this proposed shunt (Jiao & Azam 2011). Study of the oceanic MCP and its impact on carbon export and sequestration in the face of a high CO_2 world and ocean warming is currently an active research area in microbial oceanography.

Flux attenuation

Role of food webs

Direct predictions of potential global changes in sequestration flux require a better understanding of particle processing in the mesopelagic. Gravitational

settling of particles, which dominates the export of organic matter from the euphotic zone, accounts for up to 80% of the carbon reaching the deep sea (Hansell 2002, Hopkinson & Vallino 2005). Size distribution and sinking velocity of marine snow, as well as its repackaging, destruction and respiration, are regulated by food web structure within the water column (Michaels & Silver 1988, Peinert et al. 1989, Boyd & Newton 1999, Boyd et al. 1999, Steinberg & Hansell 2010). Particle transformations determine the RLS and thus the efficiency with which organic matter is transported to depth. The formation or potential reduction of fast sinking POM (i.e. marine snow) is of particular interest in this context as only large, marine snow sized (>0.5 mm) feeding structures, aggregates, or feces sink at velocities (>50 to 100 m d^{-1}) great enough to potentially escape remineralization within the upper 1000 m (Asper 1987, Alldredge & Gotschalk 1988, Knauer 1991, Berelson 2001b, Armstrong et al. 2009). The magnitude of flux attenuation varies regionally and temporally because there is significant variability in food web structure and processing over geographic and temporal scales (Boyd & Trull 2007). Below we discuss some of the expected changes to euphotic and mesopelagic food webs and explore their potential to alter sequestration flux of POC.

Increased temperatures may enhance bacterial respiration in relation to primary production (López-Urrutia et al. 2006, Sarmento et al. 2010) resulting in reduced export flux (Wohlers et al. 2009, Kritzberg et al. 2010, Vaquer-Sunyer et al. 2010). Rose & Caron (2007) demonstrated that as temperatures increased in microcosm experiments microzooplankton growth rates became greater than phytoplankton growth rates. Using their temperature relationships, they calculated that the maximal growth of herbivorous microplankton at 0°C is 60% that of phytoplankton, whereas at 15°C it is 110%, implying a reduction of large sedimentation events in a warmer ocean, especially if grazers generate fecal pellets that are recycled within the surface ocean.

An increase in ocean temperature may also result in a reduction in bloom biomass. If true, this could affect export flux as well as flux attenuation in the mesopelagic. For example, the fraction of sinking organic carbon that escapes remineralization in the euphotic and mesopelagic zone is greater during pulsed sedimentation events after phytoplankton blooms compared to periods of more continuous but lower sedimentation rates typical for recycling-type systems (Ducklow et al. 2001). The argument is that the mesopelagic community acclimates and optimizes its growth response under more constant flux conditions and, thus, increases flux attenuation and reduces sequestration flux.

Particle transformation due to zooplankton activity

Zooplankton utilize, repackage, and physically disrupt aggregates, thus transforming sinking POM, with various functional groups of zooplankton impacting sinking flux differently. For example, while large salp pellets sink rapidly (Bruland & Silver 1981), copepod fecal pellets are generally recycled within hundreds of meters of their release (Lampitt et al. 1990, Noji 1991, Noji et al. 1991). Abandoned larvacean houses may dominate sinking flux by scavenging other particles on their way (Passow et al. 2001), while a swarm of euphausids may fragment marine snow (Goldthwait et al. 2004, Dilling 1997, Dilling et al. 1998, 2004), thereby affecting its sinking velocity and potentially but not necessarily its remineralization (Goldthwait et al. 2005). Fractionation of particles due to sloppy feeding (Goldthwait et al. 2005) or swimming activity of zooplankton (Dilling et al. 1998, Goldthwait et al. 2004) as well as solubilization of POM to DOM due to the hydrolytic enzymes produced by attached bacteria (Smith et al. 1992, Grossart & Simon 1998, Ploug & Grossart 2000) effectively convert rapidly sinking particles to suspended organic matter. Zooplankton also consume marine snow (Steinberg 1995, Green & Dagg 1997, Dilling et al. 2004, Koski et al. 2005, 2007), feces (Lampitt et al. 1990, Noji et al. 1991, Poulsen & Kiørboe 2005) and appendicularian houses (Alldredge 1976), potentially recycling the majority of the fixed carbon back to DIC.

Consequently, shifts in zooplankton composition can have an impact on sequestration flux. For example, a shift from euphausids that fragment and eat marine snow to salps that produce rapidly sinking feces in Antarctic waters has appreciable ramifications for carbon cycling, including an increased sequestration flux (Loeb et al. 1997, Atkinson et al. 2004). In the Northern Bering Sea a decreased carbon supply to the benthos is causing simultaneous shifts in top predator distributions, suggesting that the whole ecosystems structure is adjusting as export is changing (Grebmeier et al. 2006, Grebmeier 2012). Increases in export flux of more degraded, biogenic material are expected in the Northern Barent Sea in the future (Wassmann et al. 2008). Such changes in ecosystem structure and functioning and thus carbon cycling are especially evident in high latitude areas, which are the most sensitive regions to change and

where the retreat of the sea ice adds an additional critical component (Wassmann 2011, Wassmann et al. 2011). The complexity of the role that marine food webs play for flux attenuation and their high spatial and temporal variability (Noji 1991, Silver & Gowing 1991, Gage 2003, Boyd & Trull 2007, Buesseler et al. 2007, Smith et al. 2008) prevents global generalizations, and effects and consequences to change will most likely have to be determined on a regional and seasonal basis before global predictions become possible. However, shifts in food webs, especially of the upper ocean, are evident in many regions including the Bristol Channel (Henderson et al. 2011) and the Irish Sea (Lynam et al. 2011), although causes are often unclear (Richardson & Gibbons 2008) and responses may be very spatially variable as observed in the NE Atlantic (McGinty et al. 2011).

Changes within the mesopelagic food webs will likely control flux attenuation between export flux and sequestration flux. Changes in any part of the mesopelagic food web, which is highly adapted to the flux of particles sustaining it, will be reflected in the type and quantity of sequestration flux. The coupling or decoupling between a sedimentation event of rapidly sinking marine snow and the mesopelagic predators will determine sequestration flux; their synchronization will result in high flux attenuation, and their decoupling in a large flux event. Spatial migration (pole wards or to greater depths) due to warming as observed for copepods in the Atlantic (Beaugrand et al. 2002) as well as temporal shifts (e.g. seasonal or ontogenetic delays) of events like the timing of the phytoplankton bloom are expected to uncouple trophic interactions (Edwards & Richardson 2004), with unforeseeable consequences for the biological pump. Accelerated bloom build-up and a forward shift of the bloom peak by ~2 d $°C^{-1}$ due to more rapid growth, as well as the amplification of microbial degradation and grazing (see 'Role of food webs' above) are anticipated to impact particle–food web interactions in the mesopelagic. Large changes within marine ecosystems have been observed in many parts of the ocean (e.g. Reid et al. 2009, Philippart et al. 2011), but the consequences for sequestration flux have rarely been assessed and will be a major emphasis in refining our understanding of the biological pump in the next decade.

To complicate matters, direct environmental changes to the mesopelagic have to be considered as well. The expected combination of decreased oxygen and increased pCO_2 in increasingly larger areas of the ocean interior may make respiration a challenge for many marine heterotrophs living at these depths (Brewer & Peltzer 2009). The resulting decreased activity of such heterotrophs would shift remineralization to organisms less sensitive to O_2. Direct impacts of ocean acidification and elevated temperature are also anticipated in some organisms, e.g. for aragonite-precipitating pteropods (Fabry 2008, Lischka et al. 2011). Environmental conditions in the Canada Basin are already at levels expected to inhibit development and growth of pteropods there (McLaughlin et al. 2011). Direct responses of ocean acidification on adult non-calcifying zooplankton have rarely been measured, but those that do exist show only subtle change in survival, growth and physiology (Hauton et al. 2009). However, planktonic larval stages of benthic organisms indicate that potentially the success of larval development of many organisms could be appreciably reduced (Dupont et al. 2008), but different organisms do not respond uniformly (Hendriks et al. 2010).

The complex interactions within marine food webs and the microbial loop, which drive both sinking velocity and degradation rate of organic matter (Noji 1991, Silver & Gowing 1991, Neuer et al. 2002, Gage 2003, Boyd & Trull 2007, Buesseler et al. 2007, Smith et al. 2008), are expected to change as ecosystems shift.

Microbial impact on flux attenuation

Cho & Azam (1990) reported that the bacterial carbon demand in the mesopelagic zone of the North Pacific was equivalent to the sinking POC flux, indicating that mechanisms responsible for the transformation of sinking POM to suspended matter or DOM could support the free-living mesopelagic bacterioplankton. Uncoupled solubilization, i.e. the solubilization of POM via the production of hydrolytic enzymes and the subsequent release of DOM to the surrounding environment, was proposed as a possible mechanism to help support the mesopelagic bacterial carbon demand (Smith et al. 1992, Azam 1998, Grossart & Simon 1998, Azam & Long 2001, Kiørboe et al. 2001). Futher work by Ploug & Grossart (2000) demonstrated that the O_2 exchange across surfaces of a sinking particle results in a larger percentage of microbial remineralization (compared to solubilization) than had been previously estimated (Ducklow et al. 1985, Karl et al. 1988) and helps to explain POC flux attenuation in the open sea. Both microbial remineralization and/or solubilization of sinking particles are important for the attenuation of POC flux in the mesopelagic.

If enhanced microbial utilization of organic matter were true for the mesopelagic bacterial community, a decrease in sequestration flux would be expected. However, the relative contributions of zooplankton and heterotrophic bacteria to the attenuation of particles and to the recycling of organic carbon remains under considerable debate. Steinberg et al. (2008b) reported that either zooplankton processes or bacterial processes could account for more than 100% of the POC flux at 2 oligotrophic and highly productive stations in the North Pacific. This discrepancy reveals that our understanding of mesopelagic biological processes or constraining these processes by measured flux estimates is still somewhat rudimentary but progress continues. Reports indicate that microbial contribution to flux attenuation may increase with depth, dominating in the lower mesopelagic and below (Steinberg et al. 2008b, Anderson & Tang 2010, Burd et al. 2010, Robinson et al. 2010). Zooplankton preferentially feed on large, fast settling particles (Bathmann et al. 1987, Lampitt et al. 1990), whereas attached Bacteria and Archaea appear to affect all size classes via solubilization or remineralization (Stemmann et al. 2004).

The few investigations that address potential impacts of environmental changes on bacterial activity and composition have indicated that while some changes are apparent, the signal is often obfuscated by factors such as fast turnover time of DOM and the complexity of shifts in the composition of both DOM and bacterioplankton (Weinbauer et al. 2011). Three mesocosm studies implied minor effects of ocean acidification on bacterial abundance (Rochelle-Newall et al. 2004, Grossart et al. 2006, Allgaier et al. 2008), but clearer shifts in bacterial community structure (Allgaier et al. 2008, Arnosti et al. 2011). Hydrolysis rates of complex polysaccharides exhibited treatment-specific effects in some cases (Arnosti et al. 2011). For example, glucosidase activity appears accelerated at higher hydrogen ion concentrations (Piontek et al. 2010), although the effect is not always visible (Grossart et al. 2006). Association with minerals are hypothesized to protect organic matter from degradation (Lee et al. 2009a), and a laboratory experiment comparing degradation of calcified and naked coccolithophores partially supported this possibility (Engel et al. 2009a). However the consequences of reduced occurrence of biomineral-forming phytoplankton in combination with degradation of organic matter during transit needs further study.

