

Biogeosciences Discuss., author comment AC3  
<https://doi.org/10.5194/bg-2022-151-AC3>, 2022  
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## Reply on RC2

Chaofeng Wang et al.

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Author comment on "Diel variations in planktonic ciliate community structure in the northern South China Sea and tropical Western Pacific" by Chaofeng Wang et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-151-AC3>, 2022

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### Reviewer 2:

The authors try to understand the diel variation of planktonic ciliates in northern South China Sea and tropical West Pacific. This investigation requires tremendous efforts and times. It must be very valuable information for understanding of plankton ecology and ciliates community. This manuscript, however, have weak point on (1) sampling design and (2) reliability of data obtained for short period.

- In your study, sampling location is fixed. You do not trace the same ciliate community conveyed by the continuous water flow. Small-scaled patchiness and small-scaled non-homogenous distribution of planktonic organisms are frequently observed in any sea areas. You had better to explain the validity of your sampling design with referring to this point.

Response: Our sampling locations were fixed and we could not trace the same ciliate community conveyed by the continuous water flow. We also recognized that small-scaled patchiness and small-scaled non-homogenous distribution of planktonic organisms are frequently observed in any sea areas. But all above items had less connection with our sampling designs. In the manuscript, we aimed to find out diel variations in planktonic ciliate community structure in the nSCS and tWP. We treated aloricate ciliates, tintinnids, and total ciliates separately. Through WMD, we wanted to recognize whether each of them perform diel vertical migration in the nSCS and tWP. During initial sampling design stage of this manuscript, we found that both the nSCS and tWP belonged to oceanic seas, and they had some similarities and differences of ciliate diel vertical distributions. Thus we put ciliate diel vertical variations together in both the nSCS and tWP.

- Sampling period at tWP station might be too short. Daytime sampling was carried out only two times in the same day. This sampling design might be unreasonable for comparing ciliate community structure between daytime and nighttime. You should explain the reliability of your results obtained under this sampling design.

Response: We admit that sampling period at tWP station is too short. But we have no

selection and opportunity to carry out a long period survey. The chance for the survey about ciliate diel variations in the tropical western Pacific Ocean is extremely precious. We also revised accordingly **in lines 84-87 in revised manuscript.**

“Despite their important role in marine microbial food webs, our knowledge of ciliate assemblage diel variations in tropical oceanic waters are limited due to their inaccessibility for oceanographic surveys”.

In ‘Results’

- Sentence-to-sentence connection is frequently not smooth; meaning of each chapter is incomprehensible. This might be mainly due to the excessive change of subject in each sentence. You had better to rewrite sentences in each chapter of result part.

Response: We appreciated your valuable suggestions and rewrote sentences in each chapter of result part throughout all “Results” part **in revised manuscript.**

- Statistical test (significant analysis in statistics) should be indispensable when you compare distributional depth of ciliates or compare standing crops of ciliates between daytime and nighttime or between nSCS and tWP.

Response: We accepted suggestion and added statistical test (Independent *t*-test, nonparametric-test, PERMANOVA analysis) to compare distributional depth of ciliates or compare standing crops of ciliates between daytime and nighttime in the nSCS **in revised manuscript.** Because of less data in the tWP, we cannot perform Independent *t*-test or nonparametric-test to testing the difference.

- Fig. 7 (three-dimensional graphs). Data points are many and complicated. You had better to improve these graphs more comprehensible.

Response: Information of Fig. 7 included tintinnid lorica length (*x*-axis), lorica oral diameter (*y*-axis) and each species abundance proportion (*z*-axis). As for the species abundance proportion in Fig. 7, each dot means variations of each species abundance proportion at day (blue dots) and night (red dots). We also revised the Fig. 7 to improve these graphs more comprehensible **in revised Fig. 7 in revised manuscript.**

- Fig. 8, Fig. S4, Fig. 5S

Data points are many and sometimes overlapped. I can not understand the difference between daytime pattern and nighttime pattern.

Response: We put data of day and night together in order to better describing their difference in Fig. 8, thus many data points were overlapped. For purpose of better visualization, we increased the transparency of each data **in revised Fig. 8 in revised manuscript.** We also accepted suggestion and revised Figs. S4 and S5 accordingly **in revised Figs. S5, S6 and S7 in Supplement-R1.**

In 'Discussion'

- Line 331, 'diel-vertical-migration'

You had better to discuss some reasonable factors affecting this 'diel vertical migration'.

You had better to check that migrating scale (or distance) of ciliates estimated from the diel variation of WMD is comparable to the reported swimming speed (or swimming ability) of marine planktonic ciliates.

Response: As for reasonable factors affecting this 'diel vertical migration', we accepted this suggestion and revised accordingly **in lines 361-371 in revised manuscript**.

"There were multiple factors that could influence diel-vertical-migration behavior of ciliate (food items concentration and quality, predator avoidance, light intensity, body metabolic rates, etc) in various seas (e.g., McLaren 1974; Loose et al., 1993; Rossberg and Wickham, 2008). In the oligotrophic seas, the phytoplankton assemblage was dominated by Prochlorococcus, Synechococcus and picoeukaryotes, and they showed different diel variations (e.g., Vaulot and Marie, 1999; Oubelkheir and Sciandra, 2008). As important food items of ciliate, heterotrophic bacteria also displayed clear daily oscillations in the oligotrophic Ionian Sea (Mediterranean) (Oubelkheir and Sciandra, 2008). Thus we speculate that diel variation of food items was possibly the main reason in determining ciliate diel-vertical-migration behavior in the oligotrophic tropical seas".

