

Viewpoints

Universal response pattern of phytoplankton growth rates to increasing CO₂

Summary

Phytoplankton growth rate is a key variable controlling species succession and ecosystem structure throughout the surface ocean. Carbonate chemistry conditions are known to influence phytoplankton growth rates but there is no conceptual framework allowing us to compare growth rate responses across taxa. Here we analyse the literature to show that phytoplankton growth rates follow an optimum curve response pattern whenever the tested species is exposed to a sufficiently large gradient in proton (H⁺) concentrations. Based on previous findings with coccolithophores and diatoms, we argue that this 'universal reaction norm' is shaped by the stimulating influence of increasing inorganic carbon substrate (left side of the optimum) and the inhibiting influence of increase H⁺ (right side of the optimum). We envisage that exploration of carbonate chemistry-dependent optimum curves as a default experimental approach will boost our mechanistic understanding of phytoplankton responses to ocean acidification, like temperature curves have already boosted our mechanistic understanding to global warming.

Introduction

Phytoplankton are responsible for about 50% of primary production on Earth (Field *et al.*, 1998). They utilize CO₂ and bicarbonate (HCO₃⁻) as the dissolved inorganic carbon (DIC) source for photosynthetic carbon fixation (Burkhardt *et al.*, 2001; Rost *et al.*, 2003). Carbon fixation rates are limited by the slow catalytic rate of the RubisCO, the enzyme which binds CO₂ (Raven & Johnston, 1991). Phytoplankton hence invest energy in cellular carbon concentrating mechanisms (CCMs), which increase the substrate (i.e. CO₂) concentration in the vicinity of the RubisCO enzyme and drive the carboxylation reaction. However, the cytosolic membrane is highly permeable to CO₂. Consequently, the higher the CO₂ concentration in the seawater outside the cell, the less CO₂ is lost by diffusion from the cell, thereby increasing net CCM efficiency (Hopkinson *et al.*, 2011).

In early studies, pH was manipulated to study the sensitivity of plankton growth to increasing inorganic carbon limitation at high pH/low CO₂ (Swift & Taylor, 1966; e.g. Goldman *et al.*, 1982;

Hansen, 2002; Søderberg & Hansen, 2007). This revealed underlying differences in carbon concentrating efficiency (Reinfelder, 2011) and RubisCO specificity between phytoplankton species (Tortell, 2000). More recently, studies have focused at the other end of the pH spectrum with low pH/high CO₂ scenarios to test the sensitivity of phytoplankton to ocean acidification (OA). At high CO₂ phytoplankton growth rates can decline in some species (e.g. Berge *et al.*, 2010; McMinn *et al.*, 2014; Sett *et al.*, 2014), presumably due to proton (H⁺) inhibition as a consequence of the increased energetic costs of maintaining pH homeostasis in the cell (Bach *et al.*, 2011). Proton inhibition has been directly confirmed for the growth response in the coccolithophore, *Emiliania huxleyi* (Bach *et al.*, 2011) and three diatom species (Shi *et al.*, 2019), and implied for dinoflagellates (Berge *et al.*, 2010).

Michaelis–Menten (or Monod) kinetics are commonly used to parameterize the phytoplankton growth response to CO₂, from the species-specific physiological response (Riebesell *et al.*, 1993; Hutchins *et al.*, 2013) up to global scales in biogeochemical models, for example, Dutkiewicz *et al.* (2015). However, growth rate inhibition by H⁺ is not considered in the commonly applied Monod model kinetics. Often a plateau in growth is assumed once a critical pH is reached as has, for example, been observed within a confined range of H⁺ for selected diazotrophic cyanobacteria taxa (Hutchins *et al.*, 2013). Investigation of the optimum curve response is also potentially constrained by the employed study design. In many studies, CO₂/pH treatments are usually selected within an OA relevant range (180–780 µatm), as suggested for OA scenario testing (Barry *et al.*, 2011). This provides specific information on selected OA scenarios tested, but limits how mechanistic information on phytoplankton growth responses could be parameterized into biogeochemical models. An understanding of broader response patterns outside of the specific OA scenarios studied can, however, be very helpful for accurate model implementation (e.g. Muller & Nisbet, 2014).

