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Effects of CO₂ on growth rate, C:N:P, and fatty acid composition of seven marine phytoplankton species

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ABSTRACT: Carbon dioxide (CO₂) is the primary substrate for photosynthesis by the phytoplankton that form the base of the marine food web and mediate biogeochemical cycling of C and nutrient elements. Specific growth rate and elemental composition (C:N:P) were characterized for 7 cosmopolitan coastal and oceanic phytoplankton species (5 diatoms and 2 chlorophytes) using low density, nutrient-replete, semi-continuous culture experiments in which CO₂ was manipulated to 4 levels ranging from post-bloom/glacial maxima (<290 ppm) to geological maxima levels (>2900 ppm). Specific growth rates at high CO₂ were from 19 to 60% higher than in low CO₂ treatments in 4 species and 44% lower in 1 species; there was no significant change in 2 species. Higher CO₂ availability also resulted in elevated C:P and N:P molar ratios in Thalassiosira pseudonana (~60 to 90% higher), lower C:P and N:P molar ratios in 3 species (~20 to 50% lower), and no change in 3 species. Carbonate system-driven changes in growth rate did not necessarily result in changes in elemental composition, or vice versa. In a subset of 4 species for which fatty acid composition was examined, elevated CO₂ did not affect the contribution of polyunsaturated fatty acids to total fatty acids significantly. These species show relatively little sensitivity between present day CO₂ and predicted ocean acidification scenarios (year 2100). The results, however, demonstrate that CO₂ availability at environmentally and geologically relevant scales can result in large changes in phytoplankton physiology, with potentially large feedbacks to ocean biogeochemical cycles and ecosystem structure.

KEY WORDS: Phytoplankton · Carbon dioxide · Ocean acidification · Elemental stoichiometry · Fatty acid composition · Diatom · Chlorophyte

INTRODUCTION

The Earth’s oceans are an important sink for anthropogenic carbon dioxide (CO₂), being the second largest after the atmosphere (Sabine et al. 2004). The removal of CO₂ from the atmosphere into the world’s oceans alters the chemical equilibrium of the seawater carbonate system, resulting in ocean acidification, i.e. a decrease in pH, and an increase in bicarbonate (HCO₃⁻) and CO₂. Continuing CO₂ emissions at current rates are predicted to increase the partial pressure of CO₂ (pCO₂) in seawater from the present value of ~400 to ~800–1000 ppm by the year 2100 (IPCC 2007, Tans 2009), resulting in a mean pH drop from 8.2 to 7.8 (Feely et al. 2004). The projected rise in pCO₂ and decline in ocean pH is about 30-fold faster than that observed during the last 300 million years (Kump et al. 2009, Hönisch et al. 2012).

Carbon dioxide and pH play critical roles in mediating physiological functions within marine organisms. Phytoplankton, single-celled photosynthetic organisms that include both calcifying and non-calcifying taxa, play a crucial role in the world’s oceans by converting CO₂ to organic C by means of photosynthesis. Laboratory experiments investigating the effects of high CO₂/low pH have revealed potentially...
negative impacts on the physiological and morphological properties of calcifying marine phytoplankton (Riebesell et al. 2000, Orr et al. 2005). The effects of elevated pCO2 upon phytoplankton also include higher cellular carbon quotas (Burkhardt et al. 1999, Riebesell et al. 2007, Hutchins et al. 2009, King et al. 2011, Reinfielder 2012) and changes in phytoplankton species composition and succession (e.g. Tortell et al. 2002, Riebesell et al. 2013). Higher CO2 availability could potentially affect phytoplankton community composition by favoring taxa that have less efficient carbon concentrating mechanisms—means of increasing the supply of CO2, the primary inorganic C source for photosynthesis, to the carboxylating enzyme Rubisco (Roberts et al. 2007b).

