Responses to Referee Comment 1

Dear Editors and Reviewers,

We thank the reviewers for their supportive and constructive comments on our manuscript. Our point–by–point responses in blue text to your comments are attached. The changed contents in the revised manuscript are underlined.

Yours sincerely,
On behalf of all coauthors
Yong Zhang

RC1

In “Combined effects of low temperature and low light intensity on elemental content and macromolecules of coccolithophores”, the authors grow three strains of the morphospecies Gephyrocapsa huxleyi at two levels each of light and temperature, and measure growth rate, stoichiometry, and various macromolecules.

In all, the dataset is exciting, and clearly reflects a lot of work, but the authors need to do more to synthesize their results and place them within the context of existing research in order to draw biogeochemically-relevant conclusions about their study species, and about the ecological role coccolithophores play at the base of the photic zone in different ecosystems.

Response: We agree and thank you for your suggestions. In the discussion section, we compared the growth rates, POC and PIC contents measured in our study with those reported in other studies, and explained the reasons for the discrepancies. Meanwhile, with reference to the actual deep–sea environment and the environments of the areas where the three strains were isolated, we discussed the potential causes of strain–specific in growth rates, POC and PIC contents, and analyzed the biogeochemical effects and ecological roles of coccolithophores. Please see the discussion section in the manuscript.

To their credit, it seems as if the authors are struggling with a good problem: too much data. It is difficult to construct a concise narrative about three strains over four treatments; however, they could very easily have written twice as long a discussion section. The results section was data-heavy and difficult to parse—I would recommend focusing on the salient, statistically-significant results which will be focused on in the discussion, rather than listing every percentage change of every measurement between every treatment, all of which are visible in the figures and could also be presented in tabular form. Similarly, in many cases, it would be more effective to simply list the statistics, and only include relative increase or decrease when it will be a focus of analysis. Similarly, for future reference in the literature, I would like to see a main text table or supplemental table with all of the measurement values (mean +/- sd), rather than just the ANOVA test statistics.

Response: We agree and thank you for your suggestion. We have simplified the results section, focused on the statistically significant data, and listed all the parameters in Table 1.

Table 1. Temperature (9 and 15 ºC), light intensity (*, 15 and 150 µmol photons m^{-2} s^{-1}), growth rate (µ in d^{-1}), and cellular contents (pg $cell^{-1}$) of chlorophyll (Chl) *a*, PIC, POC, PON and POP, the ratios of PIC : POC, POC : PON, POC : POP, and PON : POP, as well as carbohydrate (Carbo) and lipid contents (pg $cell^{-1}$). The values are expressed as the mean ± standard deviations in the brackets of three replicates.

