We thank RC2 for the very detailed, extensive and rigorous review with constructive comments. At some points RC2 suggested more discussion, either in the Introduction or Discussion. Our proposed responses therefore add more to the length of the text. We hope our proposed responses below will greatly improve the manuscript.

We intersperse our responses (bold italic for explanation, bold for and in quote marks for proposed changes) with the enumerated comments of the reviewer.

1.- However, I would like to see more discussion on the implications these findings have on global carbonate production, and what it might tell us about the response of coccolithophores to ocean acidification and global warming.

Our results do suggest that ongoing ocean changes might result in decreased coccolithophore species diversity (relatively more E. huxleyi and lower proportions or diversity of other species, even closely related ones such as the Gephyrocapsa's) and lower phenotypic diversity within E. huxleyi. We would be very cautious about extrapolating to global carbonate production, as we do not have direct measurements of total calcium carbonate per cell or rates. However, the fact that Calcidiscus leptoporus showed a markedly lower Tol than E. huxleyi in the OMI analysis might be relevant. In many environments, the more robust coccoliths of this genus means it may often contribute much more to sinking carbonate than E. huxleyi, despite much lower cell abundances (Ziveri et al., 2007; Menschel et al., 2016; Eduardo Menschel and González, 2019). Thus, a replacement of such species with E. huxleyi might decrease total carbonate export.

We propose adding the following paragraph to at end of the discussion:

"The lower values of pH and Ωcal levels observed here approached levels predicted for higher latitudes of the global ocean at the end of the century under high emission scenarios such as RCP 8.5 (Feely, Richard A et al., 2009; Hartin et al., 2016). Our results suggest that ongoing changes in ocean chemistry may result in decreases in coccolithophore diversity, leading to more numerical dominance of *E. huxleyi* compared to other coccolithophores, as well as decreased phenotypic diversity within *E. huxleyi*. The extended-domain niche analysis in the present study would suggest that *C. leptoporus* might be less adaptable than *E. huxleyi*. Some studies found that *C. leptoporus* was relatively resistant to OA in the lab (Langer et al., 2006), but others reported that it is sensitive and that its PIC/POC ratio, considered important in determining ballast effects, is especially negatively affected by OA. Species such as *C. leptoporus* can be much more important than *E. huxleyi* in carbonate export due to their production of much heavier coccolithophores which sink faster and dissolve more slowly (e.g., Ziveri et al., 2007; Menschel et al., 2016; Menschel & González, 2019). Thus, a change to more *E. huxleyi*-dominated coccolithophore communities might negatively impact carbonate export."

2.- I would also like to see more contextualization of the work with laboratory results in the discussion. Specifically in the context of potential mechanistic reasons for the observed trends.

This is an important issue. While we must be careful to avoid a discussion that becomes too much a review of the very extensive work over the past two decades, we think it might be possible to add some concise discussion of two points that are most directly relevant. 1. The results here might help in designing future lab studies. There are always large practical challenges in connecting laboratory experiments to the natural world. Among those are balancing the number and ranges of conditions that can be tested in the laboratory, the number of species (or strains of the same species) that can be tested, and whether to use established isolates already identified in culture collections or to dedicate resources and time to the obtention of new isolates of target species. When the goal is to compare many strains of different species or phenotypes, it is often necessary to decrease the number of total conditions tested. On the other hand, studies focusing on determining reaction norm shapes have used one or a small number of well-established strains easily available from culture collections, to allow testing more conditions. OMI niche analysis can help by identifying which conditions might be most important to test in the lab. This point we will address more in response also to RC's comment 3.

2. Another point is to discuss better whether any lab experiments can mechanistically explain the findings. This is much harder, but we will take a try. There still have been few lab studies comparing the responses of many different species to OA. There have been studies that included C. leptoporus (which we will discuss more in addressing the point above), but there have been no published physiological studies at all of the other three non-E. huxleyi species we could include in the OMI analysis, G. ericsonii, G. parvula, and G. muellerae, and we would caution that extrapolating to these from G. oceanica would be inappropriate (Bendif et al., 2016). Within E. huxleyi, studies that have compared different morphotypes have found different results, and these we can discuss more.