The most pressing question is thus whether the microbial population will be able to respire most of the DOM created under changed conditions. This question is important, as the remineralization of exported DOC accounts for up to half the oxygen utilized in the mesopelagic zone (Carlson et al. 1994, Doval & Hansell 2000, Hansell et al. 2002). Studies have indicated that DOM which is persistent at one geographical location or depth horizon can be bioavailable at another (Carlson et al. 2004, 2011). DeLong et al. (2006) identified a greater number of genes putatively involved in polysaccharide degradation in deep microbial populations compared to those found in the surface populations with some piezophiles capable of degrading complex organic matter due to modifications in their gene structure and protein regulation (Vezzi et al. 2005, Lauro & Bartlett 2008). These studies suggest that the deep populations of prokaryotes are better adapted to utilizing recalcitrant polysaccharides. Whether this would be altered under future climate scenarios remains unclear.

The calcium carbonate pump

In contrast to the organic matter pump, which is based on the conversion of DIC to organic carbon, the calcium carbonate pump removes carbon from surface waters in its particulate inorganic forms. Production of calcium carbonate frustules by autotrophic or heterotrophic organisms incorporates carbon in shells or exoskeletons, which sink before dissolution or burial. Under ocean acidification conditions, calcification of coccolithophores has been proposed to typically decrease, although a large variability exists even between strains of the same species (Zondervan 2007, Langer et al. 2009) and coccolithophores are to some extent able to compensate for reduced pH during growth (Fukuda et al. 2011). Interaction effects of temperature, UV and pH challenge our ability to predict future patterns for even the most well studied groups, e.g. *Emiliania huxleyi* (Xu et al. 2011), and this currently an area of active research. Experimental (Feng et al. 2009), and *in situ* (Merico et al. 2003, Cubillos et al. 2007) data on mixed populations and palaeo-reconstructions do not indicate a reduction in abundance of *E. huxleyi* in the near future. However, palaeo-reconstruction indicates that the mass of individual coccoliths decreased at times of low CO_3^{2-} on geological timescales (Beaufort et al. 2011). Assuming that production of POC and PIC remains the same, elevated temperatures that result in increased POC respiration would result in a larger PIC to POC ratio (rain-ratio), potentially supporting increased sinking velocities and flux rates.

Production of calcium carbonate by organisms affects the carbonate system in a way that may seem counter intuitive. In contrast to photosynthesis, calcification alters TA, thereby shifting the equilibrium between carbonate, bicarbonate and CO_2 in the ocean. As a consequence the ability of the water to take up dissolved CO_2 decreases, despite carbon being simultaneously removed from the DIC pool (Table 1). The reduction of the capacity of the surface ocean to take up CO_2 due to calcification explains why this calcium carbonate pump is also sometimes referred to as the carbonate counter pump. The removal of 50 µmol carbon kg^{-1} as PIC simultaneously increases CO_2 from 16.6 µmol kg^{-1} to 20.9 µmol kg^{-1} (at 15°C, 35‰), whereas a removal of the same amount by photosynthesis lowers CO_2 to 12.6 µmol kg^{-1} (Table 1). Thus, the sequestration of carbon in surface water by non-calcifying phytoplankton is appreciably higher (per mol organic matter produced) compared to that of calcifying phytoplankton. If ocean acidification results in a reduction of calcifying phytoplankton, the counter-intuitive consequence will be an increased capacity of the surface ocean to store CO_2 (Reid et al. 2009). In contrast, the simultaneous reduction in sinking velocity due to the lack of ballasting with calcium carbonate has been suggested to decrease export and thereby the ability of the ocean to take up CO_2 via the organic carbon pump (Armstrong et al. 2001). However, the importance of minerals for ballasting of sinking flux has also been challenged (Passow 2004, Lee et al. 2009b).

On timescales of millennia the carbonate minerals deposited in sediments during the past 100 000 yr will act as a buffer, as their eventual dissolution will increase the TA, resupplying the water with CO_3^{2-} and readjusting the pH. However, as this process is slow compared to the current rate of pCO_2 increase, it is fairly irrelevant with respect to changes expected within the coming 100 yr.

DELIBERATE PERTURBATION OF THE BIOLOGICAL PUMP

Several geo-engineering schemes have been proposed to increase carbon sequestration via the biological pump. An overview of the state of the art is given by Lampitt et al. (2008). Briefly, iron fertilization of the HNLC regions of the ocean is the most publicized proposal, and the only one that has been tested in *in situ* experiments. However, these experiments mainly focused on biological response and export flux (Boyd et al. 2007, Boyd 2008) and did not assess if fertilization had an impact on sequestration flux. Iron fertilization of areas with residual phosphate but deficient in nitrate and iron has also been proposed to enhance nitrogen fixation and thus input of allochthonous nutrients. Natural feedbacks to ocean acidification and increasing temperatures may have a similar effect. Piping a nutrient mix, especially macronutrients, from land to beyond the edge of the continental shelf is another suggested approach thought to result in carbon sequestration. The cost for such a scheme may be prohibitive, but as for the other 3 ideas, no detailed cost–benefit analysis has been made. The fourth suggested manipulation uses wave energy to induce artificial upwelling by pumping deep water to the surface. Comprehensive experiments are currently still lacking for this approach as well, but calculations suggest that the efficiency may be very low (Oschlies et al. 2010), and of course DIC will also be brought to the surface as well via this mechanism. Currently, the practicality of utilizing deep, cold sea water to run air conditioning systems (SWAC) is being explored as a source of renewable energy (Elsafty & Saeid 2009). Studying the effect of this nutrient-rich water after its return to the ocean to a shallower depth than its origin may provide insight into the fertilization approaches as a geoengineering scheme.

Table 1. Effect of removal of inorganic carbon due to calcification or photosynthesis (35‰, 15°C, no nutrients) on the carbonate chemistry of seawater. The fixation of 50 µmol C kg^{-1} seawater in organic matter (photosynthesis) has a significantly different result for seawater chemistry than the equivalent incorporation of carbon in carbonate, because calcification uses 2 mol total alkalinity (TA) for every mol of dissolved inorganic carbon (DIC). Whereas a decrease in carbon dioxide concentration during photosynthesis allows the water to take up more CO_2, its increase during calcification leads to relative out-gassing. pH measured as total scale

	DIC (µmol kg^{-1})	TA (µmol kg^{-1})	pH_T	pCO_2 (µatm)	CO_2 (µmol kg^{-1})	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})
Start	2100	2300	8.0	445	16.6	1937	147
Calcification	2050	2200	7.9	560	20.9	1915	114
Photosynthesis	2050	2300	8.1	339	12.6	1860	178

Although all 4 fertilization ideas have the potential to sequester carbon, the appropriate modeling and *in situ* experiments, which are necessary to assess side effects, costs and benefits, are missing (Lampitt et al. 2008). Any successful attempt to rapidly increase CO_2 input into the ocean will obviously amplify ocean acidification and its consequences. Any purposeful perturbations of the biological pump will without doubt have consequences beyond the sequestration of carbon, and informed decisions to what extent these will be considered acceptable are required.

OUTLOOK

In this review we have highlighted exciting advances in biological oceanography and ocean biogeochemistry, addressing the functioning and efficiency of the biological pump and its potential response to changing pCO_2, temperature, ocean stratification and nutrient availability. However the challenge is to predict how the biological pump will change in the face of the future climate on a global scale. This question is critical to help inform governmental policy decisions.

The expected simultaneous changes in the carbonate system, temperature, mixing and nutrient regimes of the pelagic environment will manifest themselves differently in distinct regions of the oceans (McGinty et al. 2011). Integrating modifications in the marine carbon cycle expected due to rising temperatures with those due to changes in nutrient availability and ocean acidification and extrapolating these to changes in sequestration flux will be one of the great challenges ahead. While we can predict changes due to individual forces (Table 2), or a combination of forces (Bopp et al. 2001), we are currently unable to predict with certainty if the global biological pump will strengthen or weaken in the next 100 yr.

In this review it became clear that the literature is full of contrasting results, which is largely the consequence of comparing vastly different regions, ecosystems and organisms. Our review points out that the high sensitivity of multiple parameters and their synergistic effects, which ultimately impact export flux and flux attenuation, cannot be captured as global averages: Taking global data sets and finding re-

Table 2. Examples of processes that are expected to change with global change and may impact one or more of the 3 main components of the biological pump. Up arrow: The process is hypothesized to increase new production or flux; down arrow: a decrease in the biological pump is expected. Question mark: The process is known to affect the biological pump, but the direction is not foreseeable. For detailed explanations of different processes see text. POM: particulate organic matter; DOM: dissolved organic matter; TEP: transparent exopolymer particles

New production	Carbon export flux	Carbon sequestration flux
↑ Increased N_2-fixation	? Depends on new production	? Depends on sinking velocity & packaging of POM
↑ More efficient nutrient utilization[a]	↓ Decrease in diatoms, shift towards smaller phytoplankton	? Shifts in food web structure: e.g. salps replace euphausids[d]
↓ Increased stratification[b]	↓ Fewer large blooms due to elevated respiration and grazing	? Spatial or temporal decoupling between grazers & flux events
↑ Increased nutrient input: iron in HNCL areas	↑ Decreased bioavailability of carbon-rich DOM	↓ Lack of ballasting by coccoliths and diatom frustules
↓ Prolonged periods of recycled production	? Changes in TEP formation and stickiness[c]	? Mesopelagic microbial activity
	↓ Glucosidase activity increased at lower pH	↑ Preferential remineralization of nutrients
	? Formation rate of marine snow	

[a]e.g. due to alleviation of light limitation and possibly lower supply from depths
[b]The effect is uncertain, as the supply rate, not only total annual amount, determines efficiency of utilization
[c]Both an increase or a decrease in flux due to changes in TEP dynamic have been postulated
[d]Shifts in species composition with the opposite effect are just as possible

gional trends thus meets with limited success. A promising way forward is to focus on regional scales, with the ultimate intent to address the global questions by integrating knowledge from all regions.

It may be time for a large, international effort comparable to GOFS/JGOFS (Global Ocean Flux Study/Joint Global Ocean Flux Study). Rather than focus on the euphotic zone its goal would be to understand flux attenuation in the mesopelagic. A taxa-specific understanding of environmental controls of phytoplankton (Boyd et al. 2010), zooplankton and microbial loop activity (driving flux attenuation) combined with a geographically and seasonally explicit understanding of food web structure and environmental changes will be needed to gain a predictive understanding of the biological pump. Such regional predictions may permit global predictions.

Meanwhile a starting point would be to assemble and use region-specific data sets and build a mechanistically realistic model that predicts flux attenuation based on the region-specific food web structure. In some of the most intensively studied regions of the global ocean such a database will supply sufficient data to build predictive models that allow insights into the degree of decoupling between export and sequestration flux and help guide the needed empirical studies.

