

## **RC1: Anonymous Referee #1**

We thank the reviewers for their constructive detailed, and very helpful analyses of the draft manuscript. Responses to their comments are given below. The majority of comments can be grouped into 4 themes:

1. Inadequate description of methods and their disjointed presentation in different parts of the manuscript;
2. Inadequate referencing of the (rapidly growing) literature on OAE;
3. A lack of clarity in considering pH vs DIC effects, particularly in the analysis of prior studies using pH-drift experiments; and
4. Overly narrow considerations of potential approaches (coastal vs open ocean; equilibrated vs non-equilibrated) to OAE.

We are confident that we can address the reviewers' concerns and, with their guidance, can revise and improve the manuscript. Detailed responses to their comments are given below.

### *General Comments:*

There are three main conclusions made within this study. The first is that there were no significant impacts on the viability or growth rate of the two species assessed within the short term exposure to elevated alkalinity levels. This is to be expected as levels of OAE that would likely result in an impact within 10 minutes of exposure would be significantly higher than what is logistically possible or safe in terms of secondary precipitation. The second is that longer term exposure to elevated alkalinity resulted in a decrease in growth rate. This is an interesting finding as many other studies thus far have found little evidence to suggest that there will be significant impacts to the growth of phytoplankton as a result of OAE. The third is that within the current literature evidence suggests that approximately 50% of species could be significantly impacted by pH increases in line with those expected as a result of OAE. This provides an excellent summary of the current knowledge of pH impacts however it is unclear as to whether these impacts are a result of pH ( $H^+$  concentrations) or  $CO_2$  concentrations, of which the latter is expected to be the most important factor in regard to the ecological impacts of OAE on phytoplankton.

### *Major Comments:*

The manuscript does an excellent job in referencing and discussing the influence of elevated pH on phytoplankton growth and viability. However, citations and discussion around OAE specifically are currently lacking. Further I found it difficult to disentangle exactly how the experiments were run within this manuscript and strongly recommend that the methods/results section be revised so that readers may easily understand what the authors have accomplished here.

We agree that referencing other studies on OAE needs to be updated and improved throughout the manuscript and that the structure of several of the sections (specifically sections 3 and 4) should be revised for clarity and accessibility. We provide more detail below.

### *Specific Comments:*

1. Lines 8 – 9: There are many methods for achieving OAE and only mineral based methods could be considered to “mimic” the natural weathering of alkali minerals. Furthermore, enhancing alkalinity does not lead to the sequestering of atmospheric  $CO_2$  but decreases oceanic  $CO_2$  concentrations allowing for atmospheric  $CO_2$  to be sequestered under the correct conditions. I

appreciate that this is within the abstract and concise wording is necessary but would advise the authors to consider rewording this sentence.

Suggested rewording:

“One proposed NET is Ocean Alkalinity Enhancement (OAE), in which artificially raising the alkalinity favours formation of bicarbonate from CO<sub>2</sub>, leading to a decrease in the partial pressure of CO<sub>2</sub> in the water, and a subsequent invasion of atmospheric resulting in net sequestration of atmospheric carbon.”

2. Line 11-12: This sentence is difficult to understand, consider rewording.

Suggested rewording:

“The potential impacts of OAE were assessed through an analysis of prior studies investigating the effects of elevated pH on phytoplankton growth rates in pH-drift experiments and by experimentally assessing the potential impact of short-term elevation of pH on the viability and subsequent growth rates of two representative near-shore species of phytoplankton.”

3. Line 16 – please provide the actual number of days or range of days.

Suggested rewording:

“However, there was a significant decrease in growth rates with long-term (8 days) exposure to elevated pH”

4. Lines 18-19: Within this manuscript the authors have only looked at two species from two different taxonomic groups. This statement is too broad for the results of the experiment conducted here.

We suggest deleting the final sentence of the abstract.

5. Line 31: I caution the authors against the use of the word “natural”. All methods of CDR are anthropogenically motivated and furthermore depending on the method of OAE used it may be more or less similar to the natural weathering of alkali minerals (e.g. mineral based OAE vs electrochemical based OAE). Furthermore, it is highly unlikely that OAE would result in these changes as any increase in pH would ideally be negated by an influx of CO<sub>2</sub>, resulting in an increase in DIC beyond current and pre-industrial concentrations.