This we address with the change proposed to the first paragraph of section 4.5, by adding these sentences (as in response to RC1's comment 6):

"Proper differentiation between B, B/C, and C based on coccolith length would require strict morphometrics, which we did not perform due to the difficulty in accurate measurements on full coccospheres of less common morphotypes, especially in low abundance populations (as coccospheres may lack coccoliths in a correct orientation for accurate measurement). Thus the generalist behavior of lightly-calcified morphotypes in the OMI analysis that combined fjord, coastal, and open ocean sites is likely an artefact. We suspect that lightly calcified A, B, B/C, and C morphotypes might actually each exhibit specialist behaviors in distinct but overlapping niches. In fact, a laboratory study reported that B/C morphotype strains only calcified substantially in a relatively narrow range of carbonate conditions (Müller et al., 2015). "

Next (change underlined)

"Experimental findings that the R/hyper-calcified morphotype did not perform better than the moderate-calcified A morphotype under high CO2/low pH/low Ωcal (von Dassow et al., 2018) might be explained by the OMI analysis suggesting a possible narrow unimodal response to Ωcal, that would not have been detected in the experiments of von Dassow et al. (2018), where Ωcal values of 1.4 vs. 3.3 were tested in the lab. <u>The studies of Langer et al.</u> (2009) and Müller et al. (2015) did find that R morphotype strains did seem more resistant to high CO₂/low pH than other A morphotypes, either in growth rate or in PIC production. <u>Those studies used either four-fold higher light levels (Langer et al., 2009) or continuous light (Müller et al., 2015), and low light has been shown to increase the sensitivity to OA specifically of an R morphotype strain (Rokitta and Rost, 2012), highlighting that the R/hyper-calcified morphotype might be selected <u>by interactions with other variables.</u>." *Two new paragraphs proposed below will address further the above comment and the RC2's next comment.*</u> 3.- For the OMI analysis, there should be a bit more discussion about limitations of the method. For example, how would adding parameters like MLD, light, nutrients and grazers change the results?

It is important to make a difference between the limitations of the method (the OMI analysis) and the variables that were taken into consideration. As any multivariate analysis, such as the RDA and nMDS, the "limitations" are given by the variables included in the analysis (but are nor related to the method itself). Here, we choose to focus on abiotic factors and discuss these more in two new paragraphs added below the one above:

"The present study shows that the OMI analysis can be useful for identifying how parameters may determine the realized niches of both species and genetically-determined phenotypic variants within a species. For example, in the extended domain (Fig. 7b), Ω cal, temperature, and salinity were important in defining the narrowness vs breadth of niches among the E. huxlevi A morphotype, E. huxlevi R/hypercalcified morphotype, and closely related Gephyrocapsa species, while the habitat centers (niche positions) of the different E. huxleyi morphotypes lined up approximately on a gradient of pH vs CO₂. Calcification rate has been demonstrated to vary among E. huxleyi strain according to maximum photosynthetic rate, but also [CO₃²⁻] concentration at their sites of origin, while maximum photosynthetic rate also varied among the strains with [CO₂] at the site of origin (Rickaby et al., 2016). Several recent studies have called into question the importance of Ω cal in determining the response of calcifying organisms, with both theoretical and laboratory support that the concentrations of HCO₃⁻, CO₃²⁻, and H⁺ are more important (Kottmeier et al., 2016; Bach et al., 2015; Gafar et al., 2018). However, while HCO₃⁻, CO₃²⁻ were formally excluded from the OMI analyses as redundant variables, these vary more with and CO₂ and pH, respectively, which formed an axis along which the niche of the R/hyper-calcified morphotype was broad, rather than the orthogonal gradient in Ω cal. We caution that calcification rate might not relate in a simple way to the morphotypes observed here. Nevertheless, such trade-offs offer crucial clues into how traits related to calcification may be selected by the environment, resulting in the environmental patterns observed here. It might be worth exploring experimentally if there is a role for Ω cal separate from other carbonate parameters in selecting the R/hypercalcified morphotype.

"The OMI analysis presented here was limited as we focused mostly on the carbonate system, and this may be reflected in that sometimes half of the total variability was not explained by included variables. As mentioned above, several studies have shown that light can impact sensitivity of E. huxlevi to OA (Rokitta and Rost, 2012; Jin et al., 2017), although the effect reported differs in some studies (Zhang et al., 2019). The trade-offs in energy and C balances reported by Rickaby et al. (2016) would suggest that light and mixed-layer depth might also be important parameters to consider in future studies. Nutrient limitation can also modify calcification, although a careful chemostat study suggested that nutrient and CO₂ impacts were independent (Müller et al., 2017). The impact of biotic factors were also not assessed with the OMI analysis here, though it is still not clear whether coccoliths might play roles in defense against either grazing or viruses (Harvey et al., 2015; Strom et al., 2018; Johns et al., 2019; Haunost et al., 2020)." Biotic interactions also cannot yet be discounted as playing a role in selecting among morphotypes, although evidence that they do provide defense against grazers is contradictory (Harvey et al. 2015; Strom et al. 2018), and studies disagree if they might provide protection against specific viruses (Johns et al. 2019; Haunost et al., 2020). Thus clearly biotic factors

like grazing and viruses should be considered in a larger review, but adding in a detailed discussion of them here might be too weighty and distract from the main message of the paper, as the data and analyses we present do not directly contribute to these specific issues.

