



# Growth response of *Emiliana huxleyi* to ocean alkalinity enhancement

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## Abstract.

The urgent necessity of reducing greenhouse gas emissions is coupled with a pressing need for widespread implementation of carbon dioxide removal (CDR) techniques to limit the increase in mean global temperature to levels below 2°C compared to pre-industrial times. One proposed CDR method, Ocean Alkalinity Enhancement (OAE), mimics natural rock weathering processes by introducing suitable minerals into the ocean thereby increasing ocean alkalinity and promoting CO<sub>2</sub> chemical absorption. While theoretical studies hold promise for OAE as a climate mitigation strategy, careful consideration of its ecological implications is essential. Indeed, the ecological impacts of enhanced alkalinity on marine organisms remain a subject of investigation as they may lead to changes in species composition. OAE implicates favourable conditions for calcifying organisms by enhancing the saturation state of calcium carbonate and decreasing the energetic costs for calcification. This may affect marine primary production by improving conditions for calcifying phytoplankton, among which coccolithophores play the leading role. They contribute <10% to the global marine primary production, but are responsible for a large proportion of the marine calcite deposition. While previous research has extensively studied the effects of ocean acidification on coccolithophores, fewer studies have explored the impacts of elevated pH and alkalinity. In this context, we studied the sensitivity of *Emiliana huxleyi*, the most widespread coccolithophore species, to ocean alkalinity enhancement in a culture experiment. We monitored the species' growth and calcification response to progressively increasing levels of total alkalinity (TA). Above a change in total alkalinity ( $\Delta$ TA) of  $\sim 600 \mu\text{mol kg}^{-1}$ , as CO<sub>2</sub> concentrations decreased, *E. huxleyi* growth rate diminished, suggesting a threshold CO<sub>2</sub> concentration of  $\sim 100 \mu\text{atm}$  necessary for optimal growth. The cellular calcite to organic carbon ratio (PIC:POC) remained stable over the total alkalinity range. Due to the decreasing growth rate in response to alkalinity enhancement, total carbonate formation was lower. OAE is rapidly advancing and has already reached the field-testing stage. Hence, our study contributes to the most critical part of investigations required to comprehend potential biological implications before large-scale OAE will be adapted.

## 1 Introduction

Governments have recognized the need to constrain human-induced climate change and declared to keep the mean global temperature rise well below 2°C compared to pre-industrial values to prevent dangerous consequences associated with a further



30 increase in temperature (United Nations Framework Convention on Climate Change, 2015). Currently, the rate at which  
greenhouse gas emissions are being cut is slow and insufficient to meet this target, therefore the use of negative emission  
technologies (NET) to remove atmospheric CO<sub>2</sub> is expected to be inevitable in supporting efforts to limit global temperature  
rise (IPCC, 2021). One proposed method to remove CO<sub>2</sub> from the atmosphere mimics the natural process of rock weathering,  
whereby suitable minerals are extracted and introduced into the surface ocean (Ocean Alkalinity Enhancement, OAE; Gattuso  
35 et al., 2015; GESAMP, 2019). The dissolution of the respective minerals consumes protons and conservative cations are  
released, which leads to a shift of the carbonate chemistry species from CO<sub>2</sub> towards HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>, thus to an increase in  
alkalinity (Wolf-Gladrow et al., 2007; Bach et al., 2019). As a result, the seawater becomes undersaturated with CO<sub>2</sub>, which  
is then balanced by additional absorption of atmospheric CO<sub>2</sub>. Hence through OAE, CO<sub>2</sub> is chemically absorbed and  
subsequently safely stored mainly as bicarbonate over geological timescales (e.g. Hartmann et al., 2013).

40 Theoretical studies indicate that OAE has the potential for large scale application to absorb gigatonnes of carbon from the  
atmosphere and thus contribute to global climate change mitigation efforts (e.g. Keller et al., 2014; Renforth and Henderson,  
2017; Taylor et al., 2016). This provides a basis for testing the applicability, efficiency and ecological impacts of this approach  
in practice. Key factors in this context are the impacts of the seawater chemistry changes arising from enhanced alkalinity on  
primary production and biogenic calcification. In a recent paper Bednaršek et al. (2024), offered a conceptual synthesis of the  
45 responses of marine calcifiers to OAEs, utilizing insights from multiple ocean acidification (OA) studies. This study evidenced  
that only 40% of the analysed groups show a neutral response upon alkalinity addition. For marine microbes that rely on  
dissolved carbon to fuel primary production the shifts in seawater carbonate chemistry are relevant for their physiological  
processes. Below certain thresholds, dissolved CO<sub>2</sub> concentrations can indeed limit phytoplankton growth and lead to reduced  
primary production (Riebesell et al., 1993). In addition, OAE leads to beneficial changes in carbonate chemistry for biogenic  
50 calcification due to an increased pH and carbonate saturation state and thus may promote calcifying organisms (Bach et al.,  
2019; Bednaršek et al., 2024). The formation of calcium carbonate, however, consumes alkalinity and is a CO<sub>2</sub> source (Zeebe  
and Wolf-Gladrow, 2001), so increased calcification rates would counteract CO<sub>2</sub> drawdown through OAE.

Coccolithophores are the most important calcifiers in marine phytoplankton. They contribute significantly to the marine  
primary production in the surface ocean and the deposition of calcite in deep-sea sediments (Poulton et al., 2007; Broecker  
55 and Clark, 2009). In the last decades, many studies investigated the impact of OA on the growth and calcification of  
coccolithophores (e.g. Riebesell et al., 2000 Langer et al., 2006; Bach et al., 2013; Faucher et al., 2020; Paul and Bach, 2020)  
and evidenced the sensitivity of these organisms to changes in carbonate chemistry. Increased *p*CO<sub>2</sub> and lower pH induce  
significant effects on the growth and calcification of coccolithophores (e.g. Riebesell et al., 2000; Beaufort et al., 2011; Bach  
et al., 2011, 2015). Most efforts focused on manipulating the level of *p*CO<sub>2</sub> rather than alkalinity. Only a few of them  
60 investigated the effect of higher pH and lower *p*CO<sub>2</sub> on these organisms (e.g. Bach et al., 2015; Langer et al., 2006) indicating  
a reduction in the growth rate of the species tested when *p*CO<sub>2</sub> is below ~ 100 µatm (Sett et al., 2014; Bach et al., 2011; 2015)  
and offered first insights into the potential effects of OAE on coccolithophore growth.



OAE is based on adding alkalinity through different approaches, which raises carbonate chemistry parameters in various ways. Recently, Gately et al. (2023) showed a neutral response of two phytoplankton species, the coccolithophore *Emiliana huxleyi* and the diatom *Chatoceros* sp. to OAE in an experiment where the culture media were bubbled for some days to equilibrate with the atmosphere before the algae were inoculated. This approach avoided a strong perturbation of the carbonate chemistry (i.e. avoidance of pH spikes and  $p\text{CO}_2$  limiting conditions). Their results show that even in the highest alkalinity treatment ( $\Delta\text{TA} \sim 2700 \mu\text{mol kg}^{-1}$ ), the  $p\text{CO}_2$  was above  $300 \mu\text{atm}$  and the pH only slightly increased by 0.4 unit. The apparent resilience of these species to increased alkalinity in the study from Gately et al. (2023) is encouraging considering a potential application of OAE. However, in a non-equilibrated OAE approach where atmospheric equilibration of  $\text{CO}_2$  is prevented (*sensu* Hartmann et al., 2022; Suitner et al., 2023), the carbonate chemistry shifts towards lower  $p\text{CO}_2$  and higher  $[\text{CO}_3^{2-}]$  and pH. The impact of these more extreme conditions on marine calcifiers, as coccolithophores, has not yet been sufficiently investigated in order to assess the effects of ocean OAE on marine primary production.

