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Author:
Mackey, KRM
Morris, JJ
Morel, FMM
Kranz, SA

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Abstract:
© 2015 by The Oceanography Society. All rights reserved. All phytoplankton and higher plants perform photosynthesis, where carbon dioxide is incorporated into biomass during cell growth. Ocean acidification (OA) has the potential to affect photosynthetic kinetics due to increasing seawater pCO<inf>2</inf> levels and lower pH. The effects of increased CO<inf>2</inf> are difficult to predict because some species utilize carbon concentrating mechanisms that buffer their sensitivity to ambient CO<inf>2</inf> levels and require variable energy investments. Here, we discuss the current state of knowledge about the effects of increased CO<inf>2</inf> on photosynthesis across marine photosynthetic taxa from cyanobacteria and single-celled

eukaryotes to marine macrophytes. The analysis shows that photosynthetic responses to OA are relatively small for most investigated species and highly variable throughout taxa. This could suggest that the photosynthetic benefits of high CO<inf>2</inf> are minor relative to the cell's overall energy and material balances, or that the benefit to photosynthesis is counteracted by other negative effects, such as possible respiratory costs from low pH. We conclude with recommendations for future research directions, such as probing how other physiological processes respond to OA, the effects of multiple stressors, and the potential evolutionary outcomes of longterm growth under ocean acidification.

Supporting material:

Response of Photosynthesis to Ocean Acidification

By Katherine R.M. Mackey, J. Jeffrey Morris, François M.M. Morel, and Sven A. Kranz

ABSTRACT. All phytoplankton and higher plants perform photosynthesis, where carbon dioxide is incorporated into biomass during cell growth. Ocean acidification (OA) has the potential to affect photosynthetic kinetics due to increasing seawater pCO_2 levels and lower pH. The effects of increased CO_2 are difficult to predict because some species utilize carbon concentrating mechanisms that buffer their sensitivity to ambient CO_2 levels and require variable energy investments. Here, we discuss the current state of knowledge about the effects of increased CO_2 on photosynthesis across marine photosynthetic taxa from cyanobacteria and single-celled eukaryotes to marine macrophytes. The analysis shows that photosynthetic responses to OA are relatively small for most investigated species and highly variable throughout taxa. This could suggest that the photosynthetic benefits of high CO_2 are minor relative to the cell's overall energy and material balances, or that the benefit to photosynthesis is counteracted by other negative effects, such as possible respiratory costs from low pH. We conclude with recommendations for future research directions, such as probing how other physiological processes respond to OA, the effects of multiple stressors, and the potential evolutionary outcomes of long-term growth under ocean acidification.

INTRODUCTION

Ocean acidification (OA), the process by which anthropogenic CO_2 dissolves in the ocean and forms carbonic acid, has already caused a nearly 30% (0.1 pH unit) increase in seawater [H^+], and will continue to lower pH by an additional 0.2–0.3 pH units by the end of the century (IPCC, 2014). This rapid acidification is expected to impact the health and physiology of marine organisms. To date, much of the attention on ocean acidification has focused on understanding how organisms with calcium carbonate shells will fare in the future as dissolution of their shells is increasingly favored at low pH.

In addition to lowering pH, the excess CO_2 itself also has the potential to affect organisms. CO_2 is the substrate for photosynthesis, and like other substrates necessary for growth, CO_2 can limit photosynthetic rates if it is scarce or saturate rates if it is abundant. In the ocean, photosynthetic rates affect the growth rates of populations, and hence influence competition, geochemical cycles, and the geographical distributions of species. Yet, important differences set CO_2 apart from other growth-limiting substrates and complicate our understanding of how OA will affect photosynthesis in the future.

The main complicating factor is that many phytoplankton employ a carbon concentrating mechanism (CCM; see details in Box 1). For other growth-limiting substrates like nitrogen, Monod kinetics are observed (Morel, 1987); at low concentrations of substrate, the growth rate is limited, but increases linearly as more substrate is supplied. This relationship holds until a saturating level is reached, and further additions of substrate will not increase growth rate. Although CO_2 is a substrate for photosynthesis, the CCM adds an extra regulatory step that shields photosynthetic machinery from directly sensing ambient changes in CO_2. Accordingly, photosynthetic rates may not respond directly to ambient changes in CO_2. In the presence of a CCM, the cell may be carbon saturated even when ambient CO_2 levels are low. It is therefore difficult to predict whether a cell's photosynthetic rate will increase (due to higher substrate availability and/or less energy expenditure needed to operate the CCM), decrease (due to, for example, adverse effects of lower pH), or remain the same.

Predicting the responses of marine photoautotrophs to increased CO_2 is confounded by their unique evolutionary histories and environmental contexts. As a group, phytoplankton share certain traits and perform similar biogeochemical functions, but are phylogenetically highly diverse. The first phytoplankton arose over 2.8 billion years ago and have diversified in form and function to span the bacterial and eukaryotic domains of the phylogenetic tree of life. This genetic variability exists against a backdrop of considerable environmental complexity. The pH and pCO_2 characteristics of the ocean vary widely between sites, depending on physical and biological factors. Sites with naturally higher CO_2 and low pH include seeps and upwelling regions, whereas CO_2 can be depleted and pH levels more alkaline following intense algal blooms.

In this review, we discuss the current state of knowledge on how anthropogenic CO_2 affects marine photosynthetic organisms, ranging from single-celled prokaryotes to multicellular macrophytes. Mechanistic details about the CCM in each group are discussed, and results from laboratory experiments and field campaigns are summarized. These results reveal considerable diversity in how phytoplankton species and functional groups respond to enhanced CO_2. We then discuss these findings in light of their implications for the ecology, evolution, and distribution of marine photoautotrophs. Below, we consider the different functional groups of marine phototrophs in turn, and then present some possibilities for how OA may affect these organisms in their natural communities.

CYANOBACTERIA

Common Traits of the Cyanobacterial CCM

Marine cyanobacteria are diverse in form and function, with representatives of two very different types of organisms—nitrogen-fixers and ultra-small picocyanobacteria—playing critical roles throughout the world ocean. Despite the great differences in growth characteristics, cell size, and other traits between these groups, their carbon fixation mechanisms share a number of commonalities. We provide an overview of these shared traits in this section, with traits specific to each group covered in subsequent sections focusing on each group separately.

Like all aquatic phototrophs, marine cyanobacteria express proteins that actively transport dissolved inorganic carbon (DIC) into the cytoplasm (Figure 1). Two CO_2 and three HCO_3^- uptake transporters have been identified (Price et al., 2008, and references therein), although all five transporters are not necessarily present in all species and strains. In particular, oceanic strains tend to have fewer transporters compared to estuarine strains, possibly due to the more stable DIC concentrations in the open ocean compared to freshwater and estuarine environments (Price et al., 2008). Energy sources for DIC transport can include ATP, NADPH, or reduced ferredoxin, or alternatively, transport can be coupled to an electrochemical Na^+ gradient (Badger and Price, 2003).

Cyanobacteria (as well as many chemoautotrophic bacteria) also possess inclusion bodies called carboxysomes (Figure 1). These structures have proteinaceous shells that are similar in shape and size to a bacteriophage capsid (i.e., an icosahedron ~100–200 nm in diameter) and contain almost all of the cell's rubisco (an enzyme involved in the first step of carbon fixation) and carbonic anhydrase (CA; Price et al., 1992). Cyanobacteria concentrate HCO_3^- inside the carboxysome, where it is then converted to CO_2 via CA. CO_2 that leaks

from the carboxysome to the cytoplasm can be recovered and recycled back to HCO_3^- via the same pathways described above, where it can be re-concentrated.

Regulation of cyanobacterial CCM processes involves transcriptional and allosteric regulation. In studies using model unicellular freshwater cyanobacteria, basal levels of C concentration occur, and certain CCM-related genes are constitutively expressed (Price et al., 2008). An additional high-affinity CCM exists that is induced under CO_2 limitation and involves synthesis of high-affinity transporters, an increase in rubisco activity, and up to a twofold increase in carboxysome content. Allosteric regulation (likely via a redox signal or a phosphorylation event) is responsible for initiating C uptake in response to light, thereby preventing futile C pumping in the dark when energy is not available to fix carbon.

Picocyanobacteria

By far the most abundant photosynthetic cyanobacteria are the tiny (<2 µm diameter) unicellular picocyanobacteria. These most abundant photosynthetic organisms on Earth contribute 40–80% of the gross primary production in tropical and subtropical seas (Liu et al., 1997). In the open ocean, the related genera *Prochlorococcus* and *Synechococcus* trade dominance, with *Prochlorococcus* dominating in permanently stratified oligotrophic waters between 40°N and 40°S latitude, and *Synechococcus* dominating in seasonally mixed and coastal waters. Low-light-adapted ecotypes of *Prochlorococcus* are also capable of growth at much lower light levels than *Synechococcus* and are among the only phototrophs commonly found below 100 m depth.

Marine picocyanobacteria tend to have smaller genomes than their freshwater counterparts, and this is reflected in a smaller number of genes related to the CCM. The genomes of all *Prochlorococcus* strains examined to date are particularly remarkable in that they contain no identifiable homologs to genes coding for enzymes that facilitate inorganic C import (Price et al., 2008; Scanlan et al., 2009). *Prochlorococcus* is strikingly deficient in genes required to sense changes in the external environment (e.g., histidine kinases; Scanlan et al., 2009) and has lost many genes as it has diverged from its common ancestor with *Synechococcus* (Scanlan, 2009). It is thus possible that currently extant strains of *Prochlorococcus* will be at a disadvantage relative to *Synechococcus* in a future high-CO_2 ocean (see below).