4.- Finally, I would strongly recommend that the authors upload their data to a FAIRaligned reliable public data repository such as PANGAEA (specially Tables S1-S3).

We will do this.

Specific comments:

5.- line 36: coccolithophores are the main phytoplankton group to contribute to CaCO3 production, but not necessarily the main calcifying plankton group (see Buitenhuis et al., 2019).

Good suggestion. We suggest changing to (change underlined): "Coccolithophores <u>can</u> carry out <u>a substantial portion of</u> calcium carbonate (CaCO₃) precipitation in pelagic systems (Broecker and Clark, 2009), which may enhance organic matter export by CaCO₃ ballasting (Armstrong et al., 2001; Klaas and Archer, 2002), while also contributing to alkalinity (Zondervan et al., 2001) and the carbonate counter pump (Passow and Carlson, 2012)."

6.- line 37: not sure what it is meant with functional roles?

We propose simplifying to "Thus, understanding how coccolithophores respond to environmental stressors..."

7.- line 38: expand this to add some discussion about impact of the PIC:POC ratio on global biogeochemical cycles. (e.g. Ridgewell et al., 2007; Ridgewell, et al., 2009).

We prefer not to add that here, as we are already proposing to add substantially to the text. We do not have direct measures of PIC:POC ratios and so raising them might confuse readers who are new to the subject but not add information to those who are already familiar with the issues.

8.- line 40: define calcite.

Ok. We propose to add the underlined words to the phrase: ("where Kspcal is the solubility constant for calcite, the more stable form of CaCO₃")

9.- line 50: see discussion in Kottmeier et al., 2016, Gafar., et al 2018, Gafar et al., 2019, Paul and Bach 2020.

10.- line 51: please add a citation to back up this statement.

We propose to respond to these to comments together changing the sentences to: "Which extracellular carbonate chemistry parameter most influences intracellular coccolithophore calcification is debated, e.g., whether Ω cal, or more complex relationships involving HCO₃⁻, H⁺, and or CO₂ (Bach et al., 2015; Cyronak et al., 2016; Kottmeier et al., 2016; Gafar et al., 2018). Additionally, OA can have contrasting effects, with increased CO₂ availability

potentially benefiting photosynthesis but high H⁺ negatively affecting metabolisms besides calcification (Kottmeier et al., 2016; Paul and Bach, 2020)."

11.- line 85: Fjord systems

We accept change [get rid of s on fjords]

12.- line 233: mechanistically pH, CO2, and HCO3- would have been a better choice, as calcite saturation state only indirectly influences sensitivity. See Kottmeier et al 2016, Gafar et al 2018, Gafar et al 2019, Paul and Bach 2020.

We propose this change (underlined): "Temperature, salinity, in situ pH, and Ω cal were selected as they are non-redundant based on Spearman's correlation < 0.75 (Fig. S3) and they are interpretable from a biological or cell physiological point of view. To these four, we also included CO₂. It was moderately correlated with pH (Spearman correlation = 0.8), but represents the substrate for photosynthesis and is typically incorporated as a driving variable in ocean acidification studies. <u>HCO₃⁻ may more directly impact sensitivity of coccolithophores in lab measurements (e.g., Kottmeier et al., 2016; Gafar et al. 2018) but it was redundant with the other variables."</u>

13.- line 265 "Thereby, a species having a low OMI (species score close to zero, located in the center of the multivariate space) and high Tol is one that utilizes a wider array of resources and maintains populations within a wider variety of conditions (i.e., generalist), when compared with the specialized and less resilient species with more restricted realized-niche associated to high OMI and low Tol (Dolédec et al., 2000)."

This needs clarification. Although generally true, low OMI values do not necessarily imply a large niche breadth and vice versa.

This needs clarification. Although generally true, low OMI values do not necessarily imply a large niche breath and vice versa.