Phytoplankton provide organic matter (carbon, nitrogen, phosphorous) and specific nutritional needs (such as polyunsaturated fatty acids) that support the marine food web. For optimal physiological performance of marine metazoans, the trophic transfer, assimilation, and retention of key nutrients contained within phytoplankton is critical. Phytoplankton-produced polyunsaturated fatty acids (PUFA) are considered ‘essential’ because metazoan organisms require PUFA for growth and are incapable of de novo synthesis (del R. Gonzalez-Baro & Pollero 1988, Kainz et al. 2004). For zooplankton, bivalves, and fish, these fatty acids (FA) are critical for enzyme activity, neural development, stress resistance, membrane fluidity, growth, and survival (Langdon & Waldock 1981, Sargent et al. 1999). Numerous studies have focused on enhanced growth and reproductive rates in the aquatic food web when PUFA and C, N, and P are optimized (Elser et al. 2000, Wacker & von Elert 2001, Sterner & Elser 2002). Slight changes in the nutritional quality of marine phytoplankton (higher C:P or C:N, community FA composition) can result in reduced growth rates and fecundity at higher trophic levels (Sargent et al. 1999). Elevated pCO2 has been shown to reduce the PUFA content of a cultured diatom (Thalassiosira pseudonana) and therefore reduce the PUFA content and hatching success of a copepod grazer reared on the diatom (Rossoll et al. 2012).

Here we present a series of experiments with 7 temperate phytoplankton species, previously isolated from coastal and oceanic locales, grown under carbonate system manipulations that represent the low pCO2 found in glacial maxima and modern post-bloom scenarios, pCO2 of present-day average open ocean, pCO2 predicted by year 2100, and geological maximal pCO2 levels. The phytoplankton species in this study are relevant in terms of being key species in both the coastal and oceanic marine realms and for use in shellfish aquaculture operations. We show that carbonate system variability can have significant effects upon specific growth rate and elemental stoichiometry on certain species, but this effect differs greatly between species, even between species within the same genus. Despite significant change in growth rate and elemental stoichiometry, there were no significant effects of seawater carbonate manipulation upon FA composition. Findings based upon these phytoplankton species suggest that shifts in O2 availability can potentially alter phytoplankton community structure as a consequence of variable growth rate responses, biogeochemical cycling of nutrient elements, and nutritional value in terms of elemental composition, but not PUFA content, of phytoplankton for the marine food web.

MATERIALS AND METHODS

Culture experimental setup

Laboratory cultures were grown aseptically and semi-continuously at 20 ± 2°C under growth-saturating light intensity (~120 μmol photons m⁻² s⁻¹, 4π detector; 14 h light:10 h dark cycle) in f/2 medium made with seawater collected on several occasions from Woods Hole, Massachusetts (Environmental Systems Lab, Woods Hole Oceanographic Institute, MA, USA; salinity = 32–33). After nutrient additions, the culture medium was filter-sterilized (<0.2 μm) into autoclaved 101 borosilicate glass reservoir containers and adjusted to 4 CO2 levels by sparging (<100 ml min⁻¹) for a 2 d equilibration period prior to beginning each experiment. After the equilibration period, ~500 to 1000 ml of equilibrated seawater was transferred aseptically into autoclaved, 4 l, borosilicate glass bottles and inoculated with a starter culture (Erlenmeyer flasks with f/2 medium) in log growth phase at an initial cell density of <5 × 10⁴ cells ml⁻¹. Each of the 4 CO2 treatments consisted of 3 replicates (12 experimental bottles total). The same air:CO2 mixtures were sparged gently into experimental bottles at <30 ml min⁻¹. All air:CO2 mixtures were filtered with 0.01 μm filter elements (Balston, Parker Hannifin Corp.) prior to entering experimental containers.

Growth rates were confirmed to be unaffected by comparing maximum growth rates under unbalanced and bubbled conditions, and to growth rates reported previously in literature. The experimental set up was designed based upon best practice recommendations made by the European Project on Ocean Acidifica-
tion (EPOCA) (Riebesell et al. 2010). A schematic of each experimental unit is shown in Fig. 1.

