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Comment

***Interactive comment on “Coupling of the spatial dynamic of picoplankton and nanoflagellate grazing pressure and carbon flow of the microbial food web in the subtropical pelagic continental shelf ecosystem” by K.-P. Chiang et al.***

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Main comments Q1: Although the authors mention "This study investigated the impact of the substrate supply and the grazing of nanoflagellates on picoplankton communities: : ." (P235, L24-27), it is questionable if the methods used in this study were proper to investigate the impact of the substrate supply on picoplankton community. The authors should clarify what kind of substrates (e.g., inorganic nutrients, vitamin, dissolved organic carbon, and trace metals) are potential limiting factor for picoplankton community (i.e., mainly bacteria, *Synechococcus*) in the study area, and which method

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was applied to investigate this aspect A1: I admit that the methods in our study are improper to discuss the top-down or the bottom-up control concepts. We changed goal of the study into “population growth and nanoflagellate grazing on picoplankton communities” (P 4, L15-16) and rewrote the first paragraph of Section 4.1 with a change in section title to “ Resource supply as a control of picoplankton growth in an oligotrophic ecosystem” (P 10, L12-24) Q2: The authors use the concept of "predator-prey eddy" of heterotrophic nanoflagellate -bacteria association (cf. Tanaka et al., 1997) to understand the spatial consistency or variability of the close predator-prey association between nanoflagellates and picoplankton. This concept is proposed to describe that abundances of bacteria and HNF vary seasonally over one and two orders of magnitude, respectively, overlaid by a higher frequency oscillations of smaller amplitude throughout the year in a coastal system (e.g., Thingstad, 2000). On the other hand, the compiled dataset from a variety of plankton ecosystems shows that both bacteria and heterotrophic nanoplankton vary about 3 orders of magnitude, in which the numerical relationship between them is remarkably consistent (1000 bacteria : 1 heterotrophic nanoplankton) (e.g., Sanders et al. 1992). Hence I do not understand how this concept can be applied to a dataset which has large spatial scale but much less temporal resolution. A2: The concept of “predator-prey eddy” described that the abundances of predator (nanoflagellate) and preys (bacteria or Synechococcus) were confined to a narrow range and both showed a circular orbit when both were plotted in a phase space in similar environmental conditions. The locations of the orbits in the prey-predator orbit clearly differ due to seasonal variation, such as high abundance in summer and low abundance in winter, while the predator-prey eddy was also maintained in each season or each month. In our study, we sample in a broad sea area that was occupied by the oligotrophic Taiwan Strait Warm Current Water and we also find a clear predator-prey eddy relationship. Therefore, we infer that the predator-prey eddy also existed on a spatial scale in a homogenous environment. In addition, we try to give an adequate explanation regarding the mechanism of predator-prey eddy Q3: The authors mention in the article (P235, L9-10), picoplankton generally consist of

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heterotrophic bacteria, *Prochlorococcus*, *Synechococcus* and picoeukaryotes in marine pelagic ecosystem. The authors focus on bacteria and *Synechococcus* as the important preys for nanoflagellates in this study without explaining why the other picophytoplankton groups were not investigated. A3 : In our study area, The dominant components of picoplankton community are heterotrophic bacteria and *Synechococcus*, Due to the facts that *Prochlorococcus* was not observed and picoeukaryotes were rare, an investigation was not conducted. We added two explanations in the introduction (P3, L9-11) and the method sections (P6, L22-26), respectively. Q4: Nanoflagellate community consists of autotrophic, mixotrophic, and heterotrophic flagellates. The authors should explain why they treat whole nanoflagellate community as an important predator of picoplankton community. They also should consider if abundance and/or predation rates of mixotrophic nanoflagellates were minor or if abundance of autotrophic nanoflagellates were dominant in this study. If so, how the conclusion can be modified? A4: Recently, several reports indicated that the mixotrophic pigmented nanoflagellate and the phototrophic algae both have good phagotrophic abilities and responsible for a large fraction of bacterivory in oligotrophic marine pelagic ecosystem. Therefore we used total nanoflagellates to estimate the grazing impact on picoplankton. We added a paragraph in the discussion section (Section 4.2, P. 12 L 5-9) to explain the phenomenon. Q5: The authors collected water samples at six water depths (5, 10, 25, 50, 75 and 100 m) (P237, L5-8). However because the authors use "surface", "surface water" and "surface layer" whose depth or depth range are not defined, it is very difficult to understand the results and discussion. Growth and grazing rates were measured using "surface seawater" samples at 7 stations. The authors should discuss how growth and grazing rates in surface water can be related to the data on abundance of picoplankton and nanoflagellates down to 100 m depth. A5: In our study, "surface", "surface water" and "surface layer" means the water at 5 m-depth. According to the reviewer's comment, we added an explanation in P. 6, L14. In addition, it is theoretically impossible to infer the vertical variation of picoplankton abundance using the growth rate of picoplankton and grazing rate of nanoflagellate in surface waters. Q6: Spa-