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Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes

Hans-O. Pörtner*

Integrative Ecophysiology, Alfred-Wegener-Institute, Am Handelshafen 12, 27570 Bremerhaven, Germany

ABSTRACT: Climate change effects on marine ecosystems involve various stressors, predominantly temperature, hypoxia and CO_2, all of which may combine with further anthropogenic stressors such as pollutants. All life forms respond to these drivers, following potentially common principles, which are insufficiently understood. Specific understanding may be most advanced in animals where the concept of 'oxygen and capacity dependent thermal tolerance' (OCLTT) is an integrator of various effects, linking molecular to ecosystem levels of biological organisation. Recent studies confirm OCLTT involvement in the field, causing changes in species abundance, biogeographical ranges, phenology and species predominance. At the whole-animal level, performance capacity set by aerobic scope and energy budget, building on baseline energy turnover, links fitness (within a thermal window) and functioning at the ecosystem level. In variable environments like the intertidal zone, animals also exploit their capacity for passive tolerance. While presently the temperature signal appears predominant in the field, effects may well involve other stressors, acting synergistically by narrowing the aerobic OCLTT window. Recent findings support the OCLTT concept as a common physiological basis linking apparently disjunct effects of ocean warming, acidification and hypoxia in a so-called climate syndrome. In brief, warming-induced CO_2 accumulation in body fluids links to the effects of ocean acidification mediated by the weak acid distribution of CO_2. Temperature-induced hypoxemia links to the hypoxia sensitivity of thermal tolerance. Future work will need to develop proxies for the temperature-dependent effects of climate-related stressors and also identify the principles operative in organisms other than animals and their underlying mechanisms. Mechanism-based modelling efforts are then needed to develop reliable organism to ecosystem projections of future change.

KEY WORDS: Ocean warming · Hypoxia · Acidification · Climate change · Ecosystem change · Aerobic scope · Energy budget · Mechanism-based projections

INTRODUCTION

Climate change effects on marine ecosystems are investigated by marine researchers from various, apparently disconnected angles. Empirical studies have repeatedly elaborated effects on global and regional scales (e.g. Perry et al. 2005) and have led to projections of change adopting principles like temperature-dependent biogeography or body size (Cheung et al. 2009, 2012). However, the respective cause and effect understanding (e.g. Pörtner et al. 2008) has not been fully integrated into such efforts. Benefits of such understanding are a high reliability and certainty of the knowledge and attribution of presently detected effects to climate change, and also a high certainty in the projection of future effects. Understanding cause and effect requires physiological knowledge of the mechanisms driving effect as

*Email: hans.poertner@awi.de

well as knowledge of the reasons for their compulsory responses (trade-offs and constraints) under a set of environmental conditions. Lastly, these mechanisms require testing under field conditions so as to demonstrate their involvement and their role as leverage in causing ecological effects.

Quantitative evidence linking physiological phenomena to ecosystem-level processes and change is currently still scarce. Moreover, the number of concepts suitable to integrating and linking physiological with ecological phenomena is limited. In animals, relevance of physiological effects in a climate change context at the ecosystem level has only been demonstrated for the concept of 'oxygen and capacity dependent thermal tolerance' (OCLTT). The functional capacity of oxygen supply systems, especially of cardio-circulation, to fully match demand and sustain aerobic scope is limited to a thermal window characterized by its species- and life stage-specific setting and width on the temperature scale (Fig. 1). The principles of the OCLTT concept have been elaborated in representative species from various animal phyla: sipunculids, annelids, molluscs (bivalves, cephalopods), crustaceans and vertebrates (fishes). Recent evidence shows that the OCLTT principles likely also hold in air breathers relying on convective oxygen transport or in aquatic larvae of insects (Verberk & Bilton 2011). These principles (see Pörtner 2001, 2002a, Pörtner et al. 2004a,b) have also been verified in larval stages of crustaceans (Storch et al. 2009) as well as in small zooplankton (Seidl et al. 2005). These observations tie in with a more general picture of how ambient oxygen levels shape and limit animal life through oxygen availability and the associated parameters (diffusion coefficient, concentration including gas solubility) of Fick's First Law of Diffusion. The capacity to supply oxygen is limited by the functional capacity of the circulatory system in fishes and invertebrates (Pörtner et al. 2004b, Somero 2012). Further limitation (in invertebrates) may involve the ventilatory system. In special cases, such as in adult crustaceans, ventilation of the egg masses is also limited by capacity (Cohen & Strathmann 1996, Fernandez et al. 2000, Woods & Moran 2008). The balance between oxygen supply and demand shapes the dependence of maximum body size in marine invertebrate phyla on temperature-dependent oxygen availability (Chapelle & Peck 1999, Pörtner 2002b). These principles thus appear unifying in shaping the environmental border conditions of aquatic and possibly terrestrial animal life (Pörtner & Farrell 2008). They may also help to understand the nature and changes in biotic interactions (competition, predator–prey relationships) from the physiologies of interacting species (see below, Pörtner & Farrell 2008, Pörtner 2010).

Applicability of the OCLTT concept to climate change questions would depend on its verification at the ecosystem level. Few studies have established such clear links between laboratory and field studies. Such field studies would report a climate-induced effect at the ecosystem level, and laboratory studies would establish the physiological reasons for such an effect (e.g. Anestis et al. 2007, 2008, Pörtner & Knust 2007, Katsikatsou et al. 2012). Existing findings thus corroborate the applicability of the OCLTT concept. After its verification, this concept can also be used in the interpretation of field observations even when the underlying physiology has not (yet) been established. Nonetheless, further investigations still need to identify relevant details in the complex relationships between climate-dependent physiology and ecology. In this context, more traditional concepts require reinvestigation and, if possible, integration into OCLTT. For example, studies of thermal biology have often identified characters in isolation (such as critical thermal maxima or lethal limits) which are not directly but only distantly related to field observations or the effect of climate change. Studies may also have selected individual, e.g. cellular, stress indicators (Fig. 1B) which fit the context of OCLTT, but the whole organism aspects have not been included. Arrhenius slopes and break point temperatures studied at the whole-animal level frequently remained unexplained. Integration and interpretation of such findings in the OCLTT context is warranted and often feasible. While the identification and debate of mechanistic details are going on and group- or species-specific features or twists are being identified (e.g. Pörtner 2010, Eliason et al. 2011, Schulte et al. 2011), this does not question the general applicability of the OCLTT concept as supported by a rising number of field investigations.

Overall, the physiological processes underlying OCLTT shape the performance curve of a species, commonly exemplified in the temperature-dependent growth patterns observed. Oxygen limitation under warm conditions is predominantly caused by the limited capacity of the circulatory system to maintain aerobic scope despite rising baseline energy and thus oxygen demand reflected in the standard metabolic rate (SMR). Thermal limits can thus be shifted in two ways, by increasing or decreasing capacity and by increasing or decreasing SMR. Both capacity and SMR are interdependent such that limits result as a tradeoff between the two.

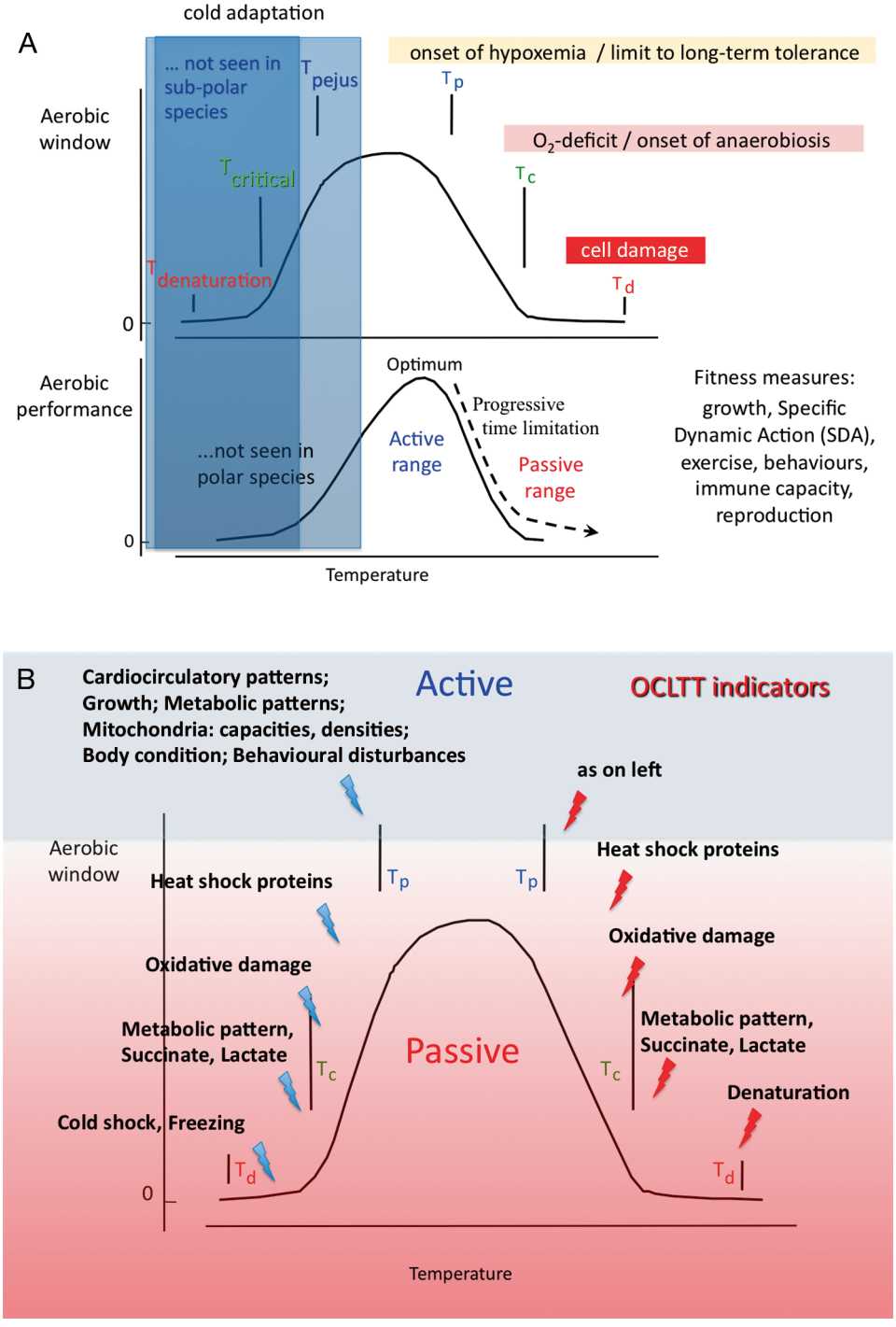

Fig. 1. OCLTT (Oxygen and capacity-limited thermal tolerance) as a concept integrating multiple stressors and various processes and their indicators (simplified from Pörtner 2010). (A) Limited thermal windows are set by (aerobic) performance capacity as the first level of thermal limitation. Optimized oxygen supply to tissues between low and high pejus temperatures (top) combined with the kinetic stimulation of performance rates by warming supports temperature-dependent performance and a functional optimum (i.e. an optimum of aerobic scope) close to upper pejus temperature. Note that in sub-polar and polar climates, due to falling oxygen demand and rising oxygen concentrations, the oxygen limitation of cold tolerance may not be seen, leaving a limitation through functional capacity (Wittmann et al. 2012). (B) According to the involvement of various physiological and biochemical processes in characterizing the various phases of thermal limitation, a set of parameters results which can be used as indicators of the thermal limitation process depending on temperature and over time. Note that the passive tolerance range is a relevant component of the niche in cases when organisms regularly experience extreme temperatures

Depending on the climate regime studied, capacity limitation in the cold may or may not go hand in hand with oxygen limitation, as metabolic rate falls and high oxygen solubility in media and body fluids causes oxygen availability to be in excess under cold conditions. While oxygen deficiency in the cold develops in warm temperate species, it is alleviated in sub-polar and polar species (e.g. Wittmann et al. 2012, Fig. 1A). This review and position paper provides the most recent update on the OCLTT concept and extends to a treatment of its roles in understanding patterns of change in the field and how the mechanistic links between thermal tolerance and other factors relevant in climate change can be identified.