The 4 CO2 levels represented a range of physiologically and environmentally relevant levels, consisting of post-bloom/glacial maxima inorganic C availability (less than ~200 ppm pCO2), present-day open ocean conditions (~350 to 400 ppm pCO2), future high CO2/ocean acidification projections (~750 to 1000 ppm pCO2) (IPCC 2007), and maximum CO2 availability estimated from the geological record (greater than ~3000 ppm pCO2) (Kump et al. 2009, Hönisch et al. 2012). Carbon dioxide in the experimental supply air was adjusted using mass flow controllers (Aalborg) that combined air sources of <10 ppm pCO2 (using a molecular sieve CO2 adsorber; Puregas) and ~100 000 ppm pCO2 (AirGas Inc.). For each experiment, each of the 4 target CO2 levels were achieved by calculating the needed flow rate of each airstream, measuring carbonate system variables, and adjusting flow rate as needed. The pCO2 in growth media was determined by calculating pCO2 using CO2SYS (Pierrot et al. 2006), with constants from Mehrbach et al. (1973, refit by Dickson & Millero 1987), and inputs of temperature, salinity, and inputs of temperature, salinity, and pCO2 using CO2SYS (Pierrot et al. 2006), with constants from Mehrbach et al. (1973, refit by Dickson & Millero 1987), and inputs of temperature, salinity, total alkalinity, total dissolved inorganic carbon (DIC), spectrophotometric pH (m-cresol purple), phosphate, and silicic acid. Carbonate system variables from the final time point of each experiment are presented in Table 1. In addition to the endpoint measurement, multiple discrete measurements were made during each experiment to confirm carbonate system manipulations. The standard deviation of pH (total scale) was on average 0.2% (between replicates), the standard deviation of total DIC was on average 0.5% (between replicates), the standard deviation of total alkalinity was on average 0.4% (across all replicates in each of the experiments), and the standard deviation of CO2SYS-calculated pCO2

![Fig. 1. Schematic of an experimental treatment unit that consists of 1 ‘reservoir’ container (10 l) for dilutions and 3 experimental replicates (4 l). (An experimental system with 4 CO2 treatments would consist of 4 of these treatment units.) Solid lines refer to tubing for growth medium that are used for making dilutions. Dashed lines refer to gas tubing used for routing different air:CO2 mixtures to the containers. Labels are as follows: A, compressed air; B, 100 000 ppm CO2; C, molecular sieve CO2 adsorber; D1, mass flow controller for low CO2 air; D2, mass flow controller for 100 000 ppm CO2](image-url)

Table 1. Carbonate chemistry at the final time point of experiments listed by species. Average and standard deviation (n = 3) of variables A and B measured in each experiment are listed (A and B are denoted as the following: ALK, total alkalinity, µmol kg⁻¹; DIC, pH, µmol kg⁻¹; total scale; DIC, total dissolved inorganic carbon, µmol kg⁻¹). Also included is pCO2 (ppm) calculated using CO2SYS (see ‘Materials and methods’)

<table>
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<tr>
<th>Species</th>
<th>CO2 condition</th>
<th>A Avg.</th>
<th>A SD</th>
<th>B Avg.</th>
<th>B SD</th>
<th>CO2SYS pCO2 Avg.</th>
<th>SD</th>
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<td>0.040</td>
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was on average <5% (between replicates). The average final time point pCO₂ of each replicate was assigned as the reported pCO₂ value of each experiment (Table 1).

The experiments were conducted with 7 species: coastal diatoms *Thalassiosira pseudonana* (CCMP 1335), *T. rotula* (GSO101; recently deposited as CCMP 3096), and *T. weissflogii* (CCMP2599); oceanic diatoms *T. weissflogii* (CCMP1010 — oceanic isolate) and *T. oceanica* (CCMP1005); and nearshore chlorophytes *Chlorella autotrophica* (CCMP243) and *Dunaliella salina* (UTEX LB200). Cell densities were kept low during the experiments to minimize changes in nutrients and availability of light and dissolved inorganic carbon. Each species was transferred from mid-exponential phase in 1/2 medium and grown for 7 to 10 generations, after which growth rates stabilized. Cultures were diluted with equilibrated medium every 2 to 3 d, depending upon growth rate. Macronutrients during the experiment always were in excess of 170 µM nitrate, 18 µM phosphate, and 21 µM silicic acid. Cell densities in the experiments were kept low at <8.5 × 10⁵ cells ml⁻¹ and were on average 3.0 × 10⁵ cells ml⁻¹.

