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Interactive comment on "Biogeochemical implications of comparative growth rates of *Emiliania huxleyi* and *Coccolithus* species" *by* C. J. Daniels et al.

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Anonymous Referee #2

We thank the reviewer for their comments and address them below.

Sparseness of information. This paper tries to make the case that lab rat species *E. huxleyi* may not be as an important contributor to calcite production in the Northern Atlantic as is commonly believed or implied. It is important that myopic viewpoints get challenged; the amount of research effort devoted to a certain species doesn't proof its importance. However, in order to make their challenge convincing, the authors should evaluate a much wider selection of published

data, e.g. including those of Bach et al., Hoppe et al., Rodriguez-Iglesias et al. and many older publications, especially since the per-capita growth rate and cellular calcite content reported for *E. hux* in this manuscript are substantially lower than those reported in many other papers. I find the author's reply to the same issue raised by the editor not compelling (most published data would not be comparable with the author's data due to a difference in growth conditions) and even contradicting their own application of lab results to estimate calcite production in field populations, as those mixed populations are highly unlikely facing growth conditions that are comparable to those maintained in the lab. Publications that have appeared in the context of OA all report on the performance of coccolithophorids at present day ocean carbonate system conditions; this voids the author's second objection. With those alternative data, the authors may come to conclusions that are qualitatively similar but quantitatively much less pronounced.

We apologise for any confusion surrounding our stated reservations about using other culture data. Our concern was that to accurately compare relative growth rates of different coccolithophore species, they must be grown in parallel, under identical conditions whereas there is a wide range of conditions (temperature, day length, irradiance level) used in the literature. For our manuscript, we require *E. huxleyi* and *C. pelagicus*/*C. braarudii* to be cultured in an identical manner, which does not exist in most of the literature, thus making a direct comparison more challenging. In the revised version we now include more reference to other literature in an attempt to put our observations in the general context of the literature. Although our maximal growth rates of *E. huxleyi* (0.85 d^{-1}) were lower than those measured in Bach et al. (2011)(1.1 d^{-1}) and Hoppe et al. (2011) (1.17-1.22 d^{-1}), they are comparable to Iglesias-Rodriguez et al. (2008) (0.6-1 d^{-1}), and faster than observed in some literature: eg: 0.7 d^{-1} (Balch et al., 1992), 0.67-0.7 d^{-1} (Müller et al., 2011) and 0.15 d^{-1} (De Bodt et al., 2010). Our growth rates also lie within the range of growth rates summarised in the seminal review of *E. huxleyi* biology by Paasche (2002) (0.43-1.94 d^{-1}). Hence, we do not consider

that our growth rates are “substantially lower than those reported in many other papers”, and consider them rather as being within the range found by other researchers. Following the recent paper by Hoffman et al (2014) who examined the coccosphere of *E. huxleyi* in minute detail and found higher coccolith numbers per cell than we previously accounted for, we have increased our estimates of *E. huxleyi* calcite by 1/3 (20-24 coccoliths per cell rather than 15-18) to 0.43 (RCC3533) and 0.52 (RCC1228) pmol C cell^{-1} for the two *E. huxleyi* strains. While these values are indeed lower than those measured by Hoppe et al. (2011)(0.8-1.1 pmol C cell^{-1}), they are very similar to the range measured by Iglesias-Rodriguez et al. (2008) (0.23-0.48 pmol C cell^{-1}), and greater than measured values from a number of other studies (eg Fritz and Balch, 1996; Paasche, 1999, 2002). There is no consensus in the literature as to the cellular calcite content of *E. huxleyi* and our values are well within the range found by other researchers-hence we do not believe that our cellular calcite content is “substantially lower” than found by others, although we have now considered a wider range of literature within our manuscript. Importantly, within the modelling exercise in the paper we already consider a wide range of *E. huxleyi* cellular calcite content and growth rates in order to examine how this influences our conclusions.

In addition, the authors consider only 2 *E. hux* strains; Read et al. (doi:10.1038/nature12221) have recently shown light on the large diversity in metabolic potential among *E. hux* strains. This makes the foundation of the author’s case rather fragile.