If alternative concepts exist that are equally powerful, similarities, differences and links to OCLTT would need to be clearly identified. However, I am presently not aware of concepts that would be equally as integrative as the OCLTT concept in animals. Some apparent alternatives can in fact be understood to emphasize various aspects that would be linked to OCLTT or where OCLTT would provide the links between the various approaches. Concepts clearly linking to the mechanistic framework of OCLTT include temperature-dependent reaction norms (Tewksbury et al. 2008), which mirror the level of aerobic scope in OCLTT (this paper). Long-term lethal limits (Peck et al. 2009) result from the exploitation of passive tolerance in OCLTT (Pörtner 2010). Thermal acclimatization and adaptation at transcriptomic and proteomic levels (Stillman 2003, Lucassen et al. 2006, Somero 2012, Tomanek 2012) link to associated shifts in pejus and critical tolerance limits (Pörtner et al. 2008). Studies of membrane or protein structure (Somero 2012) relate to whole-organism functional capacity at various temperatures and contribute to understanding how pejus and critical limits are set at the whole-organism level (Pörtner et al. 2012). Energy budgeting and efficiency (Sokolova et al. 2012) mirror the width and position of the thermal window on the temperature scale (Pörtner 2006). Changes in species interactions including predation and associated biodiversity changes (Harley 2011) may result from differential performance levels and their relative shifts in interacting species (Pörtner & Farrell 2008, see below). In general, studies at molecular and biochemical levels can be interpreted to identify the foundation of functional capacity and thus OCLTT. It therefore seems that overall, concepts should be brought together more, rather than being presented as parallel alternatives.

EVIDENCE LINKING OCLTT AND FIELD PHENOMENA

Fishes

The examples discussed here all include field data and performance indicators, thereby linking to the OCLTT concept. First evidence for the concept being applicable to the ecosystem level came from a comparison of field and laboratory data in eelpout *Zoarces viviparus* from the German Wadden Sea (Pörtner & Knust 2007). Summer extreme temperatures negatively affected population dynamics and caused a loss in the population, starting with the largest individuals. Laboratory studies confirmed that processes related to OCLTT, especially cardiocirculatory limitation and the allometry of oxygen limitation, explain these patterns. Onset of hypoxemia and capacity loss explains the loss of fitness upon warming and occurs in the largest individuals first. Reduced heat tolerance in larger specimens of a species suggests that especially spawners with their additional biomass of eggs and sperm are sensitive to warm temperatures (Pörtner et al. 2008). In Atlantic cod *Gadus morhua*, spawning may therefore occur in winter or early spring and even then, warming interferes, as indicated by the clear effect of winter warming on cod distribution (Perry et al. 2005). Early life stages may also be sensitive during the time when circulatory and ventilatory systems are not fully developed. On large latitudinal scales in the oceans, the pressure to thermally specialize may contribute to the strong differentiation of Atlantic cod into populations which specialize on the regional climate and display thermal windows different from each other, as a result of regional (i.e. local) adaptation (Pörtner et al. 2008). Local adaptation leads to genetically distinct natural populations of a species (Sanford & Kelly 2011, Sotka 2012), possibly as a result of selection from a wide inter-individual geno- and phenotypic variability in the larval population.

OCLTT is also involved in constraining the spawning migrations of Pacific salmon in the Fraser River, BC, Canada (Farrell et al. 2008). The thermal limitation of swimming capacity in the warming river prevents adult spawners from reaching their upstream spawning grounds. Differences between stocks relate to differences in cardiac capacity and functioning (Eliason et al. 2011). Atlantic salmon *Salmo salar* or Pacific sockeye salmon *Oncorhynchus nerka* do not feed during spawning migrations (Doucett et al. 1999, Cooke et al. 2004). They thereby protect their aerobic scope from being compromised by the cost of

food processing. This not only benefits their aerobic scope for swimming, but in light of OCLTT, this also maximizes their heat tolerance. However, the capacity to maximize exercise performance will be time-limited under these conditions, as high energetic demands will ultimately cause starvation and muscle wastage.

A study of the metabolic background of temperature-related tolerances in various age (0+ and 2+) and size groups of Atlantic salmon contributes to an understanding of how thermal limits relate to behavioural changes (Breau et al. 2011). A behavioural shift associated with aggregations in coldwater sites occurs in older and larger individuals (2+) when warming drives their basal metabolic rate up to its maximum and leads to an accumulation of lactate, indicating that anaerobic pathways are recruited at beyond critical temperatures. This metabolic shift might be elicited by hypoxemia, which is thermally induced inside the organism. Lactate accumulation likely acts as an alarm signal, then causing behavioural hypothermia, a shift of preferred temperature to lower values (Pörtner et al. 1994).

In light of this progress in our understanding of cause and effect in climate change impact on organisms, any field data containing information on a performance term like growth or reproduction may also become explainable with OCLTT-related hypotheses. Certainly, supporting such explanations would require laboratory investigations of the physiological background. An example emphasizing how thermal specialization explains sensitivity, productivity and large-scale ecological phenomena such as non-linear shifts in the species composition of ecosystems (regime shifts), is one of sardines and anchovies in the Japanese Sea. The thermal windows of growth and reproduction overlap in the 2 species but differ in their position on the temperature scale (Takasuka et al. 2007, 2008). This explains why warming caused anchovies to thrive and increase in abundance while sardine populations collapsed at the same time.

Specialization on regional climate associated with specific limits to thermal acclimation is also evident in field data on young banded morwong *Cheilodactylus spectabilis* and their growth and distribution in the Tasman Sea (Australia, New Zealand). Growth data from otolith readings over the range of field temperatures according to the biogeography of the species yield the species-specific thermal performance curve (growth) (Neuheimer et al. 2011). On the cold side of the thermal window, left of the optimum, the data indicate a warming-induced increase in growth and a shift in distribution range, whereas beyond the thermal optimum, a warming-induced decrease in growth rate occurs reflecting the warm side of the performance curve (Fig. 2). Interestingly, field growth data falling on the warm side indicate that the species has some limited scope to reduce its temperature-dependent performance and to still persist in the ecosystem studied before performance levels become too low. At the species distribution boundary, warm acclimation would be expected to involve trade-offs in cellular and metabolic design and energy budget and allocation (cf. Pörtner & Lannig 2009). Especially the down-regulation of mitochondrial densities and their maintenance costs under warm conditions may contribute to a lowering of SMR and, as a consequence, aerobic scope for growth with the benefit of enhanced heat tolerance. This also results through a reduction in maximum body size such that smaller individuals of a species are more heat tolerant and can persist in warmer waters (cf. Pörtner et

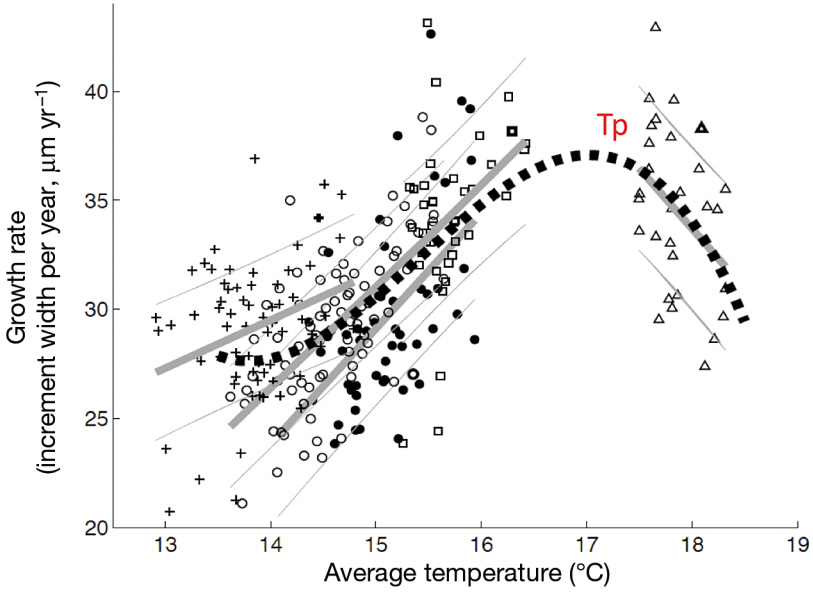

Fig. 2. *Cheilodactylus spectabilis.* Specialization on climate reflected by the thermal niche (limited by the capacity for acclimatization) according to growth rates from otolith readings in banded morwong around Australia and New Zealand in the field (after Neuheimer et al. 2011). Note the decrease in growth rates beyond apparent pejus limits under warm conditions, likely reflecting warm acclimatization, shifts of the acute thermal window and tradeoffs in energy budgets (see 'Evidence linking OCLTT and field phenomena' in the main text and Fig. 3). Tp: pejus temperature

al. 2008). Recruitment from cooler waters may support such a pattern at distribution limits.

The examples discussed provide evidence that fishes experience their acute upper thermal limits of aerobic scope in the field. Aerobic scope fuels rates of growth as in benthic eelpout and banded morwong or swimming activity as in migrating salmon. These findings suggest that species operate in the field up to their specific limits of warm acclimation capacity. These environmental conditions represent those limiting the distribution limits of the species. In essence, all of these data emphasize the usefulness of interpreting data from well-controlled laboratory experiments in the context of environmental data from the field and vice versa.

Coastal and intertidal invertebrates

Further examples come from studies in the Mediterranean. Submersed mussels (*Mytilus galloprovincialis, Modiolus barbatus*) were observed and experimentally exposed to various temperatures in the lab and in the field (Anestis et al. 2007, 2008, Katsikatsou et al. 2012). Lab findings were compared to those gained in submersed mussels at field temperatures throughout the year. Field experiments transposed some animals to water depths with different temperature regimes. These studies in permanently submersed mussels exclude the complications introduced by air exposure in the intertidal zone and allowed the researchers to clearly identify those phenomena related to the seasonal temperature regime.