**Macronutrient and carbonate system measurements**

Nitrate+nitrite, phosphate, and silicic acid were determined using a Quattro autoanalyzer (Seal Analytical). Aliquots of 40 ml were syringe-filtered (0.2 µm), and nutrients were measured within 24 h of collection. All nutrient protocols were developed by the Royal Netherlands Institute for Sea Research. Nitrate+nitrite was determined using the red azo dye method, with a detection limit of 0.02 µmol l⁻¹ and a standard deviation of 0.03 µmol l⁻¹ (Method NO Q-068-05 Rev. 4). Phosphate was determined using a phosphomolybdenum complex with a detection limit of 0.004 µmol l⁻¹ and a standard deviation of 0.01 µmol l⁻¹ (Method NO Q-064-05 Rev. 3). A silicomolybdenum blue complex was used to determine silicic acid with a detection limit of 0.05 µmol l⁻¹ and a standard deviation of 0.01 µmol l⁻¹ (Method NO Q-066-05 Rev. 3).

Sample aliquots for the total alkalinity, total DIC, and pH (total scale) analyses were collected at multiple time points from each experiment to confirm stability of CO₂ manipulations. Total alkalinity was determined using a Gran titration approach with a custom-made, open-cell alkalinity titration system (C. Langdon, University of Miami, FL, USA) equipped with a fine-step, motorized dosing burette coupled with a combination glass/reference pH electrode calibrated against a TRIS HCl buffer (Sigma-Aldrich). The alkalinity titration was performed in a jacketed cell with temperature recorded by an electronic temperature probe (Fisher Scientific Traceable). Total DIC was measured using a DIC analyzer based upon sample acidification and LI-COR CO₂ detection (Apollo SciTech). pH (total scale) was determined colorimetrically using meta-cresol purple (Sigma-Aldrich) with a Varian dual beam spectrophotometer (Agilent Technologies), 10 cm cylindrical cells (Innovative Lab Supply), and jacketed cell holders, with temperature maintained by a Peltier cooler. The 3 analytical methods in principle are described in detail by DOE (1994). All samples were analyzed within ~5 to 60 min of collection, without the addition of preservatives. Certified reference material for total DIC and total alkalinity was analyzed for accuracy comparisons and, if needed, corrections (A. Dickson, Scripps Institute of Oceanography/University of California San Diego, CA, USA). In terms of precision, replicate measurements (n = 5) on certified reference material resulted in 1 standard deviation of ±5.5 µmol kg⁻¹ for total alkalinity measurements, ±2.7 µmol kg⁻¹ for total DIC measurements, and ±0.0014 for total pH. The carbonate system analyses described here were part of an international inter-laboratory comparison exercise that consisted of low and high CO₂ test seawater samples — samples measured using these methods were within 0.5% of assigned values (Bockmon & Dickson 2015).

**Physiological measurements**

Cell density was measured during the course of each experiment using FACSScan and Accuri C6 flow cytometers (Beckton-Dickinson Biosciences). Populations were identified by adjusting voltages and thresholds for forward scatter, side scatter, and chlorophyll auto-fluorescence channels, and collecting data for approximately 1 min. Cell densities were used to make dilution calculations, and specific growth rate calculations were made from the final 3 generations. Particulate organic C, N, and P (POC, PON, and POP, respectively) samples (>200 ml) were vacuum-filtered (<100 mm Hg) onto pre-combusted 24 mm GF/C glass fiber filters (Whatman, GE Healthcare Biosciences) and were determined using conventional methods. Briefly, POC and PON samples were dried in an oven at 60°C, acidified with HCl...
fumes, and analyzed using gas chromatography with a CHNSO analyzer (Costech) (Parsons et al. 1984). POP samples were rinsed with 0.17 M Na₂SO₄, dried at 90°C in 0.017 M MgSO₄, and measured using the colorimetric molybdate method (Solorzano & Sharp 1980).

FA composition was determined for T. pseudonana, T. weissflogii (CCMP2599), C. autotrophica, and D. salina. Sample aliquots (>1.5 l) were vacuum-filtered onto precombusted, 42.5 mm GF/C glassfiber filters, purged with N₂, frozen at −80°C, and subsequently extracted in 2:1 (v/v) chloroform:methanol using a modified Folch procedure (Budge & Parrish 1999). An aliquot of the lipid sample was transmethylated, and fatty acid methyl esters (FAME) were identified on a Shimadzu GC-2014 equipped with an Omega wax 320 column (Supelco). Peak detection used Shimadzu SystemGC software with mixed and individual FAME standards (Supelco, eicosapentaenoic FAME, docosahexaenoic FAME, and PUFA No. III — Menaden Oil; 47085-U, CRM47571, and CRM47570). Individual FAs and the proportion of total FAs represented by saturated fatty acids (ΣSFA), monounsaturated fatty acids (ΣMUFA), and ΣPUFA were determined by dividing the area of each FAME peak or peak group by the total identified FAME.