Suggested rewording:

“Ocean Alkalinity Enhancement (OAE) is one promising NET that involves anthropogenically raising the alkalinity of a parcel of water causing the partial pressure of the CO<sub>2</sub> in that water to decrease. This change leads to either in-gassing of CO<sub>2</sub> from the atmosphere or a reduction in out-gassing of CO<sub>2</sub> from the ocean, depending on the initial air-sea gradient. Both scenarios result in a theoretical net reduction of atmospheric CO<sub>2</sub> through storage in the form of bicarbonate (HCO<sub>3</sub><sup>-</sup>) and carbonate (CO<sub>3</sub><sup>2-</sup>) ions in the ocean (Oschlies et al., 2023).”

6. Line 33: Ideally this would be the case however additions of alkalinity and subsequent in gassing of atmospheric CO<sub>2</sub> will lead to increases of both carbonate and bicarbonate. This will depend on

other variables, but without CO<sub>2</sub> equilibration concentrations of carbonate would increase significantly more than bicarbonate.

Suggested rewording:

“The additional carbon would be stored in the form bicarbonate (HCO<sub>3</sub><sup>-</sup>) and carbonate (CO<sub>3</sub><sup>2-</sup>) ions, with the former (which has a residence time of c. 1,000 years in the ocean) favoured after in-gassing of CO<sub>2</sub>.”

7. Line 34: “...would likely be...” . Also, it is not yet clear which method of OAE will be implemented at large scales. It is more likely that multiple methods will be implemented in different regions e.g. electrochemical in coastal regions and mineral based in pelagic regions.

Suggested rewording:

“There are currently several different methods of OAE in development, including mineral- and electrochemical-based methods, with deployment from vessels, through preexisting outfalls, or from placement on beaches. The focus of this study is the mineral-based approach from preexisting outfalls, implementation of which is likely to occur through addition of unequilibrated hydroxide minerals (OH<sup>-</sup>) to the coastal surface ocean.”

8. Line 36: Again, enhancing alkalinity does not necessarily result in bicarbonate formation. For a parcel of water with TA 2100 μmol/kg, DIC 2000 μmol/kg, T 15°C and salinity 35 an increase in alkalinity of 500 μmol/kg would result in changes of bicarbonate – 316.93 μmol/kg, carbonate + 340 μmol/kg. Also, such changes in carbonate chemistry do not necessarily lead to CO<sub>2</sub> drawdown this is dependent on several other factors.

We suggest deleting this sentence.

9. Lines 84 – 85: The authors discuss changes in pH throughout this section but was there additional criteria to ensure that manipulations of pH resulted in changes in DIC that would be expected of OAE (particularly for those articles not by “Hansen and colleagues”)? For example, using varied additions of sodium carbonate and HCl one can achieve similar pH values but drastically different concentrations of TA and DIC. Given this and the expected ranges of pH in OAE one would expect CO<sub>2</sub> and not pH to be the driving factor to influence phytoplankton.

We agree that more clarity on the roles of pH vs DIC is critical for interpreting these data. However, most of the papers we included in the analysis did not report both pH and DIC and we did not use DIC as a criterion for including the study in the analysis. Our intention was to illustrate this with the single experiment presented in Figure 3, which contrasts the changes in pH and DIC in pH-drift vs aerated cultures (i.e., without/with in-gassing) and in which the biological responses were most consistently correlated with CO<sub>2</sub> in a multivariate analysis. Even so, we argue that mineral alkalization of an outfall (our focus) is likely to have an effect on pH without a significant reduction in DIC and that pH has the potential to be an important determinant of phytoplankton metabolism and growth.

Suggested modification: “...batch cultures without ventilation to replenish CO<sub>2</sub>. It is critical to recognize that in pH-drift cultures such as these, both pH and DIC vary and that the biological response may be due to changes in CO<sub>2</sub> availability as much as, or more than, by pH (see Section 3, below).”

10. Figure 1: The y axis of figure 1a/b are very hard to discern as they overlap and it is unclear which axis label is referring to which set of units on the axis.

Agreed, the figure can be modified (and saved at a higher resolution) for clarity.

11. Figure 1d: It is very difficult to see the median value for the 90%  $\mu$  reduction. Consider changing the colour of this line and/or adjusting the alpha values in this plot.

Agreed.