During summer heat as well as winter cold in the field, even these submersed mussels exploited the heat shock response (Anestis et al. 2007, 2008, Katsikatsou et al. 2012). These findings indicate that despite slow seasonal temperature change, animals reach the extreme ends of their thermal windows, beyond their thermal range of aerobic scope and in their passive range of thermal tolerance (extreme pejus and pessimum range, Fig. 1A). The range of ambient temperatures thus matches the thermal window, reflecting tight thermal specialization and limits to acclimatization capacity. Furthermore, the exploitation of the heat shock response indicates that vertical zonation of submersed mussels is co-determined by their capacity of time-limited passive resistance to temperature extremes. This indicates that these sessile species live where ambient temperatures periodically exceed the limits of sustained aerobic scope. These findings likely also relate to the capability of these mussels to thrive in intertidal habitats. Here, the southern distribution limits of intertidal mussels (*Mytilus edulis*) correlate with their lethal limits, an observation which also emphasizes that these mussels exploit their passive range of tolerance (Jones et al. 2009). Temperature, however, shapes distribution in the intertidal zone together with wave splash, emersion time and desiccation stress (Harley & Helmuth 2003).

The capacity for acclimatization is likely very strong and the resulting shift in thermal window very wide in temperate zone species settling in the intertidal zone. Wittmann et al. (2008) and Schröer et al. (2009) demonstrated such shifts in the intertidal lugworm *Arenicola marina* according to season and climate zone. Such acclimatization capacity is reflected in the up-regulation of metabolic capacity upon cold exposure and the down-regulation of metabolic costs in warm conditions, such that residual aerobic scope remains sufficiently high. A thermal niche results, within which the acute thermal window shifts with acclimatization. The borders of the niche are characterized by the limits of acclimatization capacity (Fig. 3)

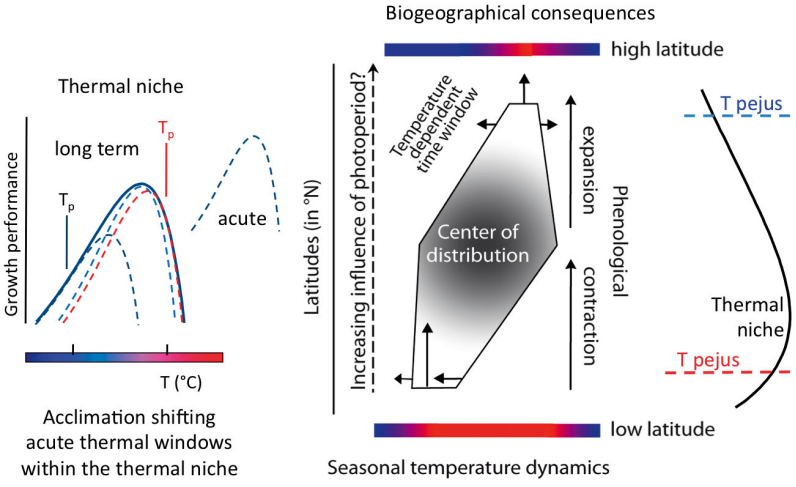

Fig. 3. The oxygen- and capacity-limited thermal tolerance (OCLTT) concept and its underlying mechanisms provide an understanding of performance shifts between seasons and the associated patterns of acclimatization, understood as a shift of acute thermal windows within the limits of the thermal niche (left, after Pörtner 2010) (see Fig. 2 legend). They also explain shifts in large-scale, temperature-dependent biogeography and climate sensitivity in the marine realm which involve a contraction of the available niche space on the warm side and a widening on the cold side of the temperature-dependent distribution range (center, after Beaugrand 2009). The temperature-dependent distribution range matches the thermal niche (right)

and would match the niche realized at the ecosystem level (cf. Pörtner et al. 2010). A recent study by Marshall et al. (2011) illustrates how intertidal snails (*Echinolittorina malaccana*) undergo acute acclimatization during low tide and down-regulate their metabolic costs acutely, upon warm exposure, possibly by exploiting strategies of metabolic depression when in air. The onset of the heat shock response is then delayed to a breakpoint temperature beyond which energy demand finally rises. These findings suggest that the down-regulation of metabolic costs under warm conditions may characterize the temperature range of constant metabolism of intertidal ectotherms already described by Newell (1969). The OCLTT concept would characterize this phase of apparent thermal insensitivity as the exploitation of a wide pejus range during which SMR remains constant upon warming. Aerobic scope becomes constrained and performance declines, but the transition to anaerobic metabolism is likely shifted to higher temperatures. The breakpoint temperature may in fact be equivalent to the critical temperature, which is characterized by the onset of anaerobic metabolism, in parallel to the onset of the heat shock response.

Phenomena seen on large latitudinal scales in the oceans are found on small scales in coastal and intertidal environments. Even within the intertidal zone, and similar to large-scale observations, local adaptation on small scales leads to genetically distinct natural populations of a species and plays an important role in governing survival and distribution patterns (Sanford & Kelly 2011).

Generalizations

The examples of pelagic fish and coastal and intertidal invertebrates discussed here indicate that there are physiological limits to the realized niches of ectothermic species (Pörtner et al. 2010), which are associated with the limits of their acclimatization capacity (Stillman 2003). Acute thermal windows are narrower than this thermal niche, and the width and position of the acute window can shift widely with seasonal acclimatization (Fig. 3). Associated with this shift are shifts in baseline energy turnover and in aerobic scope as well as reallocations from the energy budget to crucial performances like growth, reproduction and behaviour. The comparison of ectotherms from various climates suggests that thermal specialization occurs for the sake of energy savings which are highest with narrow thermal windows in the permanent or seasonal cold (Pörtner 2006).

Intertidal and coastal species exploit the range of passive tolerance more than oceanic species, as a regular add-on to the acute thermal window (Fig. 1, not differentiated in Fig. 3). On shorter, e.g. diurnal, time scales in the intertidal zone, the exploitation of metabolic depression saves energetic cost. While this may contribute to aerobic scope reduction upon warming beyond pejus limits, it would cause an upward shift in critical temperature. Then, beyond critical limits, metabolic depression reduces the degree of anaerobic energy production and extends the time limits of passive tolerance (cf. Fig. 5).

Furthermore, it needs to be emphasized that the examples reported only take a snapshot glimpse out of the whole life cycle of a species. Thermal windows are dynamic and may change from the gamete through egg, embryo, larval, juvenile and adult stages to adult spawners (Pörtner & Farrell 2008).

Providing cause and effect not only means linking ecological and physiological whole-organism phenomena but also means linking levels of biological organisation from ecosystem down to molecular and up. Unravelling the connections between levels of biological organisation, from genomic, molecular to cellular, individual and population levels, is crucial in understanding why organisms specialize on a limited range of environmental temperatures, and conversely, why and how the genomes and their intraspecific variability define the thermal sensitivity of species and their populations (cf. Kassahn et al. 2009). Scopes for acclimatization (phenotypic plasticity in physiology and behaviour) and adaptation (evolutionary shifts in morphology, physiology and behaviour) that together define species resilience require study at various life stages (eggs, larvae, juveniles, adults), as thermal windows as well as sensitivities to other factors vary between these stages.

The OCLTT concept has been proposed as a suitable umbrella integrating effects at various levels of biological organisation, from genome via molecular, cellular, whole organism to ecosystem (Pörtner 2002a, 2010). It thereby allows integrating phenomena that have been reported for one organisational level in isolation (e.g. heat shock protein expression or proteomics in general) and can now be re-interpreted in a larger context. For example, thermal specialization and associated energy savings in Antarctic stenotherms, largely exemplified in fish, typically involve reduced aerobic and anaerobic capacity, large myocytes with low capacities for ion exchange, the use of lipid body stores for low-cost neutral buoyancy and low-cost oxygen distribution by diffusion due to high density of lipid membranes. Excess oxygen availabil-

ity at high oxygen solubility and low metabolic rates in the cold allow for the loss of haemoglobin and even myoglobin in some icefishes and the loss of the heat shock response in notothenioid fishes in general (for review see Pörtner et al. 2007, 2011). The other side of the coin of such adaptations is a high sensitivity to heat exposure, which in fact correlates with blood haemoglobin content (haematocrit) of Antarctic fishes, thereby reconfirming applicability of the OCLTT concept to Antarctic species (Beers & Sidell 2011).

As a corollary, specialization on temperature likely supports maximized energy efficiency. Efficiency results from trade-offs at several hierarchical levels, from molecular structure to whole-organism functioning. For aerobic scope to be available requires staying within the aerobic thermal window; however, this may not be possible and thus may be discontinuous in some cases such as in the intertidal. Allocation of a sufficient fraction of time to staying within the aerobic temperature range appears obligatory for the sustenance of fitness and crucial life history phases (Pincebourde et al. 2008, Marshall et al. 2011 as reinterpreted here). The intervals of passive tolerance do not support growth or reproduction. Thermal stress thus causes performance losses, associated with hypoxemia and capacity limitations in warm conditions, and capacity limitations (associated with hypoxemia or not) at the cold end of the thermal envelope. The range of passive tolerance is regularly exploited in intertidal species and involves systemic and cellular stress signals like hormonal responses or oxidative stress. Stress protection is provided by mechanisms like metabolic depression, antioxidative defence or the expression of heat shock proteins. All of these indicators find their place and represent relevant components in the OCLTT window concept (Fig. 1B, cf. Fig. 5). Thermal acclimatization or adaptation cause thermal windows to shift, to change their widths or to adjust the shape of the performance curve. This happens between seasons or during adaptation to a climate regime or during local adaptation to variable local conditions.

BEST PRACTICE APPROACHES

Application of the OCLTT concept to various examples from diverse marine habitats leads one to ask about the best practice of its application, especially when it is applied by scientists from various disciplines, in this case either physiologists or ecologists. Each scientist may do this from different, apparently disconnected angles. The examples selected here indicate recent twists in the application of the concept and also the consequences once the relevance of integration is ignored.

A crucial aspect, which requires careful implementation with respect to the OCLTT concept, is the most appropriate way to test aerobic scope. This is best done by investigating the scope for aerobic performances like growth, or locomotion in steady state, including steady-state swimming or repeated spontaneous, non-stressful activities, and possibly reproductive output. These performances are those displayed by the species in nature but to various degrees, depending on the season and, most importantly, depending on its mode of life. A key element should therefore be the careful consideration of species-specific functional characteristics. For example, in highly mobile pelagic species, exercise is fuelled aerobically up to very high steady-state exercise levels, until anaerobic metabolism kicks in above critical swimming speeds (Pörtner 2002b, Lurman et al. 2007). Transition to functional anaerobiosis should be avoided in assays determining OCLTT, as stress hormones will become involved and push for non-steady state performance or affect limiting thresholds. Maximum aerobic metabolic rate can have an anaerobic component and would then be less suitable.

More sluggish demersal and benthic species display a smaller aerobic scope for exercise or do not display a capacity for continuous exercise at all. In extreme cases such as infaunal species, locomotion is mostly fuelled anaerobically, and not suitable as an indicator of aerobic scope. The resulting thermal window and optimum would apply for anaerobic metabolism and must not be the same as for aerobic metabolism. Furthermore, if experimental conditions place animals outside their preferred modes of behaviour or performance, a stress response may result, leading to unfavourable feedback on the parameters to be tested. As a corollary, aerobic scope should be tested as close as possible to the living conditions and behaviours seen in the natural habitat of the species.