*Emiliana huxleyi* is the most abundant and widespread coccolithophore species in the modern ocean (Westbroek et al., 1993) and served as a model species in plenty of studies that investigated the impact of ocean acidification on coccolithophores (see Wheeler et al., 2023). Bednaršek et al. (2024) concluded that *E. huxleyi* stood out as the sole coccolithophore species not displaying a neutral effect of increased alkalinity on calcification.

In this study, we tested the response of *E. huxleyi* in growth and calcification under progressively increasing total alkalinity (TA) levels. The increase in alkalinity was achieved by adding sodium hydroxide (NaOH) solution to the culture medium. The medium was not allowed to equilibrate with the atmosphere after NaOH addition and the response of *E. huxleyi* to decreasing  $\text{CO}_2$  concentrations and increasing pH due to enhanced alkalinity was investigated. The study aimed to test whether i) the growth rate of *E. huxleyi* decreases under reduced  $\text{CO}_2$  concentrations due to enhanced alkalinity and ii) the carbonate chemistry conditions under increasing TA (i.e. increasing  $[\text{HCO}_3^-]$ , lower  $[\text{H}^+]$ ) that are conducive to calcification, lead to physiological disbalances between calcification and growth.

## 2 Materials and Methods

### 2.1 Culture conditions and experimental design

Monospecific cultures of *Emiliana huxleyi* (B92/11) were grown in batch cultures at low biomass ( $< 50000 \text{ cell mL}^{-1}$ ) to avoid a significant impact of biological processes on the chemical conditions of the growth medium (LaRoche et al., 2010). *Emiliana huxleyi* was grown at  $15^\circ\text{C}$  in a photoperiod of 16:8 hours light to dark and a photon flux of  $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$  (measured with a Li-Cor, HeinzWalz GmbH, Effeltrich). The growth medium was based on an artificial seawater (Kester et al., 1967) with a salinity of 34, which was enriched with  $64 \mu\text{mol kg}^{-1} \text{ NaNO}_3$ ,  $4 \mu\text{mol kg}^{-1} \text{ NaHPO}_4$ , and trace metals and vitamins according to the *f/8* medium (Guillard and Ryther, 1962). In addition,  $10 \text{ nmol kg}^{-1} \text{ SeO}_2$  was added (Danbara and Shiraiwa, 1999) as well as  $2 \text{ mL kg}^{-1}$  of  $0.2 \mu\text{m}$  filtered natural seawater to prevent potential growth limitation of *E. huxleyi* due to other substances not included in the *f/8* receipt (Bach et al., 2011). The medium was filtered ( $0.2 \mu\text{m}$ ) into sterile  $0.6 \text{ L}$  polycarbonate bottles



95 where different amounts of NaOH (1M) solution were added to set up an alkalinity gradient among the replicates. The alkalinity  
gradient was set up across 16 distinct treatments, with intervals of 50-100  $\mu\text{mol kg}^{-1}$  from ambient TA levels of 2350, up to  
~3500  $\mu\text{mol kg}^{-1}$ . After the addition of NaOH, the bottles were sealed gastight and the headspace inside was kept as low as  
possible to minimize gas exchange. Small volumes of a pre-culture with exponentially growing *E. huxleyi* were pipetted into  
the bottles to meet target cell concentrations of around 100 cells  $\text{mL}^{-1}$ . The cells were acclimated for 7-9 generations to the  
100 respective alkalinity conditions and samples of 0.5 mL were taken at selected time points to monitor the concentration and  
growth of *E. huxleyi*. After the acclimation phase, a small volume of the cultures was transferred into a further set of  
polycarbonate bottles (2.7 L) filled with fresh NaOH-manipulated medium. Cells were homogenized by gentle rotation and  
samples for TA, dissolved inorganic carbon (DIC), nutrients and cell abundance were taken. Thereupon, the content of the  
bottles was gently transferred into smaller (2 L) polycarbonate bottles for the main experiment. Bottles were filled from bottom  
105 to top using a hose and a funnel to limit gas exchange and avoid bubble formation. The headspace inside the bottles was kept  
at  $< 5$  mL. Bottles were regularly gently rotated to keep the cultures in suspension. The growth period during the main  
experiment was estimated from the respective cell concentrations determined during the acclimation phase. Cells were  
harvested at low biomass to keep the change in dissolved inorganic carbon (DIC) due to the buildup of biogenic carbon below  
4% over the growth period. Samples for cell concentrations, particulate organic (POC) and inorganic carbon (PIC) content,  
110 and nutrient concentrations were taken at the end of the experiment.

## 2.2 Determination of the carbonate system

Samples for TA and DIC measurements were 0.2  $\mu\text{m}$  filtered with low pressure, poisoned with a saturated  $\text{HgCl}_2$  solution (0.5  
% final concentration) and stored at  $4^\circ\text{C}$  until analysis. TA concentrations were determined by potentiometric titration with a  
Metrohm Compact Titrosampler 862 and corrected with certified reference material (A. Dickson, Scripps Institution of  
115 Oceanography, La Jolla, California; Dickson et al., 2003). DIC concentrations were measured with an Automated Infrared  
Inorganic Carbon Analyzer (AIRICA) equipped with a LICOR (LI-7000  $\text{CO}_2/\text{H}_2\text{O}$  analyzer) detector and corrected with the  
certified reference material. The carbonate system parameters were calculated from salinity, temperature, TA, and DIC using  
the program CO2SYS (Pierrot et al., 2006) with equilibrium constants from (Mehrbach et al., 1973), refit by (Dickson and  
Millero, 1987).

## 120 2.3 Determination of nutrient and carbonate system

0.5 mL samples were transferred into Eppendorf tubes and the cell concentrations of *E. huxleyi* were measured with a flow  
cytometer (Accuri C6, Becton Dickinson), equipped with a 488 nm diode laser at a flow rate of  $66 \mu\text{L min}^{-1}$ . Cells were  
determined based on their red fluorescence signal ( $> 670$  nm) and forward light scatter. Daily growth rates ( $\mu$ ,  $\text{d}^{-1}$ ) were  
determined using Eq. (1):

$$125 \quad \mu = \ln(N_t/N_0)/\Delta t, \quad (1)$$



where  $N$  denotes the cell concentration of *E. huxleyi* and  $t$  represents the time in days.

#### 2.4 Particulate inorganic (PIC) and organic carbon (POC)

Samples for total particulate carbon (TPC) and particulate organic carbon (POC) were obtained by gentle filtration of the cultures through combusted (500°C for 5 hours) glass fiber filters (0.7  $\mu\text{m}$ ), which were stored at -20°C until analysis. POC filters were kept in a desiccator with fuming hydrochloric acid (37 %) for 2 hours to remove all particulate inorganic carbon (PIC). After that TPC and POC filters were dried overnight at 60°C and their carbon content was measured in an elemental analyzer (Euro EA, Eurovector). The amount of PIC was determined as the difference between TPC and POC. PIC and POC production rates were calculated by multiplication of the respective cellular content with  $\mu$ . The ratio of PIC to POC is indicative of the cellular degree of calcification.