Several studies have investigated the response of picocyanobacteria to OA. The first laboratory study

Box 1. Photosynthesis and Carbon Concentrating Mechanisms

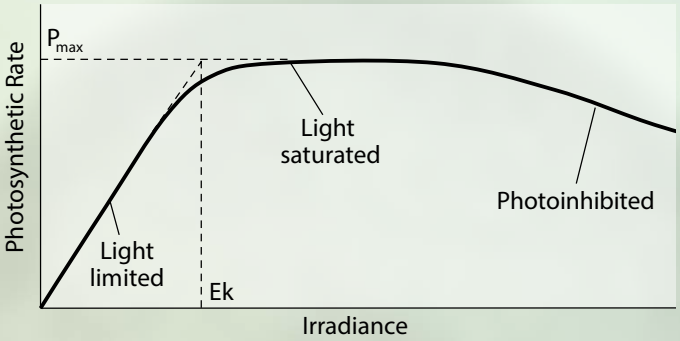
Oxygenic photosynthesis is a complex process that is remarkably conserved among plants, algae, and cyanobacteria. Photosynthetic reactions can be categorized into light-dependent and light-independent sections (often referred to as the light and dark reactions). The light-dependent reactions of photosynthesis generate energy needed to fuel the light-independent reaction as well as the carbon concentrating mechanism (CCM).

Photosynthesis–irradiance (PE) curves are a common way to describe photosynthetic characteristics of cultures and field populations in response to light intensity (see figure). At low irradiances when light limits photosynthesis, photosynthetic rates increase linearly with irradiance until the maximal photosynthetic rate (P_{max}) is reached. The slope of the light-limited portion of the curve is called alpha (α); high α values indicate that photosynthesis saturates rapidly as irradiance increases. The optimal photosynthetic rate occurs at the saturation irradiance (E_k), which is equal to P_{max}/α. Higher values of E_k indicate photosynthesis saturates at higher irradiances. Under high light, photoinhibition may lead to a decline in photosynthetic rates.

The light-independent reactions, also known as the Calvin Cycle, fix CO_2 into simple sugars using energy and electrons generated during the light-dependent reactions. The first and rate-limiting step in the Calvin Cycle is the reaction of CO_2 with ribulose bisphosphate (RuBP). The enzyme that catalyzes this reaction, ribulose bisphosphate carboxylase oxygenase (rubisco), can comprise more than 40% of the soluble protein in photosynthetic biomass (Falkowski and Raven, 2007), although lower concentrations of 3–5% have been observed in phytoplankton (Losh et al., 2013). By some estimates, rubisco is the most abundant protein on Earth.

In addition to the fixation of CO_2, rubisco also catalyzes photorespiration, the reaction of O_2 with RuBP. Rubisco has evolved in response to atmospheric CO_2 concentrations (Young et al., 2012), and at modern atmospheric levels of CO_2 and O_2, the reaction with CO_2 is favored approximately 4:1 relative to the reaction with O_2 (although this varies depending on the form of rubisco.) However, inside a photosynthetic cell, the light-dependent reactions flood the cell with O_2, while at the same time CO_2 levels are constantly drawn down via C fixation. Aquatic photoautotrophs face an additional problem: at seawater pH, almost all dissolved CO_2 is protonated to form bicarbonate anion (HCO_3^-), which cannot serve as a substrate for rubisco.

To minimize photorespiration, many plants, algae, and photosynthetic bacteria have evolved strategies to concentrate CO_2 in the vicinity of the rubisco enzyme. The CCMs of marine phototrophs are diverse but share several features in common. First, they take advantage of spatial compartmentalization to maintain high concentrations of CO_2 near rubisco. Second, they use ATP-fueled active transport to pump inorganic carbon into these rubisco-containing compartments. Finally, they almost always employ carbonic anhydrase, an enzyme that accelerates the interconversion of HCO_3^- and aqueous CO_2.



involved *Synechococcus* strain WH7803 and *Prochlorococcus* strain MED4 cultured under modern (380 ppm) or year-2100 CO_2 levels (750 ppm) (Fu et al., 2007). Photosynthesis in *Prochlorococcus* was unresponsive to pCO_2, whereas *Synechococcus* was more sensitive (Figure 2A–C). Elevated CO_2 caused higher phycobilin and chlorophyll-*a* content in *Synechococcus*, leading to a higher light harvesting efficiency (α) and a lower light saturation constant (E_k). High CO_2 alone did not increase maximal photosynthetic rates in this strain; however, when high CO_2 was combined with a warmer temperature (4°C above the control), maximal photosynthetic rates increased twofold relative to elevated temperature alone. The reason for this temperature dependence is not certain, but may be that light-saturated carbon fixation rates are enzymatically controlled and therefore have temperature-dependent kinetics. This is consistent with the observation that photosynthetic efficiency and photosynthetic protein abundance in *Synechococcus* is highly sensitive to temperature (Mackey et al., 2013). Fu et al. (2007) hypothesized that faster C fixation rates at high temperature could drive cells toward C limitation, making the high-CO_2 treatment beneficial under high temperature, but not under low temperature when CO_2 would already be saturating. The fourfold increase in the maximum photosynthetic rate yielded only a twofold increase in growth rate, possibly indicating that release of photosynthetic exudates was used to balance electron flow due to high photosynthetic rates. Interestingly, another study with *Synechococcus* WH7803 found the opposite result, with growth rate declining at low pH (Traving et al., 2013), although the reason for these different findings is not clear.

Field studies of picocyanobacteria suggest that natural populations may not respond strongly to changes in pH or pCO_2. Studies in the Sargasso Sea show that picocyanobacterial physiology acclimates rapidly to CO_2 enrichment, and

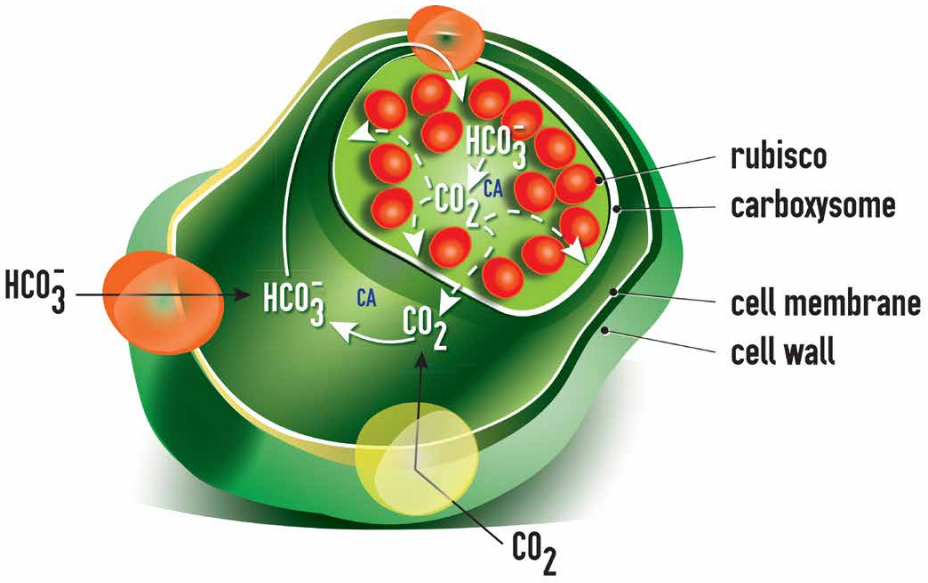


FIGURE 1. The cyanobacterial carbon concentrating mechanism (CCM). Carbon acquisition occurs via active uptake of HCO_3^- and CO_2 via transport proteins on the cell surface (an exception is *Prochlorococcus*, which appears to lack CO_2 transporters). In the cytoplasm, inorganic C is maintained as HCO_3^- via the thylakoid NDH complex, which acts as a unidirectional carbonic anhydrase (CA). This step is thought to prevent diffusive loss of C due to the lower permeability of the cell membrane for HCO_3^- than for CO_2. The HCO_3^- enters a proteinaceous microcompartment, the carboxysome, via diffusion or possibly transport. The carboxysome contains most of the cell's rubisco. Inside the carboxysome, HCO_3^- is converted into CO_2 via CA at a rate sufficient to saturate rubisco and minimize photorespiration. The CO_2 is fixed by rubisco into organic molecules as it passively diffuses through the carboxysome. Any CO_2 lost via leakage can be recovered in the cytoplasm and re-enter the concentration pathway. *Modified from Price et al. (2008)*

although photosynthetic rates were elevated under high CO_2 at the beginning of the incubations, these changes were not retained at the end of the experiment one to three days later (Lomas et al., 2012). Picocyanobacterial cell size and pigment content were also not sensitive to CO_2 enrichment, in contrast to culture studies (Fu et al., 2007). Nevertheless, it is striking that in the field study of Lomas et al. (2012), the ratio of *Synechococcus* to *Prochlorococcus* concentrations increased substantially under elevated CO_2, suggesting that *Synechococcus* may have a competitive advantage against its close relative that is not easily explained by changes in photosynthesis.

The insensitivity of these picocyanobacterial populations to elevated CO_2 could reflect their evolution under relatively stable oceanographic conditions, which has led to genome streamlining and loss of many sensory, transport, and regulatory systems present in other algae (Scanlan et al., 2009). Consistent with this explanation is the observation that certain carboxysome proteins associated with the cyanobacterial CCM are also not sensitive to CO_2 concentrations between 100 and 750 ppm (Gonzalez et al., 2005). The response of picocyanobacteria to OA may therefore be due to indirect factors like nutrient availability (Lomas et al., 2012), competition with other taxa (Paulino et al., 2008), or changes in viral activity (Traving et al., 2013), all of which may be more sensitive to pH and/or CO_2. Impacts of elevated CO_2 on grazing, which is a major cause of picocyanobacterial mortality, also need to be elucidated.