RESULTS

Specific growth rates

Over the 4 CO₂ scenarios ranging from <230 to 5100 ppm pCO₂ (Table 1), specific growth rates were found to be significantly different for Thalassiosira rotula, T. weissflogii (CCMP2599), T. weissflogii (CCMP1010), T. oceanica, and Chlorella autotrophica (1-way ANOVA; p < 0.05) (Fig. 2A, Table 2). The T. rotula growth rate was 13 and 29% greater at 690 ppm pCO₂, relative to low and present-day pCO₂ levels, respectively. T. weissflogii (CCMP2599) and T. weissflogii (CCMP1010) growth rates were unchanged between present-day pCO₂ and the geological maxima, but growth rates in the lowest pCO₂ treatment were 60 and 26% lower than growth rates at the geological maxima pCO₂, respectively, for each species (Fig. 2A, Table 2). T. oceanica specific growth rates ranged from 19 to 49% lower under high CO₂ conditions (700 and 2925 ppm) relative to growth rates at low pCO₂ and present-day pCO₂ (Fig. 2A). C. autotrophica exhibited a significantly higher growth rate at ~1000 ppm pCO₂—nearly 20% higher than at present-day CO₂ levels (Fig. 2A, Table 2). Specific growth rates for the remaining 2 species, T. pseudonana and Dunaliella salina, were unchanged across the range of experimental pCO₂ conditions (Fig. 2A, Table 2).

C:N:P composition

Molar ratios of C:P and N:P exhibited some variability between species regardless of CO₂ treatment, with the most notable difference being a several-fold higher C:P and N:P ratio in D. salina—ranging from 100 to 186 C:P and 19 to 31 N:P—in comparison with the other 6 species (Fig. 2B). The effects of CO₂ treat-
Figure 3. Fatty acid composition expressed as the contribution of saturated fatty acids (cross-hatched), monounsaturated fatty acids (hatched), and polyunsaturated fatty acids (open) to total fatty acids for *Thalassiosira pseudonana*, *T. weissflogii* (CCMP2559), *Chlorella autotrophica*, and *Dunaliella salina* grown under 4 CO2 conditions (see gray scale key). Standard errors for fatty acid fractions were on average ±2.1%.

DISCUSSION

The intention of this study was to characterize the responses of individual phytoplankton species grown under various CO2 conditions, including the ocean acidification (OA) conditions that are projected to occur by the year 2100. To this end, experiments were conducted under nutrient-replete, light-saturating, and controlled and constant inorganic carbonate system conditions. While we acknowledge that carbonate chemistry and multiple other environmental variables in most ocean regimes are dynamic over diel and longer timescales and that these factors certainly affect phytoplankton growth and physiology, characterizing the response of phytoplankton in treatments with constant CO2/pH and no limitation/co-limitation by other factors is the first step needed in detecting and addressing the potential response of phytoplankton to progressive change in the carbonate chemistry of seawater.

We also acknowledge that geological scale pCO2 estimates are based on a variety of geochemical proxies (Hönisch et al. 2012) and that evidence sug-
gests that pCO₂ may have co-varied with changes in alkalinity on geological timescales (i.e. elevated pCO₂ in the past may not have resulted in pH declines similar to those projected for the modern OA scenario). Thus, the carbonate system manipulations reported here that consisted of >3000 ppm pCO₂ and modern ocean alkalinity do not necessarily reflect the oceanic carbonate conditions of the geological past, but merely the potential of higher CO₂ availability for phytoplankton that likely evolved under high CO₂ conditions.