#### 2.5 Statistical analyses and data fitting

The data were analysed using a nonlinear regression model. Numerous treatment levels were established without replication, aiming to provide quantitative insights for ecological modelling while preserving statistical power (Cottingham et al., 2005). The coefficients of determination ( $R^2$ ) and p-values for the regression model are presented in the results section. The Monod equations were used to analyse the growth rate, chlorophyll *a* (Chl *a*) and calcification, relating to resource concentration (e.g. Riebesell et al., 1993; Rost et al., 2003). The Monod function is defined as Eq. (2):

$$V = V_{\text{max}} [R/(R + k_{\text{max}})],$$

where  $V$  represents the growth rate (or POC or PIC),  $V_{\text{max}}$  is the maximum growth rate (or POC or PIC) at high, non-limiting concentrations,  $R$  is the resource concentration in the environment, and  $k$  is the half-saturation constant, the resource concentration at which the growth rate is half of its maximum value. All statistics and curve-fitting were conducted in Rstudio 2022.12.0 (R packages “drc” and “ggplot2”; RStudio team, 2020).



### 3 Results

#### 3.1 TA manipulation and carbonate chemistry

The addition of NaOH led to a steady TA increase among the culture bottles up to a concentration of  $\Delta 1200 \mu\text{mol kg}^{-1}$  (Fig. 1a). The measured TA values at the beginning of the experiment were slightly lower than the target concentrations (Fig. 1a; Tab. 1). The deviation from target concentrations increased with increasing concentrations of NaOH added. The TA values at the end of the experiment were slightly lower in most treatments. Also, the DIC concentrations show some minor fluctuations from the beginning to the end of the experiment (Fig. 1b).

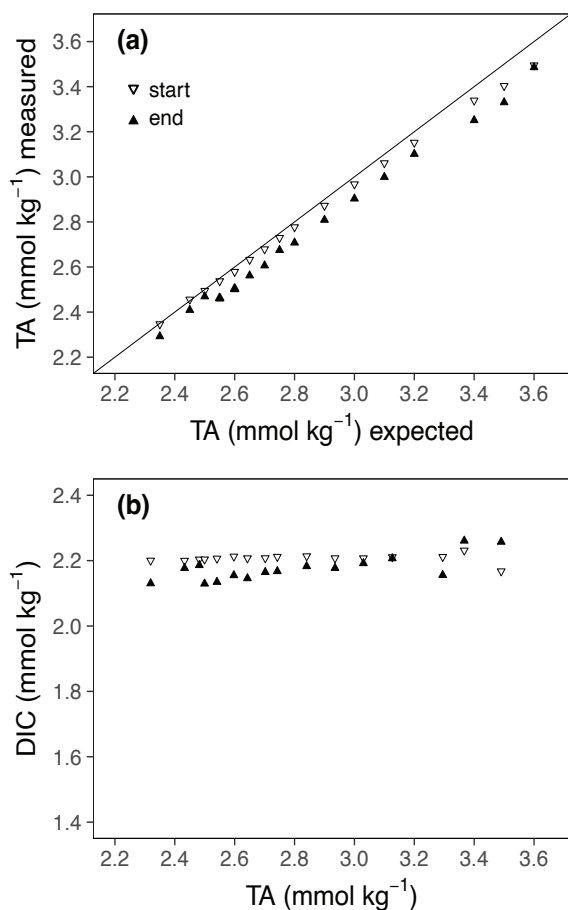


Figure 1 (a) Comparison of targeted and measured TA values at the start and end of the experiment. (b) TA:DIC diagrams at the start and end of the experiments.



The effects of increasing TA on the carbonate chemistry parameters are shown in Fig. 2 and Table 1. The concentrations of  $\text{CO}_2$  [ $\text{CO}_2$ ], and bicarbonate ions [ $\text{HCO}_3^-$ ] decreased with increasing TA (Fig. 2a, b), whereas the carbonate ion concentrations [ $\text{CO}_3^{2-}$ ] increased (Fig. 2c). The saturation state of calcite ( $\Omega_{\text{calcite}}$ ) increases with increasing TA from 2.7 in the lowest to 24 in the highest treatment (Table 1).

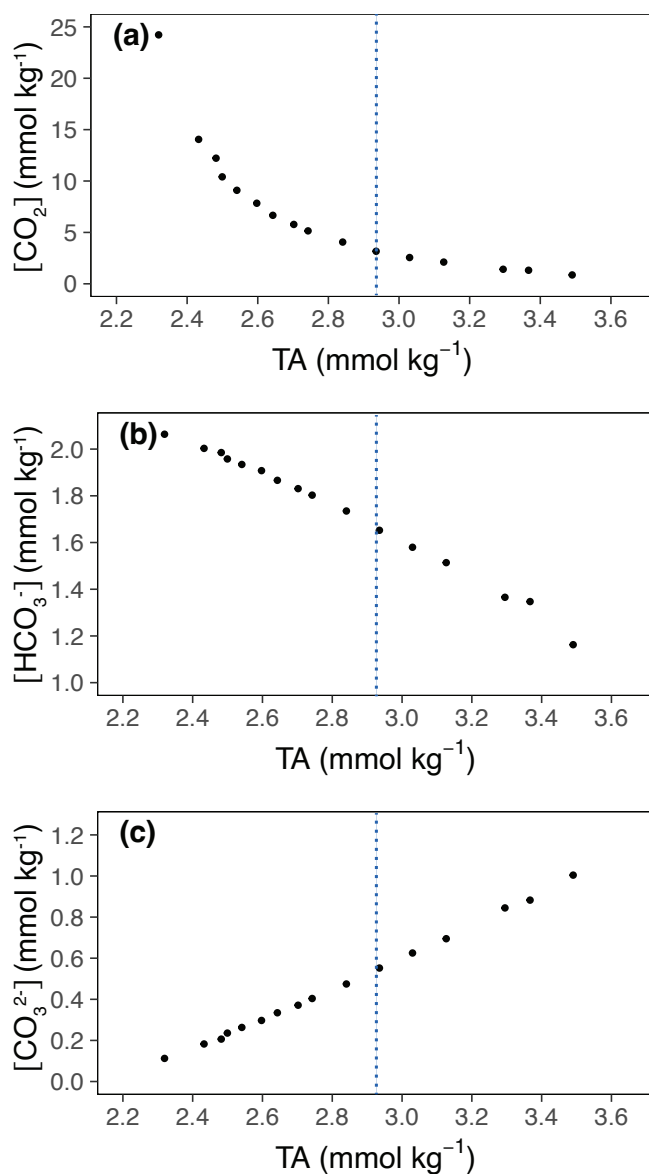


Figure 2 Relevant carbonate chemistry parameters in relation to TA. TA is the average of the values at the start and at the end of the experiment. (a) [ $\text{CO}_2$ ]; (b) [ $\text{HCO}_3^-$ ]; (c) [ $\text{CO}_3^{2-}$ ]. The blue lines represent  $\Delta\text{TA } 600 \mu\text{mol Kg}^{-1}$  which is the level of alkalinity beyond which *E. huxleyi* shows a decline in growth.

