We provide here the point-by-point responses to each reviewer, documenting and explaining all changes made. Responses to RC1 begin on p. 1, responses to RC2 on p. 8, responses to RC3 on p. 23, and a list of 4 other minor corrections is on p. 27.

Responses to RC1

We thank RC1 for the supportive and very constructive comments and suggestions. We respond to them individually below, and expect our proposed changes would lead to an improvement of 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.

Specific comments:
1. - The authors should discuss the possible origin of morphological variability within E. huxleyi (especially within the morphotype A) in more detail as it has implications for the niche analysis and the overall conclusion of the manuscript. The heavily calcified "A-CC" and "R/hyper-calcified" morphotypes have been reported as stable under different environmental conditions (e.g. Von Dassow et al. 2018) and seem to be also genetically distinct (e.g. Hagino et al. 2011). On the other hand, the "light", "moderate", and "robust" morphotypes could also represent the continuum of phenotypic plasticity within the same genotype or population, though there is evidence that they are also genetically distinct (Young et al. 2014). If they are indeed the same genotype, the results of the niche analysis suggest that E. huxleyi morphotype A has a remarkably broad niche and is highly adaptable to changing carbonate chemistry or calcite saturation state while exhibiting different phenotypes under different environmental conditions. If each morphotype represents a separate genotype or a population, then their individual niches are narrower (e.g. as seen for the "light" morphotype), and they are arguably less adaptable to environmental changes. In any case, the use of "light", "moderate", and "robust" morphotypes in this study is valid as it provides a detailed insight into the degree of calcification found under different environmental conditions. Finally, the overall conclusion on the high adaptability of E. huxleyi as a species holds regardless of the nature of its morphotypes.

This is a very interesting issue. There is clear evidence of genetic differences among the broad A and B morphotypes based on the CMM alleles of the 3'-UTR of the GPA gene, which also supports the grouping of the R morphotype (including Chilean R/hypercalcified strains) with the A morphotype and grouping together the lightly calcified B, B/C, and C morphotypes. However, genetic markers that are probably neutral (at least with respect to calcification), such as mitochondrial cytochrome oxidase 1 (cox1) and nuclear microsatellites, do not distinguish among morphotypes: The three R morphotype strains analyzed by Hagino et al. (Hagino et al., 2011) all grouped together in cox1 phylogenies, but they also all came from the same sampling site and date. A later phylogeny found the same "warm" and "cold" clades, but one R morphotype strain was found among the "cold clade" and the other among the "warm clade" (Bendif et al., 2014). We have a much larger dataset of cox1 and cox3 that we are preparing to publish where we observe this as well, that is, the R/hypercalcified strains exist in both clades. Similarly, B/C morphotypes were found dispersed among other morphotypes in

genetic groups defined by microsatellite markers (Krueger-Hadfield et al., 2014). Thus the morphotypes might not represent distinct biological species, but instead genetically determined alternative phenotypes which might be selected in diverging populations exposed to distinct conditions. This conclusion also is congruent with another population study (Cook et al., 2013), which found genetic separation of Southern Ocean morphotype A and morphotype B/C (though the small number of isolates compared is a caveat).

We propose to develop these points in the Introduction, as it is necessary for understanding subsequent organization of the analysis. Specifically, we propose to modify the sentences on lines 64-67 and expand the introduction of what is known of the genetic determination of morphotype into a complete paragraph:

Lines 60-61 (original numbering; now corresponding to the paragraph beginning on line 65) were expanded (underlined):

"Morphological variability in E. huxleyi has been reported with several morphotypes described so far with different degrees of calcification of the coccoliths, such as fusion of coccolith elements or calcite overgrowth (Young et al., 2003). <u>Morphotype A coccoliths have a grill central area and tend to be moderately calcified, while morphotypes B and C are have more lightly calcified distal shield elements and the central area is either a plate or open (type O) central (Young and Westbroek, 1991; Hagino et al., 2011). Additional morphotypes, or morphotype sub-classes, include B/C (intermediate in coccolith size between B and C) and R (*Reticulofenstra*-like), considered an A morphotype where distal shield elements are mostly or completely fused (Hagino et al., 2011).</u>"

Lines 64-67 were expanded to a new paragraph, with changes and expansions underlined (beginning on line 88 in revised manuscript):

"Nevertheless, cultured isolates maintain their morphotype classifications even under variable environmental conditions that can alter total calcite production and even lead to coccolith malformation (Young and Westbroek, 1991; Langer et al., 2011; Müller et al., 2015; von Dassow et al., 2018; Mella–Flores et al., 2018), <u>suggesting a genetic determination of coccolith morphology. One genetic marker has been associated with morphological variability in *E. huxleyi*. The calcium-binding protein GPA has been potentially associated with *E. huxleyi* coccolith deposition (Corstjens et al., 1998). Although the function of this protein is unclear, the 3' untranslated region (non-coding) showed consistent differences between morphotypes with all morphotypes A and R showing alleles (coccolith morphology motifs) CMM I, III, or IV and B, B/C and C morphotypes showing CMM II (Schroeder et al., 2005; Krueger-Hadfield et al., 2014). The uronic acid content of coccolith-associated polysaccharides also varies among strains, and the one R morphotype tested was much higher in this character than most of the other A morphotypes (Rickaby et al., 2016). It is likely that further comparative biochemical analyses following Rickaby et al. (2016) and/or associating comparative genomics analyses (e.g., studies such as Read et al., 2013; von Dassow et al., 2015; Bendif et al., 2019) with morphometric analyses may identify genetic markers associated with sub-types within the broader A and B. However, mitochondrial phylogenies classify *E. huxleyi* into a warmer-water clade and a colder water clade, and each clade contains both A (including R) morphotypes and B (or B/C or O) morphotypes (Hagino et al., 2011; Bendif et al., 2014), and B/C morphotypes also occurred in different genetic groups defined by microsatellite markers (Krueger-Hadfield et al., 2014), although another microsatellite study did find a separation between A and B/C morphotypes (Cook et al. 2013). Therefore, different morphologies likely correspond to stable genetically</u>