Picocyanobacteria share their ecological niche with a class of eukaryotic flagellates collectively called picoeukaryotes. Compared to the picocyanobacteria, much less is known about the ecophysiologies of these organisms, although culture-independent studies are beginning to shed light on their diversity (Kirkham et al., 2013). At least one genus of picoeukaryotes (*Ostreococcus*) appears to be strongly enhanced by elevated CO_2, with growth rates almost

doubling at 1,000 ppm (Schaum et al., 2013). However, among the 13 ecotypes tested by Schaum and colleagues, variability in response was similar to intergenera variation. The response of *Ostreococcus* ecotypes to enhanced CO_2 is more related to sampling location than to genetic similarity.

Diazotrophic Cyanobacteria

In marine ecosystems, phytoplankton productivity is often limited by the availability of fixed nitrogen. The fixation of atmospheric dinitrogen (N_2) by diazotrophic cyanobacteria thus plays a crucial role for primary productivity, especially in the oligotrophic regions of the ocean. In addition to the energy needed for the acquisition of DIC (see above), these specialized cyanobacteria invest a considerable amount of their photosynthetic resources in N_2 fixation:

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2$$

Similar to rubisco, the nitrogenase enzyme that catalyzes the conversion of N_2 into NH_3 is highly sensitive to O_2 (Falkowski and Raven, 2007), and additional costs are incurred to protect this enzyme from photosynthetically produced O_2.

Single-celled diazotroph species generally separate photosynthesis and N_2 fixation in time, fixing N_2 only during the night, whereas photosynthesis is carried out during the day. This day-night cycle requires concerted regulation of nitrogenase synthesis and degradation as well as respiration and photosynthesis, which is driven largely by a circadian rhythm (Sherman et al., 1998; Mohr et al., 2010). In most filamentous species, photosynthesis and N_2 fixation are separated in space, with only certain cells (heterocysts) containing nitrogenase. Heterocysts are fully differentiated cells that lack the O_2-evolving photosystem II, and they are surrounded by thick cell walls that act as O_2 diffusion barriers. Nitrogen fixed in these cells is transported along the filament as amino acids, while heterocysts are supplied with carbohydrates from the vegetative cells (reviewed in Böhme, 1998). In *Trichodesmium*, a species known for forming massive blooms in tropical and subtropical areas (Capone et al., 2005), temporal and spatial separation are combined. *Trichodesmium* employs a tightly regulated diurnal cycle with down-regulation of photosynthesis during the midday N_2 fixation peak, oxygen scavenging via the Mehler reaction, and localization of nitrogenase within specialized cells called diazocytes (Berman-Frank et al., 2001).

The CCM of diazotrophs is similar to other non-diazotrophic cyanobacteria, except that N_2 fixation competes with C acquisition for energy. For example, in *Trichodemium*, photosynthesis and CCM activity are both down-regulated when N_2 fixation rates are high (Berman-Frank

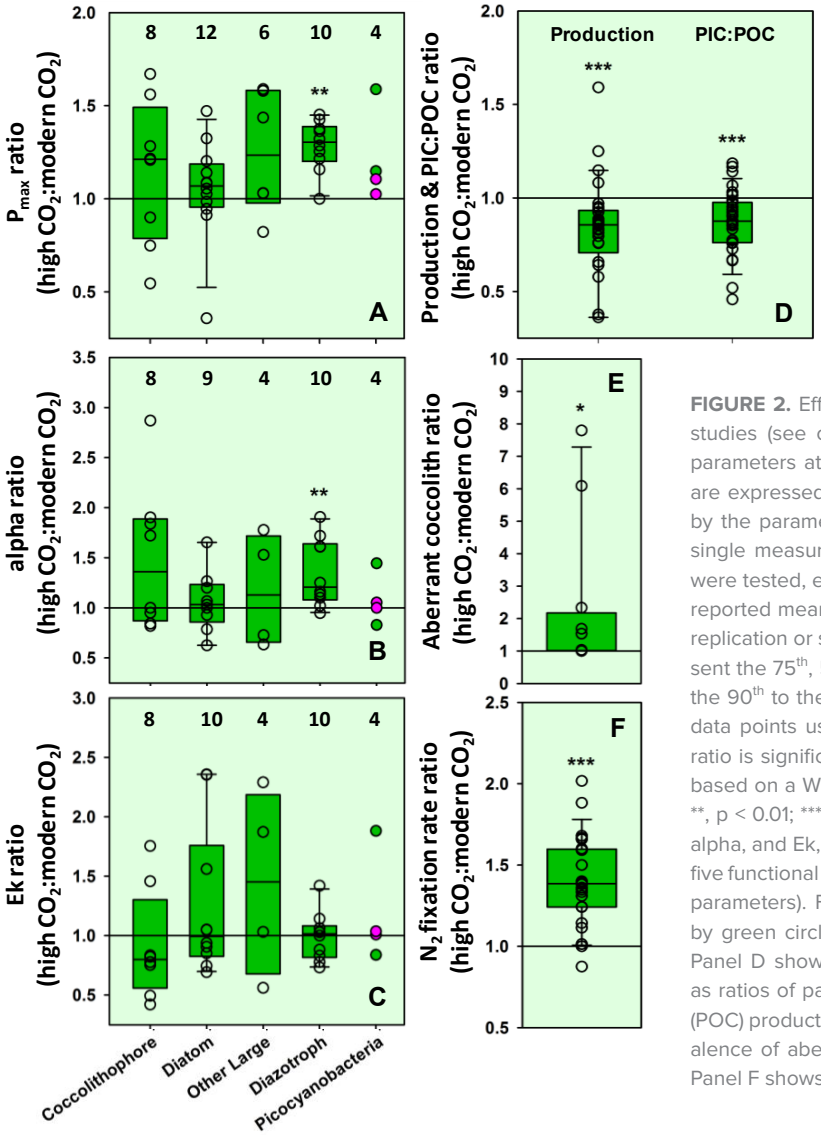


FIGURE 2. Effects of elevated CO_2 on phytoplankton. Data collected from studies (see online Supplemental Table S1) that compared the indicated parameters at modern (~380 ppm) versus elevated (650–1,000 ppm) CO_2 are expressed as the ratio of the parameter value under high CO_2 divided by the parameter value under modern CO_2. Each data point represents a single measurement; for studies where multiple environmental conditions were tested, each experiment is represented as a separate data point. Only reported mean values are shown; no attempt was made to capture level of replication or significance. Top, middle, and bottom lines of the boxes represent the 75th, 50th, and 25th percentiles, respectively. Whiskers extend from the 90th to the 10th percentiles. Values above each box give the number of data points used to construct the box. Asterisks indicate that the median ratio is significantly different than 1 (i.e., there is a significant effect of CO_2) based on a Wilcoxon Signed Rank test conducted in R v 2.15.2. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Panels A, B, and C show the fit parameters (P_{max}, alpha, and Ek, respectively) for photosynthesis-irradiance (PE) curves for the five functional groups considered in the text (see Box 1 for discussion of these parameters). For picocyanobacteria, values for *Prochlorococcus* are given by green circles and values for *Synechococcus* are given by pink circles. Panel D shows measurements of coccolithophore calcification, presented as ratios of particulate inorganic carbon (PIC) to particulate organic carbon (POC) production (left box) and cell quota (right box). Panel E shows the prevalence of aberrant or unusual coccolith morphology for coccolithophores. Panel F shows changes in the rate of nitrogen fixation by diazotrophs.

et al., 2001; Küpper et al., 2004; Milligan et al., 2007; Kranz et al., 2009, 2010, 2011). *Trichodesmium* takes up primarily HCO_3^-, although CO_2 uptake increases with increasing atmospheric CO_2 concentrations (Kranz et al., 2010). Less is known about modes of carbon acquisition in other diazotrophs like *Crocosphera, Nodularia, Calothrix* or the uncultured group UCYN. Nonetheless, multiple studies have investigated the effect of OA on these organisms (see Eichner et al., 2014, Table 3 and references therein).

Trichodesmium spp. are globally important filamentous diazotrophs in the surface mixed layer of the oligotrophic ocean. Because of their importance, they are among the most extensively studied diazotrophs in the field of OA research. Across studies, multiple species have been assayed, and their responses to CO_2 have been tested along with the effects of secondary variables such as light, temperature, and nutrient availability. Several studies on *Trichodesmium erythraeum* IMS101 found that growth rate, biomass production, C acquisition, and/or N_2 fixation were all stimulated under high CO_2 conditions (Barcelos e Ramos et al., 2007; Hutchins et al., 2007, 2013; Levitan et al., 2007, 2010a,b; Kranz et al., 2009, 2010; Garcia et al., 2011; Spungin et al., 2014). While other *Trichodesmium* species such as *T. erythraeum* and *T. contortum* were also shown to be C limited under modern CO_2 levels, growth of *T. thiebautii* saturated under much lower C concentrations (Hutchins et al., 2013).

While these studies showed, for the most part, a stimulatory effect to enhanced CO_2 on *Trichodesmium* growth and N_2 fixation (Figure 2A–C, F), Shi et al. (2012) found that under Fe limitation, N_2 fixation and growth were negatively affected by increased CO_2. They concluded that nitrogenase responded negatively to the change in seawater pH. Similarly, temperature, phosphorus availability, and light intensity all modulated the effect of CO_2 on *Trichodesmium*; however, productivity generally increased under elevated CO_2 regardless of these other factors (Kranz et al., 2010; Garcia et al., 2011; Levitan et al., 2010b; Spungin et al., 2014). The enhanced C and N_2 fixation by *Trichodesmium* under high CO_2 appears to be due to lower energy expenditure on the CCM rather than increased photosynthetic rates, as neither O_2 evolution nor relative electron transport rates increased under high CO_2 conditions (Levitan et al., 2007; Kranz et al., 2010).