### 3.2 Physiological response of *E. huxleyi*

The growth rate ( $\mu$ ) of *E. huxleyi*, the production of POC and PIC, decreased with increasing TA (Table 2; Fig. 3a-c). There was no significant effect of TA on the ratio of PIC to POC ( $R^2 = 0.003$ ,  $p = 0.806$ ) (Table 2; Fig. 3d). The growth rate declined with decreasing  $[\text{CO}_2]$  and was analyzed using the Monod equation (Fig. 4a). The fit predicts a maximum growth rate of  $1.24 \text{ day}^{-1}$  (confidence interval (CI):  $1.1 - 1.38 \text{ d}^{-1}$ ) with a residual standard error of  $0.09 \text{ d}^{-1}$  (14 df), and a half-velocity constant  $K_s = \frac{1}{2} \mu_{\text{max}} = 2.04 \mu\text{mol CO}_2 \text{ kg}^{-1}$ . POC production was a function of  $\mu$  and likewise decreased with declining  $[\text{CO}_2]$  (Table 2; Fig. 4b). The corresponding saturation function results in a maximum POC production rate of  $21.10$  (CI:  $12.43 - 29.77$ )  $\text{pg C cell}^{-1} \text{ day}^{-1}$  with a residual standard error of  $3.48 \text{ pg C cell}^{-1} \text{ d}^{-1}$ , and a half saturation constant of  $5.02 \mu\text{mol kg}^{-1} \text{ CO}_2$ . The PIC production rate generally decreased with decreasing  $[\text{HCO}_3^-]$  (Fig. 4c;  $R^2 = 0.40$ ,  $p < 0.01$ ) and followed the same pattern of the saturation function plotted over the  $[\text{CO}_2]$  (Fig. 4d). The Monod equation predicts a maximum PIC production rate of  $9.7 \text{ pg C cell}^{-1} \text{ d}^{-1}$  (CI:  $5.89 - 13.52$ )  $\text{pg C cell}^{-1} \text{ day}^{-1}$  with a residual standard error of  $2.58 \text{ pg C cell}^{-1} \text{ d}^{-1}$ .

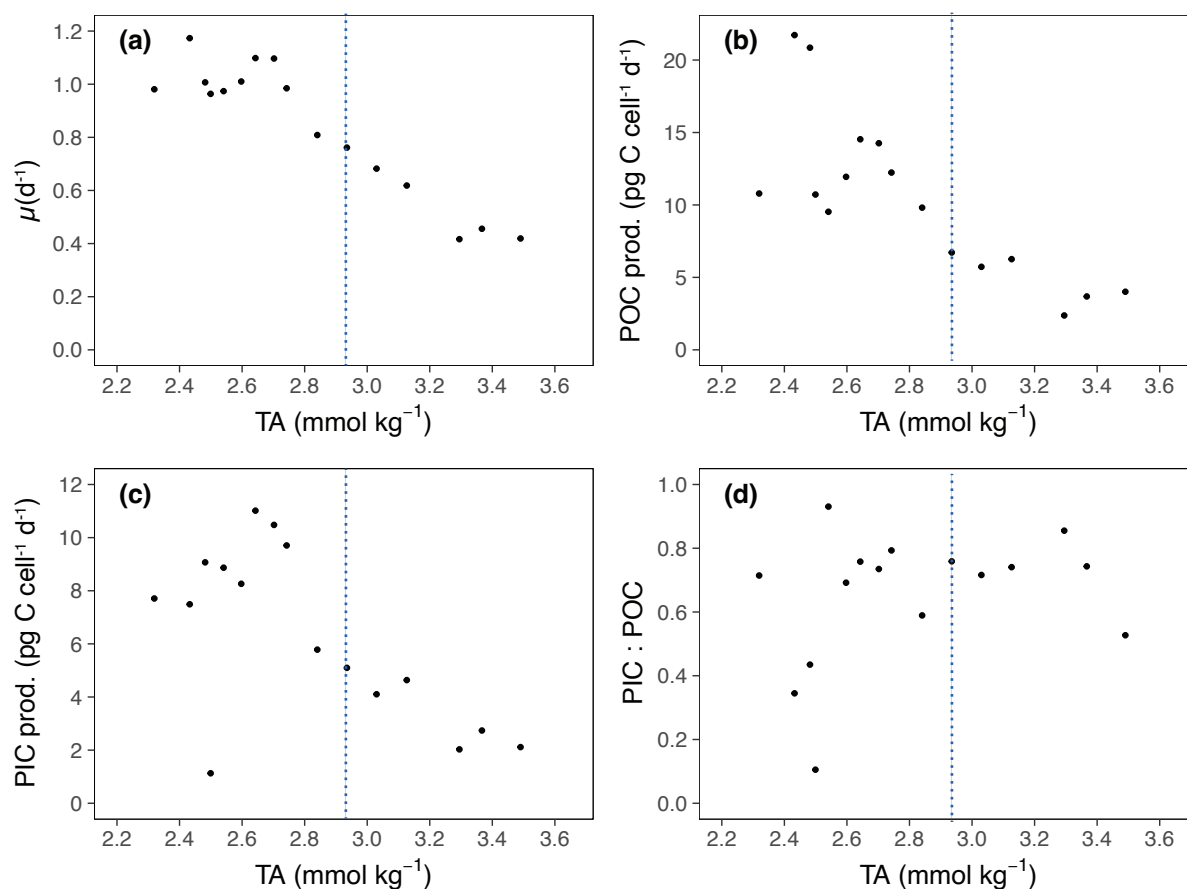


Figure 3 Physiological response parameters for *Emiliania huxleyi* in relation to TA. TA is the average of the values at the start and at the end of the experiment. (a) Growth rates ( $\mu$ ). (b) particulate organic carbon production (POC prod.). (c) particulate inorganic carbon production (PIC prod.). (d) PIC:POC ratio. The blue lines represent  $\Delta\text{TA } 600 \mu\text{mol Kg}^{-1}$  which is the level of alkalinity beyond which *E. huxleyi* shows a decline in growth.





## 175 4 Discussion

This experiment was conducted according to guidelines of best practice in Ocean Alkalinity research (Oschlies et al., 2023) that emphasise the importance of small-scale laboratory experiments (Iglesias-Rodríguez et al., 2023) to contribute to assess an environmental safety approach of OAE. Bottle experiments are considered essential to comprehend the responses of keystone species to various OAE application scenarios, enabling the understanding of the physiological performance of model  
180 species and the identification of potential tipping points in OAE.

