RC1:

Comments on "Temperature fluctuation alleviates the negative effects of warming on marine diatoms: comparison between *Thalassiosira* sp. and *Nitzschia closterium* f. minutissima" by Sheng et al.

General Comments: This manuscript addresses a compelling and timely topic by exploring how temperature fluctuations influence the physiological and biogeochemical responses of marine diatoms to ocean warming, an aspect often overlooked in studies conducted under static temperature conditions. The authors provide valuable data on two ecologically significant diatom species, *Thalassiosira* sp. and *Nitzschia closterium* f. minutissima, revealing species-specific responses in growth rate, particulate organic carbon (POC), biogenic silica (BSi), and sinking rate. These findings contribute to our understanding of how diatom-driven biogeochemical cycles may respond to future ocean conditions. The manuscript is well-structured, clearly written, and supported by robust experimental methods. However, I have several minor concerns and suggestions to enhance the manuscript's clarity, scientific rigor, and overall impact.

Response:

We sincerely appreciate the reviewer's thoughtful evaluation of our manuscript and their constructive suggestions, which have helped us improve the study's clarity and scientific rigor. Below, we address the reviewer's specific comments point by point.

Specific Comments:

Lines 92–94: Please provide details on the season when *Thalassiosira* sp. and *Nitzschia closterium* f. minutissima were isolated from the Yellow Sea, along with the corresponding mean water temperature at the time of collection. Additionally, clarify how long these species were maintained in the laboratory prior to the experiments, as this could influence their acclimation to culture conditions.

Response:

Line 98-102: The marine diatoms *Thalassiosira* sp. and *Nitzschia closterium* f. minutissima were originally isolated from surface seawater (depth 3 – 12 m; salinity 34.78) at 118° 58.055′E, 38°39.111′N (China) in August 2019. At the time of sampling,

the sea surface temperature was approximately 15 °C. Stock cultures of two diatoms were maintained in sterilized f/2 medium natural seawater, in Nalgene polycarbonate bottles in a constant temperature and illumination incubator (GXZ-280D, NingBo) at 20 °C.

Line 102: Correct the typographical error in the cell abundance notation. The number "4" in "1 × 104 cells mL-1" should be formatted as a superscript (i.e., 1 × 10^4 cells mL^{-1}).

Response:

We have made the correction in the manuscript.

Revision:

Line 113: For each temperature treatment, stock cultures in the logarithmic growth phase were inoculated into triplicate polycarbonate culture flasks (1L, Nalgene, USA), with an initial cell abundance of 1×10^4 cells mL^{-1}. The light intensity was maintained at 170-200 μmol photons m^{-2} s^{-1} with light: dark cycle of 12 h: 12 h.

Line 110: Clarify whether Figure 1 represents recorded temperature changes from the experiment or is a schematic diagram of the temperature treatments. If it is a schematic, consider including a supplementary figure with actual temperature data to validate the experimental setup.

Response:

Figure 1 is a schematic diagram illustrating the temperature treatments used in this experiment. We did monitor the temperature in the water baths using water temperature data loggers (MX2201, HOBO, USA). The measured temperature was within a range of ± 0.2 ℃ from the preset temperature.

In the revised manuscript, we have clarified in the figure legend that Figure 1 is a schematic diagram.

Revision:

Line 111: The measured temperature was within a range of ± 0.2 ℃ from the preset temperature.

Line 121: Figure 1: Temperature schematic diagram. LTCT: 20 ℃, LTFT： 20 ± 4 ℃, HTCT： 25 ℃, HTFT： 25 ± 4 ℃.

Line 115: Specify the storage conditions for samples during chlorophyll *a* (Chl *a*) extraction. Were the samples stored in the dark, and at what temperature (e.g., 4°C)?

Response:

We have added the detailed information for sample storage conditions.

Revision:

Line 128: For chlorophyll *a* (Chl *a*) measurement, 20 mL sample was filtered onto GF/F filters (Whatman, USA). After extraction with 5 mL of 90 % acetone for 24 hours (stored at -20 °C in the dark), the Chl *a* content was measured using a fluorometer (Trilogy, Turner Designs, USA).

Line 118: For the growth rate calculations, confirm whether Chl *a* fluorescence was measured directly from the algal culture or after filtration. If measured directly, discuss any potential interference from culture medium or cell aggregation that might affect fluorescence readings.