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tial variations of environmental condition between 7 stations need to be reworked. As the authors mention, T-S diagram suggests that the study area was mostly characterized as Taiwan Current Warm Water (Fig. 3). However, vertical profile of temperature was different between the stations, likely because of Kuroshio subsurface upwelling. In addition, the coastal stations could be influenced by riverine input. Although the authors present the data on concentration of nitrate and chlorophyll in Table 1, they do not really benefit from these data. Trophic status could be different between stations and depth. To better understand the data on abundance, and growth and grazing rate of the microbial community, it would be important to clarify how similar or different the trophic condition was along the coast-offshore transect and between different months/years. A6: In our study, we focused on the spatial variation of picoplankton abundance in surface waters and also hope to understand their population dynamic. Therefore we measured growth rate of picoplankton and grazing rate of nanoflagellate on picoplankton in surface waters at 6 stations. Since we only have growth and grazing rates in surface waters, we hardly discussed about the vertical profile of picoplankton abundance. In addition, the surface layer is usually occupied by the oligotrophic Taiwan Strait Water according to T-S diagrams. The upwelling water could affect Taiwan Strait Water occasions, but not be affected by the coastal water. The goal of our study is to discuss the dynamic process of picoplankton in an oligotrophic environment. The writing in our original manuscript was not good enough so that caused some misunderstandings. I changed the result section (P8, L14-25) by separating these 2 parts, and described the vertical profiles and the picoplankton and nanoflagellate distributions in different paragraphs. In addition, a sentence was added to emphasize that the growth rate and grazing rate were measured only in surface water (P8, L26). Q7: In measurement of growth and grazing rates (P238-239), the authors prepared triplicate samples at the beginning and end of each incubation. Such a set-up should allow estimating variability in cell counting between triplicate bottles, by which growth and grazing rates are determined with variability (e.g., standard deviation, standard error). The comparison of growth and grazing rates can statistically be tested. Otherwise, the objectivity in