determined phenotypes that might reflect adaptations selected to specific conditions within a taxon whose recent evolution has been as a single biological species (Filatov, 2019).”
Line 73 (now lines 110-111) then required a minor change to avoid redundancy, changing **“A morphotype”** *to* **“R morphotype”**

To our knowledge, there is not yet a genetic marker that associates with sub-types of the larger A morphotype, though we expect such markers might be discovered soon when ongoing comparative genomics and/or biochemical analysis is combined with morphometric approaches. The Young et al. (2014) morphometric study of natural samples did include what we term the A-CC morphotype (with high relative tube width, or overgrowth of the central area, but without a high degree of fusion of distal shield elements). The histograms (Fig. 5 and 7 of Young et al., 2014) show that there is indeed a small subset "heavily calcified coccoliths (relative tube width > 0.4)" which seem to form a separate mode in that parameter. This would suggest that it might be a binary character. There is some phenotypic plasticity around the different modes. We documented this in the R/hypercalcified morphotypes, where the proportion of the central area not covered by the tube (as tube width is not possible to measure when its overgrowth is irregular) varied between high and low CO_2 conditions (von Dassow et al. 2018). The degree of fusion of distal shield elements appears to be similarly a partially discontinuous character, although this will be much more difficult to quantify, especially when working with attached coccolithophores in field samples: B morphotype strains never showing any fusion, R morphotype strains always show a large degree of fusion, but then moderately calcified A morphotype or A-CC morphotypes may show plasticity around some intermediate character mode between no fusion and partial fusion (which might be best explored in laboratory studies).
To clarify this, on lines 178-180 (original; now corresponding to lines 226-230) were expanded as follows: **"This analysis assumes discontinuous traits that can be accurately assessed by qualitative analysis. A morphometric study supports this, where coccoliths of what we term the A-CC morphotype cluster well apart from other A morphotype coccoliths in the parameter relative tube length (that is, a small second mode in histograms) (Young et al., 2014). This assumption was also necessary as morphometric analyses in these characters are difficult to measure consistently in field samples and on attached coccoliths. Similarly,** due to frequent overlap in coccolith distal shield lengths and coccosphere diameters observed in moderate- and robust-calcified A-forms (Table 1), we consolidate them into one group (hereafter jointly referred to as "moderate-calcified A-morphotype") for statistical analyses.”

2. - in Figures 4 and 6, it would be useful to have station names written above the plots a), b) and c) so that the readers can immediately see which stations the series of plots are referring to. Currently, this is not immediately clear, and the information is only found in the figure caption.
We accepted this useful suggestion.

3. - Line 415-416: "The low diversity of coccolithophores assemblages, dominated by E. huxleyi, is common to both the Patagonian and Norwegian fjord systems.”
The dominance of E. huxleyi and apparent low coccolithophore diversity may also represent a seasonal feature of both systems, as is the case in well-studied areas such as the

Mediterranean Sea, where winter communities are dominated by E. huxleyi, while summer communities can have a larger proportion of other species. Detailed seasonal studies, including sampling along the vertical profiles, would likely reveal significant additional coccolithophore diversity in the Patagonian and Norwegian fjords.

RC1 is rightly concerned about how the absence of year-around in-depth records of coccolithophores assemblages in fjord systems could affect the statement. According to our literature review, there is no complete time-series available for Patagonia and Norwegian fjords systems but only spring or summer snapshots (Table 4). So, we clarified the statement adding "spring-summer feature" **to say (line 515 of present manuscript): "The low diversity of coccolithophores assemblages, dominated by *E. huxleyi*, is a common spring-summer feature in both the Patagonian and Norwegian fjord systems."** *and insert at the end of section 4.1:* **"The low diversity in southern Patagonian waters thus may partly reflect this latitudinal trend, although more detailed seasonal studies, including sampling along vertical profiles, might reveal significant additional coccolithophore diversity in the Patagonian and Norwegian fjords."**

4. - Line 111-113: "iii) does the abundance and relative composition of E. huxleyi morphotypes reflect populations in adjacent Pacific, Atlantic, or Southern Ocean waters or instead exhibit similarities to the Norwegian fjord system, suggesting it is shaped by local factors?"

The authors can consider leaving out the part: "suggesting it is shaped by local factors?" at this point while listing the aims of the manuscript. The explanations for the similar community composition in Norwegian fjords and the studied area can be addressed later in the discussion section.