Response:

For the growth rate calculations, the *in vivo* Chlorophyll *a* fluorescence was measured directly from the algal cultures without filtration. For the *in vivo* fluorescence measurements, the culture medium was always used as blank, therefore the potential interference from culture medium was eliminated. In addition, we conducted a semi-continuous incubation with cells growing at the exponential phase, and the cultures in the incubation bottles were well mixed for a 2-3 times a day, as such, there was no cell aggregation happening in the culture system.

Revision:

Line 130: 2 mL sample was kept in the dark for 20 minutes, followed by measurement of the in vivo Chl *a* fluorescence using a fluorometer (Trilogy, Turner Designs, USA). For the in vivo fluorescence measurements, the culture medium was always used as blank, therefore the potential interference from culture medium was eliminated. In addition, we conducted a semi-continuous incubation with cells growing at the exponential phase, and the cultures in the incubation bottles were well mixed for a 2-3 times a day, as such, there was no cell aggregation happening in the culture system.

Line 158: Origin 2021 is a product of OriginLab Corporation, not "Tukey", which is a statistical method.

Response:

We have made the correction as suggested.

Revision:

Line 175: Significance analysis and interaction effects were performed by two-way ANOVA using Origin 2021 software (OriginLab Corporation, USA). Differences between treatments were considered significant at level of $p < 0.05$. The pairwise tests between treatments were conducted using Tukey's multiple comparison post-hoc analysis.

Discussion Section: I recommend adding a paragraph to discuss the differential responses between *Thalassiosira* sp. (a centric diatom) and *Nitzschia closterium* f. minutissima (a pennate diatom). Highlight how their morphological and ecological differences (e.g., cell size, silica structure, or habitat preferences) might contribute to their distinct responses to warming and temperature fluctuations. This would strengthen the manuscript's ecological and taxonomic insights.

Response:

We agree that adding a paragraph to compare the differential responses between the two species will strengthen the ecological implications of our study. Now a new paragraph has been incorporated into the Discussion to clarify the differences between *Thalassiosira* sp. (a centric diatom) and *Nitzschia closterium* f. minutissima (a pennate diatom).

Revision:

Line 401:

5.4 Differential thermal responses driven by species traits

The differential physiological responses of *Thalassiosira* sp. and *Nitzschia closterium* f. minutissima to warming and temperature fluctuations are likely attributable to inherent differences in their morphology and ecological niches. Generally, algal cellular utilization of both light energy and nutrients, as well as metabolic efficiency, are intrinsically associated with cell size (Marañón, 2015; Marañón et al., 2012). As a representative centric diatom, *Thalassiosira* sp. typically has a larger cell size (~30 μm), leading to fast sinking into depth but also impose higher metabolic costs under thermal

stress. Conversely, the smaller pennate diatom *N. closterium* f. minutissima (~15 μm) exhibits a higher surface-area-to-volume ratio., promoting more efficient nutrient uptake and gas exchange, especially in variable environmental conditions. Additionally, pennate diatoms are commonly found in benthic or nearshore habitats that experience greater environmental heterogeneity (Burden et al., 2020), thus with increased adaptability and thermal resilience to temperature fluctuations observed in the present study. These morphological and geographical differences likely underpin species-specific strategies to thermal tolerance, and the consequent resource allocation and carbon export, highlighting the necessity of incorporating taxonomic and functional diversity when evaluating phytoplankton responses to climate change.

References cited:

Burden, A., Smeaton, C., Angus, S., Garbutt, A., Jones, L., Lewis, H., and Rees, S.: Impacts of climate change on coastal habitats, relevant to the coastal and marine environment around the UK., MCCIP Sci. Rev., pp:228-255, https://doi.org/10.14465/2020.arc11.chb, 2020.

Marañón, E.: Cell Size as a Key Determinant of Phytoplankton Metabolism and Community Structure. Annu. Rev. Mar. Sci., 7, 241-264, https://doi.org/10.1146/annurev-marine-010814-015955, 2015.

Marañón, E., Cermeño, P., López-Sandoval, D. C., Rodríguez-Ramos, T., Sobrino, C., Huete-Ortega, M., Blanco, J. M., Rodríguez, J., Fussmann, G.: Unimodal size scaling of phytoplankton growth and the size dependence of nutrient uptake and use. Ecol. Lett., 16, 371-379, https://doi.org/doi:10.1111/ele.12052,2012.