

Interactive comment on “Physiology regulates the relationship between coccosphere geometry and growth-phase in coccolithophores” by Rosie M. Sheward et al.

Anonymous Referee #1

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General comments: The manuscript is well written and overall clearly structured, although the results and discussion sections are not clearly separated with some results discussed in the results section and some new data (μ , PIC) introduced in the discussion. The methods are provided in sufficient detail to allow reproduction, however, some additional information on how the data was treated in creating the figures could be useful, see specific comments. The results section needs to be carefully reread. There is a lack of consistency between described results and presented data (especially table 1), see specific comments. Furthermore, not all data described in the methods/presented in table 1 are discussed e.g. POC data. The manuscript addresses a relevant scientific question. The approach of using data from modern coccolithophores

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to interpret the fossil record is highly relevant for understanding long-term trends and identifying possible future trends. In this light, intact fossil coccospheres pose an intriguing possibility to study the relationship between physiology and (palaeo-) environment and the authors add to this knowledge base by presenting data on coccosphere geometry of lesser studied (and genetically distant) species. Yet, I wonder what the range of applicability is. Do the authors have any knowledge on how common it is to find intact coccospheres rather than single coccoliths in the fossil record? Is there any data available for the species presented in the current manuscript? The conclusion that the trend observed by Gibbs et al. (2013) is a feature of coccolithophores as a whole is well founded. However, the hypothesis that coccosphere geometry can give information on population fitness is quite bold. In fact, the authors are very well aware of this fact and discuss the difference between growth phase and growth rate. Figure 5 nicely illustrates that an exponentially growing culture can be likened to a bloom situation in the field, whereas a non-bloom situation can look like a “stationary phase” culture. However, these situations are very short-lived in nature. The authors write themselves (L 301-303) “growth rates would not necessarily be expected to influence coccosphere geometry in the same way as a shift in growth phase”. I would agree, but this limits the applicability of coccosphere geometry to the fossil record. To relate coccosphere geometry and nutrient availability in a geological setting, it is necessary to (at least) look at whether nutrient-induced changes in steady state i.e. continuous growth rates have an effect on average coccosphere geometry. The authors tried to address this by growing the species at different temperatures, which failed to induce a significant range in growth rate. The concept of “growth phase in the fossil record” is difficult because growth phases are of very short duration in nature. Over long time periods, steady state or maximum exponential growth rates become important. It is therefore misleading to relate growth phase to the fitness of a population (e.g. L 68; L 378) because all populations undergo different growth phases. The presented data is intriguing and propose a framework, which may prove useful in the future. However, the conclusion that this is a proxy for “population fitness” is a bit overstated (L 375-380)

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and the discussion would benefit from including some thoughts on testing this hypothesis further e.g. by using chemostats. In a similar light, although the authors discuss this, in some parts of the text (see specific comments) it could be made clearer that it is only the average coccosphere size and CN that shifts, whereas the range is the same in both growth phases (Figure 3). Although Figure 4 looks very impressive, it creates the idea that in exponential phase “all cells” are small, whereas in stationary phase “all cells” are large. However, figure 3 makes it clear that both stages span the same size range and there is only a slight (though significant) shift in the average size.

Specific comments: L 14-16: “however, to realize the potential of this archive requires an understanding” needs to be rephrased. L 36: why do the authors use a threshold size of 63 μm , instead of e.g. 200 μm , the threshold for microplankton? Does this relate to the max. size of coccolithophores? L 37-38: what do the authors mean by “the biomass that sustains the wider diversity of marine life at higher trophic level”? I would argue that the (genetic) diversity of photosynthesizing plankton surpasses that of higher trophic levels. L 93: should it be “classified into separate families” rather than “in”? L 105: please check the calculation of daily photon flux; it should be half that value; maybe the calculation was carried out using 24-h light? L 106: the authors could consider mentioning up front that the different temperatures failed to give a significant range in cell division rates and therefore all data was pooled. L 108-110: I would add a table as supplementary information with μ (rather than just mentioning the range in the discussion) and coccosphere geometry parameters for each temperature experiment. The authors state that there were no (significant?) differences among the temperature treatments. However, they have used only two replicates for each temperature. How was this tested? L 114-115: please give nitrate and phosphate concentrations of the medium as this is important for interpreting final cell concentrations. The authors give references to the K/20-medium, but following Daniels et al. (2014), nitrate concentrations would be 28.8 μM and phosphate 1.8 μM , following Gerecht et al. (2014), concentrations would only be 16 μM nitrate and 1 μM phosphate. Šupraha et al. (2015) presented data on the same strain of *Helicosphaera* used in the present experiment