Description of the phytoplankton response to CO₂

Here, we tested the applicability of optimum growth curve response pattern found in calcifying phytoplankton (Swift & Taylor, 1966; Langer *et al.*, 2006; Bach *et al.*, 2011) to a broader range of phytoplankton taxa, primarily using the growing body of literature on the response of phytoplankton to changes in seawater carbonate chemistry. This may also reveal if CO₂ optima in growth rates of individual phytoplankton species are systematically different between taxa, for example, coccolithophores and diatoms as this may have implications for shifts in phytoplankton community structure under changing seawater pH.

Data was compiled from literature searches for available raw datasets using the PANGAEA data depository collection from the Ocean Acidification International Coordination Centre (OA-ICC,

Yang *et al.*, 2016) and citing literature available up until June 2019. An additional unpublished dataset using the diazotrophic cyanobacteria, *Crocospaera watsonii*, from our laboratory was also included as it fitted the required criteria detailed in the following. Datasets on phytoplankton were selected for model fitting based on four criteria. Firstly, five or more distinct pH/CO₂ treatments are provided. Secondly, the range of pH/pCO₂ treatments is wider than OA relevant range (pH_F (free scale) < 7.4 or > 8.2; pCO₂ < 200 μatm or > 1200 μatm; OA relevant range pCO₂ = 400–1000 μatm). Thirdly, growth rates are reported for individual strains. Finally, pH was reported or alternatively data was available to calculate [H⁺]. An overview on all studies considered, culturing information about *Crocospaera watsonii*, and those studies selected for model fitting for each phytoplankton group is provided in Supporting Information Methods S1 & S2 and Tables S1–S3. Due to the over-proportionally large representation of coccolithophore genotypes fitting these criteria (mainly *E. huxleyi* strains), only five coccolithophore species were considered in our figures.

All growth rates (μ) were normalized to the maximum measured rate for each taxa in each study and then fitted to a modified Monod model (Eqn 1) by Han & Levenspiel (1988). This modified equation considers the substrate concentration as a rate limiting factor below a certain concentration (i.e. Monod kinetics, Monod (1949)) and as an inhibitor above this critical concentration.

$$\mu(\text{d}^{-1}) = k \left(1 - \frac{[\text{H}^+]}{[\text{H}^+]^*}\right)^n \cdot \frac{[\text{H}^+] \cdot [\text{cell}]}{[\text{H}^+] + C_M \left(1 - \frac{[\text{H}^+]}{[\text{H}^+]^*}\right)^m} \quad \text{Eqn 1}$$

Here, *k* is the reaction rate constant or maximum possible growth rate (i.e. *V*_{max} equivalent), [cell] is the cell concentration, [H⁺]^{*} is the critical inhibitor concentration above which no growth occurs, *n* and *m* are fitting constants which if ≠ 0 indicate inhibition behaviour of H⁺, and *C*_M is the Monod constant, that is, the substrate concentration below which no growth occurs.

We assume that at low CO₂, the optimum curve response is driven by the stimulating influence of the substrate (CO₂). Evidence to show CO₂ and not pH is dominant driver of growth rates at low CO₂ is compiled in the (Figs S1–S4; Notes S1). At high CO₂, the inhibitory effect of H⁺ controls the growth response (Bach *et al.*, 2015; Kottmeier *et al.*, 2016). Under constant total alkalinity (OA scenario where TA = 2300 μmol l⁻¹, *T* = 15°C), [H⁺] and CO₂ concentration are linearly correlated, provided that pH_F is below 8.3, with small deviations from linearity above pH_F 8.3 (Fig. 1). Due to the quasi-linear relationship between CO₂ and H⁺, we could still extract information on the general response pattern, without any necessary approximation of CO₂ from the datasets where only pH was reported. Hence, for consistency across all datasets used and mathematical simplicity, the optimum curves were fitted only with [H⁺] as this still implicitly considers inorganic carbon substrate supporting phytoplankton growth due to the correlation with CO₂ (Fig. 1). Nevertheless, for orientation we add the approximate OA relevant pCO₂ range to Fig. 1 as grey shaded area.