We agreed with the suggestion and made this change (see lines 155-160).

5. - Conclusion point 5 – "Niche analysis shows that the moderate A morphotype and A-CC morphotypes are generalists, whereas the R/hyper-calcified morphotype has a more marginal (specialized) realized niche."

Can this observation indicate that the R/hyper-calcified morphotype is truly genetically distinct (as was shown earlier, e.g. by Hagino et al. 2011), while A-CC is a part of the same population as the "light", "moderate" and "robust" morphotypes (i.e. morphotype A)?

The realized-niche differentiation of the R morphotype might suggest that it is indeed behaving as a distinct population. However, in consideration of the evidence we discuss in response to RC1's first point, we suggest great caution. A phenotype can be genetically determined and the allele or alleles determining that phenotype can be selected for in particular populations, but those populations might still exchange genes (or at least be able to exchange genes) with other populations where other phenotypes are prevalent. We don't know enough about the life cycle (see, e.g., (von Dassow et al., 2015; Frada et al., 2017) or population genetics/genomics of E. huxleyi, so prefer to avoid speculating in this paper whether those could represent incipient speciation.

6. - The lightly calcified genotype (LC) should be addressed in the conclusions, as it shows a narrower niche than the other ("moderate" and "robust") type A-related morphotypes.

This is identifies a couple very important points that we clarified with the following modifications (underlined):
Lines 601- (new numbering): **"The broader niche-breadth by the moderate-calcified A morphotype contrasted with the marginal niche of the R/hyper-calcified forms in Patagonia (Fig. 9a). <u>The lightly calcified A morphotype also showed a low tolerance (more specialist), but this was not statistically significant</u>."**
[note figure number changed due to accepting RC3 suggestions]
New lines 608-618 (old numbering 482-485):
"The lightly-calcified morphotype also appeared to be a generalist in the extended domain. However, we caution that while the lightly calcified E. huxleyi were almost exclusively lightly-calcified A morphotype in Patagonia, there was a continuum of lightly-calcified A, B, and B/C morphotypes (and some lightly calcified cells were difficult to classify among these types) in some of the coastal and oceanic sites. 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). "
These caveats are why we avoid concluding about the niche or niches of the lightly-calcified morphotypes.

7. - It would be interesting to include the other E. huxleyi morphotypes (B, O and B/C) into the expanded niche analysis (Figure 7b) to show how their niches compare with the different type A morphotypes addressed in this study. Of course, if the data on their distribution and abundance is available in the expanded dataset when coastal/oceanic sites are included.

Yes, this would be very interesting to do. However, as we discuss in the point above, we did not have the confidence to do that at this stage. The distinction between B, B/C, and C morphotypes is only based on coccolith length, so can only be distinguished by morphometrics. However, this is often hard to do consistently on coccospheres (our focus) when the total E. huxleyi abundance was low and the relative abundance of lightly calcified cells was also low. That's because sometimes no coccolith on a coccosphere is correctly oriented and also not covered by another coccolith for permitting length measurement. Therefore, we focus on the difference among the A, A-CC, and R/hypercalcified morphotypes, and the comparison with closely related Gephyrocapsa's, where we can draw robust conclusions.

8. - Line 509: "Our study of how E. huxleyi populations and morphotypes respond to the highly dynamic physical and chemical environments"

The authors can omit the term "populations" here, as the populations in the genetic sense were not studied in this work.

***We agree, and substituted* "abundances" *for* "populations" (line 709 of present manuscript).**

9. - Line 451: "4.4 Comparison of E. huxleyi morphotypes in Patagonia to nearby oceans vs. Norwegian fjords"
Rephrase, e.g. "to nearby oceans and Norwegian fjords"
We accepted the suggestion (line 563 now).

10. - Line 490: "…eastern South Pacific (Beaufort et al., 2011; Alvites, 2016; von Dassow et al., 2018), although it has seen (and reported as rare)"
Should read "although it has been seen…"
We accepted the correction (line 647 now)

11. - Line 502: "…a genetic underpinning of E. huxleyi morphotype (Krueger-Hadfield et al., 2014)…"
Should read "morphotypes"
Thanks! Corrected (line 691)

Cited references (some new to be incorporated):

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Responses to RC2

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 added the following paragraph to at end of the discussion (lines 697 of revised manuscript):

"**The lower values of pH and Ω_{cal} 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 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 and 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 to the first paragraph of section 4.5, by adding these sentences (as in response to RC1's comment 6) (starting at line 612):
"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 (from line 648, changes underlined)
"Experimental findings that the R/hyper-calcified morphotype did not perform better than the moderate-calcified A morphotype under high CO_2/low pH/low Ω_{cal} (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. The studies of Langer et al. (2009) and Müller et al. (2015) did find that R morphotype strains did seem more resistant to high CO_2/low pH than other A morphotypes, either in growth rate or in PIC production. 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 by interactions with other variables. "
Two new paragraphs below address further the above comment and the RC2's next comment.