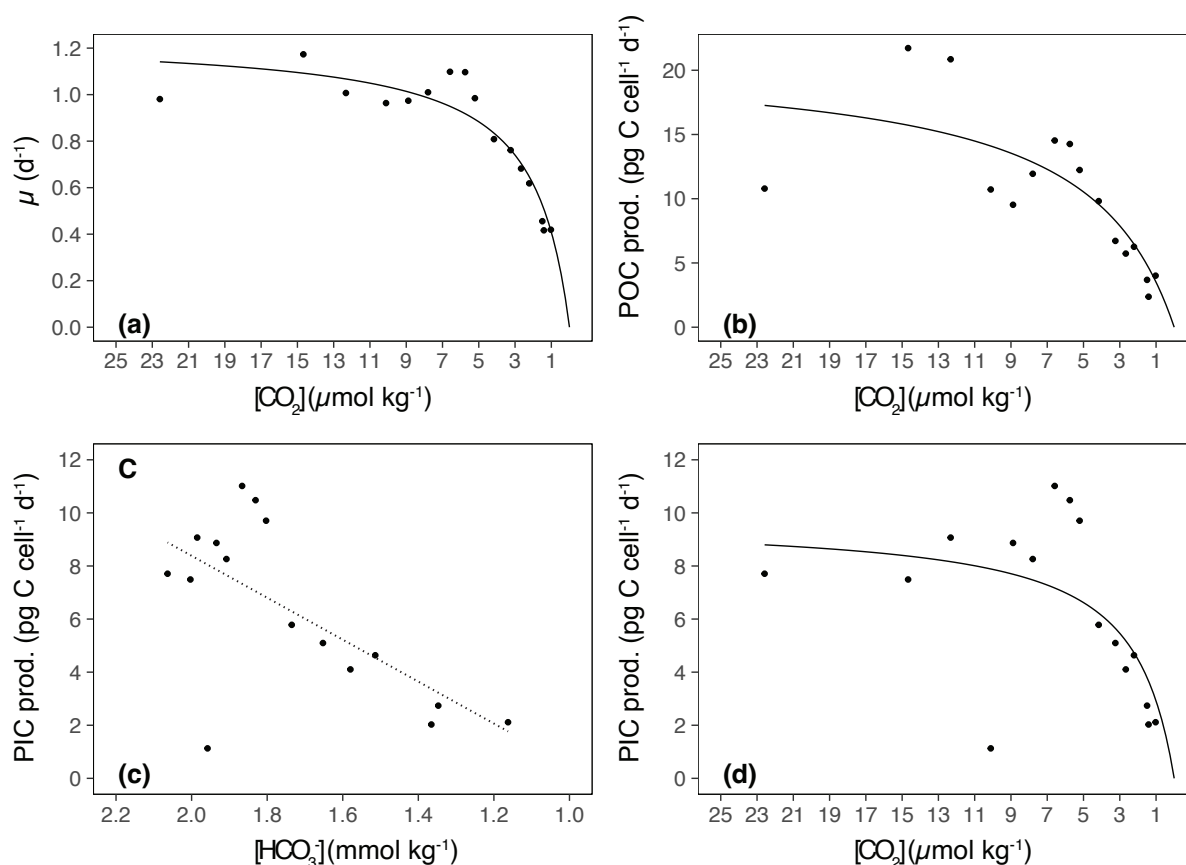


Figure 4 Growth rates, cellular POC and PIC productions in relation to  $[\text{CO}_2]$  and  $[\text{HCO}_3^-]$ . (a) growth rate ( $\mu$ ) in relation to  $[\text{CO}_2]$ . The solid line represents the Michaelis-Menten fit in the form of  $V = V_{\text{max}} * f\text{CO}_2 / K_{\text{max}} + f\text{CO}_2$  where  $V$  is the growth at a specific  $f\text{CO}_2$ ,  $V_{\text{max}}$  is the theoretical maximum of growth or production,  $K_{\text{m}}$  is the  $f\text{CO}_2$  at which the maximum is half saturated ( $R^2 = 0.87$ ,  $p < 0.0001$ ). (b) POC production (POC prod.) in relation to  $[\text{CO}_2]$ , using the Michaelis-Menten fit (in this case  $V$  is the POC at a specific  $f\text{CO}_2$ ,  $V_{\text{max}}$  is the theoretical maximum of production,  $K_{\text{m}}$  is the  $f\text{CO}_2$  at which the maximum is half saturated; ( $R^2 = 0.65$ ,  $p < 0.05$ )). (c) PIC production (PIC prod.) in relation to  $[\text{HCO}_3^-]$ . The fit according to the linear regression is given ( $R^2 = 0.40$ ,  $p < 0.01$ ). (d) PIC production (PIC prod.) in relation to  $[\text{CO}_2]$ , using the Michaelis-Menten fit (in this case  $V$  is the PIC at a specific  $f\text{CO}_2$ ,  $V_{\text{max}}$  is the theoretical maximum of production,  $K_{\text{m}}$  is the  $f\text{CO}_2$  at which the maximum is half saturated; ( $R^2 = 0.40$ ,  $p = 0.12$ )). The biological response data are plotted against the means of the initial and final  $[\text{CO}_2]$  and  $[\text{HCO}_3^-]$  values.



Among the different sources of alkalinity, the addition of NaOH in a non-equilibrated way increasingly gains attention due to its smaller environmental footprint and high dissolution rates (Hartmann et al., 2023; Iglesias-Rodríguez et al., 2023; Riebesell et al., 2023). The addition of alkalinity leads to a perturbation of the carbonate chemistry, the extent of which depends on the amount of alkalinity added.

185 In this study, the response of the biogeochemically important coccolithophore species, *E. huxleyi*, to increasing alkalinity was tested in a system, where the carbonate chemistry was changed towards the decline of the  $[\text{CO}_2]$  while the total concentration of DIC remained constant. Any gas exchange and equilibration with the atmosphere were excluded, simulating the pronounced carbonate chemistry perturbations that occur in the non-equilibrated transient state immediately after alkalinity addition.

Previous studies showed that the growth rate and POC production of coccolithophores are dependent on  $\text{CO}_2$  concentrations (e.g. Krug et al., 2011; Bach et al., 2011; 2013; 2015). Most of these studies provided limited data for conditions where pH is 190 (e.g. higher than 8.4 and  $[\text{CO}_2] < 6 \mu\text{mol kg}^{-1}$ ). These values are not unrealistic, as in natural environments, *E. huxleyi* can occasionally experience  $\text{CO}_2$  limitation in post-bloom phases when  $[\text{CO}_2]$  falls below  $7.5 \mu\text{mol kg}^{-1}$  (Bach et al., 2013). Similarly, some OAE scenarios predict even stronger  $\text{CO}_2$  limitation with values below  $5 \mu\text{mol kg}^{-1}$  (Hartmann et al., 2023; Suitner et al., 2023).

195 In our study, *E. huxleyi* exhibited strong reductions in growth and production of POC (below  $7 \text{ pg cell cell}^{-1} \text{ d}^{-1}$ ) starting at  $\sim \Delta\text{TA}600 \mu\text{mol kg}^{-1}$  ( $f\text{CO}_2 < 86 \mu\text{atm}$ ;  $[\text{CO}_2] < 3.1 \mu\text{mol kg}^{-1}$ ), suggesting a threshold below which *E. huxleyi* primary production was limited by  $[\text{CO}_2]$ . However, there was only a weak relationship between calcification rates and  $\text{HCO}_3^-$  concentration. Furthermore, the PIC:POC ratio remained stable with values always lower than 1.

Our study doesn't have the purpose of conceptually understanding the acquisition of DIC and its subsequent use in 200 photosynthesis and calcification under the perturbation induced by OAE. However, the knowledge gained from ocean acidification studies can help link some physiological mechanisms when *E. huxleyi* is subjected to low  $[\text{CO}_2]$ . Under low  $[\text{CO}_2]$ , the phytoplankton, to overcome the constraints inflicted by an inefficient carbon uptake and to maintain higher photosynthetic efficiency, have developed the so-called carbon concentration mechanisms (CCMs) that function to enhance the  $\text{HCO}_3^-$  concentration inside the cell and promote its conversion into  $\text{CO}_2$  (Badger et al., 1998). Bach et al., (2013) stated 205 that CCM might have evolved to help *E. huxleyi* deal with low rather than high  $\text{CO}_2$  levels. Indeed, several studies indicate that  $\text{CO}_2$  is the primary source of growth and photosynthesis and *E. huxleyi* upregulates its CCMs when  $\text{CO}_2$  decreases to avoid a shortage in  $\text{CO}_2$  (Rost et al., 2003; Bach et al., 2013).  $\text{HCO}_3^-$  is an additional inorganic carbon source for growth and Chl *a* production of *E. huxleyi* (Paasche, 1964; Rost et al., 2003; Schulz et al., 2007) under low  $[\text{CO}_2]$ . The system demonstrates constrained effectiveness, primarily due to elevated  $\text{CO}_2$  loss rates from leakage (Rost et al., 2006): observations indicate that 210 in coccolithophores,  $\text{CO}_2$  leakage escalates as  $[\text{CO}_2]$  levels fall below 20 PA (Trimborn et al., 2006) concomitant with a pronounced decline in growth rates and POC production. This pattern could explain the reduction in growth and POC observed in our study when  $[\text{CO}_2]$  is lower than  $100 \mu\text{mol kg}^{-1}$  ( $\Delta\text{TA} 600$ ).

