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

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

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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

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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

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(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 &

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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

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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.

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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)

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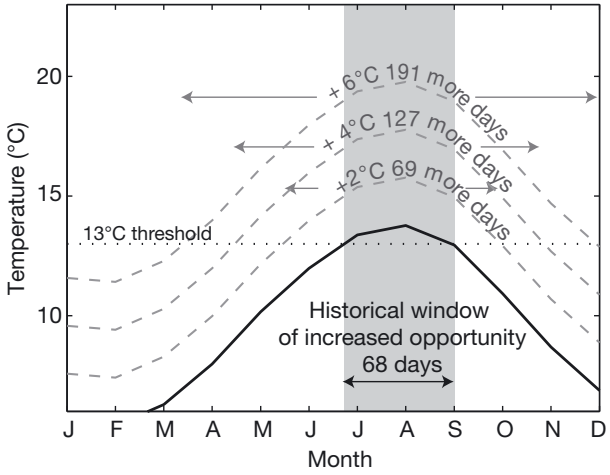

Fig. 1. *Alexandrium catenella.* Expansion of the annual temporal window for blooms of the saxitoxin-producing dinoflagellate in Puget Sound, Washington, USA, at projected sea surface warming levels of +2, +4, and +6°C. The species is limited to blooming at temperatures over 13°C; with a 6°C average 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-*

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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).

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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

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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.

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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).

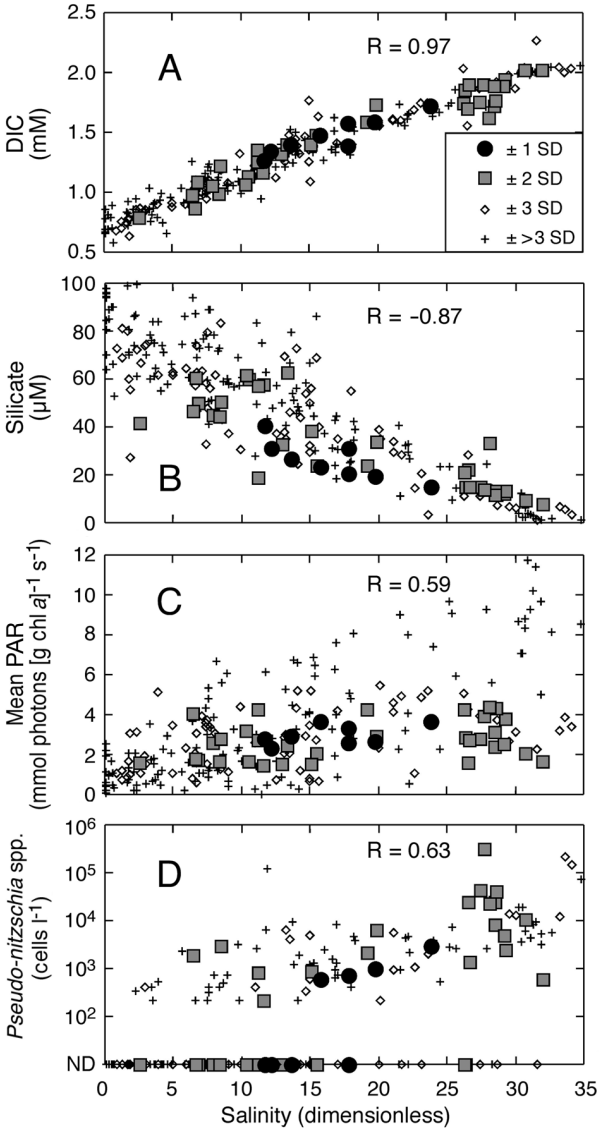

Fig 2. *Pseudo-nitzschia* spp. Environmental correlations with abundance of domoic acid-producing diatoms in samples in which domoic acid was detected across an estuarine salinity gradient in the Gulf of Mexico. Plotted against salinity are (A) total dissolved inorganic carbon (DIC), (B) silicate concentrations, (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

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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.