Crocosphaera is a unicellular diazotroph that grows in the tropical Atlantic and Pacific Oceans and contributes substantial amounts of fixed N in many oligotrophic regions. Contrary to *Trichodesmium*, *Crocosphaera* fixes N_2 during the night using energy generated from photosynthesis during the day. Due to the temporal separation in N_2 and C fixation, the effect of OA on N_2 fixation by *Crocosphaera* is caused by the lower pH (Shi et al., 2012) and the potential for diel variability in cellular photosynthate availability. The CCM of *Crocosphaera* includes two HCO_3^- transporters (an inducible, high affinity Na^+-dependent transporter, SbtA, and a low affinity, high flux, Na^+-dependent transporter, BicA), as well as a CO_2 uptake system located in the thylakoid ($NdH1_4$; Price et al., 2008). The genetic diversity of high and low affinity inorganic carbon transport systems shows that *Crocosphaera* is well equipped to adjust its CCM to variable CO_2 concentrations (Gradoville, 2014). The ability to use a variety of transporters depending on the availability the substrate likely allows the cell to reallocate energy toward other metabolic processes such as N_2 fixation, leading to enhanced growth under high CO_2.

Similar to studies on *Trichodesmium*, OA effects on the single-celled diazotroph *Crocosphaera* seem to be strongly modulated by light and nutrient availability. Fu et al. (2008) showed that in *Crocosphaera* WH8501 growth, N_2 fixation, and cellular pigment content all increased with high CO_2, but only under Fe replete conditions. The α value was also lower in high-CO_2 conditions, indicating that more light was required to increase photosynthetic rates by the same amount under high-CO_2 conditions compared to low-CO_2 conditions. Later studies tested other strains in addition to WH8501 (Garcia et al., 2013a; Hutchins et al., 2013). These studies show that all strains tested (WH8501, WH0401, WH0003, and WH0402) have lower growth and N_2 fixation when acclimated to CO_2 concentrations below modern levels (<390 ppm CO_2). However, the responses under elevated CO_2 (>390 ppm CO_2) were more variable among strains, where growth and N_2 fixation were unaffected in strains WH0003 and WH0402, but increased in strains WH0401 and WH8501 (in contrast to Fu et al. [2008], where Fe limitation was required to see this effect). Additionally, the growth and N_2 fixation response of WH0401 was stronger under low light (Garcia et al., 2013a). Garcia et al. (2013b) further investigated the response of *Crocosphaera* WH0003 to a matrix of CO_2, light, and phosphorus concentration. The phosphorus use efficiency increased with increasing CO_2, which could indicate that the cells require less energy (ATP) to fuel the CCM under OA scenarios.

The uncultured unicellular diazotrophic cyanobacteria (UCYN) also contribute a significant share to marine N_2 fixation (Zehr et al., 1998; Moisander et al., 2010). While the physiologies of *Crocosphaera* (UCYN-B) and *Cyanothece* (closely related to group UCYN-C) have been investigated in laboratory experiments, most of the newly discovered species are uncultivated and thus poorly characterized. Cyanobacteria belonging to group UCYN-A lack the genes for PSII and rubisco and therefore are assumed to acquire fixed C symbiotically (Zehr et al., 2008). Their global nitrogenase gene abundance exceeds that of *Trichodesmium* (Luo et al., 2012). The only study on the response of these organisms to enhanced CO_2 found no stimulation of N_2 fixation (Law et al., 2012). This study also posited that due to substantial physiological differences from other diazotrophs (e.g., lack of PSII and rubisco), UNCY-A cyanobacteria may gain no benefit from elevated CO_2. Yet, not much is known

about combined effects of CO_2 and light, Fe, or P, or how a change of pH might affect this group.

The single-cell diazotroph *Calothrix* lives in symbiosis with the diatom *Chaetoceros* as well as the diazotroph *Cyanothece*. *Calothrix* showed no significant change in growth or primary production, but N_2 fixation was strongly stimulated under elevated CO_2 (Eichner et al., 2014). In contrast, *Cyanothece* increased production rates but not N_2 fixation under enhanced CO_2.

The filamentous heterocyst-containing diazotrophic species *Nodularia spp*. is a common bloom-forming diazotroph in the Baltic Sea. Studies on how enhanced CO_2 will affect this species have yielded mixed results for *N. spumigena*, including reduced growth and enhanced N_2 fixation (Eichner et al., 2014), reduced growth and reduced N_2 fixation (Czerney et al., 2009), and increased growth and C and N_2 fixation (Wannicke et al., 2012).

To date, almost all studies suggest that N_2 fixation will increase in response to enhanced CO_2 (Figure 2F). Yet, the observed responses of diazotrophic cyanobacteria to elevated CO_2 show species and strain-specific responses that are further modulated by light, Fe, P, and temperature. Therefore, particular emphasis should be placed on understanding the effects of multiple parameters on diverse types of diazotrophs.

EUKARYOTIC MICROALGAE

Eukaryotic microalgae such as diatoms, dinoflagellates, and coccolithophores have developed different C acquisition mechanisms than the cyanobacteria. Three major constituents comprise the CCM of eukaryotic algae: (1) inorganic C transporters located in the plasma and chloroplast membranes, (2) a suite of internal and external carbonic anhydrases, and (3) in many species, a proteinaceous microcompartment within the chloroplast where rubisco is located, the so-called pyrenoid. Due to the subcompartmentalization in eukaryotic cells, inorganic carbon has to pass three to five membranes before reaching rubisco, and therefore active transport of HCO_3^- is even more critical for these organisms than it is for cyanobacteria. However, only a fraction of this carbon is fixed photosynthetically, and the rest is lost through diffusion back to the cytoplasm and eventually to the external seawater, if not recovered (Price and Badger, 1989; Tchernov et al., 2003; Giordano et al., 2005; Hopkinson et al., 2011). Both the passive influx of CO_2 from the external medium and the recovery of leaked CO_2 are achieved by CA activity combined with maintenance of low HCO_3^- concentrations in the cytoplasm. (For more comprehensive reviews on the CCM in eukaryotes, see Raven, 1997; Beardall and Giordano, 2002; Giordano et al., 2005; Hopkinson et al., 2011; Raven et al., 2014). In the following sections, we briefly describe the CCMs of different groups of eukaryotic phytoplankton and their responses to ocean acidification.

Diatoms

Diatoms are responsible for 40% of oceanic primary production (Nelson et al., 1995). Because of their ecological importance and role in the C cycle, diatoms have been intensively studied with respect to their modes of C acquisition and response to changes in seawater CO_2 (Giordano et al., 2005; Rost et al., 2008, and references therein). Due to the diverse and often extreme habitats they inhabit, it is not surprising that diatoms have evolved multiple ways to acquire inorganic C for growth. Several reviews focus on the function and diversity of diatom CCMs, including Roberts et al., 2007b; Matsuda et al., 2011; Matsuda and Kroth, 2014.

Compared to other photoautotrophs, the form of rubisco found in diatoms has a relatively high specificity for CO_2 vs. O_2 (Badger et al., 1998; Tortell, 2000); nevertheless, the low equilibrium concentration and low diffusion coefficient for CO_2 in water would limit photosynthesis without a CCM. Numerous studies that measured C fluxes across external diatom membranes show that CO_2 as well as HCO_3^- can be taken up by the cells (see below). Until recently, less was known about internal carbon concentration and the respective fluxes. A study combining C flux measurements with modeling shed light on the modes of C transport, including flux rates and internal C concentrations in the diatom *Phaeodactylum tricornutum* (Hopkinson et al., 2011; Figure 3). However, specific details of the CCMs differ considerably between diatom species and under different environmental conditions (Trimborn et al., 2013).While most of the CCMs follow so-called C3 photosynthetic pathways (i.e., the first C fixed is a C3 metabolite, e.g., 3-phosphoglycerate), the possibility of C acquisition in diatoms via a C4-like pathway has been discussed (Reinfelder et al., 2000, 2004; Granum et al., 2005; Roberts et al., 2007a,b; Kroth et al., 2008; McGinn and Morel, 2008; Trimborn et al., 2009). The C4 pathway uses an additional step, fixing HCO_3^- via the PEPC (phosphoenolpyruvate carboxylase) into a C4 sugar (oxaloacetate) that subsequently is de-carboxylated close to rubisco. This pathway is a mechanism by which cells avoid photorespiration by storing CO_2 in an intermediate molecule that cannot diffuse out of the cell, thereby enhancing the CO_2/O_2 ratio close to rubisco. While evidence for such a pathway has been shown in *Thalassiosira weissflogii* (Reinfelder et al., 2000, 2004; Morel et al., 2002), Roberts et al. (2007a) characterize it as an intermediate C3–C4 photosynthetic pathway. Inconclusive results for *Thalassiosira pseudonana* cast doubt on the existence of a C4 metabolism in this species (Granum et al., 2005; Roberts et al., 2007a; McGinn and Morel, 2008), although a recent study showed that low CO_2 induces C4 assimilation in this species (Kustka et al., 2014). The existence of C4 metabolisms in diatoms is controversial, but it would theoretically provide an evolutionary advantage during bloom conditions when CO_2 becomes limited. It has also been suggested that a C4 metabolism might help to dissipate excess light energy and keep internal pH constant (Haimovich-Dayan et al., 2013). However,

because C4 pathways require more energy per mole of C fixed than C3 pathways, it is possible that organisms using solely the C4 pathway will be less economical under high-CO_2 OA conditions and might be outcompeted due to slower growth.

Many experiments have studied the modes of carbon acquisition in diatoms in pure cultures, mixed in vitro communities, and in the field. While the laboratory studies reveal strong species-specific differences (Figure 2A–C), field responses are more complex. Due to the high number of OA studies involving diatoms, we have grouped the studies as follows: CO_2 effects on (1) laboratory model species, (2) bloom-forming vs. non-bloom-forming species, (3) potentially toxic species, (4) benthic species, and (5) mixed field populations.