This is indeed very important to clarify, as there are a couple possible exceptions. We modified to acknowledge these possibilities (underlined):

"Thereby, a species having a low OMI (species score close to zero, located in the center of the multivariate space) and high Tol is <u>likely</u> one that utilizes a wider array of resources and maintains populations within a wider variety of conditions (i.e., generalist), when compared with the specialized and less resilient species with more restricted realized-niches <u>that are expected to show lower Tol and may also be associated with lower OMI</u> (Doledec et al., 2000)."

14.- line 270: which environmental parameters were used for the OMI analysis?

This was informed in section 2.4.1: "Temperature, salinity, pCO2, pH, and Ω cal were selected to be used in all subsequent statistical analyses". To make it clear, we propose to add the following text in line 259 (underlined): "We used the outlying mean index (OMI) analysis (Dolédec et al., 2000) to assess how the different physical-chemical variables (selected in section 2.4.1) were associated..."

15.- line 273: where does the number of this correction factor come from?

Proposed change (underlined): "A 1.84× correction factor <u>(determined as informed in section 2.2)</u> was applied to these data "

16.- line 372: Add another section here summarizing the main trends from Sections 3.1 and 3.2.

We prefer not to add another section here, as a qualitative summary is done in the Discussion (and wouldn't be new "Results"), and the main trends are statistically analyzed together in a quantitative way in the subsequent sections (which are Results as they are outcomes from formal analyses).

17.- line 387: Patagonian fjords.

Change accepted

18.- line 398: What about the Moderate A morphotype? %Rtol suggests that over half of variance is not explained by the OMI analysis?

We incorporated the response to this observation in response to RC2's comment 3.

19.- line 399: This analysis is very interesting and an important part of the manuscript. Consider replacing 'complementary' with 'interspecies'.

We propose replacing "complementary" with "expanded" here.

20.- line 414: Add some biomass numbers here.

We can add "> 89% of total biomass"

20.- line 426: Is there any satellite data for the Fjords? Why not?

There is satellite data covering the fjords, although they are often covered by clouds. Processed Level-3 data is available from the Nasa Ocean Color website, among other sites, and we have looked at this (and we offer some figures below). We caution that it is not simple to know how well the automatic algorithms will apply to such case 2 waters. While in most of the ocean coccoliths are presumed to be dominant contributors to the PIC signal, that has been demonstrated to not be the case in some continental shelf waters, e.g., the Bay of Biscay (Daniels et al., 2012). The Bay of Biscay is a special case where the sediments resuspended have a high concentration of fossil coccoliths and other lithogenic material. Nevertheless, certain parts of the current study area could also present such conditions, such as the Archipelago Isla Madre de Dios, where sediments carried in by run-off and rivers would be expected to be very high in PIC. Properly quantifying and ground-truthing satellite signals from such waters would be another paper in and of itself. We offer here figures from MODIS Aqua climatologies of SST, PIC, and Chl-a for late winter through late summer for the relevant areas, to illustrate the patterns.

We would propose to place the PIC figures in the main manuscript for context (e.g as a first figure, with the SST and Chl-a figures as supplementary material), making clear the caveats we highlighted above. It is interesting for drawing attention to the contrast that the well-known dense E. huxleyi blooms seen in the North Sea and Norwegian fjords, which now have also been well described in the Atlantic Patagonian shelf, do not seem to be matched in the Patagonian shores or on the adjacent Pacific Coast. While there clearly is evidence for offshore increase in PIC characteristic of seasonal blooms (presumably E. huxleyi) the Pacific

waters in front of Patagonia, with seasonal timing that is not greatly different from the North Sea, these blooms seem to be of substantially lower average intensity.



Spring-summer Sea Surface Temperature (SST) monthly climatologies (2002-2020) for Patagonia (left) and northern fjords systems (right) and nearby coastal/ocean locations obtained

from the MODIS-Aqua satellite (NASA Goddard Space Flight Center, Ocean Ecology Laboratory, and Ocean Biology Processing Group, 2018).



Spring-summer Particulate Inorganic Carbon (PIC) monthly climatologies (2002-2020) for Patagonia (left) and northern fjords systems (right) and nearby coastal/ocean locations obtained from the MODIS-Aqua satellite (NASA Goddard Space Flight Center, Ocean Ecology Laboratory, and Ocean Biology Processing Group, 2018).



Spring-summer chlorophyll-a (Chl-a) monthly climatologies (2002-2020) for Patagonia (left) and northern fjords systems (right) and nearby coastal/ocean locations obtained from the MODIS-Aqua satellite (NASA Goddard Space Flight Center, Ocean Ecology Laboratory, and Ocean Biology Processing Group, 2018).

21.- line 439: What are some of these potential environmental and biotic factors?