It is also possible that the insufficient  $\text{CO}_2$  supply for growth and photosynthesis indirectly limits calcification. In principle the alkalinity addition, increased  $\Omega_{\text{calcite}}$  (Table 2) which should be a favourable condition that reduces the metabolic costs for



215 calcification in coccolithophores (Bach et al., 2019). Our experiment shows a decrease in calcification with increasing  
alkalinity but the data cannot prove weather calcification is indirectly limited by the insufficient  $\text{CO}_2$  and/or  $\text{HCO}_3^-$  supply for  
photosynthesis and growth. Since it is known that the formation of the coccosphere in *E. huxleyi* is closely related to the growth  
of the individual cell (Kottmeier et al., 2020), we speculate that the observed reduction in PIC in our data can be explained by  
the the reduction in growth rate under a  $[\text{CO}_2]$  limited scenario.

220 Gately et al. (2023), recently investigated the effect of “Moderate” ( $\Delta\text{TA} \sim 700 \mu\text{mol kg}^{-1}$ ) and “High” ( $\Delta\text{TA} \sim 2700 \mu\text{mol kg}^{-1}$ )  
alkalinity enhancement on the coccolithophore *E. huxleyi* in comparison to the diatom *Chaetoceros* sp. The manipulation  
allowed atmospheric equilibration of  $\text{CO}_2$  after TA manipulation and only mild fluctuations in pH were tested. The study  
evidenced that an equilibrated limestone-inspired alkalinity enhancement has little effect on the physiological performance of  
*E. huxleyi* suggesting that calcifiers may be relatively resilient to OAE when  $\text{CO}_2$  is not a limiting factor. On the other hand,  
225 our study evidenced that the increase in alkalinity without or incomplete  $\text{CO}_2$  equilibration affects the growth rate of *E. huxleyi*  
due to  $\text{CO}_2$  limitation beyond a TA increase of  $600 \mu\text{mol kg}^{-1}$  seawater. Thus, within the period after the addition of alkaline  
materials when the  $\text{CO}_2$  is not yet equilibrated with the atmosphere, *E. huxleyi* may experience a competitive disadvantage  
compared to other species or phytoplankton groups that have more effective CCM strategies. Since the cellular calcification  
(PIC:POC) remains stable under increasing TA, the overall calcite production will decrease along with decreasing growth rates  
230 in this scenario.

To conclude, to consider the applicability of OAE at a large scale, it is important to investigate the potential effects of enhanced  
alkalinity on both, entire marine communities as well as relevant key taxa since even small effects on single species could alter  
phytoplankton community composition with significant impacts on their population size that translates into their ecological  
and biogeochemical relevance. Given that numerous studies on ocean acidification have demonstrated species- and even strain-  
235 specific reactions to changes in carbonate chemistry (e.g., (Langer et al., 2006) the complexity of this pattern is further  
emphasized. Hence further physiological data are urgently required to indicate the relevant processes that need to be  
investigated in community-level studies before OAE application can be considered.

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### Conflict of interest

Allanah J. Paul has been employed by the non-profit organisation Bellona as a CDR Research and Technology Advisor since  
October 2023. The research reported in this manuscript was completed prior to starting this role. Allanah is also an external  
245 scientific advisor to "Seafields" (<https://www.seafields.eco/>), an aquaculture business for CDR using seaweed.



**Author contribution:** MH designed the experiments with CAUT. CAUT carried out the experiment. GF analysed the data and prepared the manuscript with contributions from all co-authors.

## References

- 250 Allan, R. P., Hawkins, E., Bellouin, N., and Collins, B.: IPCC, 2021: Summary for Policymakers, edited by: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M. I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J. B. R., Maycock, T. K., Waterfield, T., Yelekçi, O., Yu, R., and Zhou, B., Cambridge University Press, 3–32, 2021.
- Bach, L. T., Riebesell, U., and Schulz, K. G.: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliana huxleyi*, *Limnology and Oceanography*, 56, 2040–2050, <https://doi.org/10.4319/lo.2011.56.6.2040>, 2011.
- 255 Bach, L. T., Mackinder, L. C., Schulz, K. G., Wheeler, G., Schroeder, D. C., Brownlee, C., and Riebesell, U.: Dissecting the impact of CO<sub>2</sub> and pH on the mechanisms of photosynthesis and calcification in the coccolithophore *Emiliana huxleyi*, *New Phytologist*, 199, 121–134, 2013.
- 260 Bach, L. T., Riebesell, U., Gutowska, M. A., Federwisch, L., and Schulz, K. G.: A unifying concept of coccolithophore sensitivity to changing carbonate chemistry embedded in an ecological framework, *Progress in Oceanography*, 135, 125–138, 2015.
- Bach, L. T., Gill, S. J., Rickaby, R. E., Gore, S., and Renforth, P.: CO<sub>2</sub> removal with enhanced weathering and ocean alkalinity enhancement: potential risks and co-benefits for marine pelagic ecosystems, *Frontiers in Climate*, 1, 7, 2019.
- 265 Badger, M. R., Andrews, T. J., Whitney, S. M., Ludwig, M., Yellowlees, D. C., Leggat, W., and Price, G. D.: The diversity and coevolution of Rubisco, plastids, pyrenoids, and chloroplast-based CO<sub>2</sub>-concentrating mechanisms in algae, *Can. J. Bot.*, 76, 1052–1071, <https://doi.org/10.1139/b98-074>, 1998.
- Beaufort, L., Probert, I., de Garidel-Thoron, T., Bendif, E. M., Ruiz-Pino, D., Metzl, N., Goyet, C., Buchet, N., Coupel, P., Grelaud, M., Rost, B., Rickaby, R. E. M., and de Vargas, C.: Sensitivity of coccolithophores to carbonate chemistry and ocean acidification, *Nature*, 476, 80–83, <https://doi.org/10.1038/nature10295>, 2011.
- 270 Bednaršek, N., Pelletier, G., Van De Mortel, H., García-Reyes, M., Feely, R., and Dickson, A.: Unifying framework for assessing sensitivity for marine calcifiers to ocean alkalinity enhancement identifies winners, losers and biological thresholds – importance of caution with precautionary principle, <https://doi.org/10.5194/egusphere-2024-947>, 17 April 2024.
- Broecker, W. and Clark, E.: Ratio of coccolith CaCO<sub>3</sub> to foraminifera CaCO<sub>3</sub> in late Holocene deep sea sediments, *Paleoceanography*, 24, <https://doi.org/10.1029/2009PA001731>, 2009.
- 275 Cottingham, K. L., Lennon, J. T., and Brown, B. L.: Knowing when to draw the line: designing more informative ecological experiments, *Frontiers in Ecology and the Environment*, 3, 145–152, [https://doi.org/10.1890/1540-9295\(2005\)003\[0145:KWTDTL\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2005)003[0145:KWTDTL]2.0.CO;2), 2005.
- 280 Danbara, A. and Shiraiwa, Y.: The requirement of selenium for the growth of marine coccolithophorids, *Emiliana huxleyi*, *Gephyrocapsa oceanica* and *Helladosphaera* sp. (Prymnesiophyceae), *Plant and cell physiology*, 40, 762–766, 1999.