> 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 (beginning on line 609):
"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), Ωcal, temperature, and salinity were important in defining the narrowness vs breadth of niches among the *E. huxleyi* A morphotype, *E. huxleyi* 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 CO_2. Calcification rate has been demonstrated to vary among *E. huxleyi* strain according to maximum photosynthetic rate, but also $[CO_3^{2-}]$ concentration at their sites of origin, while maximum photosynthetic rate also varied among the strains with $[CO_2]$ at the site of origin (Rickaby et al., 2016). Several recent studies have called into question the importance of Ωcal in determining the response of calcifying organisms, with both theoretical and laboratory support that the concentrations of HCO_3^-, CO_3^{2-}, and H^+ are more important (Kottmeier et al., 2016; Bach et al., 2015; Gafar et al., 2018). However, while HCO_3^-, CO_3^{2-} were formally excluded from the OMI analyses as redundant variables, these vary more with and CO_2 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 Ωcal. 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 Ωcal 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. huxleyi* 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 CO_2 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 FAIR-aligned reliable public data repository such as PANGAEA (specially Tables S1-S3).

We have done this. We are waiting for acceptance in PANGAEA. Here is a screen-capture of the email from PANGAEA confirming that it is in process. Nevertheless, we have also deposited the scanning electron microscope data and Tables S1 and S3 are in Zenodo, another public data repository (EC funded through CERN in the OpenAIRE project), where it is already available: https://doi.org/10.5281/zenodo.4292020.

Lars Möller on 2021-07-29 10:04

Dear Francisco Díaz,
Your data submission has been initially checked and was approved for the next steps in our editorial workflow. Our issue tracking system will inform you as soon as your data submission is further processed by one of our data editors. In general, all data and metadata are quality checked, harmonized, and processed for machine readability, which allows efficient and reliable re-usage of your data according to the FAIR principles (Wilkinson et al, 2016; https://doi.org/10.1038/sdata.2016.18).
Please be aware that we are currently facing a high rate of data submissions to PANGAEA and thus the editorial process and minting of DOI names might take up to 12 weeks.
In case you have questions or additions, you can reply to this email (not changing the email subject). Alternatively, you can edit or comment on your submission using the web interface: https://issues.pangaea.de/browse/PDI-29185
With kind regards,
the PANGAEA Editorial Team

> 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 made several changes to the first paragraph accordingly.

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

We simplified 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 now define in the first sentence (see lines 37-38)

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 expanded to respond to these two comments together changing the sentences to: **"Which extracellular carbonate chemistry parameter most influences intracellular coccolithophore calcification is debated, e.g., whether Ωcal, or more complex relationships involving HCO_3^-, H^+, and CO_2 (Bach et al., 2015; Cyronak et al., 2016; Kottmeier et al., 2016; Gafar et al., 2018). Additionally, OA can have contrasting effects, with increased CO_2 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

Thanks! [We got rid of s on fjords, now line 129]

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 made this change (lines 300-305 now, addition underlined): **"Temperature, salinity, pH, and Ω_{cal} were selected as they are non-redundant based on Spearman's correlation < 0.75 (Fig. S3) and they are easiest to interpret from a biological or cell physiological point of view. We also included CO_2. 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. HCO_3^- 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."**

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 the current lines 336-340 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 Ωcal were selected to be used in all subsequent statistical analyses". To make it clear, we added the following text in lines 327-328 of revised version (underlined): **"We used the outlying mean index (OMI) analysis (Dolédec et al., 2000) to assess <u>how the different physical-chemical variables (selected in section 2.4.1) were</u> associated…"**

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

Change on line 345 (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 (now line 496)

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 replaced **"complementary"** *with* **"extended domain"** *(line 511 now)*