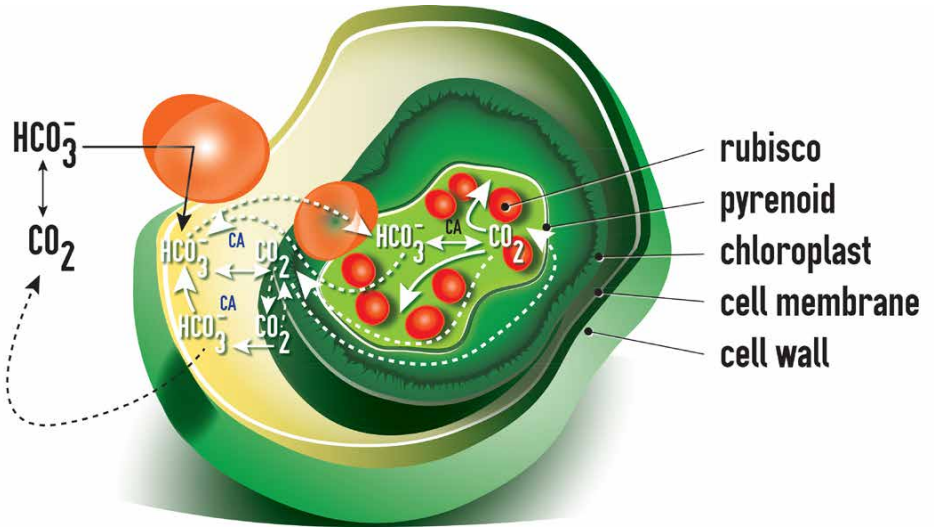


FIGURE 3. The diatom CCM. Diatom cells acquire C through the active transport of HCO_3^- and passive diffusion of CO_2. All of the cell's membranes are highly permeable to CO_2, which diffuses between the compartments. The main driver of the CCM is active transport of HCO_3^- from the cytoplasm into the chloroplast. The HCO_3^- in the chloroplast then diffuses into the pyrenoid, a proteinaceous microcompartment enclosing rubisco. Carbonic anhydrase (CA) in the pyrenoid generates CO_2 from HCO_3^-, saturating rubisco and minimizing photorespiration. CO_2 lost through diffusion at any step in the concentration process can be recovered through conversion back into HCO_3^- by CA in the cytoplasm and stroma. *Modified from Hopkinson et al. (2011)*

Laboratory Model Diatom Species

"Model species" are organisms widely studied in laboratories that are typically easy to cultivate and generally have fully sequenced and annotated genomes. *Phaeodactylum tricornutum*, although not individually relevant in the global carbon cycle, was one of the first two fully sequenced diatoms. This species has been subject of numerous carbon uptake studies, but only a few of them have investigated its response to OA.

P. tricornutum takes up CO_2 preferentially over HCO_3^- from seawater (Burkhardt et al., 2001; Cassar et al., 2006); thus, one would expect a pronounced response in photosynthetic C fixation under enhanced CO_2. However, studies have provided different findings. Under enhanced CO_2, *Phaeodactylum* shows increased photosynthetic electron transport rates, but no change or very modest increases in growth (5–13%; Wu et al., 2010; Li et al., 2014) or C fixation (Burkhardt et al., 2001). Even at very high CO_2 concentrations (5,000 ppm), photosynthesis was not stimulated (Matsuda et al., 2011). The CCM of this species is down-regulated under high CO_2, showing lower cellular affinities for inorganic carbon (Burkhardt et al., 2001; Wu et al., 2012; Li et al., 2014). This could potentially save energy to fuel other metabolic processes but may result in the loss of photoprotection (Yang and Gao, 2012; see below).

Thalassiosira pseudonana is another diatom with a fully sequenced and annotated genome. Compared to *P. tricornutum*, *T. pseudonana* takes up CO_2 and HCO_3^- in similar proportions and exhibits much lower CA activity (Trimborn et al., 2009). The cellular affinity for inorganic C is relatively constant under a range of CO_2 concentrations. Despite the low CA activity compared to other diatoms, several CAs are down-regulated under enhanced CO_2 concentrations (McGinn and Morel, 2008; Trimborn et al., 2009; Crawfurd et al., 2011). *T. pseudonana* showed an increased maximum photosynthetic rate (P_{max}) and an increased PE curve half-saturation constant (E_k) under high CO_2, suggesting that more light was required to saturate photosynthesis (Sobrino et al., 2008). Growth rate also increased by 20% under high CO_2 in this experiment, and the PE curve suggests that the experimental light conditions (~ 250 µE m^{-2} s^{-1}) were no longer saturating after CO_2 increased.

Bloom-Forming vs. Non-Bloom-Forming Diatoms

It has been proposed that the ability of certain diatom species to form intense blooms depends on their capacity to overcome C limitation at high population densities using an efficient and highly plastic CCM. Thus, bloom-forming diatoms would be expected to show major variations in their modes of C acquisition within the range of CO_2 concentration of a bloom. For example, the bloom-forming diatom *Skeletonema costatum* efficiently increased C acquisition under low CO_2 by increasing the affinity of its CO_2 and HCO_3^- transporters, increasing its HCO_3^-:CO_2 uptake ratio, and increasing CA activity (Rost et al., 2003). Other bloom-forming diatom species also down-regulated CA activity and CO_2 half saturation constants for photosynthesis under enhanced CO_2, whereas CCM parameters were not tightly regulated in non-bloom-forming species like *T. pseudonana* (Trimborn et al., 2009, 2013). It has been widely speculated that tight regulation of CCM parameters could facilitate bloom-forming behavior by saving energy that could instead

be invested in growth or other metabolic processes. However, the bulk of CCM energy cost is due to active HCO_3^- transport from cytoplasm to chloroplast to make up for passive loss of CO_2 by diffusion. CCM energy demand is therefore roughly proportional to the gradient of CO_2 from the site of fixation to the bulk seawater. Hopkinson et al. (2011) calculated that a doubling of ambient CO_2 levels would translate to an energy savings of ~20% of the energy expended on the CCM. However, growth rate depends on many factors in addition to energy allocation, and factors like photoprotection and nutrient uptake ability will likely modulate the growth responses of bloom-forming diatoms in the future.

Cell size may also be an important factor determining whether diatoms will benefit from elevated CO_2. In contrast to other phytoplankton groups, diatom volumes span more than five orders of magnitude, and a recent study showed a strong positive correlation between cell size and the dependence of growth rate on CO_2 supply (Wu et al., 2012). Because larger cell volumes are associated with smaller surface area:volume ratios, larger diatoms are less able to rely on passive diffusion of CO_2 to meet their DIC needs and must expend more energy to transport DIC into the cell. Thus, they save more on energy than smaller cells when CCMs are down-regulated at elevated CO_2. While this finding is intuitively satisfying, it is not an absolute rule: at least one large diatom, the psychrophile *Proboscis alata*, exhibited profound reduction in growth rate under elevated CO_2, reaching almost 67% when light was limiting growth (Hoogstraten et al., 2012).

Toxic Diatoms

Among the most widely distributed and environmentally destructive harmful bloom species are diatoms in the genus *Pseudo-nitzschia,* which produce the neurotoxin domoic acid (DA). DA production is regulated by nutrient and trace metal availability as well as extreme pH increase such as might be experienced during a bloom event (Pan et al., 1996; Lundholm et al., 2004; Wells et al., 2005). DA production in *P. fraudulenta* increased with increasing CO_2 concentration under silica-limiting conditions, whereas the stimulatory CO_2 effect was much weaker under silica replete conditions (Tatters et al., 2012). In contrast, less DA production occurred in *P. multiseries* and *Nitszchia navis-varingica* under OA conditions (Trimborn et al., 2008), although another study demonstrated that *P. multiseries* under phosphorus limitation increased DA production by over threefold under OA conditions (Sun et al., 2011). Thus, OA appears to generally increase the production of DA when nutrients are limiting, although the reasons for this are as yet unclear. The effects of multiple stressors, such as nutrient availability, will clearly play an important role in determining the frequency and intensity of *Pseudo-nitzschia* blooms in the future.

Benthic Diatoms

Marine benthic diatoms grow on sediments in biofilms or biological crusts (periphyton) and provide a major food source for small and large grazers (Underwood, 1984; Thompson et al., 2004). Unfortunately, not much is known about C acquisition in benthic diatoms because culture experiments are difficult to conduct without changing the complex substrate composition for these species. Field research on the effects of CO_2 on diatoms in microbial mats has recently been conducted along a CO_2 gradient at a shallow water cold vent system near Sicily, Italy (Johnson et al., 2013). This study revealed a significant increase in diatom abundance at high CO_2, whereas the associated cyanobacterial biomass remained constant. Interestingly, the composition of the diatom community changed dramatically under enhanced CO_2, with some genera being stimulated (*Toxarium, Grammatophora, Bacillaria, Navicula, Cocconeid, Amphora*), some species losing biomass (*Cyclophora, Neosyndedra, Rhabdonema, Nitzschia*), and some species remaining unaffected (*Lincomophora, Striatella*). A study conducted in biological mats at the Great Barrier Reef determined that enhanced CO_2 led to small changes in relative taxon abundances or O_2 fluxes of benthic diatoms, whereas their cellular C and N contents increased significantly (Witt et al., 2011).

Natural Assemblages

Diatoms living in the upper water column are major drivers of the biological carbon pump. While responses to OA have mainly been described based on pure cultures of pelagic species (see above), recent mixed culture and field experiments (many in high-latitude regions) have found pronounced floristic shifts in diatom-dominated assemblages under OA (Tortell et al., 2008, 2002; Hoppe et al., 2013). For example, enhanced CO_2 increased diatom abundance, while the abundance of *Phaeocystis* decreased (Tortell et al., 2002). Interestingly, despite the community shift, total biomass and primary productivity did not differ significantly between the CO_2 treatments. In a separate study, elevated CO_2 led to an increase in phytoplankton productivity, promoting the growth of larger chain-forming diatoms, suggesting that OA could enhance C export by the growth of larger, faster sinking cells (Tortell et al., 2008). Another study in the Bering Sea showed that OA conditions caused a community shift away from dominance by diatoms toward a variety of flagellate species (Hare et al., 2007).