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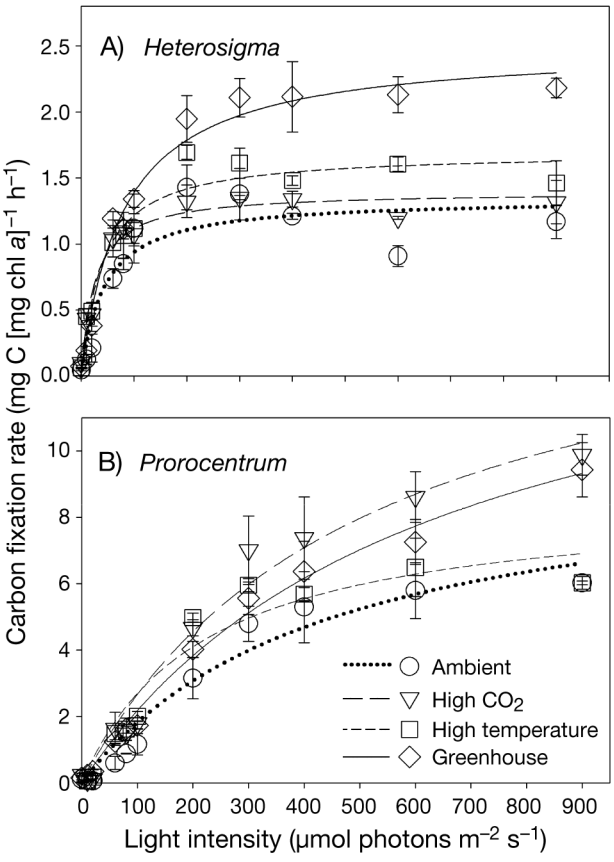

Fig. 3. *Heterosigma akashiwo* and *Prorocentrum minimum.* Photosynthesis versus irradiance curves for (A) the raphidophyte *H. akashiwo* and (B) the dinoflagellate *P. minimum* in a CO_2/temperature interaction experiment. Treatments included ambient (20°C and 375 ppm CO_2), high CO_2 (20°C and 750 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)

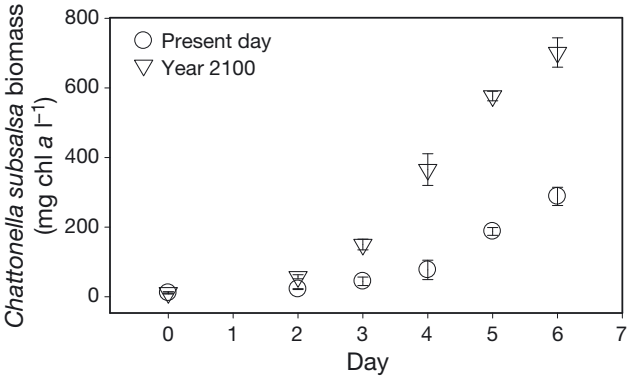

Fig. 4. *Chattonella subsalsa.* Growth of an estuarine harmful algal bloom raphidophyte (mean ± SD, n = 3) at present-day temperature and pCO_2 (20°C and 375 ppm CO_2), and at levels projected for the year 2100 (24°C and 750 ppm CO_2) (F. X. Fu & D. A. Hutchins unpubl. data)

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-

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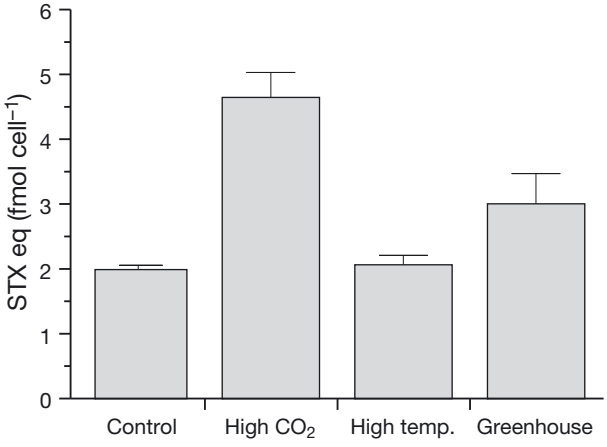