12. Lines 149 – 148: Is this anticipated maximum pH value for OAE based on changes in pH at the point of alkalinity addition or after dilution within a region?

This pH is the anticipated maximum at the point of addition, and suggest this modification for clarity.

“For all of the species investigated (Figure 1), the median threshold pH is above 8.5, suggesting about 50% of the species would not be impacted by the anticipated maximum pH increase at the point of addition anticipated for a mineral-based addition of OAE from a land-based point source.”

13. Lines 174: I recommend the authors adjust the wording of this sentence as OAE does not change DIC concentrations initially so there can be no “resupply” of DIC. More accurately this would be an influx of CO<sub>2</sub> increasing DIC beyond what it was prior to OAE.

Suggested rewording:

“Most of the studies analyzed in Section 1 did not permit CO<sub>2</sub> in-gassing, as they were conducted using a closed-bottle, batch-culturing method. However, for OAE to successfully function as a NET, in-gassing of CO<sub>2</sub> is required to increase the DIC pool.”

14. Lines 190 -192: If these observations are made on the basis of the Metric multidimensional scaling plots, please state this.

Suggested rewording:

“There is clear separation in the mMDS (Figure 3) between the aerated and pH-drift cultures following the period of exponential growth (Days 0-1), both in the carbonate system parameters (Figure 3c) and in the biological parameters describing abundance and physiological status (3d)”.

15. Lines 195 – 196: This is a strong statement to be made from a relatively small comparison. Furthermore, there are various approaches to OAE that would not necessarily result in CO<sub>2</sub> influxes similar to those seen here e.g. in areas where CO<sub>2</sub> outgassing occurs, or where OAE is added in an equilibrated form. Could the authors provide some references to support this statement?

Suggested rewording:

“These results demonstrate that the test organism, the biological responses to alkalization depend in large part on CO<sub>2</sub> in-gassing, so the response to mineral-based OAE and subsequent in-gassing could not necessarily be inferred from pH drift experiments.”

16. Lines 209 – 213: Please make it clear to the reader at the start of this section whether the cultures were grown in filtered seawater with the addition of nutrients and trace metals? Also, it would be beneficial to disclose the exact location of water collection i.e. xxx kms offshore via boat or pump. In addition, it is stated that "...fresh media in mid-exponential phase..." is this for the experimental cultures or the maintenance of culture pre experiment?

Suggested rewording:

"The cultures were maintained in 40-mL volumes of sterile-filtered f/2 (Guillard, 1975) or L1 (Guillard & Hargraves, 1993) seawater medium, and diluted into fresh media in mid-exponential phase in a laminar flow hood. The seawater was collected by pump about 100 m offshore from the National Research Council of Canada's Marine Institute at Ketch Harbour, NS, and tangential flow filtered on collection. It was refiltered through a 0.2- $\mu$ m capsule filter (Cytiva Whatman Polycap Disposable Capsules: 75TC) and nutrient-enriched in autoclaved glassware or in sterile cell culture plates. Prior to experimentation, parent cultures were fully acclimated to the experimental growth conditions, continuous illumination at c. 190  $\mu$ mol photons  $m^{-2} s^{-1}$  at a temperature of  $18 \pm 1^\circ C$ , by maintaining them in balanced growth in semi-continuous culture (MacIntyre & Cullen, 2005)"

Comments 18 and 29 make it clear that a major reorganization of the Methods would be helpful to the reader. We propose to address this by consolidating all of the experimental methods into a new, separate section, with the differences in set-up between the different experiments tabulated for ease of comparison. The table would include the experimental ID, treatment (alkalization as concentration and duration of exposure), culture volume, presence/absence of aeration, measurement parameters and frequency. The revised Methods would also include a better explanation of the rationale for picking the studies compared in Section 2: a literature survey in which our search terms included "phytoplankton AND (alkalinization OR "high pH")". Studies found in the search were gated to include those in which cultures were maintained under pH drift conditions, with culture medium ensuring that the media would have DIC:DIN below the Redfield Ratio, and with time-series (growth curves) of phytoplankton abundance and pH.

17. Line 250: The authors previously used a dark acclimation of 30 minutes, is there a reason for the change to 20 minutes?

This was a typographical error. The dark acclimation time was 30 minutes.