As for migrating scale (or distance) of ciliates, we referred some references and revised accordingly **in lines 349-360 in revised manuscript**.

"In contrast, other studies provided evidence that ciliates indeed perform diel vertical migration (Dale 1987; Jonsson 1989; Pérez et al., 2000; Rossberg and Wickham, 2008). e.g., in the eutrophic lakes with steep light gradients, ciliate have been observed to move vertically at speeds of 1–2.5 m/h (Jonsson 1989). The weighted mean depth (WMD) was used to test diel vertical migration in copepods (Frost and Bollens, 1992). To our knowledge, we are the first to use the WMD for testing ciliate diel-vertical-migration. We found that the WMD of aloricate ciliates and tintinnids differed between day and night in both the nSCS and tWP with migration distance less than 20 m (Fig. 3), supporting our hypothesis that they do perform diel vertical migration, and this distance was consistent with the speed of ciliate (1-2.5 m/h) in the eutrophic lakes (Jonsson 1989) and the northwestern Mediterranean Sea (migrate distance: 20-30 m) (Pérez et al., 2000)".

- Line 335, 'The ciliate vertical distribution patterns were the same between day and night'

This sentence must be in contradiction with the sentence of 'WMD of aloricate ciliates and tintinnids differed between day and night (line 332)'. It must be also in conflict with the sentence of 'ciliate abundance and biomass were higher in night than in day (line 342)'.

Response: We revised this paragraph accordingly **in lines 372-384 in revised manuscript**.

"The ciliate abundance was high in surface and DCM layers in both day and night of both the nSCS and tWP. These results were similar to previous ones established in the western Pacific Ocean (Yang et al., 2004; Sohrin et al., 2010; Wang et al., 2019, 2020, 2021b).

However, the studies that previously investigated the ciliate vertical distribution, did not assess potential differences between day and night in vertical direction. Therefore, our study provides more accurate data on ciliate diel-vertical-migration in the nSCS and tWP. Additionally, our results in the upper 200 m provide evidence that ciliate abundance were higher at night than at day in both the nSCS and tWP (Fig. 2). Zooplankton distribution in waters mainly depends on phytoplankton presence (Daro 1988; Ursella et al., 2018). Thus, it is possible that the availability of more food items (flagellates, picoeukaryotes, *Prochlorococcus*, *Synechococcus* and heterotrophic bacteria) at night than at day explains the higher ciliate abundance at night (Olli 1999; Oubelkheir and Sciandra, 2008; Li et al., 2022)".

- Line 378, 'Heterotrophic tintinnids feed on ----- in the ocean.'

I can not understand the meaning of connection between this sentence and the previous sentence. This discontinuity might make the chapter unclear. You had better to rewrite the sentence more clearly.

Response: We revised this sentence accordingly **in lines 414-422 in revised manuscript.**

"As for heterotrophic microzooplankton tintinnids, photosynthetic organisms, e.g., nanoplankton (nanoflagellates), are important food items influencing their abundance and composition in the oligotrophic seas (Pitta et al., 2001; Weisse and Montagnes, 2022). Our study showed that tintinnid abundance at night was higher than at day for two possible reasons: 1) oceanic tintinnid species have stronger cell division in midnight than at day in tropical Pacific waters (Heinbokel 1987); and 2) predation on picoplankton, nanoplankton and heterotrophic bacteria occurred primarily at night (Tsai et al., 2005; Ribalet et al., 2015; Connell et al., 2020)".

- Line 394, 'nutrient loaded'

You had better to describe theoretical processes from this nutrient load to ciliate increase. In Fig. 2, chl-a concentration in the surface layer was very low and phytoplankton must not be promoted by the loaded nutrients.

Response: We accepted suggestion and revised this sentence accordingly **in lines 434-439 in revised manuscript.**

"Nutrients are material basis for the growth of microphytoplankton and heterotrophic bacteria. High nutrient concentrations always accompanied with high abundance of microphytoplankton and heterotrophic bacteria in surface waters in the oligotrophic tropical seas, which further affected and determined microzooplankton abundance and composition (Caron, 1994; Kirchman, 1994; Song, 2011; Zhang et al., 2016; Ma et al., 2020)".

- Line 404, 'We speculate that ----- could be the main reason of the disagreement.'

You had better to describe the theoretical reason on your speculation.

Response: We accepted suggestion and revised this sentence accordingly **in lines**

**447-452 in revised manuscript.**

“Low sampling frequency is often accompanied by low species richness (Dolan et al., 2007, 2009). The total samples in the tWP (45 samples) and nSCS (72 samples) were much lower than in previous studies ( $\geq 100$  samples) (Li et al., 2018; Wang et al., 2019, 2020). Thus we speculate that low sampling frequency in our results could be the main reason for the disagreement”.