Fig. 5. *Alexandrium catenella.* Preliminary experiment showing cellular paralytic shellfish toxin contents (fmol cell^{-1} saxitoxin equivalents, STX eq) in a southern California isolate of the dinoflagellate grown at control (15°C and present-day CO_2 at 380 ppm), high CO_2 (15°C, 800 ppm CO_2), high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

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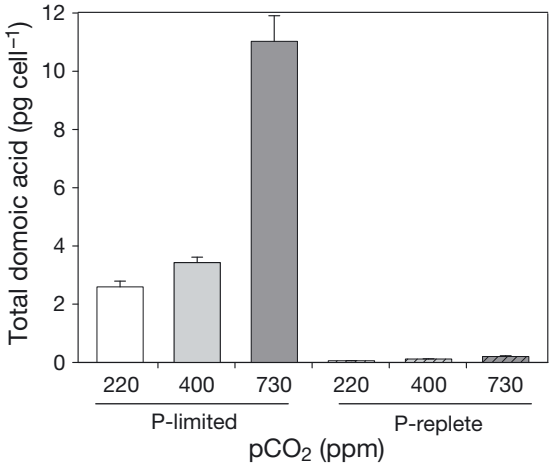

Fig. 6. *Pseudo-nitzschia multiseries.* Domoic acid production (mean + SD, n = 3) by the Canadian east coast isolate CCMP 2708 grown at 3 pCO_2 levels (220, 400, and 730 ppm) under both P-limited (open bars, 0.5 µmol l^{-1}) and P-replete (cross-hatched bars, 20 µmol l^{-1}) conditions (data from Sun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

