**Responses to comments of Prof. Kunshan Gao**

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

**CC1:**

The paper entitled "Combined effects of low temperature and low light intensity on elemental content and macromolecules of coccolithophores" by Shang et al. reports about the growth and elemental compositions of several coccolithorphore strains. It presents a potentially valuable dataset on the physiology of coccolithophores under conditions relevant to the bottom layer of euphotic zone in the oceans. The experimental approach is sound, and the core findings are clear. However, several key aspects require clarification and expansion to fully contextualize the results and strengthen the paper's conclusions before it can be considered for publication.

**Major Comments**

1. The results are generally straightforward and presented clearly. However, some data presentations, particularly in Figures [mention specific figures, e.g., 2 and 3], could be more concise. The current expressions, while detailed, are occasionally repetitive. Streamlining this would improve readability and impact. The values (%) should be mentioned without any digits after the decimal points.

   Response: Thank you for your suggestions. We have recreated the figures, simplified the icons, and converted all percentage values in the manuscript to integers, i.e., removing the digits after the decimal point.

2. The finding about the coccolithophores beyond 100 meters is intriguing. The authors should carefully consider and explicitly discuss the physiological state of these cells. It is highly plausible that the low light (near or lower than compensating light point) and temperature levels at these depths are insufficient to support sustained growth, and cells may merely be surviving in a dormant or maintenance state. The interpretation of the data should be nuanced to distinguish between active growth and simple persistence, which has significant ecological implications.

   Response: Thank you for your suggestions. In lines 417–423, we have added "Under laboratory conditions (9 °C and 15 μmol photons $m^{-2}$ $s^{-1}$), the three coccolithophore strains maintained growth rates of 0.24 to 0.46 $d^{-1}$, equivalent

to a division cycle of approximately 2–3 days. In contrast, within the actual ocean at depths of 100–200 m, cells may cease division entirely, opting instead to expand light–harvesting antenna protein or the functional cross–sectional area of PSII reaction centers to ensure survival (Pierangelini et al., 2015). This strategy preserves their capacity for rapid recovery and reproduction once favorable conditions return."

Pierangelini, M., Stojkovic, S., Orr, P. T., and Beardall J.: Photo-acclimation to low light–Changes from growth to antenna size in the cyanobacterium *Cylindrospermopsis raciborskii*, Harmful Algae, 46, 11–17, http://doi.org/10.1016/j.hal.2015.04.004, 2015

3. The discussion would be significantly strengthened by addressing the gaps between the laboratory conditions and the real-world environment at 100-150 meters. Specifically, please discuss how factors not replicated in your experiment—such as spectral light quality, pressure, nutrient pulses, and microbial interactions—might influence coccolithophore physiology *in situ*. Acknowledging these limitations will provide a more realistic framework for applying your findings to the natural ocean.

   Response: Thank you for your suggestions. In lines 426–437, we have added "Because other environmental factors, such as hydrostatic pressure, nutrient pulses, microbial interactions, and spectral light quality, can affect these physiological characteristics of phytoplankton. In the dysphotic zone, high hydrostatic pressure may inhibit tubulin polymerization and membrane fluidity, thereby suppressing photosynthesis and cell division (Potts and Friedmann, 1981). Conversely, nutrient pulses provide the essential substrates for synthesizing key proteins even under depressed metabolic rates (Sui et al., 2019), while microbial interactions dictate the balance between survival or decomposition (Zweifel et al., 2025). Furthermore, spectral light quality–specifically the dominance of blue light at 100–150 m–acts as a critical signal.

Coccolithophores adapt to this “blue light regime” by enriching their antennae with chlorophyll c and fucoxanthin to maximize absorption efficiency (Shen et al., 2025b).”

Potts, M., and Friedmann, E.: Effects of water stress on cryptoendolithic cyanobacteria from hot desert rocks, Arch Microbiol., 130, 267–271, http://doi.org/10.1007/BF00425938, 1981.