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which entered stationary phase at ca. half the cell concentration (15500 cells mL⁻¹) as in the current study (growing on 1 μM initial phosphate; I therefore assume phosphate concentration was 1.8 μM ? L 115-119: Daniels et al. (2014) grew dilute batch cultures and harvested in exponential phase, whereas the authors have used the same medium (?), but harvested in stationary phase. In fact, the cultures reached much higher cell concentrations (for *C. braarudii* 25.000 cells mL⁻¹ vs. max. 8.700 in Daniels et al. (2014)). The authors do not give information on the carbonate chemistry, but based on the high final cell concentrations, I would assume that there was a significant consumption of DIC (for comparison, in Gerecht et al. (2014), *C. braarudii* reached max. cell concentrations of 17550 cells mL⁻¹, which reduced DIC down to 1200 μM . A rough calculation and assuming a proportional response, at 25000 cells mL⁻¹, DIC would be reduced to ca. 900 μM which is assumedly limiting for growth (Bach et al., 2013). How much can this really be compensated for by passive diffusion into a bottle and then into the medium, which are both presumably slow processes? Similarly, Šupraha et al. (2015) presented data on a Mediterranean strain of *Helicosphaera* that entered stationary phase at 41.000 cells mL⁻¹ vs. the 30.000 in this study. At 41.000 cells mL⁻¹, carbonate chemistry was severely altered (and PIC quota of the single coccoliths was affected). A significant change in carbonate chemistry and ensuing DIC limitation does not directly affect the conclusions of the manuscript, also because PIC quota was not directly measured, but inferred from coccolith length measurements. However, L 115-119 is misleading in suggesting that carbonate chemistry was not affected as no data is presented to confirm this. On the contrary, the available data indicate that there was a significant impact (as outlined above). L 278: POC production could have been affected by DIC limitation. L 127: assumedly, formaldehyde was also added to *Helicosphaera* cultures for size measurements? Although the coccosphere is probably relatively stable, formaldehyde may lead to shrinking of the cell membrane, which could influence morphology measurements. Did the authors consider/check this? L 135: no need to mention that half of the filters were stored for SEM, if the data is not presented. Did the authors do any control measurements under SEM? L 143: If coccosphere size

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is used as a (realistic) proxy for cell size, the terminology should be used more clearly. In the methods, coccosphere and cell size are presented as two different parameters, whereas in the abstract “cell size” is used more loosely. L 170: as daily growth rates ranged between max. and zero, it is confusing to write “resulted in a modest range of daily and mean exponential growth rates”; remove daily. L 170: which days were used to calculate mean exponential growth rates? L 182: none of the values reported in the text for coccosphere diameter correspond to those presented in table 1 i.e. max value for *H. carteri* is 21, not 15 μm . L 187: mean CN for *C. braarudii* is 14, not 11-12 μm . L 188-189: move information on large coccosphere in *C. leptoporus* up one sentence. L 211: according to table 1, CL varies by 8.0 μm in *C. quadriperforatus*. L 217-218: I agree that there is no relationship between CN and cell diameter. However, I do not understand this sentence. The range in both coccosphere diameter and CL is very broad. Also, the cultures are not synchronized in regard to cell division as large cells (“about to divide”) are also present in exponential phase and small cells (“just divided”) also in stationary phase; only the peak of the mean shifts (see figure 3). L 235-239: the way this is phrased is misleading; it sounds as if “all cells” in stationary phase contain numerous coccoliths whereas you still have small, recently-divided cells. L 254: in the results the shift in coccosphere diameter is 0.55-0.7 μm (1.75 μm for *C. braarudii* (L 229); this does not amount to ca. 2 μm . According to my calculations, using mean diameter presented in L 227-228 and the above mentioned increases, there is a 3% increase in *Helicosphaera* coccosphere diameter, 4-5% in *Calcidiscus* and 9% in *Coccolithus*. Here, however, the authors write 10-12%. If the authors calculated differently/used different values, this needs to be made clearer in the text. L 300: in results, max. μ for *H. carteri* is listed as 0.45 d⁻¹. L 300-303. This argument is not convincing. If coccosphere geometry is to be a tool for looking at long-term (as this is the fossil record) changes in growth, then it is necessary to look at reduced physiological rates due to e.g. lower nutrient availability. It is not possible to look at long-term shifts in growth phase in the fossil record. If there is an increased percentage in large cells in a certain sediment layer, what does this mean? That this was a time of “nutrient

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limitation” so that more cells grew larger and stopped dividing? Although this hypothesis is intriguing, it is necessary to look for a link between reduced physiological rates and larger cells if this is to be applied as a proxy to the fossil record. L 310: in this study, coccolith length is independent of growth phase so this factor of coccosphere geometry should be removed here. L 416: Gerecht et al. (2015) show that growth rate determines calcite production in *C. braarudii*: PIC production is reduced by ca. 50% due to the 50% reduction in growth rate. Table 1: is this all combined data i.e. exponential and stationary phase? Table 1: check values Max PIC for *C. braarudii* (lower than Mean) Table 1: there is no mention in the methods as to how POC per cell was calculated, nor is it presented in the results or discussed in the rest of the paper. Figure 2: check frequency values for right y-axis. Figure 3: How is “early stationary-phase growth” defined i.e. which daily growth rate is still considered exponential? Figure 4 provides convincing evidence that coccosphere geometry data can be obtained by POL. I wonder, however, why SEM was not used? This would supply more accurate measurements of coccosphere diameter and especially of CL and equally adequate estimates of CN. It would not provide cell diameter, but the authors do not discuss cell diameter in the text. Cell diameter should be mentioned in the results as it is included in table 1, even if only as a short sentence e.g. cell diameter followed the same trend as coccosphere diameter (?). The authors present calcite production rates in Figure 6. I would appreciate a sentence on how this was calculated in the Methods section i.e. was this an average of all PIC contents x growth rates in exponential phase or just from one specific day? Likewise for the “stationary phase”-values.

Technical corrections: Please check the bibliography closely. I have found at least 3 errors: de Vargas 2004 and Keller et al. 1987 are missing, whereas Young 2003 is in the citation list, but not cited in the text. Ziveri 2007: check journal name L 420: (*Toweius*) *pertusus* needs to be cursive

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