Shifts within communities of diatoms also occur at the species level. In mixed communities, high CO_2 stimulated growth of *Chaetoceros debilis* over *Pseudo-nitzschia subcurvata*, even though high C uptake capacities were measured for both species (Trimborn et al., 2013). Both temperature and CO_2 increase were important drivers of community change in mixed cultures of six diatoms, and the competitive ability of the individual species in these cultures was not significantly improved by a 12-month period of solo adaptation to their changed environment

(Tatters et al., 2013b). Species-specific differences in CO_2-dependent regulation of individual CCM components, especially in the regulation of external carbonic anhydrase activities, may be responsible for the different growth rate effects, although the possible phenotypes contributing to these competitive differences extend far beyond the CCM.

A mesocosm experiment conducted in Bergen, Norway, reported that net community carbon consumption and net photosynthesis increased under increased CO_2 for a mixed community of diatoms and coccolithophores (Riebesell et al., 2004). In a later mesocosm study with a different initial community composition, diatoms were outcompeted at high CO_2 while picoplankton were strongly stimulated (Riebesell et al., 2013). A third mesocosm experiment with two different diatom species (*Skeletonema costatum* and *Nitzschia* spp.) under nutrient replete conditions showed that only *S. costatum* increased growth rate under enhanced CO_2 (Kim et al., 2006). Therefore, the effect of enhanced CO_2 on natural diatom populations depends on the species of diatoms, as well as on the overall composition of the phytoplankton community.

Additional factors like temperature (Feng et al., 2008; Tatters et al., 2013b) and Fe availability (Hoppe et al., 2013) also modulate the effects of enhanced CO_2. Hoppe et al. (2013) showed that productivity was not stimulated under Fe limitation, while Fe-replete cultures increased primary production and showed a floristic shift from *Pseudo-nitzschia* sp. to *Fragilariopsis* sp.

Coccolithophores

Coccolithophores are haptophyte algae that extrude calcium carbonate plates, called coccoliths, during the diploid stage of their haplodiplontic life cycle. The coccolithophore *Emiliania huxleyi* forms conspicuous seasonal blooms in the North Atlantic. How coccolithophore blooms terminate affects the fate of the inorganic and organic carbon contained by their cells, with important biogeochemical consequences. Large blooms often crash due to viral infection (Jacquet et al., 2002; Wilson et al., 2002), which tends to release much of the organic C back into the surface mixed layer where it may be remineralized such that only the inorganic $CaCO_3$ is exported. Alternatively, grazers can consume the cells and package them into fecal pellets where the coccoliths act as ballast to increase sinking rates, resulting in enhanced export of both organic and inorganic C to the deep ocean.

Whether coccolithophores serve as a source or sink of CO_2 depends on the amount of calcification versus photosynthesis that occurs and on the relative export of organic and inorganic carbon. The process of calcification converts approximately two HCO_3^- anions into one molecule of $CaCO_3$ and one molecule of CO_2, and thereby acts as a net source of CO_2 in the surface ocean. In contrast, photosynthesis, which fixes CO_2 into organic carbon, acts as a net sink of atmospheric CO_2. The "rain ratio" of $CaCO_3$ to organic C in sinking material therefore determines whether coccolithophores are net exporters of CO_2 to the deep ocean.

Coccolithophores appear to rely primarily on aqueous CO_2 for photosynthesis, while preferring to access HCO_3^- for calcification (Buitenhuis et al., 1999; Bach et al., 2013). Consequently, rising CO_2 tends to promote photosynthesis over calcification and reduce the ratio of inorganic to organic carbon in these organisms (Figure 2D; Riebesell et al., 2000; Zondervan et al., 2002; Langer et al., 2006, 2009; Feng et al., 2008; Iglesias-Rodriguez et al., 2008; Shi et al., 2009; De Bodt et al., 2010; Müller et al., 2010; Fiorini et al., 2011a,b; Lefebvre et al., 2012; Rokitta and Rost, 2012). The effect of elevated CO_2 on calcification and photosynthesis could affect C export by altering the rain ratio. Although certain species and strains within the same species calcify more under enhanced CO_2 (Iglesias-Rodriguez et al., 2008; Shi et al., 2009), the majority of coccolithophores reduce their level of calcification when growing at elevated CO_2 (Figure 2D,E; Langer et al., 2009; De Bodt et al., 2010; Fiorini et al., 2011a,b; Langer, 2011; Richier et al., 2011; Lefebvre et al., 2012; Rokitta and Rost, 2012; Beaufort et al., 2011), and the fossil record suggests that eras with reduced CO_2 levels (e.g., glacial maxima) have favored more heavily calcified cells (Beaufort et al., 2011). Lower calcification rates tend to decrease the rain ratio and lead to greater C export, which in turn could indicate that these organisms will continue to be a net sink for CO_2 in the near future (Zondervan et al., 2001). Moreover, one experimental evolution study suggests that coccolithophore mutants that calcify even less will arise and spread in a future high CO_2 ocean (Lohbeck et al., 2012).

In addition to lower calcification, numerous experimental studies show that coccolithophores growing at elevated CO_2 have a tendency to produce degraded or aberrant coccoliths (Figure 2E; Riebesell et al., 2000; Langer et al., 2006; Feng et al., 2008; Iglesias-Rodriguez et al., 2008; De Bodt et al., 2010; Müller et al., 2010, 2012; Ramos et al., 2010; Langer, 2011). Problems related to calcification may cause coccolithophores to experience elevated CO_2 as a stress in some cases. One study showed that coccolithophores produced more of the compound dimethylsulfoniopropionate (DMSP, a precursor of the cloud-forming, climate-influencing gas dimethyl sulfide, DMS) when grown under predicted year 2100 CO_2 levels, and the CO_2 effect on DMSP production was more pronounced when temperatures were also elevated (Spielmeyer and Pohnert, 2012). This increase in DMSP production was accompanied by increased growth rates in the same cultures.

The genetic machinery for DIC acquisition is different in coccolithophores than in other phytoplankton. Most algae express a number of different DIC transporters, including inducible high-affinity transporters. In contrast, the core genome of the most abundant coccolithophore species, *E. huxleyi*, contains only low-affinity DIC transporters (Read

et al., 2013). While *E. huxleyi* clearly retains the capacity for a robust CCM, it is only induced at CO_2 levels lower than present-day (Bach et al., 2013). This observation is consistent with *E. huxleyi* never being limited by DIC availability in the modern ocean. However, other coccolithophores are C limited under modern conditions (see below).

The ability of organisms to calcify can be affected by the pH of their environments. Under current conditions, most ocean waters are above the saturation point for calcite (the form of $CaCO_3$ precipitated by coccolithophores), which means that it is energetically favorable for organisms to precipitate this mineral. However, as CO_2 increases and ocean pH decreases, high-latitude regions (e.g., the Southern Ocean) are predicted to become undersaturated in calcite by the year 2100 (Orr et al., 2005), which could result in the dissolution of coccoliths, potentially impacting the competitiveness of this group.

Many studies show that photosynthetic rates of coccolithophores are not saturated at ambient CO_2 (Figure 2A; Zondervan et al., 2001, 2002; Langer et al., 2006, 2009; Iglesias-Rodriguez et al., 2008; Shi et al., 2009; De Bodt et al., 2010; Ramos et al., 2010; Fiorini et al., 2011b; Langer, 2011; Richier et al., 2011; Lefebvre et al., 2012; Müller et al., 2012; Rokitta and Rost, 2012), and some studies also show increases in calcification and net growth rate at elevated CO_2, at least in certain environmental conditions (Feng et al., 2008; Iglesias-Rodriguez et al., 2008; Zondervan et al., 2002; Shi et al., 2009; De Bodt et al., 2010; Fiorini et al., 2011a,b; Lefebvre et al., 2012; Spielmeyer and Pohnert, 2012). Light harvesting efficiency (the α-parameter of the PE curve) tends to increase with elevated CO_2, whereas saturating irradiance (E_k) decreases, suggesting that elevated CO_2 improves photosynthetic rates for these organisms at low irradiances (Feng et al., 2008; Rokitta and Rost, 2008, 2012). However, the effect of elevated CO_2 on maximum photosynthetic rates (P_{max}) is inconsistent, with different strains of *E. huxleyi* exhibiting opposite responses (Feng et al., 2008; Rokitta and Rost, 2012). Together, these responses suggest that future elevated CO_2 conditions could increase light harvesting efficiency, and allow maximal photosynthetic rates to be reached at lower irradiances.

In some cases, whether CO_2 enhances coccolithophore growth appears to depend on the energy requirements for growth. For instance, CO_2 enhanced growth rate in *E. huxleyi* cultures growing on nitrate (which requires energy to be converted to ammonium prior to incorporation) but not on a mixture of nitrate and ammonium (Lefebvre et al., 2012). Another study showed that high CO_2 enhanced growth rates in light-limited cultures, but actually depressed growth rates when light was saturating (although neither of these differences was statistically significant; Rokitta and Rost, 2012). However, in another study, CO_2 enhanced growth at both saturating and subsaturating light intensities, and the effect was more pronounced in high light (Feng et al., 2008). Thus, it is clear that the effects of CO_2 on coccolithophores are dependent on other environmental factors, but more research is necessary to understand these synergies and how they differ among strains and species.