Another key facet and potential dilemma involved in studying such a concept is the human factor. Individual scientists may be motivated to add their own twists to the interpretation of an overarching concept or to focus on selected aspects without being aware of relevant implications and their own biases. This may have the consequences of being too reductionist or of testing and discussing these selected aspects out of comprehensive context. Erroneous conclusions and misleading implications may result. Comprehensive testing of an overarching concept such as OCLTT in relevant case studies is important. Also,

research needs to address the question of how to best integrate new findings. Such an approach would then also allow discovering new and relevant aspects and thereby developing the concept further.

The recent case study by Marshall et al. (2011) tested the OCLTT concept in littorinid intertidal snails. Their findings can successfully be integrated, with no need to classify them as being 'in contrast' to existing theory. In fact, their paper concludes that metabolic resting rate per se is important in shaping thermal tolerance. The principle role of resting or standard metabolic rate (SMR) is in fact a relevant component of the OCLTT concept. A low SMR in the thermal optimum characterizes sluggish species like the intertidal snail which exploits metabolic depression and passive tolerance more than active species. In contrast, a high SMR in the thermal optimum characterizes highly mobile, energetic species and supports high net aerobic scope as seen for example in squids or active teleosts. Due to high energy demand, their capacity for passive tolerance is extremely limited.

In general, if baseline oxygen demand or SMR rises from the thermal optimum to beyond upper pejus and then to critical limits, this indicates the development of constraints on aerobic scope. During temperature change, the adjustment of such resting rates thus is an integral part of the warm-acclimation process. This involves the down-regulation of mitochondrial cost (through proton leakage) and capacity as well as cost savings at the level of the cellular membrane. Thereby, the otherwise limiting increment of cost and associated SMR in warm conditions can be delayed and occur at higher temperatures. However, this likely occurs at the expense of aerobic scope such that the down-regulation of resting rate will also cause down-regulation of maximum aerobic metabolic rate (see Fig. 5). The key issue is that processes are intertwined between levels of biological organisation and between functional states. Looking at a level or process in isolation may be misleading with respect to how the full picture and the associated trade-offs develop. Specific modes of life as in the intertidal zone may include interesting modifications of individual processes contributing to OCLTT but not to the extent that alternative concepts are needed. The example of the banded morwong (Fig. 2) indicates that acclimatization or evolutionary adaptation to warm temperatures would not necessarily lead to beneficial acclimation in terms of maximized performance, but the decrease in growth seen in warm conditions likely results from trade-offs between all performances undertaken and/or from a decrease in maximum performance achieved in terms of aerobic energy turnover (see above).

ROLE OF HABITAT CHARACTERISTICS: MULTIPLE FACTORS

Experimental biology has traditionally focused on analysing responses to one environmental factor at a time and has treated the responding processes and mechanisms in isolation. However, palaeo- and ongoing climate changes involve changes and effects of various factors, making it difficult to disentangle the overall response and attribute elements thereof to individual factors. As a precondition, a comprehensive understanding of the full response of organisms to environmental change will only be possible from an integrative understanding of the interaction between factors and the synergistic, additive or antagonistic nature of effects.

Variability of environmental factors, especially temperature, differs between habitats. In the open ocean, temperature changes develop together with progressive hypoxia caused by enhanced stratification and oxygen demand of warming oceans (Stramma et al. 2008). Progressive carbon dioxide accumulation leads to ocean acidification, the degree of which depends on emission scenarios (Caldeira & Wickett 2005, Cao & Caldeira 2008). At increasing depths, the anthropogenic CO_2 signal adds to already enhanced CO_2 levels originating from microbial respiration of organic matter, which leads to the development of oxygen minimum zones (Brewer & Peltzer 2009, Brewer 2009, Hofmann et al. 2011). The stochastic occurrence of extreme changes in any of these factors would exacerbate the effects of these progressive trends. However, this projection may be premature, as variability may have positive feedbacks on resistance (hardening). Regular extremes in temperature, hypoxia and hypercapnia characterize the seasonal and diurnal variability in intertidal zones.

Beyond changes in temperature, CO_2 and hypoxia levels, salinity may change due to freshening of surface layers during enhanced river runoff or during ice melt in Arctic oceans (Denman et al. 2011). In coastal areas, pollution, e.g. by heavy metals, may interact with the other factors (Lannig et al. 2008, Sokolova & Lannig 2008). For an integration of effects of various environmental factors, the concepts elaborated for the effects of individual factors should merge, preferably on a common denominator. As a basis for such integration, temperature, hypoxia and

CO_2 all affect energy turnover in relation to oxygen supply and demand. Temperature is the most ubiquitous, overarching and pervasive of these factors, as it shapes key characteristics of all marine life and ecosystems. For a comprehensive picture, it has therefore been suggested (Pörtner 2010) that temperature can be used as a matrix indicator such that the effects of the other factors occur on a landscape of temperature and its variability. Furthermore, interactions of the other factors with temperature change key temperature-dependent functions relevant for fitness and at the ecosystem level (see above). Therefore, the OCLTT concept has been proposed as a suitable integrator for specific effects of other climate-related stressors like ocean acidification and hypoxia or the effects of some pollutants (Pörtner 2010). Building on the causality emerging from such integration, the levels and changes of performance and resistance can be quantified. Such changes then provide a link between physiology and ecology and thus an understanding of ecosystem level processes as needed for realistic estimates and projections of species and ecosystem sensitivities to environmental change.

It needs to be emphasized that the aerobic window of an animal may not encompass the full range of temperature variability in its habitat, for reasons of energy efficiency or because temperature extremes coincide with other stressors like hypoxia, hampering the full use of aerobic scope. All of this would decide whether the aerobic performance window only, or the passive sections of the thermal window, are also regularly exploited (as in the intertidal zone). This would involve the use of whole-organism to molecular mechanisms supporting passive tolerance (see Fig. 5). In the intertidal zone, especially during daytime heat exposure at low tide, oxygen deficiency may develop rapidly and regularly for some species, e.g. due to oxygen deficiency or emersion from sea water. Constraints on respiration cause CO_2 to accumulate internally under such conditions. Animals then go into metabolic depression and with rising temperatures exploit the mechanisms of passive tolerance, by using anaerobic metabolism, the heat shock response and antioxidative defence mechanisms beyond critical temperatures. Use of these mechanisms and their species-specific capacities will extend the time period of survival (cf. Pörtner 2010) but would not involve activities like foraging and reproduction or performances like growth and, therefore, is time limited. Foraging, growth and reproduction would largely take place during high tide periods and within the aerobic window (see above).

Various other environmental factors like CO_2 (ocean acidification), pollution or hypoxia are also subject to variability, partly depending on habitat characteristics like stratification or microbial oxygen demand or on the degree of anthropogenic influence, for example eutrophication. All of these pose additional stressors, which have been interpreted to constrain aerobic performance and fitness and narrow the thermal window. The scarcity of data suggests that CO_2, hypoxia and heavy metals like cadmium display synergistic interactions with temperature at thermal extremes, whereas effects may be antagonistic in the central section of the thermal window. For example, warming from lower pejus temperature (T_p) to the thermal optimum may aid ion exchange capacity and thereby improve CO_2 resistance. Upon warming to higher than upper pejus and critical limits, however, aerobic scope and associated active CO_2 resistance fall, as concluded from a downward shift of critical temperatures under elevated CO_2 levels (Walther et al. 2009).

Hypoxia decreases oxygen availability and thereby causes a narrowing of thermal windows due to exacerbated body fluid hypoxemia. Similarly, CO_2 accumulation causes a narrowing of thermal windows, likely through a pH-induced decrease in tissue functional capacity, strongest at thermal extremes. Some pollutants cause an increase in SMR and, thereby, a downward shift in upper thermal limits. These processes function as links between the effects of individual factors as outlined below. Weak acid distribution of CO_2 (Fig. 4) provides access to an integrated understanding of apparently disconnected effects of ocean acidification on whole-organism processes. Disturbances in acid–base status and differential acid–base regulation in various compartments lead to different levels of pH, bicarbonate and carbonate and affect cellular, including neuronal, functioning or calcification, the latter set by the resulting calcium carbonate saturation at calcification sites (cf. Pörtner 2008). As an example, this principle mediates the correlation between calcification rates and calcium carbonate saturation levels in the water, the pattern of correlation being influenced by pH compensation capacity (e.g. McCulloch et al. 2012). Overall, hypoxia and CO_2 constrain active tolerance to thermal extremes and rather stimulate the mechanisms supporting passive tolerance by enforcing metabolic depression (Fig. 5), for example via a decrease in body fluid pH or an accumulation of adenosine in nervous tissues (Reipschläger & Pörtner 1996, Reipschläger et al. 1997, Pörtner et al. 2000). This may be useful for extending passive tolerance periods as in