**Variability in specific growth rates**

In the context of phytoplankton community structure and projected success of species under different CO₂ conditions, specific growth rate is a strong determining factor and has therefore been considered a metric of evolutionary fitness (e.g. Collins et al. 2014). There were 4 observed specific growth rate responses to pCO₂ manipulation within the 7 phytoplankton species: no change across all CO₂ treatments (*Thalassiosira pseudonana*, *Dunaliella salina*), higher growth rate at elevated pCO₂ (*T. rotula* and both strains of *T. weissflogii*), lower growth rate at low pCO₂ (*T. oceanica*), and optimal growth rate at an intermediate pCO₂ (*Chlorella autotrophica*) (Fig. 2A). Previously published growth rate responses to CO₂ are in agreement for *T. pseudonana* (no response; Clark & Flynn 2000, Roberts et al. 2007a, Crawfurd et al. 2011, Reinfelder 2012, Yang & Gao 2012, Wynn-Edwards et al. 2014) and *T. weissflogii* (higher growth rate at elevated CO₂, 1999, Shi et al. 2010, Wu et al. 2014). In the case of *T. weissflogii* (CCMP2599 and CCMP1010), specific growth rate is limited only under the lowest CO₂ treatment (<100 ppm pCO₂) and apparently at maximal present-day pCO₂ (~350 to 380 ppm) and at pCO₂ representative of future projections and the geological past.

The positive response of *T. rotula* and *C. autotrophica* specific growth rates to high CO₂ availability in OA scenarios suggests that these 2 species could benefit from the projected increase in oceanic pCO₂ within the next century. Although elevated CO₂ conditions would provide an evolutionary advantage if competition were dictated purely by CO₂ availability, it remains unclear how this response would play out under natural conditions in which these species may respond differently to multiple environmental variables (e.g. temperature, light, and nutrient availability), in addition to ecological interactions including grazing and allelopathy. Indeed, a number of natural community CO₂ manipulation experiments ranging from liters to 1000s of liters in scale have shown a variety of different responses in terms of phytoplankton community structure (Tortell et al. 2002, Feng et al. 2009, Riebesell et al. 2013).

The growth rate responses among the 7 species also provide clues as to the degree to which each species could have been carbon limited prior to the industrial revolution. The slower growth rates of *T. rotula* and *T. weissflogii* (both strains) under the low CO₂ treatment indicate that these species could have been limited by low CO₂ availability in the past, while the growth rates of *T. pseudonana*, *T. oceanica*, and both chlorophytes appear to be unaffected by low CO₂ availability. A possible explanation could be that the latter species possess carbon concentrating mechanisms that are active and/or efficient enough to supply adequate CO₂ to the chloroplast. Another possible mechanism that could explain possible C limitation is cell size and the resulting limitation of CO₂ diffusion and/or the density of inorganic C transporters on the cell surface arising from the low ratio of surface area: volume (Finkel et al. 2010, Wu et al. 2014). *T. rotula* and *T. weissflogii* (both strains) both had enhanced growth rates at higher pCO₂ and are relatively large diatoms with maximal cell lengths ranging from ~18 to 60 µm. While *T. pseudonana* (no growth response) and *T. oceanica* (negative growth response) are morphologically similar to *T. rotula* and *T. weissflogii*, their maximal cell lengths range between ~5 and 12 µm. Neither *C. autotrophica* nor *D. salina* growth rates exhibited apparent limitation by C availability, and both are also relatively small with maximal cell lengths ranging from ~5 to 15 µm.

The negative growth rate response of *T. oceanica* to high CO₂/low pH conditions was not observed in any of the other species in the present study. Shi et al. (2010) observed a positive response of *T. oceanica* growth rate to pCO₂ enrichment ranging from ~100 to 680 ppm pCO₂ (same strain used in the present study; CCMP1010). Some diatoms, however, in other studies (*Phaeodactylum tricornutum*, *T. pseudonana* [also CCMP1335], and *Skeletonema costatum*) have been reported to exhibit between ~10 and 16% lower growth rates when grown under high CO₂ conditions (~1000 ppm pCO₂) in combination with high light intensities (>200 µmol photons m⁻² s⁻¹) (Gao et al. 2012). Hypothetically, a lower growth rate under high light intensities and elevated carbon availability could be a result of reduced energy requirements for inorganic C acquisition combined with the lack of alternate energy-shunting mechanisms (Gao et al. 2012) or the downregulation of carbon concentrating mechanisms.
and reduction in inorganic carbon availability, resulting in a reduction in photosynthetic saturation irradiance (Hoppe et al. 2015). Although the experimental light intensity used in the present study was well below 200 µmol photons m⁻² s⁻¹, reduced growth rates could potentially be a manifestation of the interactive effects of reduced energy requirements for carbon-concentrating mechanisms under high CO₂/low pH and the cell’s inability to cope with excess light (i.e. lack of photoprotective mechanisms) (Wu et al. 2010). Indeed, *T. oceanica* has been shown to perform well at light intensities that are lower relative to those of neritic species (e.g. Sakshaug et al. 1987).