- Dickson, A. G. and Millero, F. J.: A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media, *Deep Sea Research Part A. Oceanographic Research Papers*, 34, 1733–1743, [https://doi.org/10.1016/0198-0149\(87\)90021-5](https://doi.org/10.1016/0198-0149(87)90021-5), 1987.
- 285 Dickson, A. G., Afghan, J. D., and Anderson, G. C.: Reference materials for oceanic CO<sub>2</sub> analysis: a method for the certification of total alkalinity, *Marine Chemistry*, 80, 185–197, [https://doi.org/10.1016/S0304-4203\(02\)00133-0](https://doi.org/10.1016/S0304-4203(02)00133-0), 2003.
- Faucher, G., Riebesell, U., and Bach, L. T.: Can morphological features of coccolithophores serve as a reliable proxy to reconstruct environmental conditions of the past?, *Climate of the Past*, 16, 1007–1025, 2020.
- 290 Gately, J. A., Kim, S. M., Jin, B., Brzezinski, M. A., and Iglesias-Rodriguez, M. D.: Coccolithophores and diatoms resilient to ocean alkalinity enhancement: A glimpse of hope?, *Science Advances*, 9, eadg6066, <https://doi.org/10.1126/sciadv.adg6066>, 2023.
- Gattuso, J.-P., Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., Allemand, D., Bopp, L., Cooley, S. R., Eakin, C. M., Hoegh-Guldberg, O., Kelly, R. P., Pörtner, H.-O., Rogers, A. D., Baxter, J. M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., Sumaila, U. R., Treyer, S., and Turley, C.: Contrasting futures for ocean and society from different anthropogenic CO<sub>2</sub> emissions scenarios, *Science*, 349, aac4722, <https://doi.org/10.1126/science.aac4722>, 2015.
- 295 GESAMP: High level review of a wide range of proposed marine geoengineering techniques, *Rep. Stud. GESAMP No. 98*, p-144, 2019.
- Guillard, R. R. L. and Ryther, J. H.: Studies of marine planktonic diatoms: i. *Cyclotella nana hustedt*, and *Detonula confervacea* (cleve) gran., *Canadian Journal of Microbiology*, 8, 229–239, <https://doi.org/10.1139/m62-029>, 1962.
- 300 Hartmann, J., West, A. J., Renforth, P., Köhler, P., De La Rocha, C. L., Wolf-Gladrow, D. A., Dürr, H. H., and Scheffran, J.: Enhanced chemical weathering as a geoengineering strategy to reduce atmospheric carbon dioxide, supply nutrients, and mitigate ocean acidification, *Reviews of Geophysics*, 51, 113–149, 2013.
- Hartmann, J., Suitner, N., Lim, C., Schneider, J., Marín-Samper, L., Aristegui, J., Renforth, P., Taucher, J., and Riebesell, U.: Stability of alkalinity in ocean alkalinity enhancement (OAE) approaches – consequences for durability of CO<sub>2</sub> storage, *Biogeosciences*, 20, 781–802, <https://doi.org/10.5194/bg-20-781-2023>, 2023.
- 305 Iglesias-Rodríguez, M. D., Rickaby, R. E. M., Singh, A., and Gately, J. A.: Laboratory experiments in ocean alkalinity enhancement research, *State of the Planet, 2-oae2023*, 1–18, <https://doi.org/10.5194/sp-2-oae2023-5-2023>, 2023.
- Keller, D. P., Feng, E. Y., and Oschlies, A.: Potential climate engineering effectiveness and side effects during a high carbon dioxide-emission scenario, *Nature communications*, 5, 1–11, 2014.
- 310 Kester, D. R., Duedall, I. W., Connors, D. N., and Pytkowicz, R. M.: Preparation of artificial seawater, *Limnology and Oceanography*, 12, 176–179, <https://doi.org/10.4319/lo.1967.12.1.0176>, 1967.
- Kottmeier, D. M., Terbrüggen, A., Wolf-Gladrow, D. A., and Thoms, S.: Diel variations in cell division and biomass production of *Emiliana huxleyi*—Consequences for the calculation of physiological cell parameters, *Limnology and Oceanography*, 65, 1781–1800, <https://doi.org/10.1002/lno.11418>, 2020.
- 315 Krug, S. A., Schulz, K. G., and Riebesell, U.: Effects of changes in carbonate chemistry speciation on *Coccolithus braarudii*: a discussion of coccolithophorid sensitivities, *Biogeosciences*, 8, 771–777, 2011.



- Langer, G., Geisen, M., Baumann, K.-H., Kläs, J., Riebesell, U., Thoms, S., and Young, J. R.: Species-specific responses of calcifying algae to changing seawater carbonate chemistry, *Geochemistry, geophysics, geosystems*, 7, 2006.
- LaRoche, J., Rost, B., and Engel, A.: Bioassay, batch culture and chemostat experimentation, in: Guide for best practices for ocean acidification research and data reporting., edited by: Riebesell, U., Fabry, V., Hansson, L., and Gattuso, J. P., Publications Office of the European Union, Brussels, 81–94, 2010.
- 320 Mehrbach, C., Culbertson, C. H., Hawley, J. E., and Pytkowicz, R. M.: Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure, *Limnology and Oceanography*, 18, 897–907, <https://doi.org/10.4319/lo.1973.18.6.0897>, 1973.
- Oschlies, A., Stevenson, A., Bach, L. T., Fennel, K., Rickaby, R. E. M., Satterfield, T., Webb, R., and Gattuso, J.-P.: Guide to Best Practices in Ocean Alkalinity Enhancement Research, Copernicus GmbH, <https://doi.org/10.5194/sp-2-0ae2023>, 2023.
- 325 Paasche E.: A tracer study of the inorganic carbon uptake during coccolith formation and photosynthesis in the coccolithophorid *Coccolithus huxleyi*, *Physiol Plant Suppl*, 0, 5–82, 1964.
- Paul, A. J. and Bach, L. T.: Universal response pattern of phytoplankton growth rates to increasing CO<sub>2</sub>, *New Phytologist*, 228, 1710–1716, <https://doi.org/10.1111/nph.16806>, 2020.
- 330 Pierrot, D., Lewis, E., and Wallace, D. W. R.: MS Excel program developed for CO<sub>2</sub> system calculations, ORNL/CDIAC-105a, Carbon Dioxide Inf. Anal. Center, Oak Ridge Natl. Lab. US Dep. Energy, Oak Ridge, Tennessee, 2006.
- Poulton, A. J., Mark Moore, C., Seeyave, S., Lucas, M. I., Fielding, S., and Ward, P.: Phytoplankton community composition around the Crozet Plateau, with emphasis on diatoms and Phaeocystis, *Deep Sea Research Part II: Topical Studies in Oceanography*, 54, 2085–2105, <https://doi.org/10.1016/j.dsr2.2007.06.005>, 2007.
- 335 Renforth, P. and Henderson, G.: Assessing ocean alkalinity for carbon sequestration, *Reviews of Geophysics*, 55, 636–674, <https://doi.org/10.1002/2016RG000533>, 2017.
- Riebesell, U., Wolf-Gladrow, D. A., and Smetacek, V.: Carbon dioxide limitation of marine phytoplankton growth rates, *Nature*, 361, 249–251, 1993.
- Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E., and Morel, F. M.: Reduced calcification of marine plankton in response to increased atmospheric CO<sub>2</sub>, *Nature*, 407, 364–367, 2000.
- 340 Riebesell, U., Basso, D., Geilert, S., Dale, A. W., Kreuzburg, M., and Meysman, F.: Mesocosm experiments in ocean alkalinity enhancement research, <https://doi.org/10.5194/sp-2023-11>, 2023.
- Rost, B., Riebesell, U., Burkhardt, S., and Sültemeyer, D.: Carbon acquisition of bloom-forming marine phytoplankton, *Limnology and oceanography*, 48, 55–67, 2003.
- 345 Rost, B., Riebesell, U., and Sültemeyer, D.: Carbon acquisition of marine phytoplankton: Effect of photoperiod length, *Limnol. Oceanogr.*, 51, 12–20, <https://doi.org/10.4319/lo.2006.51.1.0012>, 2006.
- Schulz, K. G., Rost, B., Burkhardt, S., Riebesell, U., Thoms, S., and Wolf-Gladrow, D. A.: The effect of iron availability on the regulation of inorganic carbon acquisition in the coccolithophore *Emiliania huxleyi* and the significance of cellular compartmentation for stable carbon isotope fractionation, *Geochimica et Cosmochimica Acta*, 71, 5301–5312, <https://doi.org/10.1016/j.gca.2007.09.012>, 2007.
- 350