18. It is not clear how the transient exposure cultures discussed in section 4.3.2 are setup. Are these setup the same as those for the chronic exposure experiment? The authors also mention cultures measured after 1-2 hours, are these the same cultures or separate to the chronic and transient exposure cultures? This confusion may come from the layout in which the methods/results are presented. I am hesitant to suggest changes to this but it would be beneficial to the reader if the authors could be overly obvious in the explanation of how cultures were setup and whether cultures measured after 1-2 hours, several days and/or 10 minutes are the same cultures or separate cultures.

Please see our response to Comment 16.

19. Lines 294 - 296: The authors state "...there was no significant trend with the transient elevation in pH transient elevation in pH (Figure 4c)." However, the figure caption for figure 4 states "...measured after exposure to elevated alkalinity and pH for 1-2 hours...". Is the transient elevated alkalinity assessing the effect after 10 minutes as stated in line 286 or 1-2 hours?

We agree that the presentation is confusing. The cultures were exposed to the elevated alkalinity for 10 minutes before being diluted with the SDC-MPN method into untreated media. However, due to the time needed to finish the dilution series, and the constraint on personnel, the subsample of culture used to measure  $F_v/F_m$  could not be read immediately following the 10 minute exposure and thus were read between 1-2 hours following exposure.

Suggested rewording in the legend to Figure 4:

"Measurements of (c) the quantum yield of PSII electron transport,  $F_v/F_m$ , a measure of the proportion of functional reaction centres, and (d) the maximum fluorescence-based specific growth rate,  $\mu_m$ , following 10-minute exposure to elevated alkalinity and pH in *T. pseudonana* and *D. lutheri*. Fluorescence was measured 1-2 hours following exposure. Estimates of maximum growth rates are based on fits of the growth curve in samples that were diluted to  $10^{-3}$  in the SDC-MPN assay. There was no significant trend in the data for  $F_v/F_m$  in *D. lutheri* nor for  $\mu_m$  in either species. The dashed lines are the mean values. The reduction in  $F_v/F_m$  in *T. pseudonana* was fit to Equation 2 (dashed line). The estimated threshold pH for reduced  $F_v/F_m$  is  $8.86 \pm 0.24$ ."

20. Figures should be introduced in the order they appear in the text e.g. figure 5 is introduced in line 287 while figure 4c is introduced in line 296.

We thank the reviewer for pointing out this placement error and will switch the order of Figures 4 and 5. We believe that keeping all subfigures in figure 4 is beneficial for the reader to make visual comparisons in the data.

21. Lines 310-311: This sentence needs to be revised in line with comment 8.

Suggested rewording:

"The mechanism of unequilibrated OAE in regions of the ocean where the initial air-sea gradient favours in-gassing of  $\text{CO}_2$ , allows for the invasion of  $\text{CO}_2$  to occur and re-equilibrate across the air-sea interface. The additional  $\text{CO}_2$  is then stored in the form of bicarbonate and carbonate (Oschlies et al., 2023)."

22. Lines 311 – 314: It would be beneficial if the authors could provide some reasoning behind why there are differences between these methods and how to abate these differences.

The differences presumably reflect a difference in the physiology of the different species, but notably occur within a single genus, so don't fit on high-level taxonomic differences (e.g., diatoms vs dinoflagellates). We have no data from which to infer a mechanistic basis and prefer not to make completely unsupported suggestions of cause.

23. Lines 354 – 356: This sentence is confusing particularly "...the dominant carbon species would be carbonate and calcite would begin precipitating...". Do the authors mean that carbonate is the dominant form of carbon and would precipitate into calcite? Traditionally when discussing

precipitation omega values are provided, this would be beneficial here as many other articles discuss calcite precipitation in this form see Moras et al. (2022) and Schulz et al. (2023).

The intention of this sentence was to state that carbonate is the dominant form of carbon and would precipitate into calcite at this pH. We agree that the wording here is slightly confusing and have reworded to get our point across more clearly, as well as including reference to crossing the omega thresholds. We believe that specific omega values are outside the scope of this work.

Suggested rewording:

“We note that at the pH range in our experiments, the dominant carbon species would be carbonate and would be at a greater risk of precipitating into calcite, thus crossing the threshold for the saturation states for calcite ( $\Omega_{\text{calc}}$ ) and/or aragonite ( $\Omega_{\text{arag}}$ ; Schulz et al., 2023). This could lead to reductions in the availability of bicarbonate for the CCM and might account for the reductions in growth rates observed in Figure 4.”