Evidence for an optimum curve response pattern across diverse phytoplankton taxa

In general, an optimum curve response pattern was observed in growth rates across the species in this study. Growth rates increase with increasing CO₂ (or H⁺ using the quasi linear CO₂ to H⁺ correlation detailed in the previous section) but taper off at high CO₂ concentrations (Fig. 2). One exception was *Trichodesmium* spp., where the metabolic limits for this taxon did not appear to be reached within the experimental H⁺ range studied (equivalent to *c.* 1500 μatm pCO₂, Hutchins *et al.*, 2015). Although a large number of studies were found in our literature search, in particular for coccolithophores and diatoms (52 and 122 responses of individual species or strains found, respectively), only 28 datasets out of 251 across all species and groups fulfilled all selection criteria (see Table S3a–h). Comparatively high interest in the response of calcifying haptophytes and diatoms compared to other phytoplankton groups is likely due to their biomineralization processes and prominent bloom formation in the ocean.

A model fit was made with the data in Fig. 2, which provides an idea as to how the taxa sensitivity can be illustrated by the Monod constant (*C*_M) for the lower end of substrate availability, and by the critical substrate concentration [H⁺]^{*} for the higher end of substrate concentrations where inhibition occurs. Alternatively, the sensitivity of each taxa could also be conceptualized in terms of half-saturation or half-inhibition constants (Gafar *et al.*, 2018). Different [H⁺] tolerance ranges were detected at both the taxa level (e.g. between diatoms and coccolithophores) and on a species level (e.g. between coccolithophore species, Fig. 2). *Crocospaera watsonii* (cyanobacteria), *Coccolithus pelagicus*, *Gephyrocapsa oceanica* and *Scyphosphaera apsteinii* (all three are large, heavily calcified coccolithophores and therefore particularly H⁺ sensitive (Bach *et al.*, 2015; Gafar *et al.*, 2019)) were only able to grow up until *c.* 70 nmol l⁻¹ H⁺ (Table S2). By contrast, three diatoms species could grow at H⁺ up to 170 nmol l⁻¹ H⁺ (Table S2). Hence, diatoms species considered in our analysis were considerably less sensitive to seawater pH_F than many other phytoplankton taxa. No parameterization of the modified Monod equation was applied to *Polarella glacialis* (dinoflagellate) because of the highly variable response between the winter and summer populations. The winter population appeared to be considerably more sensitive to [H⁺] than the summer population with a much smaller [H⁺] window of maximum growth. Reaction norm variability between strains was also observed in *Ceratium* and *Prymnesium* (Fig. 2). The three *Prymnesium parvum* strains (haptophyte) and *Chlorella vulgaris* (chlorophyte) appeared to be robust up until at least 100 nmol l⁻¹ H⁺ (*c.* pH_F = 7, Fig. 2), with peak growth rates in *Chlorella vulgaris* reported at 1000 nmol l⁻¹ H⁺ (i.e. pH_F = 6, Mayo, 1997).

Universality of optimum curve reaction norm

Calcifying haptophytes are thought to be particularly sensitive to OA because of the formation of their calcium carbonate shells becomes increasingly energetically costly as seawater [H⁺] increases (Monteiro *et al.*, 2016). Here, highly taxonomically distinct