We propose to replace the phrase in question with : "possibly affected by factors not assessed in this study, such as nutrients supply and mixed layer depth (Margalef, 1978; Cermeño et al., 2011), or predation (Nejstgaard et al., 1997)."

22.- line 440: briefly define sigma calcite.

It was defined in the Intro, but we will re-state here: "The Ω cal – the saturation state of calcite, which has often been assumed to constrain calcification in calcifying organisms – ..."

23.- line 556: clarify what you mean with 'more subtle patterns'.

We assume line 456. Proposed change: "but use of vertical profiles might have permitted a higher ability to explain variability in the OMI analysis"

24.- line 458: morphologically distinct.

Change accepted.

25.- line 463: "Although the moderate-calcified and robust-calcified A morphotypes have also been shown to be present in eastern South Pacific coastal and open ocean waters (von Dassow et al., 2018), the dominance of these A morphotypes was particular to Patagonian interior waters, as revealed by the IndVal analysis (these A moderate-calcified and robust-calcified A morphotypes were consolidated for final statistical analyses as they are not easily distinguished by objective morphological characters and were present in all samples, and preliminary analysis revealed completely overlapping realized niches). "

This is a run-on sentence which should be split for readability.

Proposed change:

"Although the moderate-calcified and robust-calcified A morphotypes have also been shown to be present in eastern South Pacific coastal and open ocean waters (von Dassow et al., 2018), the dominance of these A morphotypes was particular to Patagonian interior waters. This conclusion was supported by the IndVal analysis (Table S5), where moderatecalcified and robust-calcified A morphotypes were consolidated for final statistical analyses as they are not easily distinguished by objective morphological characters, were present in all samples, and preliminary analysis revealed completely overlapping realized niches. Both the moderate-calcified and robust-calcified A morphotypes are also observed as dominant in the Norwegian fjords (Table 4) (Young, 1994)."

26.- line 497: This is a interesting observation. How does it fit in with coccolithophore calcification state? The first OMI analysis suggest that the calcification state might influence tolerance, but this result seems not to support that? Why? What are the implications of this wide niche breath on global *E. huxleyi* abundance relative to other species?

We hope this is now addressed within the responses to RC2's comments 2 and 3.

27.- line 504: clarify that the study specifically considers temperature and carbonate chemistry, but future environmental change will also include shifts in nutrient availability, mixing rates, and shallowing of the phototic zone. There may also be community shifts which could change biotic pressures like grazing.

We hope this comment will also be adequately addressed by our proposed paragraphs in response to RC2's comment 3.

28.- line 508: Add a section here contextualizing the results with previous work on coccolithophore calcification and potential biogeochemical implications. How do the omega calcite and pH parameters observed in the Patagonian fjords compare to the RCP 8.5 2100 ocean?

We hope this comment would now be adequately responded in our response to RC2's comment 1.

29.- line 513: Remove or define SS for readers skipping straight to the conclusion.

We accept the proposed change.

30.- line 514: How small? What is meant by moderate abundances? What are typical Norwegian stocks?

Proposed change (underlined): "2. Although *E. huxleyi* never reached more than a small fraction of total phytoplankton <u>carbon</u> biomass (< 13 %), it reached moderate abundances (range: 12-276 x10³ cells L⁻¹) comparable to adjacent coastal and oceanic areas, and within the lower range of stocks reported from Norwegian fjords (1-115,000 x10³ cells L⁻¹)."

31.- line 518: replace important with abundance and generally rephrase. Why does high E. hux abundance in lower macronutrients imply that E. hux is most abundant when large diatoms are absent?

Proposed change (underlined): "3. *E. huxleyi* abundance was highest ($\geq 100 \times 10^3 \text{ cells } \text{L}^{-1}$) when assemblages of large diatoms were lowest ($\leq 10 \times 10^3 \text{ cells } \text{L}^{-1}$), in <u>late-spring</u> waters with lower macronutrients, consistent with it being most important in the absence of large diatoms."

32.- line 518: briefly mention what the difference is.

Proposed new version: "4. In terms of morphotypes, the *E. huxleyi* populations in the southern Patagonian fjords/channels were similar to Norwegian fjords (dominated by moderately-calcified and robust-calcified A morphotype) and very distinct from populations previously documented in the Southern Ocean/Drake Passage, and the Patagonian Shelf of the Atlantic (where C or B/C morphotypes were reported as dominant) and from the Eastern South Pacific coastal upwelling zone, where the R/hypercalcified morphotype dominated."

33.- line: 525: dominance in.

Proposed change accepted.

Cited references (some new to be incorporated):

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