Because coccolithophores are potentially affected by both pH effects on calcification and physiological effects of elevated CO_2, attempts have been made to manipulate these parameters separately in culture experiments (Bach et al., 2011). High CO_2 reduced *E. huxleyi* growth rates when pH was allowed to decrease naturally, but this growth rate effect was not seen when pH was held constant using the organic buffer HEPES. Decreases in calcification at high CO_2 and the formation of aberrant coccoliths were also apparently caused by pH, and did not occur when pH was held constant. This suggests that, at least for *E. huxleyi*, the effects of rising CO_2 are primarily dependent upon pH and associated shifts in carbonate equilibrium. Similar studies performed on other functional groups may help distinguish between pH and CO_2 effects; however, it has been reported that organic buffers can produce undesirable side effects in phytoplankton culture, including production of hydrogen peroxide (Morris and Zinser, 2013), which may adversely affect some strains.

The ecological consequences of elevated pCO_2 on the competitiveness of coccolithophores in mixed phytoplankton communities remains unclear. When natural populations in mesocosms were enriched with CO_2 to predicted year 2100 levels (~750 ppm), the abundance of coccolithophores increased relative to that of diatoms (Riebesell, 2004). While this observation suggests that coccolithophores have a competitive advantage over their closest eukaryotic competitors under future conditions, the long-term reduction in calcification predicted by experimental evolution (Lohbeck et al., 2012) raises questions about pleiotropic effects. Future studies should focus on synergies between elevated CO_2 and other environmental factors and how they influence competitive interactions between coccolithophores and other phytoplankton.

Dinoflagellates

Dinoflagellates are among the most primitive eukaryotes, first emerging in the fossil record during the Triassic Period. They are flagellated motile protists that reproduce both vegetatively and sexually and also form resting spores. Most dinoflagellates are photosynthetic, and many are able to shift toward mixotrophic growth, during which they obtain nutrients by ingesting other cells. Endosymbiont dinoflagellates, such as those living within corals, are photosynthetic and can exist in a free-living state or within the coral host, where they receive nutrients and protection from predation while providing the coral animal with photosynthate (sugar) for growth. Obligate heterotrophic dinoflagellate species also exist, including both predatory and parasitic species. Bloom-forming dinoflagellates contribute to harmful algal blooms

known as "red tides," and they can be responsible for fish kills and toxin production in coastal areas.

Dinoflagellate C Fixation

Several CCM features set dinoflagellates apart from other phytoplankton. First, many dinoflagellates possess a Form II rubisco with low CO_2/O_2 discrimination ability (Whitney et al., 1995). This form of rubisco is found in anaerobic nonsulfur purple bacteria, but not in other photoautotrophs. The low affinity of Form II rubisco for CO_2 necessitates a CCM as well as enhanced enzymatic activity to mitigate photorespiration. The CCM of some dinoflagellates does not include microcompartments such as the pyrenoid found in other eukaryotes (Ratti et al., 2007), whereas the pyrenoid may exist in other species, including the zooxanthellae of corals (Leggat et al., 1999). Even in species with a CCM, CO_2 accumulation in the cell is variable (from negligible to 70-fold increase over ambient; Berman-Frank et al., 1998; Leggat et al., 1999; Nimer et al., 1999; Ratti et al., 2007). Some species (*Prorocentrum micans, P. minimum*) are able to utilize HCO_3^-, while others (*Amphidinium carterae, Heterocapsa oceanica*) lack external carbonic anhydrase enzymes and have limited capacity for direct HCO_3^- uptake (Dason et al., 2004). The low C concentrating capacity of cells and the low selectivity of the Form II rubisco both contribute to high photorespiration rates in dinoflagellates. Accordingly, Crawley et al. (2010) showed that enhanced activity of the phosphoglycolate phosphatase (PGPase) enzyme was critical to preventing PGP accumulation from photorespiration in the cell, which would otherwise inhibit C fixation.

Endosymbiotic dinoflagellates are affected by ocean acidification differently from free-living cells because they reside within a compartment in the host called the symbiosome, where the host regulates the chemical environment. For example, in *Symbiodinium,* photosynthesis relies on HCO_3^- actively taken up by the host rather than via diffusion of CO_2 through the host tissues (Marubini et al., 2008). Once inside the host cell, the HCO_3^- is converted to CO_2 and carbonate by carbonic anhydrase in a CCM that helps concentrate CO_2 around the Form II rubisco. In turn, C fixed during photosynthesis is exported to the host and can supply up to 100% of the host's energy requirements. Importantly, endosymbiotic dinoflagellates are also vulnerable to the effects of OA on their host organisms, many of which potentially suffer from the same problems with calcification described above for coccolithophores (Kleypas et al., 2006).

Free-living Dinoflagellates

Several studies have investigated the responses of free-living dinoflagellates to CO_2 enrichment, with divergent results (Figure 2A–C). Early studies on *Amphidinium carterae* and *Heterocapsa oceanica* indicated that both species have very limited capacity for HCO_3^- utilization, perhaps due to their lack of external carbonic anhydrase (Dason et al., 2004). Moreover, photosynthetic O_2 evolution was stimulated by the supply of bovine carbonic anhydrase to the medium, suggesting that the cells are CO_2 limited and that the exogenous CA reversed this limitation by providing CO_2 for uptake. Similar results were found by Ratti et al. (2007) for *Protoceratium reticulatum*, in which the CCM and synthesis of CA was repressed under very high CO_2 levels (5,000 ppm).

Bloom dynamics are an important aspect of dinoflagellate ecology because harmful algal blooms can affect ecosystem services by altering food web dynamics and producing toxins. Under high CO_2 conditions, the growth rate of the brevetoxin-producing dinoflagellate *Karenia brevis* increased significantly, although toxin production per cell was unaffected (Errera et al., 2014). Growth of the red tide forming raphidophyte *Heterosigma akashiwo* likewise increased under high CO_2, although maximal photosynthetic rates remained unaltered (Fu et al., 2008). In contrast, *Prorocentrum minimum*, a common coastal bloom former, showed increased maximal photosynthetic rates but not increased growth under high CO_2 (Fu et al., 2008). *Karlodinium veneficum* exhibited elevated growth rates at high CO_2, but only under nutrient-replete conditions; however, these organisms became dramatically more toxic when P was limiting and CO_2 was high (Fu et al., 2010). *Alexandrium catanella* dramatically increased its production of saxitoxin under elevated CO_2, irrespective of other stressors such as nutrient limitation and high temperature (Tatters et al., 2013a). Rost et al. (2006) also examined *Prorocentrum minimum*, along with *Heterocapsa triquetra* and *Ceratium lineatum*, and all were found to have internal CA and utilize use HCO_3^-. In these species, the affinity for and uptake of HCO_3^- increased with increasing pH. This could be an adaptation to bloom conditions where high pH shifts the carbonate equilibrium toward carbonate, making HCO_3^- less available. Therefore, it seems that dinoflagellates have C acquisition strategies for surviving bloom conditions and as a group may tend to benefit from enhanced CO_2.

Endosymbiotic Dinoflagellates

These organisms, for example, *Symbiodinium* sp., show strain-specific responses to OA and may have different photosynthetic strategies depending on whether they are free-living or reside within the host (Brading et al., 2011). Free-living *Symbiodinium* isolated from the giant clam *Tridacna gigas* initially relied upon CO_2 for 45–80% of its photosynthesis, but within two days of host-free culture, this decreased markedly and HCO_3^- became the preferred C source, supporting 35–95% of photosynthesis (Leggat et al., 1999). Carbonic anhydrase of the free-living cells was also shown to be light activated.

Much of the work involving *Symbiodinium in hospite* (living in a host cell) has examined the interactions between host and symbiont. The combined effects of high light, high temperature,

and high CO_2 can lead to bleaching and decreased productivity in crustose coralline algae and in *Acropora* and *Porites* corals, possibly due to alteration of photoprotective mechanisms in the algal symbionts (Anthony et al., 2008). Additionally, photosynthesis and calcification in corals are highly correlated, but elevated CO_2 or nutrient enrichment can cause decoupling of the two processes by increasing photosynthesis and decreasing calcification (Ferrier-Pages et al., 2000). However, several studies suggest that when high CO_2 and elevated nutrient or food supply occur together, the negative effect on calcification is mitigated (Langdon and Atkinson, 2005).

MACROPHYTES

Seagrasses and macroalgae, collectively referred to here as marine macrophytes, are multicellular autotrophs that populate shallow and deep coastal waters. While similar in size and structure to land plants, marine macrophyte taxonomic diversity is comparatively vast. There are approximately 60 seagrass species, all in the monocot lineage, and thousands of macroalgal species that are classified into three divisions, Rhodophyta (red algae), Chlorophyta (green algae), and Ochrophyta (brown algae). Seagrass meadows, which account for 30% of the marine net primary production stored in sediments (Duarte and Cebrian, 1996), have been on the decline for decades due to both natural causes and human activity. Macroalgae perform a range of ecological functions, including biogenic calcium carbonate formation (Wefer, 1980), deep-sea productivity (Nelson, 2009), sediment production (Chisholm, 2003), and larval recruitment (Ritson-Williams et al., 2009).

Macrophyte C Fixation

Whether terrestrial or aquatic, three pathways of carbon fixation occur in macrophytes: C3, C4, and CAM (Crassulacean acid metabolism). The first of these is C3 photosynthesis, so called because of the 3-carbon molecules of 3-phosphoglycerate in the Calvin Cycle (Box 1). The majority of marine macrophytes use the C3 photosynthetic pathway without any CCMs, although most are able to use HCO_3^- as a source of C (Koch et al., 2013). Because the photosynthetic rates of C3 macrophytes are more directly influenced by external CO_2 supply, elevated CO_2 levels are expected to benefit these species most. The second pathway is C4 photosynthesis (Figure 4), which is a C concentrating strategy that uses spatial isolation of rubisco to limit photorespiration, as described above in the diatom section. CAM is the third C fixation pathway. Aquatic CAM plants take up CO_2 at night when competition with other photosynthetic organisms is lower and O_2 evolution from photosynthesis has ceased. Because most marine macrophytes have the ability to utilize HCO_3^-, the CAM pathway is less common in the ocean where HCO_3^- is abundant than in freshwater where HCO_3^- can be scarce.