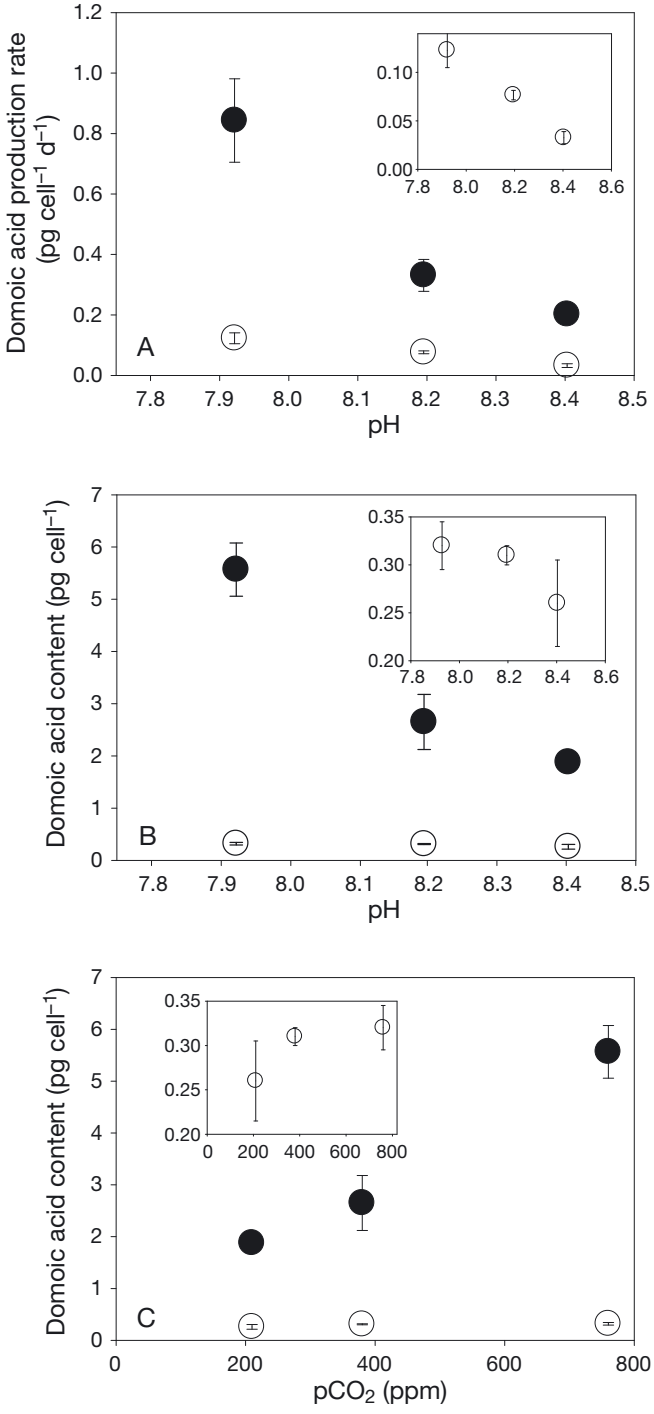

Fig. 7. *Pseudo-nitzschia fraudulenta.* Domoic acid production (mean ± SD, n = 3) by a Southern California isolate grown under $Si(OH)_4$-limited (●) and nutrient-replete (○) conditions at seawater CO_2 concentrations of 210 ppm (preindustrial atmospheric levels), 380 ppm (modern levels), and 760 ppm (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)

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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.

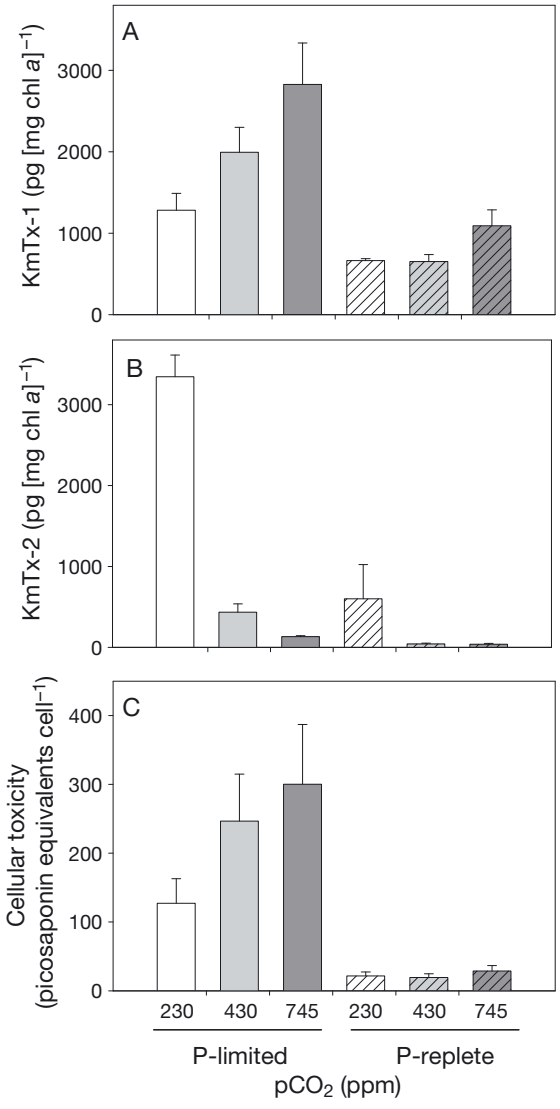

Fig. 8. *Karlodinium veneficum.* Chlorophyll *a*-normalized karlotoxin production (mean + SD, n = 3) by estuarine harmful algal bloom dinoflagellate (isolate CCMP 2936) grown at 3 pCO_2 levels (230, 430, and 745 ppm) under both P-limited (open bars, 0.5 µmol l^{-1}) and P-replete (cross-hatched bars, 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-

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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