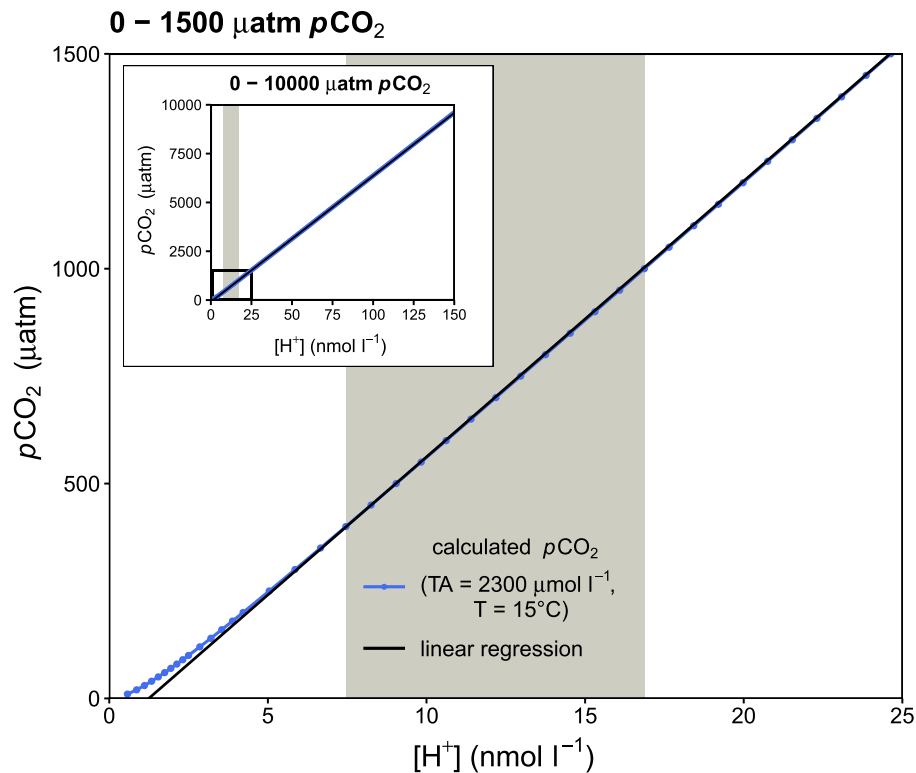


Fig. 1 Relationship between hydrogen ion concentration $[H^+]$ and pCO_2 below 1500 μatm with the ocean acidification (OA) relevant range highlighted in grey ($pCO_2 = 400\text{--}1000 \mu\text{atm}$). The inset shows a wide range of pCO_2 . This shows that the relationship between H^+ and pCO_2 is generally linear apart from small deviations below 5 $\text{nmol l}^{-1} H^+$. The carbonate system was calculated with $T = 15^\circ\text{C}$, $S = 35$, $[PO_4^{3-}]$ and $[SiO_4^{2-}] = 0 \mu\text{mol kg}^{-1}$, and total alkalinity (TA) = 2300 $\mu\text{mol kg}^{-1}$ using Co2sys software (Pierrot *et al.*, 2011) with the dissociation constants of Millero *et al.* (2006).

phytoplankton taxa showed the same optimum curve reaction norm over a large range of H^+ concentrations. Sensitivity between phytoplankton groups with respect to inorganic carbon substrate and H^+ inhibitor was, however, highly variable (Fig. 2). Based on the evidence provided herein we argue that the optimum curve reaction norm will be found in every phytoplankton tested given that the applied pCO_2 range (under constant TA) is wide enough. Therefore, we suggest that the optimum curve response should be regarded as universal and be the underlying default reaction norm for phytoplankton growth under increasing CO_2 at constant TA.

Growth rates can be considered an integrative indicator of organism fitness and cell function (Boyd *et al.*, 2013). Until a certain threshold, growth increases with increasing CO_2 (left side of the optimum) until the inhibitory behaviour presumably of H^+ becomes the dominant driver of the reaction norm (right side of the optimum). This response has been shown for coccolithophores and to some extent for diatoms and dinoflagellates (Bach *et al.*, 2011; McMinn *et al.*, 2014; Kottmeier *et al.*, 2016; Shi *et al.*, 2019) but it still needs confirmation in other species. Furthermore, the reaction norm is likely expandable to many other responses of physiological rates such as calcification (Langer *et al.*, 2006; Bach *et al.*, 2011, 2015; Gafar *et al.*, 2019), maybe even to other biota such as corals (Ries *et al.*, 2010), as H^+ is an essential molecule for signalling and metabolic control in cells (Taylor *et al.*, 2012). It is intriguing to think this response pattern in marine autotrophic organisms in general may be present for the same reason as coccolithophores.