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

*We add* **"<u>> 89% of total biomass</u>"** *(line 514 now)*

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. In fact, most published analyses (e.g., Hopkins et al. 2019, which we cite) specifically ignore areas where depth is < 200 m or cloud cover is too persistent. 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.
Finally we did not include the satellite figures in the main manuscript. Simply describing the caveats associated with the satellite data from the fjords would greatly add to the length of the manuscript, while the offshore data would extend beyond the focus of the manuscript. We decide to add only the following statement on lines 531-534:
*"__Shallow water depth and frequent cloud cover limit satellite observations of PIC within the Patagonian shelf and fjords, but moderate coccolithophore blooms (of lower intensity compared to the North Sea) may occur later in the summer in the Pacific sector offshore of the latitudes sampled here (Hopkins et al. 2019).__"*
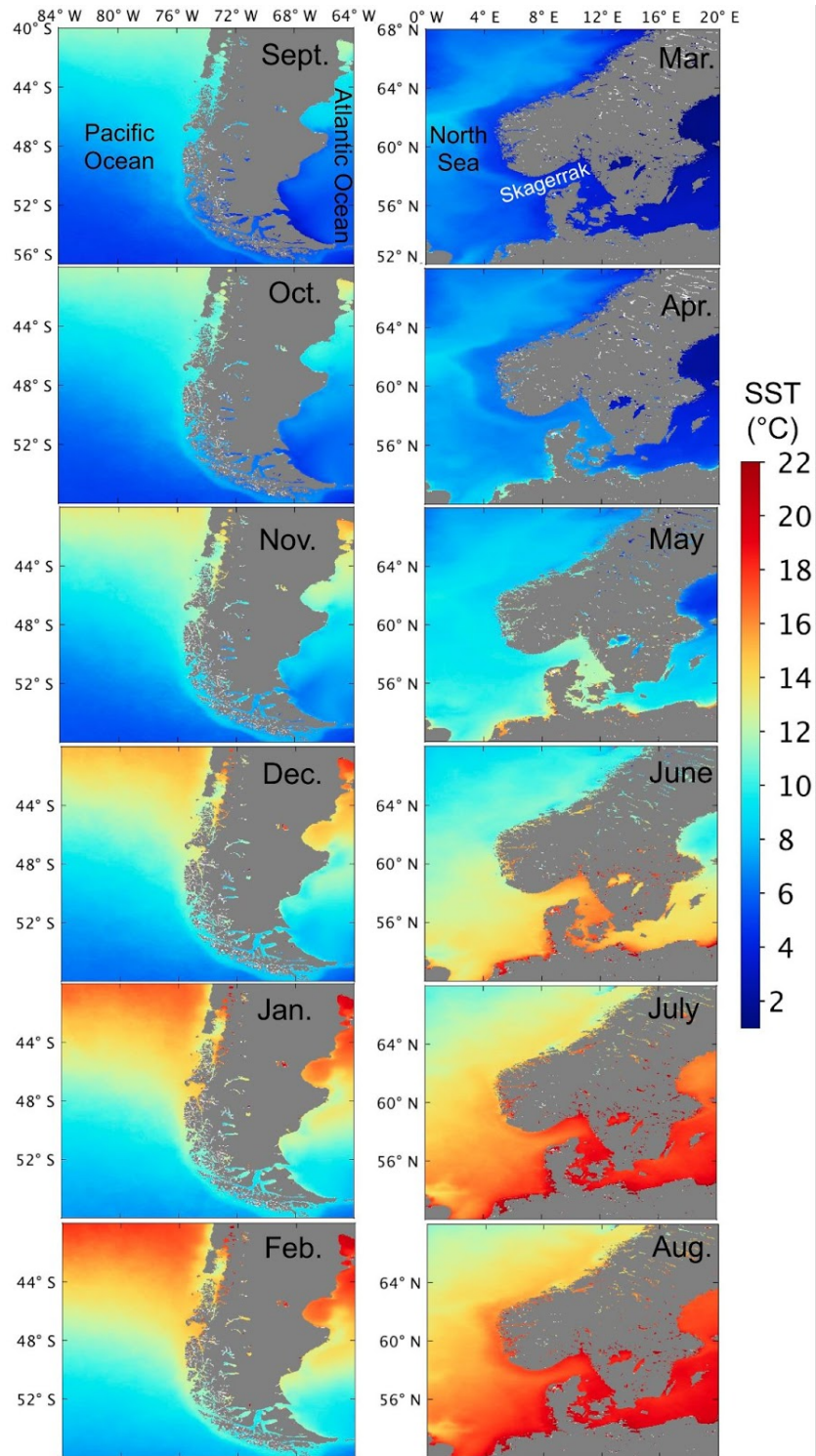
Spring-summer Sea Surface Temperature (SST) monthly climatologies (2002-2020) for Patagonia (left) and northern fjords systems (right) and nearby coastal/ocean locations obtained from the MODIS-Aqua satellite (NASA Goddard Space Flight Center, Ocean Ecology Laboratory, and Ocean Biology Processing Group, 2018).
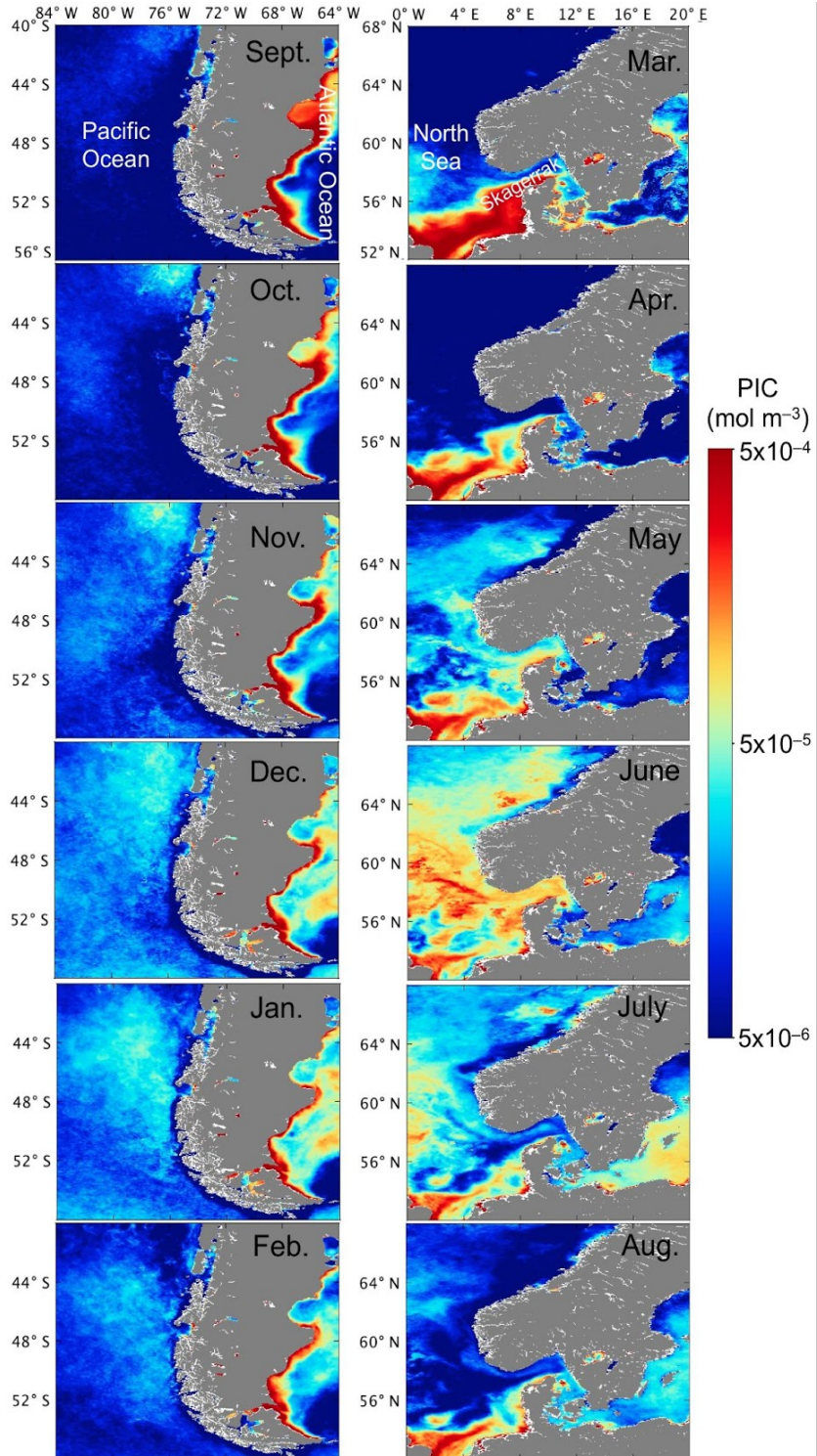
Spring-summer Particulate Inorganic Carbon (PIC) monthly climatologies (2002-2020) for Patagonia (left) and northern fjords systems (right) and nearby coastal/ocean locations obtained from the MODIS-Aqua satellite (NASA Goddard Space Flight Center, Ocean Ecology Laboratory, and Ocean Biology Processing Group, 2018).
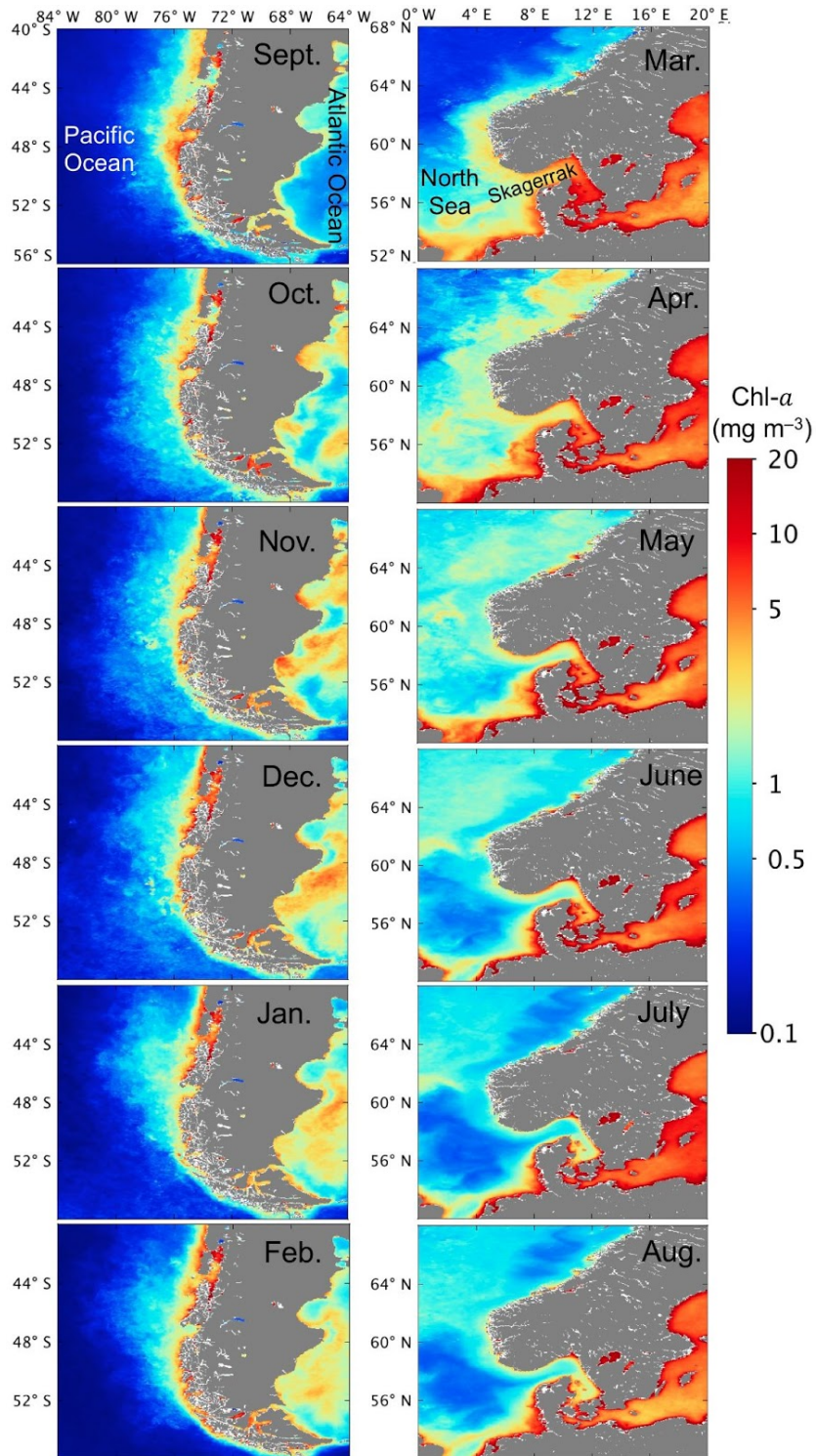
Spring-summer chlorophyll-a (Chl-a) monthly climatologies (2002-2020) for Patagonia (left) and northern fjords systems (right) and nearby coastal/ocean locations obtained from the MODIS-Aqua satellite (NASA Goddard Space Flight Center, Ocean Ecology Laboratory, and Ocean Biology Processing Group, 2018).