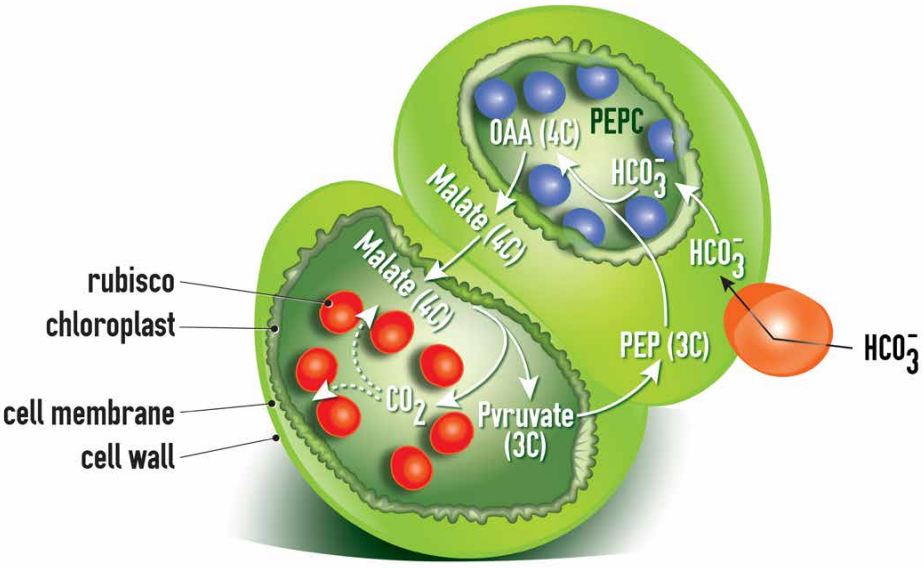


FIGURE 4. The C4 photosynthetic pathway. In C4 carbon fixation, CO_2 is incorporated with phosphoenolpyruvate (PEP) into the 4-C molecule oxaloacetate by the enzyme phosphoenolpyruvate carboxylase (PEPC), which may be located in the chloroplast or the cytoplasm. PEPC has a higher affinity for CO_2 than rubisco and does not interact with O_2, so the efficiency of C fixation is high in this step. The C is transported as malate between cells to the other chloroplast, where decarboxylation liberates CO_2 in the vicinity of rubisco, thereby saturating the enzyme and preventing photorespiration. Pyruvate is also generated in that step, and is converted back into PEP by ATP-dependent phosphorylation (not shown). Carbon fixation then proceeds via the Calvin Cycle as in the C3 pathway. Due to the additional C fixation step, the C4 pathway requires an extra 12 molecules of ATP in addition to the 18 molecules required in C3 photosynthesis.

Macrophyte Responses to OA

Koch et al. (2013) recently reviewed the responses of various marine macrophytes. In their assessment of over 100 experiments with different species, they found that >85% of macrophytes utilized the C3 pathway and that most could utilize HCO_3^-. Additionally, they found that photosynthesis and growth in most species were not C saturated under present-day CO_2 concentrations. Because C3 photosynthesis is more sensitive to the direct effects of CO_2 concentration, future increases in CO_2 will most likely increase photosynthetic rates and growth in marine macrophytes, though the extent of the effect is uncertain.

Seagrasses are particularly limited by DIC under current conditions due to slow CO_2 diffusion into their leaves and less efficient utilization of HCO_3^- relative to other macrophytes (Beer, 1994). Moreover, C limitation during photosynthesis is intensified under low light conditions for the species *Halophila ovalis* and *Cymodocea serrulata*. The difference is attributed to a greater reliance on CO_2 in deep water compared to the high light

intertidal zone, because light limitation would preclude use of HCO_3^- because of its higher energetic cost (Schwarz et al., 2000). Seagrass C allocation also changes under high CO_2, where carbohydrate synthesis increases relative to protein synthesis. This shifts the C:N ratio of the biomass produced by seagrasses, potentially altering its nutritional quality.

The potential effects of elevated CO_2 on coralline algae are complex because photosynthesis and calcification can both be affected, as in coccolithophores. Coralline algae in the Rhodophyta lineage typically form calcite crystals, whereas species in Chlorophyta typically form aragonite crystals (Koch et al., 2013), the latter being more sensitive to dissolution from acidification. Photosynthesis and calcification are highly coupled; photosynthesis takes up CO_2, causing an increase in pH and a shift in the DIC equilibrium toward CO_3^-, which favors $CaCO_3$ precipitation (De Beer and Larkum, 2001). While short-term CO_2 enrichment experiments suggest increased growth for coralline species under high CO_2, the majority (82%) of longer-term enrichment studies report a decline in calcification, growth, photosynthetic efficiency, electron transport rate, and recruitment under high CO_2 for the major calcifying divisions (Chlorophyta and Rhodophyta; Koch et al., 2013).

Field studies in naturally low pH environments, such as CO_2 seeps at Ischia, Italy, and Milne Bay, Papua New Guinea, have also shed light on how elevated CO_2 affects marine macrophytes (Fabricius et al., 2011; Porzio et al., 2011). In these sites, fleshy macroalgae and seagrasses tended to dominate macrophyte populations closest to the seeps, whereas most types of calcareous algae and crustose coralline algal epiphytes declined near the seeps. However, the responses were not universal across groups, and exceptions were often noted. For example, fleshy macroalgae growing in short tufts were most sensitive to CO_2 enrichment, whereas some large macroalgae ochrophyte species were found to grow along the entire pH gradient (e.g., *Dictyota dichotoma, Hildenbrandia rubra*), while still others were more abundant closest to the seeps (e.g., *Sargassum vulgare, Cladostephus spongiosus, Chondracanthus acicularis*).

MOVING FORWARD IN PHOTOSYNTHESIS–OA RESEARCH

Photosynthetic responses to enhanced CO_2 under OA are remarkably diverse, and variability exists both between and within taxonomic groups (Figure 2). As the substrate for photosynthesis, elevated CO_2 would be expected to increase photosynthetic rates either directly by relieving carbon limitation or indirectly by lowering the energy required to concentrate CO_2 against a smaller concentration gradient. Nevertheless, despite the growing body of literature on the topic, clear trends in the photosynthetic responses of phytoplankton to elevated CO_2 have not emerged, and the positive effects, if any, are small (Figure 2). Additionally, many studies finding "no effect" of OA are likely not published, resulting in a bias in the literature. That no significant difference is apparent even in light of this bias suggests the net effects of OA on photosynthesis are minor for a large proportion of phytoplankton species. The small effect could indicate that the benefits afforded by high CO_2 are small relative to the cell's overall energy and material balances. Alternatively, the small effect of OA could indicate that its expected benefit to photosynthesis is counteracted by other negative effects, such as possible respiratory costs from low pH. Moving forward in OA research, experiments should encompass a broader suite of measurements to probe how different physiological processes in addition to photosynthesis respond to OA.

As information on the physiological effects of high CO_2 becomes available, efforts should be made to understand how these effects will translate to ecological and evolutionary processes. For example, understanding how the combined effects of OA on photosynthesis, respiration, and other physiological processes affect growth rate and competition between species will increase our understanding of how phytoplankton biogeography and productivity patterns could change in the future.

The effect of multiple stressors is critically important for understanding how OA will affect photosynthesis in the dynamic marine environment. Modulation of OA effects by multiple stressors (e.g., nutrient availability, temperature, light) has been investigated most in experiments with diazotrophs, most likely because their stressors are already known and well characterized (e.g., diazotrophs are commonly limited by Fe or P). Competition for resources is another type of external stressor, and field-based experiments have shown mixed results for mixed assemblages under enhanced CO_2. An improved understanding of the proximal factors that will limit photosynthesis in the future is needed to inform global biogeochemical models and constrain the growth responses of different phytoplankton functional groups. Suggested methods for exploring the effects of multiple stressors are discussed in this issue by Andersson et al. (2015).

The vast majority of studies to date have investigated responses in cells acclimated to high CO_2 over time scales too short for evolution to produce major changes. Assessing how phytoplankton could adapt to elevated CO_2 over longer time scales (months to years) is an important next step in experimental evaluation and prediction of OA effects on marine photosynthesis and productivity (Collins et al., 2014). Moreover, competition for and biogeochemical cycling of nutrients in the ocean will be directly influenced by adaptive changes in photosynthetic traits. Experimental evolution with cultured phytoplankton will be a tool for predicting how important taxa will change in the future ocean.

SUPPLEMENTARY MATERIALS. Supplemental Table S1 of data used for Figure 2, is available online at http://www.tos.org/oceanography/archive/28-2_mackey.html. The raw data from the literature review, as well as methods used to compile and process those data, are archived at BCO-DMO (http://www.bco-dmo.org/dataset/554221/data).

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AUTHORS. **Katherine R.M. Mackey** (kmackey@uci.edu) is Assistant Professor, Earth System Science, University of California Irvine, Irvine, CA, USA. **J. Jeffrey Morris** is Assistant Professor, Department of Biology, The University of Alabama at Birmingham, Birmingham, AL, USA, and BEACON Center for the Study of Evolution in Action, Michigan State University, East Lansing, MI, USA. **François M.M. Morel** is Professor, Department of Geosciences, Princeton University, Princeton, NJ, USA. **Sven A. Kranz** is Assistant Professor, Earth, Ocean and Atmospheric Science, Florida State University, Tallahassee, FL, USA.

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