The physiological explanations for this H^+ inhibition of growth may be similar between phytoplankton groups, even if the sensitivity to H^+ is different. For example, voltage gated proton (H_v) channels are protein complexes which can regulate cell pH homeostasis under rapid H^+ production in coccolithophores (Taylor *et al.*, 2011) and their function is highly sensitive to the cross-membrane electrochemical, or H^+ concentration, gradient (DeCoursey, 2008). These channels are also present in the genomes of a wide variety of phytoplankton taxa including diatoms, dinoflagellates, chlorophytes (Taylor *et al.*, 2012) although absent in prasinophytes (Taylor *et al.*, 2011). Hence their relative ubiquity may explain the general H^+ growth inhibition response observed in diverse phytoplankton taxa. Nevertheless, cells may adopt different physiological strategies to maintain H^+ efflux across the plasmalemma. Two possible mechanisms are (1) increased energetic investment in active H^+ efflux to maintain cytosol pH or (2) adjustment of the cytosol pH or membrane potential to maintain the passive cross-membrane electrochemical gradient (Taylor *et al.*, 2012). The latter has been identified in diatom species under a moderate pH decrease from 8.1 to 7.8 (Shi *et al.*, 2019). These species (*Phaeodactylum triconutum*, *Chaetoceros muelleri*) also demonstrated the largest increase in growth to decreasing medium pH. This suggests that different strategies in maintaining H^+ efflux may also underlie species-specific differences in growth rates inhibition by H^+ . Particularly high $[H^+]$ tolerance was observed for taxa within chlorophytes and noncalcifying haptophytes (here,