Shen, Y., Jiang, R., Chang, J., Cai, L., Zhu, Y., Yin, Y., Shao, L., Wu, M., Zhang, J., and He, P.: The effect of temperature on the photosynthetic carbon fixation efficiency of sessile macroalgae in the mussel farming area of Guoqi Island through stable isotope, Mar. Environ. Res., 209, 107190, doi: 10.1016/j.marenvres.2025.107190, 2025b.

Sui, Y., Muys, M., Van de Waal, D. B., D’Adamo, S., Vermeir, P., Fernandes, T. V., and Vlaeminck, S. E.: Enhancement of co–production of nutritional protein and carotenoids in *Dunaliella salina* using a two–phase cultivation assisted by nitrogen level and light intensity, Bioresour. Technol., 287, 121398, http://doi.org/10.1016/j.biortech.2019.121398, 2019.

Zweifel, S. T., Henshaw, R. J., Müller, O., Keegstra, J. M., Charlton, S. G. V., Pioli, R., Martínez-Pérez, C., Alcolombri, U., Clerc, E., and Stocker, R.: Bacteria induce an amoeboid phase in coccolithophores that persists after bloom collapse, Sci. Adv., 11, eadw7280, http://doi.org/10.1126/sciadv.adw7280, 2025.

4. A critical point concerns temperature. The deep at 100-150 m to ocean is typically around 4°C, yet the culture experiments were conducted at 9°C. The authors must address this discrepancy. Based on the growth response shown in Figure 1, which indicates slower growth at lower temperatures, it is essential to extrapolate or model the expected growth rates and physiological responses at 4°C. Without this, the direct applicability of the results to the deep populations is uncertain. Please include a discussion on how the key findings (e.g., growth rate, calcification) would likely be different at the *in-situ* temperature of ~4°C.

Response: Thank you for your suggestions. In lines 403–409, we have 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 $cell^{-1}$."

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

5. When comparing "POC content differences across temperatures", the authors not only mention the core result that "POC content at 9°C is lower than at 21°C" (corresponding to Result 3.2) but also unnecessarily list detailed percentage data such as "a 28.78% reduction in POC for G. oceanica NIES–1318 under HTLL and a 38.53% reduction for E. huxleyi PML B92/11" (detailed data from Result 3.2). These detailed data have already been fully presented in the "Results" section via tables and text; the discussion only needs to discuss that "low temperature and low light significantly reduce POC content" without repeating specific values.

When describing "growth rate changes", the authors repeat strain-specific data such as "an 81.39% decrease in growth rate for G. oceanica under LTLL and a 63.18%

decrease for E. huxleyi PML B92/11" (corresponding to Result 3.1). However, they do not subsequently analyze the reasons for "strain-specific differences in growth rates", making these data mere result restatements without adding new argumentative value.

Response: Agreed. Following your suggestion, we have simplified the corresponding contents in line 335–336. For example, “which shows decreased POC contents at 9 ºC than 21 ºC” was changed to “our results demonstrate a significant decline in POC at 9 ºC compared to 21 ºC”.

In lines 409–413, we have 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.”

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.

1. **Mechanistic Exploration:** The discussion proposes the hypothesis that "stable POP content under low temperature and low light is due to phosphorus being preferentially allocated to nucleic acids and membrane phospholipids, with reduced investment in non-essential metabolic pathways" (corresponding to the result that POP shows no significant change in Result 3.2). However, it only cites Shemi et al. (2016)’s research on "membrane remodeling under phosphorus starvation" as indirect support, without providing direct data from this study on "changes in nucleic acid/membrane phospholipid content" (e.g., detecting RNA content or phospholipid composition via molecular biology methods). The weak connection between the hypothesis and the study’s own data reduces the persuasiveness of the mechanistic explanation.