24. Lines 356 – 357: It would be beneficial to cite some of the many article’s discussing the efficiency of OAE and impacts of calcite precipitation here.

Agreed. We will refer to Schulz et al. (2023), Moras et al. (2022), and Hartmann et al. (2023).

25. There have been numerous studies assessing the impact of OAE on natural assemblages of phytoplankton and cultures in recent years e.g. Gately et al. (2023) and Guo et al. (2023). Although the authors discuss changes in pH there is no significant discussion surrounding the numerous papers on the impact of OAE on phytoplankton. The manuscript is significantly lacking in this regard and inclusion and discussion of such articles within the introduction and/or discussion would help to improve this manuscript.

Agreed. We will refer to: Gately et al. (2023), Iglesias-Rodríguez et al. (2023), Guo et al. (2023), Paul et al. (2024), Hutchins et al. (2023), Ferderer et al. (2022), and Subhas et al. (2022).

26. The authors discuss initial pH values, were there also end measurements for pH or prior to media refreshment? Volumes used were relatively low and cultures were grown into stationary phase, as such a significant change in pH over the duration of the experiment would be expected. I understand that during the SDC – MPN assay it would be expected that in gassing occurred maintaining pH at a relatively stable level, however this is not clear for the other experiments.

We did not collect measurements for pH at the end of the experiments conducted in tubes because, as noted, there would be a significant change in pH over the experiment caused by the phytoplankton themselves. These changes would be more gradual as a consequence of the phytoplankton growth.

27. Line 241-242: Was alkalinity measured or was it calculated? If it was calculated was this based on the additions of NaOH or another measured carbonate chemistry parameter? It would be beneficial to the manuscript to add measured or alkalinity values calculated from a second carbonate chemistry parameter, as many articles discuss OAE in terms of alkalinity increases ( $\mu\text{mol/kg}$ ) and not pH increases.



The alkalinity values reported are calculated based on the measured pH and known DIC values.  
Suggested rewording (line 241-242):

“These additions increased the initial concentration of total alkalinity, 2168  $\mu\text{mol L}^{-1}$ , by 0-1084  $\mu\text{mol L}^{-1}$ . (All reported values of alkalinity are calculated from measured values of pH and DIC.)  
The culture and NaOH...”

28. The quality of Figure S1 is extremely poor and makes it difficult to read. In addition, Figures S1 c,d appear to be concentrations of carbon species ( $\text{CO}_2$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ) not DIC as stated in the figure caption.

We apologize for the quality of the figure, which was degraded in successive file conversions. It can be restored to original resolution and the caption corrected to accurately distinction between the carbon species and DIC.

29. It was difficult to distinguish between the experiments, variables measured, and methods used here. I strongly recommend the authors increase the clarity of the text discussing the experimental methods. For example, lines 209 – 210 “The cultures were grown in 40-mL volumes of f/2 (Guillard, 1975) or L1 (Guillard & Hargraves, 1993) seawater medium, and diluted into fresh media in mid-exponential phase in a laminar flow hood.” Was this done for all experimental culture’s transient, chronic and those in the SDC-MPN assay? Or was this only prior to the experiment during the acclimation? Am I correct to understand that there are separate cultures with separate maintenance methods i.e. the SDC-MPN cultures, the chronic and transient? A strict section or table outlining the experiments conducted and their common and/or differences in methodology would be greatly beneficial, as it is currently difficult to tease apart exactly how this experiment was conducted.

We agree that the current structure of Section 4 would benefit from revisions for clarity and thank the reviewer for the suggestions for doing so. We believe that the change in structure recommended in response to Comment 16 will aid in the reader’s comprehension of the methods/variables for each experiment and that a summary tabulation of differences would facilitate this.

30. Lines 10-12: The authors introduce the analysis of prior studies here but fail to state any results from this within the abstract. This is a major component of the manuscript and requires a section detailing the results within the abstract.

Suggested addition to the abstract:

“The analysis of prior studies indicates wide variability in the growth response to elevated pH within and between taxonomic groups, with about 50% of species expected to not be impacted by pH increase expected unequilibrated mineral-based OAE. To the extent that the growth responses reflect (largely unreported) parallel reductions in DIC availability, the susceptibility may be reduced for OAE in which  $\text{CO}_2$  in-gassing is not prevented.”



## References:

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