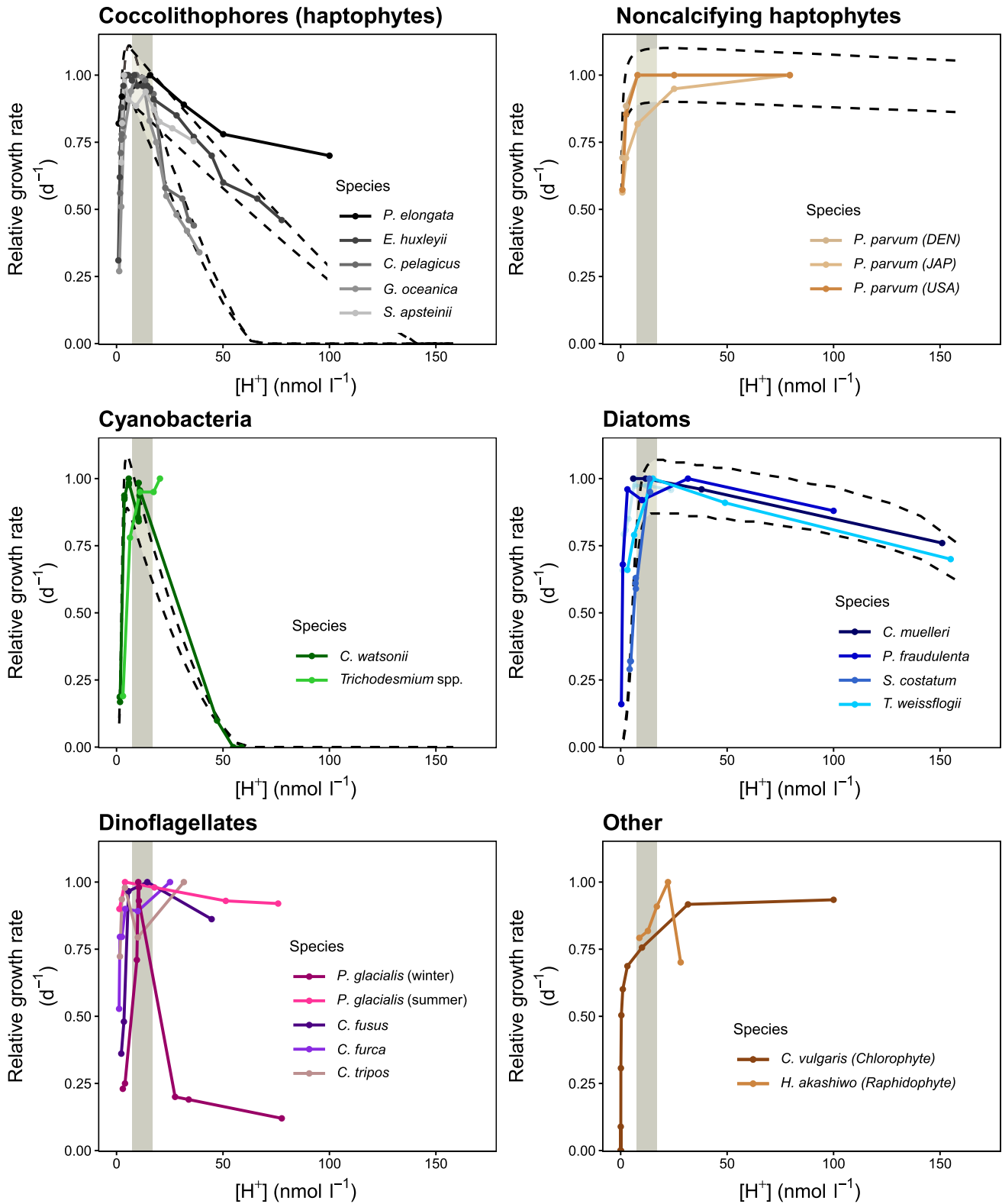


Fig. 2 Relative growth rate of different phytoplankton species from six phytoplankton groups across a wide range of H⁺ concentrations ([H⁺]). The grey vertical bar indicates the range of [H⁺] within the ocean acidification relevant range (pCO₂ = 400–1000 μatm). The black dotted lines represent the model fit of the modified Monod equation to selected species with a range of ± 10% (Supporting Information Table S2). Note that two of three replicates of *Crocospaera watsonii* (cyanobacteria) died in the highest [H⁺] treatment. Data sources are reported in Table S3(a–h).

Prymnesium parvum) groups. Variable sensitivity within the dinoflagellate taxa to both low and high $[H^+]$ is perhaps not surprising due to their diverse evolutionary history and trophic strategies (Taylor *et al.*, 2009). Indeed, a link was recently established between carbon concentrating mechanism plasticity and the evolution of phytoplankton groups (Van de Waal *et al.*, 2019). While there is still insufficient data, we also expect such a link between the organism evolutionary histories or physiological traits and the reaction norm shape (Fig. 3). This hypothesis warrants further investigation.

Potential application of the optimum-curve response pattern to predict phytoplankton growth rates in the future ocean

This literature compilation reveals variability in taxon-specific sensitivities that could be described in the simple empirical model (Fig. 2). Similar descriptions of taxon-related differences phytoplankton growth optima across large ranges in temperature (Eppley, 1972) and light (Goebel *et al.*, 2008) have a long history and have had a large influence on planktonological research. For example, temperature optimum curves from individual species form the basis of a generalized growth rate–temperature relationship ('Eppley Curve', Eppley, 1972), knowledge of considerable value to the modelling community (e.g. Thomas *et al.*, 2012). Indeed, most scientists would probably agree that a complete temperature curve is more informative than comparing two temperature treatments, even though large parts of the response curve may be outside of the range organisms experience in their natural habitat. This underlines the value of our proposed

experimental approach to explore physiological boundary conditions for phytoplankton groups in response to seawater pCO_2 , in contrast to selecting two or three CO_2 levels within the OA relevant range. We would expect taxon-specific ranges in CO_2 optima (Fig. 3) could be used to better understand shifts from one taxon to another in an acidifying ocean, as has been proposed for coccolithophores (Ridgwell *et al.*, 2009). For example, using the approach of Dutkiewicz *et al.* (2015), this framework for phytoplankton taxa sensitivity could be integrated into ecological models as an extension of the Monod model currently employed. Alternatively, the static model could be embedded in a dynamic energy budget to scale the response of individual phytoplankton physiology to population dynamics at an ecosystem level, similar to Muller (2011).

We suggest this approach may be a particularly beneficial strategy for predicting the response of primary producers to projected changes in the marine environment. Studies investigating a CO_2 range well beyond what is relevant for OA could also be designed to probe interactive effects of CO_2 with other environmental factors. Addition of light, temperature, or macro/micro-nutrient treatments would reveal how these parameters modify the CO_2 response curve. While these environmental drivers may change the shape, the general optimum curve response pattern appears conserved (Sett *et al.*, 2014; Bach *et al.*, 2015; Gafar *et al.*, 2019) and applicable to strains tested from different populations (Zhang *et al.*, 2018).