		G. oceanica NIES–1318		*E. huxleyi* PML B92/11		*E. huxleyi* RCC1266	
		15*	150	15	150	15	150
µ	9ºC	0.24(0.04)	0.67(0.01)	0.46(0.04)	0.60(0.01)	0.45(0.03)	0.65(0.02)
	21ºC	0.85(0.03)	1.29(0.01)	0.79(0.01)	1.26(0.02)	0.84(0.04)	1.25(0.01)
Chl *a*	9ºC	0.30(0.04)	0.25(0.01)	0.25(0.02)	0.23(0.02)	0.30(0.07)	0.24(0.03)
	21ºC	0.34(0.05)	0.33(0.01)	0.39(0.02)	0.27(0.03)	0.32(0.06)	0.30(0.03)
PIC	9ºC	4.29(0.51)	2.69(0.38)	4.38(1.19)	3.39(1.01)	4.10(1.11)	1.99(0.66)
	21ºC	4.94(0.25)	8.01(0.40)	7.01(2.09)	9.82(0.37)	10.91(1.53)	14.18(1.35)
POC	9ºC	10.41(0.68)	12.01(1.09)	10.32(0.78)	11.78(0.86)	9.67(0.97)	11.78(0.78)
	21ºC	22.64(2.27)	31.74(1.32)	21.21(1.49)	34.63(1.62)	21.45(3.10)	31.31(1.59)
PON	9ºC	4.71(0.62)	3.60(0.05)	3.09(0.29)	2.40(0.32)	3.40(0.85)	2.26(0.12)
	21ºC	5.65(0.80)	6.21(0.30)	4.55(0.11)	5.60(0.15)	4.17(0.44)	5.14(0.51)
POP	9ºC	0.39(0.11)	0.46(0.04)	0.38(0.03)	0.45(0.04)	0.38(0.07)	0.39(0.01)
	21ºC	0.51(0.08)	0.53(0.03)	0.50(0.05)	0.54(0.06)	0.45(0.06)	0.50(0.02)
PIC:POC	9ºC	0.41(0.05)	0.23(0.03)	0.42(0.10)	0.29(0.08)	0.42(0.08)	0.17(0.05)
	21ºC	0.22(0.02)	0.25(0.02)	0.33(0.10)	0.28(0.02)	0.51(0.05)	0.46(0.06)
POC:PON	9ºC	2.60(0.21)	3.89(0.36)	3.94(0.66)	5.75(0.33)	3.45(0.83)	6.09(0.54)
	21ºC	4.74(0.84)	5.97(0.42)	5.45(0.51)	7.23(0.52)	5.98(0.32)	7.13(0.42)
POC:POP	9ºC	72.1(18.7)	67.9(3.2)	71.1(10.3)	68.0(3.0)	65.8(6.5)	77.9(5.0)
	21ºC	116.9(23.4)	154.6(1.5)	111.3(16.8)	165.5(12.7)	122.5(5.7)	163.3(14.1)
PON:POP	9ºC	27.80(6.76)	17.54(1.67)	18.09(0.39)	11.86(0.67)	19.81(5.09)	12.82(0.66)
	21ºC	24.62(0.52)	25.98(2.05)	20.36(1.58)	23.05(3.16)	20.49(0.58)	22.99(2.83)
Carbo	9ºC	0.37(0.03)	3.01(0.07)	0.41(0.08)	2.65(0.14)	0.38(0.06)	2.98(0.05)
	21ºC	0.98(0.05)	5.66(0.16)	1.10(0.37)	5.43(0.58)	0.97(0.15)	5.18(0.21)
Lipid	9ºC	1.38(0.24)	8.07(0.33)	1.03(0.11)	4.36(0.79)	0.87(0.09)	6.42(2.02)
	21ºC	4.17(0.21)	13.37(0.62)	4.23(0.20)	12.12(0.57)	4.69(0.36)	12.82(0.39)

In their discussion, the authors miss valuable opportunities to place their own measurements with those in the literature. For instance, how do the strains responses under different treatments relate to their ecological niche (where they were isolated)? What implications does this have for their behavior and biogeochemical impact in the open ocean? Furthermore, recent research has shown evidence of mixotrophy/osmotrophy potentially playing an important role in carbon acquisition for coccolithophores living at extremely low light levels—what are the implications of the results from the current study with regards to G. huxleyi metabolism and carbon demand in this light? Several times, the authors make a conjecture, or

hypothesis, about the mechanism of one of their measurements (e.g. in the PIC discussion, lines 398-404), then leave the idea there without supporting it with other evidence or data. Additionally, the authors cite papers which do not support (or, at least, are totally tangential to) the author's point - e.g. Shemi et al., Tangunan et al.

Response: We agree and thank you for your suggestion. By comparing the previous research results with our data, in lines 403–409, we added "Previous studies reported growth rates of *E. huxleyi* ranging from 0.09 d^{-1} (strain CCMP371 at 8.5 ºC) to 0.12–0.19 d^{-1} (strains RCC1710, RCC1252, and RCC1710 at 6.5 ºC) (Rosas–Navarro et al., 2016; Wang et al., 2019). Additionally, PIC contents of *E. huxleyi* strain CCMP371 were observed at 3.2–4.8 pg $cell^{-1}$ at 8.5 ºC (Wang et al., 2019). Synthesizing these findings with our results, we estimate that the growth rates of the three coccolithophore strains in this study would be approximately 0.1 d^{-1} at 4 ºC, with PIC contents around 3–4 pg $cell^{-1}$."