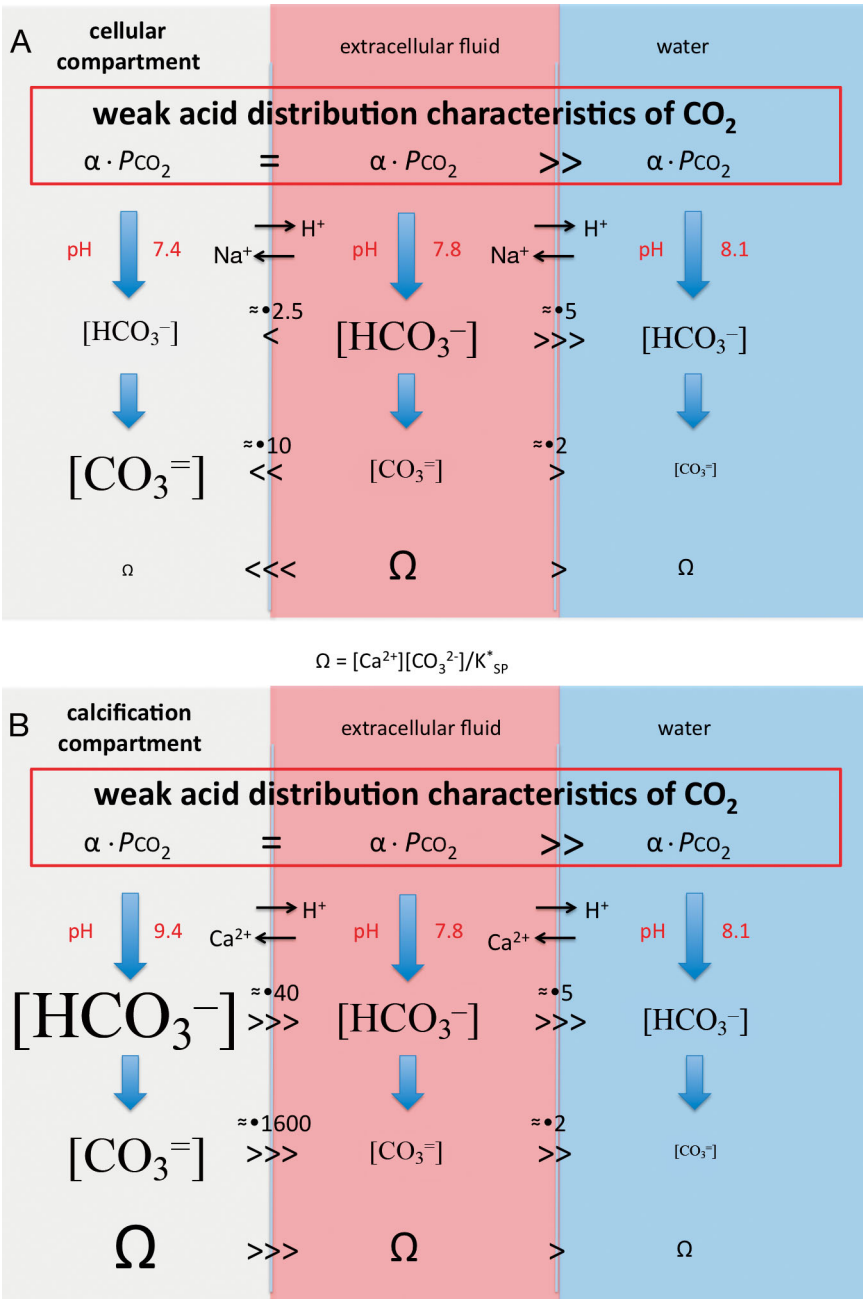

Fig. 4. Consideration of weak acid distribution of CO_2 leads to an integrated understanding of apparently disconnected effects of ocean acidification on whole organism processes like (A) cellular, including neuronal, functioning or (B) calcification at internal sites, elicited via disturbances and compensatory adjustments in acid–base status. In (B), the correlative relationship between water properties and compartmental effects depends on the capacity for pH regulation associated with the compensation of compartmental pH disturbances mediated by increasing bicarbonate levels •-fold. Carbonates are formed depending on bicarbonate concentration and the dissociation constant of bicarbonate pK_2', in relation to the pH reached. Omega (Ω) is also heavily influenced by the level of calcium set in various compartments. Note the amplification of carbonate levels and calcium carbonate saturation resulting at calcification sites (factorial ion ratios [black dots] across compartmental barriers are valid for marine invertebrates, assuming similar calcium levels across extracellular compartments and in water). α: solubility coefficient for CO_2. Note that the depiction of ion exchange mechanisms is incomplete

the intertidal. The dimensions of the thermal windows thus appear flexible, not only due to acclimation or local adaptation under a changing seasonal temperature regime but also in response to acute and progressive (climate related) stressor effects (shifting regimes of temperature, hypoxia, CO_2, biotic stressors). This flexibility and the mechanisms used are covered by the OCLTT concept in animals as outlined above. Certainly, further research needs to complement the details such as the chain of signalling events. Again it remains to be explored whether the associated principles may also work for organisms other than animals, based on similar or specific sets of mechanisms.

Recent examples that confirm the hypothesis of a narrowing thermal window under the effect of additional stressors (Pörtner & Farrell 2008, Pörtner 2010) in lab and field studies include CO_2 effects on thermal limits in crustaceans (Metzger et al. 2007, Walther et al. 2009, Findlay et al. 2010), on aerobic scope at thermal limits in fishes (Munday et al. 2009) and on performance under thermal extremes in

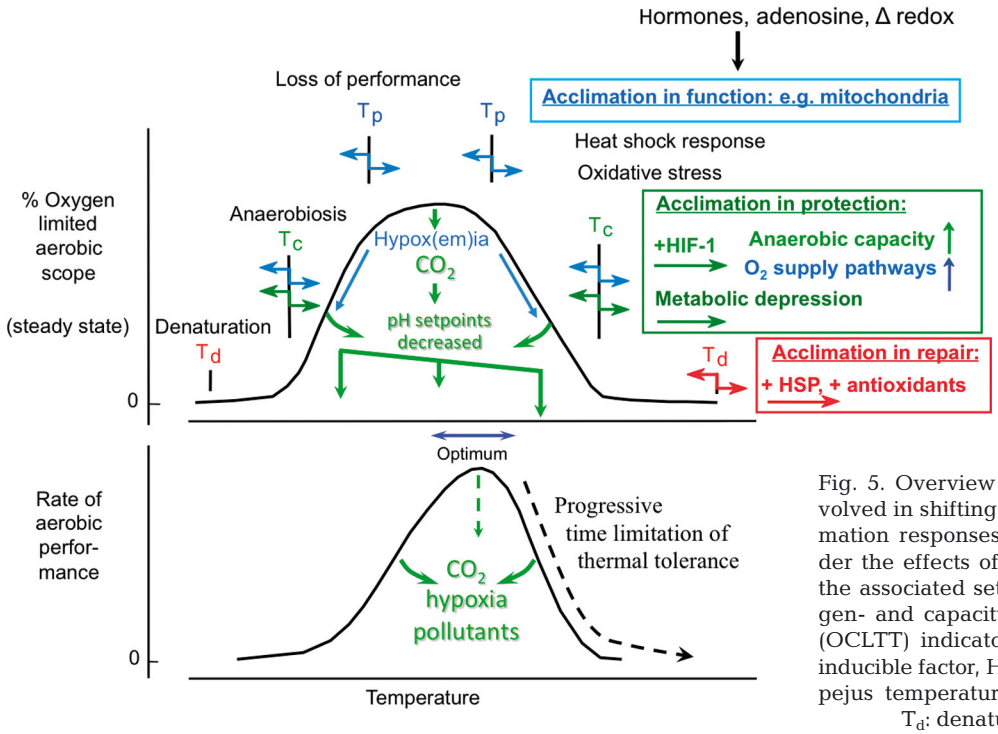

Fig. 5. Overview of various mechanisms involved in shifting thermal limits during acclimation responses (horizontal arrows) or under the effects of hypoxia and/or CO_2. Note the associated set of active and passive oxygen- and capacity-limited thermal tolerance (OCLTT) indicators (Fig. 1B). HIF: hypoxia-inducible factor, HSP: heat shock proteins, T_p: pejus temperature, T_c: critical temperature, T_d: denaturation temperature

corals (via CO_2-enhanced bleaching, Anthony et al. 2008). In a climate change scenario, with thermal tolerance likely limited and not sufficiently dynamic on relevant time scales, animals are then brought to the long-term edges of their thermal window earlier. Furthermore, CO_2 sensitivity is thermally enhanced such that warming oceans combined with progressive CO_2 accumulation would lead to an earlier effect of CO_2. At the borders of the thermal envelope, hypoxemia and associated CO_2 accumulation in body fluids would exacerbate the stress response. These aspects of enhanced sensitivity to CO_2 remain insufficiently explored in organisms from all climate zones.

Evidence for changes in the field induced by anthropogenic CO_2 is largely lacking for extant marine ecosystems, possibly because specific effects of CO_2 on marine ecosystems may still be small. A decrease in calcification rates has been observed in some coral reefs (De'ath et al. 2009); however, this observation likely includes stressful effects of ocean warming, which has repeatedly elicited bleaching and consequently a decrease in coral cover starting in the late 1970s to early 1980s. Elevated CO_2 tensions were found to enhance the bleaching response (Anthony et al. 2008), but the contribution of CO_2 to the present trends of decreased calcification has not been quantified. Nonetheless, the performance principles of the OCLTT concept also seem applicable to the special case of reef corals and will in the future likely include effects of rising ambient CO_2 levels.

The width of the thermal window is crucial in this context. It has been hypothesized that it is wider in animals displaying high resting metabolic rates (in the thermal optimum) and high functional capacities of activity and associated metabolic pathways. These features are displayed by animals exposed to high climate variability in (sub-)polar areas like the (sub-) Arctic (Pörtner 2006). Within an ecosystem, these features are also displayed by the more agile species. Animals with an elevated SMR are also those possessing a high capacity in acid–base regulation to handle respiratory CO_2 accumulation and anaerobic metabolic disturbances of acid–base status once energy demand increases beyond aerobic metabolic rates. These patterns would be associated with high levels of aerobic scope and energy turnover as seen in active compared to sluggish species in general, or in sub-Arctic species or populations compared to their temperate relatives. This differentiation may be the result of local adaptation from within-species genetic variability, which then also influences sensitivity to ocean acidification. Variable responses to CO_2 may result within the thermal optimum and thus reflect species- or population-specific CO_2 sensitivities in accordance with metabolic capacity. Sensitivity is likely higher overall at the edges of the thermal window but may also vary in a species-specific manner. Accordingly, variability between individuals would depend on where in the thermal window the response occurs. A resulting hypothesis to be tested

by meta-analyses would be that both extended eurythermy and low sensitivity to ocean acidification may go hand in hand, in the same climate zone (according to active versus sluggish mode of life) and when comparing related species or their populations from various climates. Such effects are likely strongest towards the cold end of the temperature scale (Pörtner 2004, 2006); however, examples from temperate zones with variable temperatures (such as in temperate continental seas, e.g. the Black Sea or the Mediterranean) need to be included in such comparisons.

The combined and synergistic effects of various stressors and the assessment of the overall sensitivities may in fact lead to a clearer distinction between functional groups and their response to climate change. Such categorization has been carried out by Knoll et al. (1996, 2007) and Knoll & Fischer (2011), for the Permian Triassic mass extinction event, indicating an apparent role for CO_2 in line with functional differentiations between affected groups. Contributions by other stressors have not been as prominent, although this phenomenon does not exclude the effects of other stressors. It may just be that among synergistic stressors, effects of ambient CO_2 are only apparently selective between groups, for various reasons. Present evidence suggests that effects of extreme temperatures, hypoxia and CO_2 develop synergistically; extreme temperatures also come with an inherent CO_2 and hypox(em)ia effect (by causing hypoxemia and associated internal CO_2 accumulation; Figs. 5 & 6). CO_2 effects on physiological processes like calcification (cf. Pörtner 2008) will likely also become involved under any condition causing CO_2 to accumulate internally in the organism. Respiratory constraints at extreme temperatures or during hypoxic hypercapnic exposure in oxygen minimum zones or in the intertidal cause CO_2 accumulation and associated acid–base disturbances eliciting metabolic depression (Pörtner 1982, Pörtner et al. 1998, Zittier et al. 2012; Fig. 6). Extreme temperatures at the edges of the thermal envelope in fact include effects on organismal physiology via hypoxemia and CO_2 accumulation. Such integration easily illustrates that the synergistic interactions of ambient hypoxia and CO_2 with extreme temperatures exacerbate any effects developing internally at higher temperatures and are essentially an exacerbation of OCLTT.

Temperature, hypoxia and CO_2 signals can thus not easily be distinguished, as the temperature signal will strengthen the CO_2 and hypoxia signals and vice versa. Sensitivity thresholds to ocean acidification (OA) will change depending on temperature and thermal limits; conversely, sensitivity thresholds to temperature will change depending on exposure to OA and hypoxia. Accordingly, while the fossil record witnesses a categorization according to OA effects, these would also develop under long-term temperature change and hypoxia and be most expressed once OA, temperature extremes and hypoxia develop concomitantly. These considerations would explain observations where temperature extremes cause internal CO_2 accumulation and, thereby, phenomena similar to those caused by OA (cf. De'ath et al. 2009). These concerns lead to the question of whether a climate-related syndrome should be defined which encompasses the effects of factors changing in a parallel or interdependent way in both the water and in body fluids (e.g. excess respiration causing hypoxia and hypercapnia in stratified or isolated water bodies, freshening exacerbating acidification, temperature extremes causing organismal hypoxemia and hypercapnia). These considerations emphasize that one of the clearest future impacts of OA will be the enhancement of sensitivity to temperature extremes (see above). Only when organisms are within their thermal optimum and have access to normoxic and normocapnic water will they have typically low internal CO_2 levels, and only for organisms under these conditions are specific effects of anthropogenic OA conceivable (cf. Pörtner 2008). Under all other conditions, combined effects of various environmental factors are likely.