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interpretation of the data remains unclear A7: According to the reviewer's comment, we added the standard variation of growth and grazing rates in Figures 6. Q8: It appears that the authors are confused between correlation analysis and linear regression analysis. When the significant relationship between two parameters is discussed, it is necessary to show statistical significance (e.g.  $P < 0.05$ ). A8: According to the reviewer's comment, we corrected these mistakes. Q9: There are many reports on growth rate of picoplankton and grazing rate on picoplankton in oligotrophic marine waters. The authors should compare their data with previous data reported from oligotrophic waters. Fig. 10 suggests that nanoflagellate biomass turnover is faster  $(4.32/(9.42+5.22)=0.3 \text{ d})$  than bacteria biomass turnover  $(21.81/16.09=1.4 \text{ d})$  and *Synechococcus* biomass turnover  $(13.78/12.12=1.1 \text{ d})$ . If one considers autotrophic production in the nanoflagellate community and other carbon supply through other preys (*Prochlorococcus* and picoeurykotes), the biomass turnover of nanoflagellates will be much faster. Are these values consistent with previously reported values? The bacterial carbon content (20 fg C/cell: Lee & Fuhrman 1987) is derived from culture bacteria, and could result in overestimation of bacterial biomass and production in oligotrophic water A9: The content in fig 10 might not be correctly understood by the reviewer, the turnover rate of nanoflagellate is  $4.32/2.62=1.6 \text{ d}$ , it is very close to the value of bacteria (1.4 d) or *Synechococcus* (1.1 d). I think these figures are reasonable. Other comments Q1: P234, L11-15: If the upwelling water supplies nutrients, by which bacterial growth is stimulated, one may expect that bacterial control shifts from bottom-up to top-down. A1: Replied in the A1 of Main Comments. Q2: P234, L21: I do not think that the concept of "predator-prey eddy" is well known. A2: Replies in the A2 of Main Comments Q3: P234, L25: "increasing number of sizes" needs to be clarified. A3 we changed "the increasing number size...size-fractionation experiment" into "successive size-fractionation experiment" (P2, L23) Q4: P235, L1: "nanoflagellate" should be "nanoflagellate". A4: It is canceled in revised version Q5: P245, L1-2: The authors measured predation rate of nanoflagellates on bacteria and *Synechococcus* but not other picoplankton groups. Hence it is not logic to mention "the diet of nano flagel-

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late is composed of 64% bacteria and 36% *Synechococcus* spp." A5: Replies in the A3 of Main Comments Q6: P237, L13: "-75\_ for later analysis" should be "-75\_C for later analysis". A6: According the reviewer's comment, it was corrected (P5, L13). Q7: P240, L15: Seven thousands of bacterial cells per ml and <22 *Synechococcus* cells per ml in the marine pelagic water appear too small. Please verify these numbers. A7: I checked these numbers, these are correct numbers in that deep sample. Q8: P240, L23-24: Nanoflagellate ranged from  $6.2 \times 10$  to  $1.04 \times 10^3$  cells/ml. So it varied two orders of magnitude A8: According the reviewer's comment, 1 order was changed into 2 orders (P8, L23). Q9: P241, L21-28: Please explain the methods in Materials and methods. As mentioned above, the trophic cascade effect is not evident without proper statistical comparison. A9: According the reviewer's comment, we add a paragraph in method (P 7, L22-30) to describe the methods of successive size-fractionation and of the statistical test.. Q10:P242, L18: "Ferrier-Pagés" should be "Ferrier-Pagès". A10: It is canceled in revised version Q11:P246, L16: "Hofle" should be "Höfle"(P14, L16). A11: According the reviewer's comment, this mistake was corrected Q12:P247, L6: "nonoflagellate" should be "nanoflagellate" A12: It is canceled in revised version Q13: P252, Table 1: - "surface" should be defined here. "surface" means which depth? - Values in the table should be defined (means \_ standard deviation or standard error or else?). - Although the data on concentration of nitrate and chlorophyll a were presented in Table 1, there is no description for these parameters in Materials and methods. A13, We added standard deviation and the depth of surface water in Table 1, also added the measuring methods of nitrate and Chlorophyll a in the method section (P.5, L3-8) Q14: P255, Fig. 3: All data points are within TCWW and KW whose space occupies only a small area of Fig. 3. Is it really important to show the other water masses (CDW, YSMW and YSCCW)? A14, We redrew the Fig. 3 and narrowed down the field of view. The areas occupied by CDW, YSMW and YSCCW water massed were removed. Q15: P262, Fig. 10: Production rate of nanoflagellates is not explained in the text, but presented in Fig. 10. A15: We added a sentence to describe nanoflagellate production on P14, L23 Q16: P263, Fig. 11: "1796-st1", "1809-st1", "1816-st1", and vertical bars

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need to be explained A16: We changed the explanation in Fig11

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