   Response: Thank you for your comments. Although we did not measure DNA,

RNA or phospholipid contents in this study, 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 °C and 26% lower (0.43 to 0.58 pg cell$^{-1}$) at 15 than 280 µmol photons m$^{-2}$ s$^{-1}$. In lines 374–376, we have added "Notably, low temperature–rather than light–appears to be the primary driver for increased RNA investment, a strategy that preserves core POP pools despite a sluggish metabolism (Zhang et al., 2021)."

Zhang, Y., Li, Z., Schulz, K. G., Hu, Y., Irwin, A. J., and Finkel, Z. V.: Growth-dependent changes in elemental stoichiometry and macromolecular allocation in the coccolithophore *Emiliania huxleyi* under different environmental conditions, Limnol. Oceanogr., 66, 2999–3009, doi: 10.1002/lno.11854, 2021.

**Ambiguous Mechanism for the Temperature-Dependent Reversal of Calcification**

Regarding the counterintuitive result that "high light reduces PIC content at low temperatures", the discussion proposes two hypotheses: "photoinhibition disrupts ion transport" and "coccoliths act as microlenses for light concentration". However, it fails to clarify the primary-secondary relationship or synergistic effect between these two hypotheses:

- If "ion balance disruption by photoinhibition" is the main cause, supplementary data on "changes in intracellular $Ca^{2+}$/$HCO_3^-$ concentrations under low temperature and high light" should be provided. Otherwise, such speculation should only be focused on low enzymatic activities related to photosynthesis and calcification, the latter demands energy from the former.
- If the "microlens effect" is the main cause, an explanation is needed for "why the light-concentrating effect of coccoliths suddenly becomes prominent at low temperatures but not at high temperatures".

  The simultaneous proposal of two hypotheses without targeted data support leads to a superficial mechanistic explanation.

  Response: Agreed, and thank you for your suggestion. In lines 393–396, we

have added "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)."

We deleted "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)."

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.

**Strain-Specific Differences: Inadequate Analysis of Causes and Lack of Universal Discussion**

The study involves three coccolithophore strains (G. oceanica NIES–1318, E. huxleyi PML B92/11, RCC1266), and results show strain-specific variations in multiple indicators (e.g., the PIC:POC ratio only increases significantly for G. oceanica under LTLL, Result 3.3). However, the discussion only mentions at the end that "strain diversity helps coccolithophores adapt to different habitats" and does not further analyze the causes of these differences:

- It fails to link the original habitat differences of the strains (e.g., NIES–1318 isolated from coastal waters of Japan, PML B92/11 from coastal waters of Norway) to explore whether "habitat adaptability causes differences in strain responses to low temperature and low light";

  Response: Agreed, and thank you for your suggestion. Under low temperature and low light intensity, the three strains showed similar PIC contents.

  The latitudinal location, from which *G. oceanica* is isolated, is much lower than that of strains RCC1266 and PML B92/11, and 22ºC is already the optimal temperature for growth rates of two *E. huxleyi* strains and below the optimal

growth temperature of *G. oceanica* (Buitenhuis et al., 2008; Zhang et al., 2014). In lines 413–416, we added "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*."

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.

Zhang, Y., Klapper, R., Lohbeck, K. T., Bach, L. T., Schulz, K. G., Reusch, T. B., and Riebesell, U.: Between– and within–population variation in thermal reaction norms of the coccolithophores *Emiliania huxleyi*, Limnol. Oceanogr., 59, 1570–1580, doi:10.4319/lo.2014.59.5.1570, 2014.

- It does not explain strain specificity from a genetic background perspective (e.g., sequence differences in calcification-related genes or photosynthetic genes), resulting in "strain differences" being merely a descriptive feature of the results rather than a deep insight into "the diversity of coccolithophore adaptation strategies".