In lines 409–416, we added "Notably, the lower latitudinal origin of *G. oceanica* compared to strains RCC1266 and PML B92/11 suggests a superior adaptation to warmer waters (Buitenhuis et al., 2008). Consequently, *G. oceanica* exhibits a more pronounced decline in growth rate under cold stress, driving the observed strain–specific divergence. Furthermore, rising temperatures stimulate PIC accumulation more effectively in *E. huxleyi* than in *G. oceanica*. As a result, thermal warming leads to a decreased PIC : POC ratio in *G. oceanica*, whereas the ratio remains relatively stable in *E. huxleyi*."

For the PIC discussion in lines 393–396, we changed "Alternatively, under low temperature and low light intensity, more coccoliths might act as micro–lenses to concentrate light on chloroplasts, making them beneficial (Young et al., 1999)." to "Furthermore, the observed restriction of ribosomal proteins and the potential downregulated of calcium–binding proteins–despite an abundance of photosynthetic machinery at low temperatures–may further depress PIC accumulation (Dedman et al., 2023)."

For osmotrophy discussion, in lines 437–445, we added "Most notably, recent findings by Balch et al. (2023) indicate that at depths where light is as low as 2 µmol photons m^{-2} s^{-1}, coccolithophores can utilize osmotrophy–the uptake of dissolved organic

carbon (DOC) such as acetate, mannitol and glycerol–to sustain both POC and PIC production. Although DOC–supported growth rates remain low, this osmotrophic capability serves as a vital survival strategy under extreme light limitation. Such mixotrophic behavior underscores that coccolithophore–mediated DOC uptake is a significant yet underappreciated component of the biological carbon pump (Balch et al., 2023)."

Balch, W. M., Drapeau, D. T., Poulton, N., Archer, S. D., Cartisano, C., Burnell, C., and Godrijan, J.: Osmotrophy of dissolved organic compounds by coccolithophore populations: Fixation into particulate organic and inorganic carbon, Sci. Adv., 9, eadf6973, doi: 10.1126/sciadv.adf6973, 2023.

Buitenhuis, E. T., Pangerc, T., Franklin, D. J., Le Quéré, C., and Malin, G.: Growth rates of six coccolithophorid strains as a function of temperature, Limnol. Oceanogr. 53, 1181–1185, doi:10.4319/lo.2008.53.3.1181, 2008.

Dedman, C. J., Barton, S., Fournier, M., and Richaby, R. E. M.: The cellular response to ocean warming in *Emiliania huxleyi*, Front. Microbiol., 14, 1177349, doi: 10.3389/fmicb.2023.1177349, 2023.

Rosas–Navarro, A., Langer, G., and Ziveri, P.: Temperature affects the morphology and calcification of *Emiliania huxleyi* strains, Biogeosciences, 13, 2913–2926, doi: 10.5194/bg-13-2913-2016, 2016.

Wang, X., Fu, F., Qu, P., Kling, J. D., Jiang, H., Gao, Y., and Hutchins, D. A.: How will the key marine calcifier *Emiliania huxleyi* respond to a warmer and more thermally variable ocean? Biogeosciences, 16, 4393–4409, doi: 10.5194/bg-16-4393-2019, 2019.

Finally, I would recommend the authors consider standardizing the structure of their discussion, and of each paragraph therein. There are several disconnected ideas and/or hypotheses in many paragraphs, which frequently left me confused, and I suggest they take the time to iterate the presentation of each idea with its supporting evidence and context in the literature.

Response: We agree and thank you for your suggestion. We carefully re–edited the discussion section and added substantial supporting evidence and literature background to strengthen the article's arguments, making the content more coherent and logical.

As an aside, to improve readability, I suggest the authors include a native English speaking co-author or use an AI tool to correct grammar errors, misspellings, e.g. add definite articles like "the", which may not exist in their first language.

Response: We agree and thank you for your suggestion. We have used AI tool to edit the full text and correct grammatical and spelling errors.