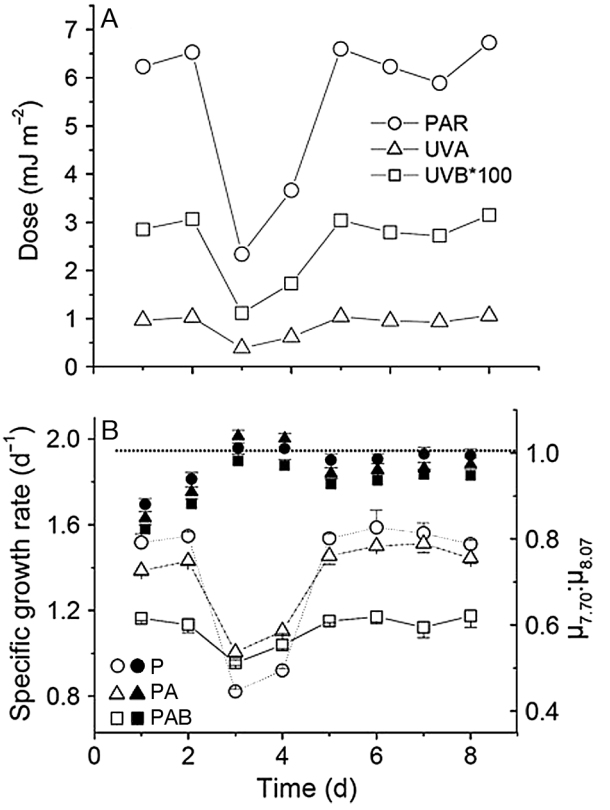

Fig. 9. *Phaeocystis globosa*. Effects of solar irradiance on growth of harmful algal bloom prymnesiophyte. (A) Daily doses of solar photosynthetically active radiation (PAR), ultraviolet radiation A (UVA, 315–400 nm), or ultraviolet radiation B (UVB, 280–315 nm) during the experimental period of 1 to 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)

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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

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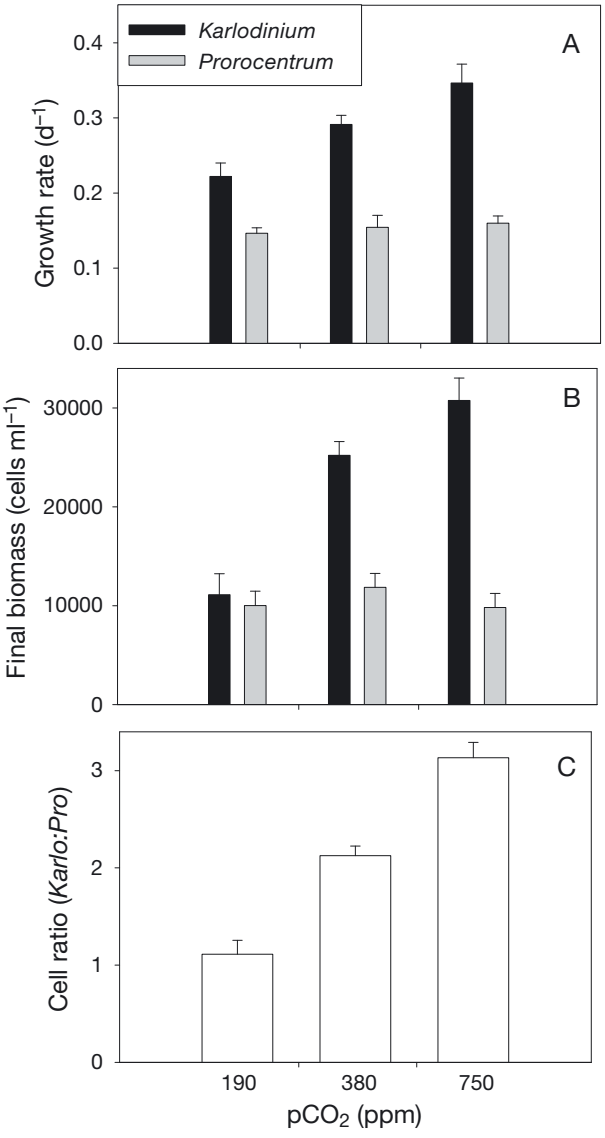

Fig. 10. *Karlodinium veneficum* and *Prorocentrum minimum*. Outcome of an ocean acidification interspecific competition experiment using mixed cultures of the dinoflagellates *K. veneficum* (black bars) and *P. minimum* (grey bars), isolated from the same estuary in Delaware Inland Bays, USA, at various 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

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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

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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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