The workload using this experimental approach due to the higher number of treatment levels required may be considered impractical. Yet, most studies in our literature analysis used three or four treatments in triplicate. Distributing the same number of

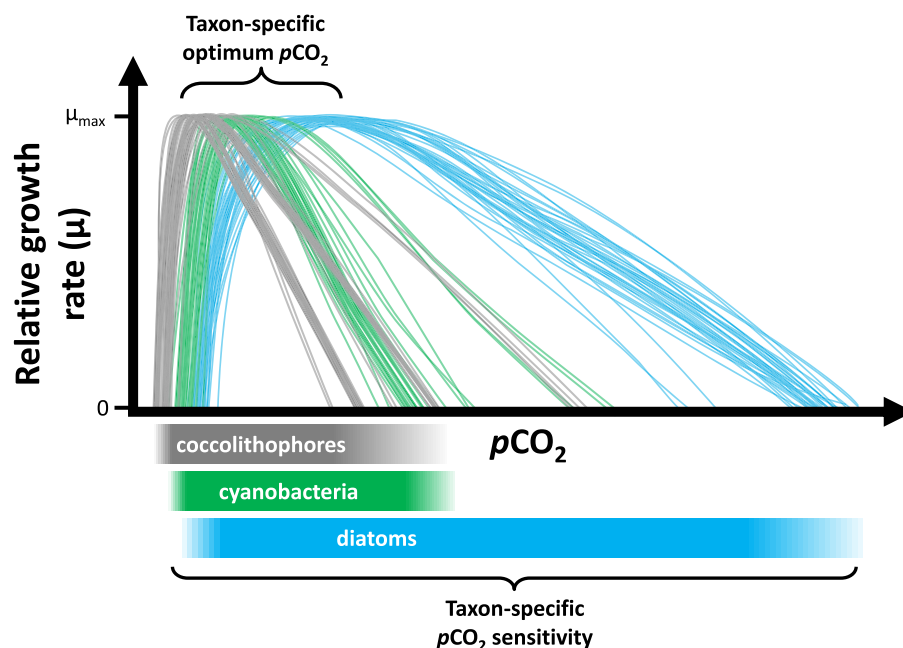


Fig. 3 Conceptual figure of what multiple pCO_2 reaction norms for a variety of species/taxa could look like if there were sufficient datasets. Each line represents the reaction norm (optimum curve) of a different species or strain and shows intra-taxon variability, where some genotypes may be more or less sensitive to CO_2 . The reaction norms from the three taxa are shown here are used to demonstrate the optimum response curve concept, an Eppley curve-like equivalent for carbonate chemistry (Ridgwell *et al.*, 2009), and are loosely based on results from the literature presented in Fig. 2.

bottles across individual treatment levels would give nine to 12 distinct treatments, which would have been much more informative with minimal difference in work load. Hence, here we hope to provide an impetus to reignite enthusiasm for this mechanistic approach to characterize phytoplankton reaction norms to CO₂. By increasing the number of treatments, here CO₂, at the expense of replication enables quantification of the response as well as allowing for statistically robust description of nonlinear response patterns (Cottingham *et al.*, 2005) thereby deepening our understanding of carbonate chemistry control of phytoplankton physiology.


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Author contributions



AJP carried out the data analysis and the culture study on *Crocospaera watsonii*. AJP and LTB discussed and developed the concept of this manuscript, completed the literature search and wrote the manuscript.

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Data availability

All data sources used in this study are compiled in the Supporting Information (Table S3a–h) or are available in the original literature. Previously unpublished data for *Crocospaera watsonii* included in this study is openly available in the PANGAEA Data Publisher website (<https://doi.org/10.1594/PANGAEA.919891>).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Growth rates of *Emiliana huxleyi* as a function of CO₂ concentration.

Fig. S2 Growth rates of three dinoflagellate species as a function of dissolved inorganic carbon (DIC) concentration.

Fig. S3 Growth rates of three dinoflagellate species as a function of CO₂ concentration from constant pH experiments.

Fig. S4 Growth rates of three dinoflagellate species as a function of CO₂ from pH drift experiments.

Methods S1 Culture conditions and growth rate determination for *Crocospaera watsonii*.

Methods S2 Carbonate chemistry (total alkalinity, dissolved inorganic carbon concentration)

Notes S1 Evidence for CO₂ as driver of growth rates at low pCO₂.

Table S1 Summary of final and initial cell densities, total incubation time, calculated growth rate and pH in *Crocospaera watsonii* culture experiment.

Table S2 Summary of modified Monod equation parameters determined for selected phytoplankton taxa.

Table S3 List of studies and corresponding experiment design details found in the literature reporting on the response of phytoplankton taxa to CO₂/pH.

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Key words: carbonate chemistry, Monod kinetics, ocean acidification, optimum response curve, phytoplankton growth, reaction norm.

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