Read et al. used genomic sequencing of 13 strains of *E. huxleyi* to identify genomic plasticity which they suggest may explain physiological variation, but they do not explicitly examine how this genomic plasticity explains metabolic potential or make any link to that observed or inferred. While it is it not feasibly possible to capture this variability using culture experiments (individual strains of *E. huxleyi*number in their hundreds), culture based experiments remain our primary tool for examining physiologies of individual species of phytoplankton (e.g., Schluter et al., 2014. Adaptation of a globally im-

portant coccolithophore to ocean warming and acidification. Nature Climate Change, doi: 10.1038/nclimate2379). We designed our experiment to minimise this issue by using strains of *E. huxleyi* from the same regions from which the *C. pelagicus* and *C. braarudii* strains were acquired. Therefore, we could reasonably expect their metabolic potential to be more closely matched to in situ populations than culture strains isolated from elsewhere.

The authors also provide too little information about the growth conditions in their lab experiments. I couldn’t find the composition of “enriched seawater K/20 medium (modified from Keller et al., 1987)” anywhere-what are the concentrations of P and N species?

This was a slight oversight on our part. The modified seawater K/20 medium based on the modified K/2 culture medium used by the Roscoff Culture Collection for growing coccolithophores (http://roscoff-culture-collection.org/sites/default/files/MediaRecipesPDF/K2-Ian.pdf). We have added further details on the composition of the medium.

Which is the nutrient that ultimately becomes limiting for growth? This is a serious omission, albeit easily remedied.

As stated in our manuscript, the cultures were harvested in mid-exponential phase, and therefore neither nutrient was limiting growth at the time of harvesting.

In the same vein, the manuscript lacks a physico-chemical characterization of the samples from the North Atlantic.

In the manuscript, we only use samples collected from the North Atlantic to demonstrate the potential for *C. pelagicus* to be a major calcite producer based on the abundance relative to *E. huxleyi*, rather than to determine the exact contribution of *C. pelagicus*. As we state at the end of the manuscript, to do this we would require accurate measurements of differential growth rates and/or the calcite production rates from

these samples. Therefore, we have not gone into the details of the physicochemical environment in the present paper. Such details are included in Ryan-Keogh et al. (2013), which we have now added as a reference. Ryan-Keogh et al. (2013) examined the limiting nutrients for the phytoplankton community as a whole through bioassays (nutrient replete during spring (D350), with iron and/or nitrate limiting during summer (D354)), not what specifically limited the coccolithophore component of the community. Hence, we feel that adding physiochemical information would tell us little about which species could potentially dominate calcite production.

The authors probably didn't find statistically significant differences in the measures among treatments in Table 1 and therefore decided to give means and SDs instead of individual measurements. Frustratingly, this is a too common practice, which can seriously limit the value of results for readers with different research questions either now or later when insights in a field will have evolved. You did the work, so why limit the credit you could potentially receive for it?

We choose to present our data in Table 1 in the form of averages with standard deviations as this was the format of the data used within the model. While the individual data are available, they are part of another ongoing project and therefore if the reviewer/reader requires this data in more detail, we will gladly provide it on request.

Those data could easily be included in Table 2 once 3 unnecessary columns are deleted. The column with daily irradiance should be deleted because it is redundant (and presented with reduced precision-cf. significant figures of column 2) and the columns with standard deviations are potentially misleading (the SDs refer to the variability in instrumental readings, not biological quantities).

Instead of removing daily irradiance we have removed instantaneous irradiance as we feel daily irradiance is more important (see our later response). We have now increased the precision of this data in the table. The standard deviations do not refer to the variability in instrument readings, but reflect the variability in the duplicate culture

experiments performed. âĂČ

Finally, the presentation of the computational method needs elaboration. Keep Equation 1 (in which numerator and denominator should change places!), but add the form that is actually used, with , and , in which the subscripted 'c' and 'e' stand for *Coccolithus* and *Emiliania*, respectively. rc is the growth rate of *Coccolithus* relative to that of *Emiliania* (expressing relative growth rates and species abundances as percentages instead of fractions is not only ugly but also confusing). Based on this recast equation, I'd suggest (1) to use corresponding measures, i.e. nc instead of ne (=1-nc) with rc, and (2) to simplify Figure 3 (but see next section), since varying rc, nc or cc gives identical results (hence, the contour curves in Figure 3 are straight lines). Unfortunately, the nonlinear relationship between %CPc and any of these relative measures is obscured in Figure 3. I think a plot with ccnc on the x-axis, %CPc on the y-axis and rc representing contour curves is more informative, while the number of panels is reduced from 6 to 2 (you could add dotted curves to display the information of Table 1 including the +/1 SD curves).