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

We replaced the phrase in question with (lines 548-550): "<u>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).</u>"

22.- line 440: briefly define sigma calcite.

It was defined in the Intro, but we will re-state here (now lines 551-552): "**– the saturation state of calcite, a parameter often assumed to constraint calcification (e.g., Zondervan et al., 2001; Kleypas et al. 2006; but see Cyronak et al. 2016) – …**"

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

We assume RC2 meant line 456. Proposed change (now to lines 568-569 in new numbering): "<u>but use of vertical profiles might have permitted a higher ability to explain variability in the OMI analysis</u>"

24.- line 458: morphologically distinct.

Change accepted (line 570 now)

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.

Thanks for the help! Changed to (lines 588-594):
"**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 moderate-calcified 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 accepted the suggested change (line 716 now)

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

Change (underlined) affected to lines 717-720: **"2. Although *E. huxleyi* never reached more than a small fraction of total phytoplankton <u>carbon</u> biomass <u>(< 13 %)</u>, it reached moderate abundances <u>(range: 12-276 x10^3 cells L^{-1})</u> comparable to adjacent coastal and oceanic areas, and within the lower range of stocks reported from Norwegian fjords <u>(1-115,000 x10^3 cells L^{-1})</u>."**

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?

Change (underlined) to lines 721-723: **"3. *E. huxleyi* abundance was highest <u>(> 100 x10^3 cells L^{-1})</u> when assemblages of large diatoms were lowest (< 10 x10^3 cells 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 (lines 724-728): **"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.

Change accepted (line 732 now)

19

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Responses to RC3

Thanks a lot to Dr. Saavedra for the very positive and helpful feedback. This reviewer has suggested moving some supplementary materials to the main text, and we agree with the suggestion in the case of two figures, where the information and analysis outcomes both support the main messages of the study and could be broadly useful to other phytoplankton ecology in the region. That change will not lead to an increase in the text length, and we consider will substantially 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.

Specific comments / minor suggestions

1.- Line 76: Specify where was this observed.

We now explain (lines 114-117) it was observed in the Bay of Biscay: **"Likewise, Smith et al. (2012) observed an increase in the proportion of *E. huxleyi*, corresponding to an "over-calcified" morphotype (with complete overgrowth of the coccolith central area but without fusion of distal shield elements, referred to hereon as A-CC for covered central area) that occurred during the <u>winter decline of Ω_{cal} in the Bay of Biscay (North Atlantic)</u>."**

2.- Line 181: I find interesting the information regarding the malformed coccospheres, although they are just marginally mentioned in the introduction and here. Maybe the supplementary material Figure S2 (i.e. the plate) could be moved to the main text and the authors could mention a bit more about malformations in the results section.

This is something interesting that we have struggled with. We prefer to keep this in the supplementary information because it is a poorly defined "group" so much more difficult to interpret or speculate about. As malformed coccoliths or coccospheres may be the result of a range of processes from the physiological to even post-mortem (in contrast with other morphotypes which are thought to be principally genetically determined), it creates the complication that it cannot be included in a simple way in the statistical analyses we later do (such as the OMI analysis) and could detract from the paper's main focus. For example, if we were to try to put examples of all the malformation types, the Fig. 2 might end up with as many or more malformed "types" than the morphotypes we could classify, even though they actually represent a small fraction of the total. Thus it would provide a lot more "information" that would not actually be clearly interpretable with respect to the main questions.

3.- Lines 202-205. I am not a diatom specialist, but I am aware that in the Southern Ocean, many diatoms are colonial and chain-forming, and thus it is difficult to enumerate at a quick glance. Can the authors specify if the diatoms were counted (or semiquantitatively estimated) as frustules or valves? can they specify if the diatoms were broken or intact? I think that only frustules (= cells) should be compared to coccospheres. Maybe the authors can elaborate more on this point.

