

We thank RCI 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 would be 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). 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 (*Reticulofenestra*-like), considered an A morphotype where distal shield elements are mostly or completely fused (Hagino et al., 2011).”

Lines 64-67 would be expanded to a new paragraph, with changes and expansions underlined

“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), 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 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 then would require 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 CO₂ 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 we propose to add the following: **“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 will accept 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 should clarify the

statement adding “spring-summer feature” to say: “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 agree with the suggestion.

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 identifies a couple very important points that we must clarify with the following modifications (underlined):

Lines 476-477: “The broader niche-breadth by the moderate-calcified A morphotype contrasted with the marginal niche of the R/hyper-calcified forms in Patagonia (Fig. 7a). The lightly calcified A morphotype also showed a low tolerance (more specialist), but this was not statistically significant.”

Lines 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 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 proposed to substitute “abundances” for “populations”.

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 will accept the suggestion.

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 accept the correction.

11. - Line 502: "...a genetic underpinning of *E. huxleyi* morphotype (Krueger-Hadfield et al., 2014)..."

Should read "morphotypes"

Thanks!

Cited references (some new to be incorporated):

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