The reviewer is correct to note that we accidently inverted our Equation 1. This was a mistake in the manuscript rather than a mistake in the model implementation, and this has now been corrected in the revised version. The new equation suggested by the reviewer is a rearranged version of our Equation 1. As we implemented our model using Equation 1, using the new equation would not affect the model and therefore we do not feel that we need to add this equation into our manuscript. Furthermore our equation is adaptable to communities consisting of more than 2 coccolithophore species whereas the reviewers is limited to 2 and hence in the future, when examining calcification rates in multi species communities, our equation is more relevant. We have produced the figure suggested by the reviewer (see attached). However, because the output of the model (%CP) is now the y axis of the figure, this constrains the plot such that a large amount of white space exists in the figure, and more importantly the

relationship between %CP, relative growth rate, and relative abundance is harder to elucidate. The reviewer is correct that the relationship between the relative variables and %CP is nonlinear; they are inversely related. We have now made this relationship explicit in the text but retain the original format of Figure 3. For comparing with natural communities, using the ratio of *E. huxleyi* to *Coccolithus*, rather than the relative abundance of *Coccolithus* (to *E. huxleyi* plus *Coccolithus*) is more applicable and less ambiguous, as we have not considered other coccolithophore species in our current work, and we are interested in realistic communities where *Coccolithus* forms only a small fraction (<10%) of the community. Therefore we feel that our original figure is most suitable for this purpose. We have not expressed relative growth as a fraction as we are concerned that such fractions (0.2-1.1) could be mistaken for actual growth rates. We found that adding +/- 1 SD dotted lines to the figures also made them too complicated to be informative.

Relative contribution to calcite production. The value of the authors' method for the estimation of species contributions to calcite production in mixed field populations depends on the reliability of 4 assumptions, of which 3 are implied; these must be made explicit and evaluated. First, the relative abundance of species in mixed field populations is constant. I would like to see some back up with literature references showing that community dynamics are sufficiently slow to warrant the assumption approximately holds for a meaningful time interval.

Our meaningful time interval is a day, as now stated in the revised manuscript. Our primary interest is in the species which dominates daily calcite production as this time period neatly incorporates both gross production of calcite and net changes in cell abundance through division and mortality losses.

(It is very confusing to mention 'steady state' in this context, as there isn't a dynamic model; e.g. with relative population densities as state variables, a steady state means populations are growing exponentially, whereas with absolute population densities, it means that the loss rate (mortality, sinking, grazing) equals

the population growth rate. Also, it is confusing to link 'steady state' with a variable relative growth rate.)

In the sense that we use the term (and much of the literature cited), steady state refers to the period when the 'specific production rates of all cellular constituents are proportional to the rate of cell division' see Leynaert et al. (2001). Steady state has to be assumed to calculate calcite production from growth rate and cell calcite. We have rephrased this sentence to clarify the context in light of the reviewers comments.

Second, the authors implicitly assume that the strains in mixed field populations are the same or behaving physiologically similar to the ones in their lab studies. The authors try to overcome the constraints of this assumption by considering cellular calcite contents that may differ up to 1 SD from the means in their calculations. However, they do not provide support that this level of variation would cover the variability in cellular calcite content among strains and environments (I doubt it does, as 1 SD corresponds to only 15-30% of the mean.

The reviewer is correct that we have varied the cellular calcite in order to account for differences between our cultures and natural populations. However, we effectively manipulated our model by more than 1 SD as we concurrently varied both *E. huxleyi* and *Coccolithus* cellular calcite in opposite directions resulting in a cumulative manipulation. If we had held *Coccolithus* constant, the equivalent variation in *E. huxleyi* would be 0.23-0.75 pmol C cell^{-1} for RCC3533 and 0.33-0.79 pmol C cell^{-1} for RCC1228, a range that easily encompasses much of the literature values for *E. huxleyi*. We have added further detail about this in the revised manuscript.

Third, the authors implicitly assume that lab and field growth conditions (the reader remains uninformed about the latter) are similar, thought those conditions are likely very different.

The temperature and light regimes used in the culture experiments were chosen to reflect realistic conditions found in the North Atlantic where the species co-occur. We

have now added references to make this more explicit.