This is an important question we can clarify. The work in 2015 was made from counting the valves. In 2017, it was only at qualitative and semi-quantitative manner from SEM, where proper abundance counts were not possible with the resources we had for costly SEM time. We replaced "cells" with "valves" to be clear (lines 262 now).

4.- Lines 374-375: I am aware that supplementary material Figure S10 contains a lot of information. It is up to the authors, but I consider that it could be also moved to section 3.3 of the manuscript.

We are glad to know this information is found to be valuable (they represent quite a lot of work both in counting and in the analyses). We have moved both S10 and S11 to the main manuscript. This did not require changes to the text other than renumbering the figures.

5.- The following point has already been mentioned by #Reviewer 2. The authors already uploaded all the scanning electron micrograph image datasets in https://doi.org/10.5281/zenodo.4292020 and they state that all data resulting from this study are available from the corresponding author upon request, which is great for the scientific community. However, I think it would be worthy to also upload the rest of the valuable data shown in some of the tables from the supplementary material in an Open Access library/repository such as PANGAEA.

We are putting the data in PANGAEA, as suggested also by another reviewer. We are still awaiting the final acceptance (see the message from PANGAEA). We have put pictures used to measure carbon biomass up, as well as SEM. All the rest of the data is in the supplementary tables, but they are being included data submitted to PANGAEA as well for convenience. Additionally, all the SEM data as well as Tables S1 and S3 are in the Zenodo public data repository (EC-funded OpenAIRE project partnered with CERN; see https://about.zenodo.org/)

Technical corrections

6.- Line 69: I recommend using either CO_3^{2-} or $[CO_3^{2-}]$, just for consistency (e.g., see lines 40, 449, Table 2…)

In line 40 (now line 43), we have to use $[CO_3^{2-}]$ because it is in a chemical formula. However, from then on, to make reading simpler, we prefer to use without brackets, and have corrected $[Ca^{2+}]$ to Ca^{2+}, $[CO_3^{2-}]$ to CO_3^{2-}, etc..

7.- Line 100: "and" instead of "but".

We changed to "however" (now line 144) because the point is that blooms have not been reported, however, that might be just because that there haven't been observations of coccolithophores. Nevertheless, in our response to RC2 we offer figures of monthly PIC climatologies, showing that blooms of the intensity seen in the North Sea at least do not appear to be regular phenomenon.

8.- Line 111: "and" instead "Focusing on the cosmopolitan E. huxleyi"

Suggestion accepted (now line 155)

9.- Line 233: Double check if you need "in situ" here.

Suggestion to remove "in situ" accepted (now line 300).

10.- Line 235: "We also included CO_2, which was moderately correlated with pH (Spearman correlation = 0.8)…"

We eliminated "To these four," (line 304)

11.- Line 244: Are diatom abundances cells/L? Can the authors specify?

We added "(in cells L^{-1})" (line 312)

12.- Lines 277-278: Either: "the potential biases from comparing data from both SEM and Utermöhl counts were minimized" or "the potential bias from comparing data from both SEM and Utermöhl counts was minimized".

We accept the first suggestion and keep plural… Thanks!

13.- Line 308: "were corrosive to calcite" sounds rather dramatic. I would use: "where undersaturated occurred".

We changed to "where conditions were undersaturated with respect to calcite" (line 385 now)

14.- Line 309: Here and elsewhere in the text. Because it is the beginning of a sentence the whole name (i.e."Emiliania huxleyi") should be written instead of the abbreviation ("E. huxleyi")

We accepted the corrections.

15.- Line 344: Add "taxa" or "species" after coccolithophore.

We accepted the correction and used "taxon" (line 424)

16.- Line 475: "Niche analysis of E. huxleyi morphotypes…"

Done (added "of" in what is now line 600)

17.- Line 564: E. huxleyi (in italics)

Done.

18.- Figure 1: Is it possible to make the station numbers lighter to see them more clearly?

Yes… We did as suggested.

19.- Figure 2 (caption). Since there is a coccosphere of E. huxleyi morphotype O in this figure, I guess the authors mean "The main E. huxleyi morphotypes recorded…" (delete A)

Good catch! We changed.

20.- Figures 4 and 6: In the text of the manuscript, salinity does not have any units, I would delete "(psu)" for consistency.

Done.

<div align="center">Other final corrections</div>

We found four minor errors in the Díaz-Rosas et al. 2021 submitted manuscript that were not marked by referees, but which we corrected.

- Table 2 headline: replaced "late-winter" by "early-spring"

- Table 4: added the R morphotype to Menschel et al. 2016 study

- Line 543: Replaced Hopkins 2015 quote by Smith et al., 2017

- Correction of conclusion #2: "Although E. huxleyi never reached more than a small fraction of total plankton carbon biomass (< 13 % of nano- and microplankton assemblages counted by microscopy), it reached moderate abundances (range: 12-276 $\times 10^3$ cells L^{-1}) comparable to adjacent coastal and oceanic areas, and within the lower range of stocks reported from Norwegian fjords (1-115,000 $\times 10^3$ cells L^{-1})."