In addition to the potential light–CO₂ interaction, *T. oceanica* and other oceanic isolates might be less likely to experience large diel/seasonal fluctuations in comparison to coastal isolates, whereby pCO₂ could be more variable due to coastal upwelling of high pCO₂ waters and low pCO₂ post-bloom periods. While *T. oceanica* growth rates did show sensitivity to elevated pCO₂, the growth rate response of *T. weissflogii* (CCMP1010—oceanic isolate) was not different than that of the coastal isolate of *T. weissflogii* (CCMP2599) (Fig. 2).

### Elemental composition

Elemental composition of each of the 7 species can be compared with the molar ratios reported for a number of species from similar genera grown under uncontrolled CO₂ conditions in the artificial seawater medium Aquil (Quigg et al. 2003). The molar ratios of C:P of ~200 and N:P of ~30 that were observed for *D. salina* were similar to those observed for *D. tertiolecta*, and the C:P molar ratios <100 and N:P <15 were also observed in diatoms, including a diatom from the genus *Thalassiosira* (Quigg et al. 2003) (Fig. 2B,C). Similar to the growth rate response to varying CO₂ levels, several response patterns were evident in C:P and N:P molar ratios of the 7 species: no statistical difference across all 4 CO₂ treatments (*T. weissflogii* CCMP2599, *T. oceanica*), higher C:P and N:P molar ratios under high CO₂ conditions (*T. pseudonana*), and higher molar ratios under low CO₂ conditions (*T. rotula*, *C. autotrophica*, and *D. salina*) (Fig. 2B,C; Table 2). The positive relationships between C:P and N:P molar ratios and CO₂ for *T. pseudonana* are consistent with previously published results from other studies involving *T. pseudonana* (Reinfelder 2012) and various other diatoms (Burkhardt et al. 1999, King et al. 2011). Significantly higher C:P under low CO₂ observed in *T. rotula* is consistent with C:P molar ratios reported for *T. weissflogii* (unknown strain) (Burkhardt et al. 1999). With regards to the OA scenarios expected within the next century, we did not detect any significant differences in C:P and N:P molar ratios between present-day pCO₂ and elevated pCO₂ treatments.

Consistent with previous work, the present study suggests that phytoplankton have nutrient requirements that are quite plastic (even within the same genus, as is shown here for *Thalassiosira*). For instance, changes in growth rate (and CO₂ availability) can occur with no change in elemental stoichiometry (both strains of *T. weissflogii* and *T. oceanica*), or no change in growth rate can occur with significant differences in elemental stoichiometry (*T. pseudonana*, *D. salina*). Knowing that C, N, and P are required for basic cell functions associated with growth (proteins, amino acids, RNA, phospholipids, etc.) and that bulk measurements constitute an integration of a multitude of cell functions, it remains a challenge to predict how elemental requirements (either absolute or as ratios) might change in response to growth rate and/or CO₂ availability (Burkhardt et al. 1999, Reinfelder 2012).

The plasticity and variability in elemental stoichiometry shown here for these 7 phytoplankton species are relevant for marine ecosystem structure in 2 respects. First, phytoplankton elemental stoichiometry combined with growth rate, to a large extent, influences nutrient cycling through uptake in the euphotic zone and the distribution of nutrients in the deep ocean through export and subsequent remineralization. Phytoplankton elemental stoichiometry, especially N:P molar ratios, can also influence temporal patterns in phytoplankton community structure in relation to the potential limitation/co-limitation associated with nitrate and phosphate availability—2 elements that often are limiting in marine systems. Second, phytoplankton with higher C:P molar ratios have been shown to be lower in nutritional quality for grazers because C tends to be in excess relative to growth-limiting P (Elser et al. 2000, Urabe et al. 2003, Schoo et al. 2013). It is hypothesized that the effort involved in respiring food sources with excess C reduces the potential for somatic growth, and the decline in phytoplankton C:P molar ratios within the last ~500 million years, largely associated with the proliferation of diatom and dinoflagellate lineages, has been linked to the rise of modern metazoans (Martin et al. 2008). The influence of CO₂ upon phytoplankton elemental stoichiometry must also be considered in concert with other environmental factors such as nutrient and light availability which can likewise affect stoichiometry significantly (Finkel et al. 2010).
Fatty acid composition