Related to this, the authors also implicitly assume that the relative growth rate is independent of the availability of the limiting nutrient, i.e. the relative maximum growth rate equals the ratio of the growth rates of the 2 species involved regardless of environmental conditions as long as the relative abundance is constant. This assumption is too heroic for my taste. A species can have a relatively low maximum growth rate and relatively high growth rate at low resource densities, and vice versa. Taking the Monod or Holling type 2 model as an example (other models such as Droop's cell quota model lead to analogous results) implying that rc ranges between (high nutrient concentrations;) and (very low nutrient concentrations;). Since K values could differ by more than an order of magnitude, the range of relative growth rates that should be considered in the authors' evaluation is much wider than the range they consider to be relevant (e.g. in Figure 3). Assuming values for Ksp are unknown, this undermines a major line of reasoning in the manuscript, unless the authors have additional information, such as estimates for loss rates through sediment trap data (in steady state, the relative growth rate is equivalent to the ratio of the loss rates of the 2 species

The reviewer is correct that there is a strong potential for relative growth rates to be different in replete and depleted nutrient conditions, however we have no experimental or field data to support this assumption to any greater degree than the assumption that they do not. Without knowing the relative nutrient requirements (K) of different species, or the ability of different species to obtain nutrients, application of the Monad/Holling/Droop models remain theoretical. Accounting for the reviewers comments we have now explicitly discussed this assumption and incorporated a much greater range of relative growth rates in the revised manuscript (revised Figure 3 now ranges down to 10%). This highlights that despite potentially large differences in growth rates, the 50-100 times higher calcite content of the *Coccolithus* species still enables them to be significant (if not dominant) in calcite production. Furthermore we have now

included a caveat in the discussion that in situ growth rates of the different species need to be made to confirm or refute our assumptions. That *C. pelagicus* dominates calcite export in much of the North Atlantic and is often seen in very high cell densities (1000 cells ml^{-1}) indicates to us that even in the event that relative growth rates are vastly different to our culture experiments, this species remains highly significant for calcite production in the North Atlantic Ocean.

Other comments

line 157-160: a difference in irradiance between this study and that of others cannot explain the relatively low growth rate found in this study. Beyond 100 mumol photons sec m^2 irradiance is ad libitum for all 4 strains species: there is no increase in temperature corrected growth rates as irradiance levels increase beyond this level (I did a crude temperature correction with the parameters estimated from growth rate vs temperature by linear regression (each strain/species separately); growth rates at irradiance levels <100 mumol/sec m^2 and 244 mumol/m^2 excluded from the fit for obvious reasons). I would give an explanation based on strain variability more credibility. The fact that the temperature response curves appear linear rather than exponentially increasing (as with Q10, Arrhenius) is interesting and might merit a bit of thought and elaboration.

We agree with the reviewer that there is likely to be significant strain variability in maximum growth rates (see Langer et al., 2009). However, beyond this, day length and the daily dose of irradiance will have a significant effect on growth rates. While coccolithophores will become light saturated at a given instantaneous irradiance, the length of time for which they are exposed to this irradiance will affect their growth rates, with a general increase in growth rate as day length increases (Paasche, 1967). It has been shown that day lengths shorter than 16 hours will reduce phytoplankton growth, however there is no consensus in the literature as to which day/night cycle is recommended (Probert and Houdan, 2004). As our study used a day length of 12 hours, we would expect our growth rates to be lower than studies that have used 16 hour day lengths

(eg Bach et al., 2011; Hoppe et al., 2011; Langer et al., 2009). However, our maximum growth rates are similar to Iglesias-Rodriguez et al. (2008) who used a 12 hour day length. We have reworded and extended our discussion of the influence of day length to improve the clarity of our discussion.

line 136. Langer (also) gives growth rates for *C. pelagicus*.

Langer et al. (2006) used *C. braarudii* rather than *C. pelagicus*, however it is referred to in Langer et al. (2006) as *C. pelagicus*. The strain used by Langer is maintained in the Roscoff Culture Collection who have confirmed that it is a strain of *C. braarudii* not *C. pelagicus* http://roscoff-culture-collection.org/rcc-strain-details/1200.

Please define 'relative growth rate' the first time it appears in the text.

We have added in a definition of relative growth rate.

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Please also note the supplement to this comment:
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