As a corollary, the responses of energy turnover and budget, tissue functional capacities and the width of thermal windows related to environmental stressors support an integrative understanding of specialization on climate and, on the other side of the coin, of sensitivity to climate-related factors under the framework of OCLTT.

MICROBIAL ORGANISMS: BACTERIA AND PHYTOPLANKTON

The same set of questions as relevant for animals would likely apply to microbial organisms. The comparison of thermal limits across organism kingdoms supports 2 hypotheses: (1) limits fall with increasing levels of organisational complexity between prokaryotes and metazoans and (2) limits are set at the highest levels of functional integration, e.g. circulation, ventilation and neural control in animals (Pörtner 2002a). Although maximum heat limits of some unicellular eukaryotic species are found above metazoan heat limits, ongoing warm-

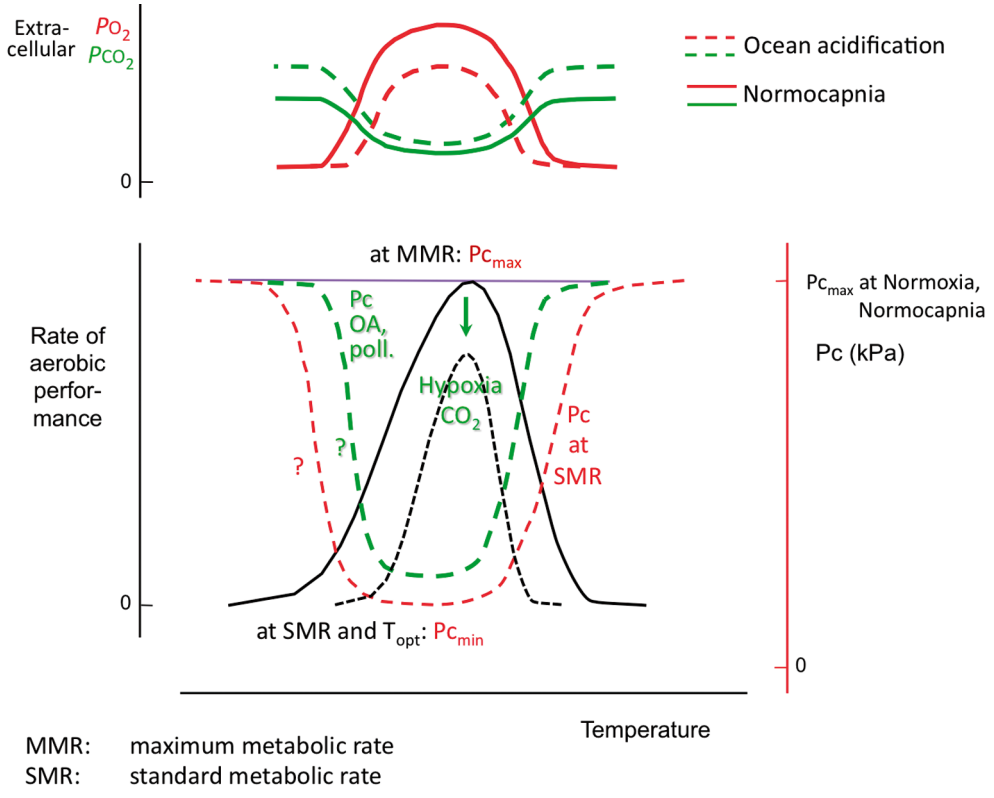

Fig. 6. Warming-induced hypoxemia and CO_2 accumulation as well as changes in energy cost provide the principal links between synergistic effects of temperature, hypoxia, ocean acidification and pollutants (see 'Role of habitat characteristics: multiple factors') in a warm-temperate animal species (cf. Fig. 1). Ambient hypoxia acts via reducing systemic oxygen tensions, thereby causing an upward shift in critical oxygen tension, Pc (indicating earlier transition to anaerobic metabolism in hypoxia), reducing performance capacity and aerobic scope and eliciting a narrowing of thermal windows. Elevated ambient and, thereby, elevated internal CO_2 levels according to weak acid distribution patterns and diffusion gradients (Fig. 4) also cause a narrowing of thermal windows. Once pH compensation remains insufficient at thermal extremes and, in some cases, in the active thermal range, lower functional capacities and reduced systemic oxygen tensions result, causing lower performance capacities. Such constraints are exacerbated by any increase in energy demand as elicited during exposure to pollutants (poll.) (expanded from Pörtner 2010)

ing causes shifts of eukaryotic phytoplankton to higher latitudes, thereby indicating lower heat limits and that large-scale biogeography of these organisms is also influenced by temperature (Thomas et al. 2012). Furthermore, the growth rates of phytoplankton and bacteria follow the typical shape of temperature-dependent performance curves or reaction norms (Eppley 1972, Ratkowsky et al. 1983). Accordingly, species succession in communities is possibly co-determined by differential thermal windows of species and their plastic responses to other factors like light and nutrient availability. In line with the present focus on animals, when addressing the impact of cold or warmth on complex cellular or organismal functions, it is important to consider that both heat and cold sensitivity are intertwined. It is also important to focus on where in the thermal window and on the performance curve effects of additional drivers are being looked at. At unicellular levels, suitable (highest) functions shaping thermal limits would be (in prokaryotes) metabolic complexes, e.g. in membranes. In unicellular eukaryotes, an additional or key element would be the coordination of function between subcellular compartments (cytosol, mitochondria, chloroplasts). Accordingly, photosynthetic capacity also follows such reaction norms (Claquin et al. 2008) and may well shape the thermal consequences for the whole organism. By disturbing pH setpoints and challenging pH regulation mechanisms (Taylor et al. 2012), OA may interact with temperature-dependent performances of unicellular algae in similar ways as outlined here for animals. Future research is needed to detail and complement this emerging picture.

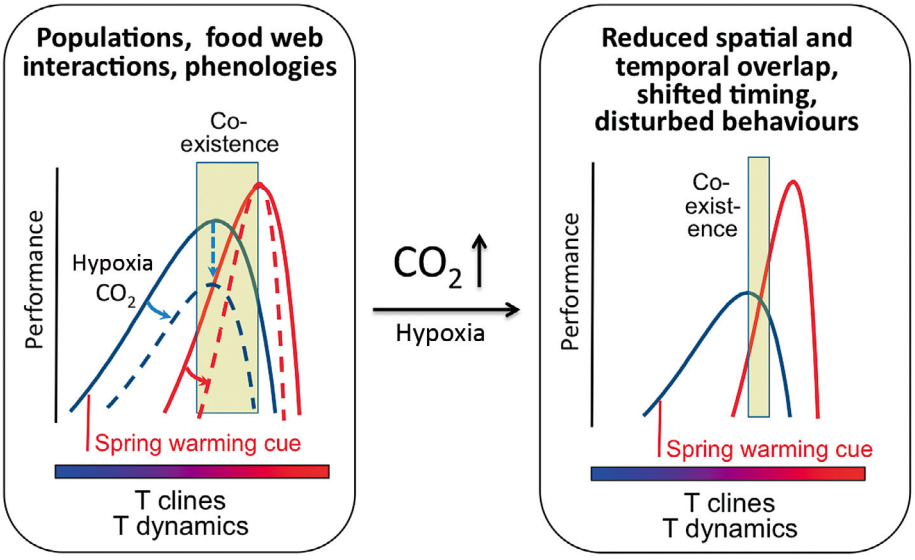

Fig. 7. Conceptual model of how species interactions are affected by the synergistic effects of shifting temperatures, elevated CO_2 and exacerbating hypoxia levels (arrows, broken curves, left) on phenology and performance, at the ecosystem level (right). Depending on species-specific lifestyles and life stages, performance windows differ between species in an ecosystem where they show spatial (e.g. on latitudinal temperature clines, cf. Fig. 3) and temporal (e.g. during time-dependent, dynamic temperature changes) overlap. The model builds on a mechanistic understanding of how effects of hypoxia and CO_2 on performance combine on a thermal matrix defined by the OCLTT principles (dashed lines, left) and shape differential changes in temperature-dependent performance, e.g. under elevated CO_2 partial pressures (upward arrow in center) or hypoxia. Species-specific sensitivities and the resulting differential changes in performance define the range of temporal and spatial overlap of interacting species as well as their changes in interactions due to shifts in relative performance (modified from Pörtner & Farrell 2008). These may involve changes in phenology, competition or susceptibility to predation

THE NEXT LEVEL UP

The question arises how to implement these conceptual developments, which firmly rest on an integrative understanding of existing data, into an ecosystem level understanding. Cheung et al. (2009) adopted the thermal window approach using climate envelope modelling and, based on the relationships between present temperature and distribution ranges, drew a picture of the projected climate-induced shifts in species distributions across wide latitudinal clines. The dynamics of the thermal window through interaction with other factors, through acclimatization or through local adaptation was not taken into account. Development of such a mechanism-based approach would enhance certainty in the simplified projections of biogeographical consequences at the species level.

Such projections remain simplified, because shifts in distribution, population dynamics and competiveness at the ecosystem level might include climate-induced changes in species interactions and thus, community responses at the ecosystem level. The productivity of a species, including its reproductive output and the growth rates of its various life stages, is determined by a complex set of factors. As a further complication, these factors may shift over time. This not only includes the seasonal changes of abiotic factors and their feedback on performance, but also biotic interactions. The latter have traditionally been viewed separately and as another complexity level; however, they should also be integrated into the present generalized picture. The relative phenology and performance of species would be crucial in this context. Availability of prey may differ over time and follow different optima from that of the predator (Fig. 7). This complexity may become accessible through knowledge of the performance curves of interacting species and their phenologies, which also define the temporal and thermal range of coexistence (Pörtner & Farrell 2008). In line with differential thermal ranges of organism groups or functions (e.g. heterotrophic bacteria versus photoautotrophic phytoplankton), this may also extrapolate to a temperature-dependent shift in functions from photoautotrophs dominating at higher (cooler) latitudes to heterotrophic bacteria progressively dominating at lower (warmer) latitudes (Hoppe et al. 2002). At least for animals, the effects of various stressors can then be projected to affect the thermal range of coexistence and species interactions.

Commercial exploitation of marine species also modulates these relationships and can be integrated as just another interacting species with variable predation (catch) efficiency and success. Any resulting changes in the population structure of predator or prey species would feed back on these relationships and thereby influence the sensitivity of marine ecosystems to climate change. It appears from these theoretical considerations that the OCLTT concept as calibrated against field phenomena and possibly developed into a mechanistic framework characterizing the climate syndrome provides a suitable framework for disentangling some of the complexities in these relationships at the ecosystem level.

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