- Sett, S., Bach, L. T., Schulz, K. G., Koch-Klavsen, S., Lebrato, M., and Riebesell, U.: Temperature modulates coccolithophorid sensitivity of growth, photosynthesis and calcification to increasing seawater p CO<sub>2</sub>, *PloS one*, 9, e88308, 2014.
- Suitner, N., Faucher, G., Lim, C., Schneider, J., Moras, C. A., Riebesell, U., and Hartmann, J.: Ocean alkalinity enhancement approaches and the predictability of runaway precipitation processes – Results of an experimental study to determine critical alkalinity ranges for safe and sustainable application scenarios, *Earth System Science/Response to Global Change: Climate Change*, <https://doi.org/10.5194/egusphere-2023-2611>, 2023.
- Taylor, L. L., Quirk, J., Thorley, R., Kharecha, P. A., Hansen, J., Ridgwell, A., Lomas, M. R., Banwart, S. A., and Beerling, D. J.: Enhanced weathering strategies for stabilizing climate and averting ocean acidification, *Nature Climate Change*, 6, 402–406, 2016.
- 360 United Nations Framework Convention on Climate Change: Adoption of the Paris Agreement. Report No. FCCC/CP/2015/L.9/Rev.1, 2015.
- Westbroek, P., Brown, C. W., Bleijswijk, J. van, Brownlee, C., Brummer, G. J., Conte, M., Egge, J., Fernández, E., Jordan, R., Knappertsbusch, M., Stefels, J., Veldhuis, M., van der Wal, P., and Young, J.: A model system approach to biological climate forcing. The example of *Emiliana huxleyi*, *Global and Planetary Change*, 8, 27–46, [https://doi.org/10.1016/0921-8181\(93\)90061-R](https://doi.org/10.1016/0921-8181(93)90061-R), 1993.
- 365 Wheeler, G. L., Sturm, D., and Langer, G.: *Gephyrocapsa huxleyi* (*Emiliana huxleyi*) as a model system for coccolithophore biology, *Journal of Phycology*, n/a, <https://doi.org/10.1111/jpy.13404>, n.d.
- Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Körtzinger, A., and Dickson, A. G.: Total alkalinity: The explicit conservative expression and its application to biogeochemical processes, *Marine Chemistry*, 106, 287–300, 2007.
- 370 Zeebe, R. E. and Wolf-Gladrow, D.: *CO<sub>2</sub> in Seawater: Equilibrium, Kinetics, Isotopes*, Gulf Professional Publishing, 382 pp., 2001.

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mean TA	mean DIC	mean pH	mean fCO <sub>2</sub>	CO <sub>2</sub> mean	HCO <sub>3</sub> <sup>-</sup>	CO <sub>3</sub> <sup>2-</sup>	omega ar	omega ca
2319	2165	7.97	599.49	22.58	2026	116	1.79	2.79
2433	2188	8.15	389.46	14.67	1999	174	2.68	4.18
2482	2195	8.22	327.17	12.32	1979	203	3.13	4.88
2499	2167	8.30	268.47	10.11	1923	234	3.60	5.61
2541	2171	8.35	235.88	8.89	1901	260	4.01	6.25
2597	2184	8.40	207.01	7.80	1885	291	4.48	6.99
2643	2177	8.46	174.85	6.59	1841	329	5.07	7.90
2702	2186	8.52	152.72	5.75	1814	366	5.63	8.79
2742	2190	8.55	138.38	5.21	1791	394	6.06	9.45
2840	2198	8.64	110.50	4.16	1733	461	7.10	11.07
2935	2192	8.73	86.06	3.24	1651	538	8.28	12.91
3030	2200	8.79	70.82	2.67	1590	607	9.33	14.56
3127	2209	8.86	58.84	2.22	1530	676	10.40	16.23
3295	2184	9.00	37.48	1.41	1353	830	12.76	19.91
3367	2246	8.99	39.70	1.50	1403	842	12.95	20.20
3490	2213	9.10	27.19	1.02	1244	967	14.88	23.21

**Table 1. Carbonate chemistry speciation.** The values represent the mean of the measurements taken at the beginning and at the end of the experiment. TA, DIC, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup> are given in μmol kg<sup>-1</sup>; fCO<sub>2</sub> in μatm; Ω aragonite and calcite are dimensionless.

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mean TA	Growth rate ( $\mu$ )	PIC/POC	TPC production	PIC production	POC production
2319	0.98	0.71	18.50	7.71	10.79
2433	1.17	0.34	29.21	7.49	21.72
2482	1.01	0.43	29.92	9.07	20.85
2499	0.96	0.11	11.85	1.13	10.72
2541	0.97	0.93	18.40	8.87	9.53
2597	1.01	0.69	20.20	8.26	11.94
2643	1.10	0.76	25.55	11.01	14.53
2702	1.10	0.73	24.74	10.48	14.26
2742	0.98	0.79	21.94	9.71	12.24
2840	0.81	0.59	15.60	5.78	9.81
2935	0.76	0.76	11.81	5.10	6.72
3030	0.68	0.72	9.83	4.10	5.73
3127	0.62	0.74	10.90	4.64	6.26
3295	0.42	0.86	4.40	2.03	2.37
3367	0.46	0.74	6.42	2.74	3.68
3490	0.42	0.53	6.12	2.11	4.01

**Table 2 Cellular element quotas and production.** Growth rate ( $\mu$ ) are given as  $d^{-1}$ , PIC:POC is dimensionless; TPC, POC and PIC productions are given as  $pg\ cell^{-1} \cdot d^{-1}$ .