- Lines 124-130 – capitalization of culture collections?
  Response: Thank you for your comments. We have changed the first letter of the culture collections to lowercase.

- Line 331—I wouldn't characterize cultures grown at 9°C as "extreme low temperature"—high latitude phytoplankton, for instance, all grow under much lower temperatures.
  Response: Agreed. We deleted "extreme" in line 317.

- Lines 335-337 – The authors say carbohydrates and lipids were primarily light dependent. However, in all strains grown under high light, the lower temperature cultures express significantly lower carbohydrates and lipids than the higher temperature cultures. What physiological changes contribute to this change in expression?
  Response: Thank you for your comments. As shown in Figure 4, for all three strains, regardless of temperature, carbohydrates and lipids levels are lower under low light than under high light conditions. Thus, carbohydrates and lipids are primarily light dependent.

- Line 367 – are these findings inconsistent? 9 and 15C are at very different points along a PAR response curve--perhaps on opposite sides of the apex?
  Response: Thank you for your comments. In lines 333–336, we changed "These findings are inconsistent with our results of low POC contents at 9 ºC" to "While previous studies observed stable or even increased POC contents at moderate temperature decreases (e.g., from 18–24 ºC down to 14–20 ºC) (Feng et al., 2008; Borchard et al., 2011; Tong et al., 2019; Torres–Romero et al., 2024), our results demonstrate a significant decline in POC at 9 ºC compared to 21 ºC (Figure 2)."

Borchard, C., Borges, A. V., Händel, N., and Engel, A.: Biogeochemical response of *Emiliania huxleyi* (PML B92/11) to elevated CO_2 and temperature under phosphorus limitation: A chemostat study, J. Exp. Mar. Biol. Ecol., 410, 61–71, https://doi.org/10.1016/j.jembe.2011.10.004, 2011.

Feng, Y., Warner, M. E., Zhang, Y., Sun, J., Fu, F., Rose, J. M., and Hutchins, D. A.: Interactive effects of increased pCO_2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae), Eur. J. Phycol., 43, 87–98, https://doi.org/10.1080/09670260701664674, 2008.

Tong, S., Hutchins, D. A., and Gao, K.: Physiological and biochemical responses of *Emiliania huxleyi* to ocean acidification and warming are modulated by UV radiation, Biogeosciences, 16, 561–572, https://doi.org/10.5194/bg-16-561-2019, 2019.

Torres–Romero, I., Clark, A. J., Wijker, R. S., Jaggi, M., Zhang, H., and Stoll, H. M.: Temperature–dependent carbon isotope fractionation in coccolithophores, Front. Earth Sci., 12, 1331179, doi: 10.3389/feart.2024.1331179, 2024.

- Line 386 – I don't believe there is any discussion of secondary metabolites in Shemi et al? The study focused rather on intracellular recycling, no?
  Response: We agree and thank you for your comments. Zhang et al. (2021) reported that in *E. huxleyi* RCC1266, RNA content was 2.7-fold higher (2.16 to 0.58 pg cell^{-1}) at 11 to 22 ºC and 26% lower (0.43 to 0.58 pg cell^{-1}) at 15 than 280 µmol photons m^{-2} s^{-1}. Thus, we changed "Shemi et al., 2016" to "Zhang et al., 2021" in line 376.

- Line 393 – is there any evidence connecting this claim with the citation?
  Response: Thank you for your comments. We deleted "This adaptability may be one of the kay factors that allowed them to survive multiple glacial–interglacial cycles throughout geological history (Tangunan et al., 2021)."

- Lines 411-424 – unnecessary to cite the same paper five sentences in a row
  Response: Agreed and thank you for your comments. We made appropriate deletions and cited Balch et al., 2023 in line 466.

Balch, W. M., Drapeau, D. T., Poulton, N., Archer, S. D., Cartisano, C., Burnell, C.,

and Godrijan, J.: Osmotrophy of dissolved organic compounds by coccolithophore populations: Fixation into particulate organic and inorganic carbon, Sci. Adv., 9, eadf6973, doi: 10.1126/sciadv.adf6973, 2023