  Response: We agree and thank you for your suggestions. We have gathered relevant data and interpreted strain specificity from the perspective of gene sequence difference. To the best of our knowledge, mitochondrial genes can distinguish between *E. huxleyi* and *G. oceanica* (Bendif et al., 2014). However, few studies have reported sequence differences in calcification–related genes or photosynthesis–related genes among different coccolithophore strains. This will be the focus of our future research.

Bendif, E. M., Probert, I., Carmichael, M., Romac, S., Hagino, K., and de Vargas, C.: Genetic delineation between and within the widespread coccolithophore morpho–species *Emiliania huxleyi* and *Gephyrocapsa oceanica* (Haptophyta), J. Phycol.,

50, 140–148, doi: 10.1111/jpy.12147, 2014.

**Extension of Ecological Significance: Insufficient Specific Linkage to Deep-Sea Biogeochemical Cycles**

The discussion proposes that "the adaptation strategies of coccolithophores affect deep-sea biogeochemical cycles" but only generally mentions "enhanced carbon sequestration" and "underestimation of carbonate production", without establishing specific quantitative or process-based connections:

- It does not combine the "changes in PIC:POC ratio" in this study (e.g., a 63.15% increase in PIC:POC for G. oceanica under LTLL) to assess the "potential increase in deep-sea carbon sinking rate ";

  Response: We agree and thank you for your suggestions. We changed “could promote sinking and enhance” to “while an elevated PIC : POC ratio enhances carbon sedimentation and sequestration in the deep sea” in lines 397–398.

- It does not explore the impact of "stable POP content" on deep-sea food chains (e.g., phosphorus is a limiting factor for deep-sea plankton; whether stable POP affects the feeding efficiency or trophic transfer of zooplankton), leading to ecological significance discussions remaining at a macro level without specific integration with deep-sea ecological processes.

  Response: We agree and thank you for your suggestions. In lines 378–381, we added “Given that phosphorus often limits deep–sea phytoplankton, stable cellular POP levels could influence zooplankton grazing efficiency, thereby modulating energy transfer and nutrient cycling within deep–sea ecosystems (Gerecht et al., 2014).”

Gerecht, A. C., Šupraha, L., Edvardsen, B., Probert, I., and Henderiks, J.: High temperature decreases the PIC / POC ratio and increases phosphorus requirements in *Coccolithus pelagicus* (Haptophyta), 11, 3531–3545, doi: 10.5194/bg-11-3531-2014, 2014.

**Literature Comparison:** When comparing with previous studies, the discussion has gaps in explaining "result differences":

- Regarding the discrepancy that "this study finds decreased POC at 9°C, while previous studies reported unchanged or increased POC at 14°C/15°C", the authors only attribute it to "stronger inhibitory effects at 9°C" but ignore whether "light intensity conditions in previous studies (e.g., 60–480 μmol photons $m^{-2}s^{-1}$ in Feng et al., 2008, vs. 15 μmol photons $m^{-2}s^{-1}$ in this study) are also contributing factors", neglecting cross-study comparisons of "temperature-light interaction effects". Cellular quota of POC depends on C assimilation and cell division, so better to focus on POC production rate.

  Response: We agree and thank you for your suggestion. Typically, high temperature and high light intensity synergistically increase POC content (Feng et al., 2008). Our results showed that low temperature (9°C) dominantly limits POC content (Figure 2). We have added "The stability of POC contents across both light intensities at 9 °C further supports this threshold effect." in lines 339–340.

In summary, the manuscript addresses an interesting topic but requires revisions to fully realize its potential. The most critical issues are the interpretation of deep-water populations as "growing" versus "surviving," the discussion of experimental limitations relative to the deep ocean environment, and the crucial temperature extrapolation to 4°C. Addressing these points will greatly improve the manuscript's robustness and ecological relevance.

Response: We agree and thank you for your suggestion. We discussed the "growth" of coccolithophores in laboratory cultures and the "survive" in deep–water environments, as well as the experimental limitation relative to the deep ocean environment. Under 4 °C condition, key parameters such as growth rate and PIC contents were estimated.