In the present study, we did not detect significant CO2/pH-driven effects upon the fraction of total FAs accounted for by PUFA in 2 diatom species or in 2 chlorophyte species, nor did we detect a significant omega-3 FA contribution to total FAs in the 2 diatoms (Fig. 3). No change in PUFA content at elevated pCO2 (~960 to 1000 ppm) was found for the Antarctic prymnesiophyte Phaeocystis antarctica, the dinoflagellate Gymnodinium sp. (Wynn-Edwards et al. 2014), or for the sea-ice diatom Nitzschia lecointei grown at 2.5°C (this was not true at −1.5°C) (Torstensson et al. 2013). A recent study with T. pseudonana, however, reported a ~38% decline in total FAs cell−1 at 915 ppm pCO2 relative to 365 ppm pCO2, and a ~20% decline in the PUFA fraction of total FAs (Rossoll et al. 2012). Similar trends in declining PUFA content at elevated pCO2 were reported for the Antarctic prasinophyte Pyramimonas gelidicola (Wynn-Edwards et al. 2014), the sea-ice diatom N. lecointei when grown at ~1.5°C (but not at 2.5°C) (Torstensson et al. 2013), and the diatom Cylindrotheca fusiformis (Bermúdez et al. 2015). In a mesocosm CO2 experiment with natural phytoplankton assemblages, PUFA were found to increase under high CO2 primarily, but this was identified to likely be caused by a shift in community structure from diatoms to dinoflagellates, the latter of which had a relatively higher PUFA content (Leu et al. 2013). Although the mechanisms behind CO2/pH-driven effects upon phytoplankton FA composition remain elusive, there does appear to be a relationship between low pH, reduced FA synthesis, and FA desaturation (Sato et al. 2003), in addition to a temperature−CO2/pH relationship and the degree of saturation in proposed interactions between membrane fluidity, temperature, and maintenance of internal pH (Teoh et al. 2004, Mayzaud et al. 2013, Torstensson et al. 2013). Growth phase (logarithmic or stationary) is often difficult to control in dense cultures and is likely a confounding factor in findings to date.

CONCLUSIONS

In a series of culture experiments with controlled carbonate chemistry and nutrient-replete and light-saturating conditions, we have shown that the response of growth rate and elemental composition of 7 phytoplankton species (5 diatoms and 2 chlorophytes) to CO2/pH differs between species. For a subset of these species in which FA composition was examined, there were no detectable effects of elevated CO2 upon FA composition, including the fraction of FAs accounted for by PUFA. In terms of OA scenarios projected for the next century, significant findings are limited to increased growth rate of T. rotula and C. autotrophica, and decreased growth rate of T. oceanica. A number of other growth rate and elemental stoichiometry effects were found for other species under very low (<215 ppm pCO2) and very high (>2900 ppm pCO2) CO2 conditions. The responses found in these CO2 treatments are of potential utility when examining phytoplankton biogeochemical processes during transient post-bloom conditions in modern oceans, as well as over geological timescales.

Characterizing the effects of CO2 availability upon phytoplankton growth and physiology is crucial for several scientific needs: (1) improving our understanding of variability in oceanic primary production on numerous spatial and temporal scales, (2) understanding potential effects of this variability upon global and ocean nutrient cycles, and (3) determining resources available to higher trophic levels that depend upon phytoplankton for nutrition. Notably, CO2-driven changes in growth rate were not necessarily reflected in significant changes in elemental composition or FA composition, and vice versa. This is exemplified by the nearly 2-fold range of plasticity that was found in C:P and N:P molar ratios, despite a relatively unchanged specific growth rate. Generalizations regarding the effects of variable CO2 availability upon phytoplankton in general are difficult to make because of the observed occurrence of both bidirectional (or perceived positive or negative) responses to changes in CO2. Predictions of future ocean biogeochemical and ecosystem structure responses to the oceanic uptake of anthropogenic CO2 are dependent upon characterizing the response and sensitivity of phytoplankton to elevated CO2 (and other environmental factors). This clearly requires further work towards identifying and understanding the mechanism(s) responsible for change and towards parameterizing and constraining oceanic biogeochemical models.

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