MS No.: bg-2012-521

Title:Seasonal variations of viral- and nanoflagellate-mediated mortality of heterotrophic bacteria in the coastal ecosystem of subtropical western Pacific

Special Issue: Biogeochemistry and ecosystems in the western north Pacific continental margins under climate change and anthropogenic forcing

I would like to thank the reviewers very much for the comment and suggestions. These thoughtful opinions and insight have helped up improve this manuscript a lot. In the revised version, we redrew figures 2, 3, 4, 5, 6 and Table 2. We have indicated the revised parts with red text in the revised manuscript.

Reviewer 1

General Comment: **I would like to point out that the discussion is not sufficient about the imbalance between the total bacterial production and the loss by nanoflagellate grazing and viral lysis. The authors attributed it to grazing by ciliates, but the discussion went half way. I request more information and profound discussion on a possibility of other grazers on bacteria in the sea such as appendicularia (Oikopleura) etc. PNFs (photosynthetic nanoflagellates) are also the influential candidates of bacteria grazer due to their myxotrophic behavior.**

Thank you very much. We have made necessary adjustments and reworded this paragraph in the discussion section (p. 15, line 7 to p. 16, line 3). Furthermore, we agree the comments of PNF are the influential candidates of bacterial grazer. However, in the dilution method, PNF are present in all culture bottles with HNF, we think the nanoflagellate grazing combines with HNF and PNF grazing in the present study.

Comment: **Since the population dynamics of bacteria are in the time scale of hour to day, and you need almost every day measurement about bacteria in order to understand the entire picture. This is an important problem, and I hope you conduct the work of short period of 1 - 2 weeks in your area in the future.**

Thank you very much. You are right. Further studies are recommended to assess the wider applications of the dilution protocol developed in this study for diel variations.

Reviewer 2

Comment: **Authors compared primary productivity or DOC released rate by lysis with bacterial growth rate to discuss an importance of released DOC by viral lysis. Because dimensions of primary productivity and DOC released rate (mass per unit volume per time) are completely different from bacterial growth rate (per unit time), authors should compare primary productivity or DOC released rate with bacterial carbon production rate (BP) rather than bacterial growth rate (or compare viral mortality with bacterial growth rate).**

Thank you. In the revised version, we redrew figures 6 to compare primary productivity or DOC released rate with bacterial carbon production rate (BP). We made necessary adjustments in p. 13, line 23 to p. 14, line 6, which now read: However, as shown in Fig. 6, this study found a significant relationship between bacterial production (BP) and released dissolved organic carbon by lysis (V), but no significant relationship between BP and primary production ($p = 0.1$). This observation suggests that viral infection is an important mechanism in C recycling in the sea. If carbon released due to virus-induced lysis is converted to new bacterial cells with an efficiency of 31% (Kristiansen et al., 1992), the fraction of BP potentially sustained by viral lysates was on average only 19% (ranged from 4% to 43%). Although virus-mediated lysis of bacteria did not contribute quantitatively to bacterial nutrition, the released material could be qualitatively important to nutrient regeneration, because cell components released by virus-mediated lysis are rich in organic nitrogen and phosphorus, can be highly labile, and are utilizable for growth by non-infected bacteria (Middleboe et al., 1996; Noble and Fuhrman, 2000).

Comment: Still more there is a similar problem in a comparison mg+mv/BP (%) with bacterial abundance (cells per unit volume) is also not appropriate. In this comparison, authors should use differences between sum of loss rate due to nanoflagelates and viluses (G+V) and BP and between bacterial abundance among two months. I, **however, think that BP determined by dilution method might not be appropriate to explain a monthly change of bacterial abundance because abundances of rapid growing microbes will fluctuate within a month.**

Thank you. We agree BP determined by dilution method might not be appropriate to explain a monthly change of bacterial abundance because abundances of rapid growing bacteria will fluctuate within a month. We sorry for not thinking this through clearly and deleted this figure in Fig. 5 in the revised version.

Comment: The second problem is insufficient procedure of statistical analysis for their conclusion. As described in abstract, authors emphasis the importance of temperature as controlling factor of the seasonal variation of bacterial growth. **If my understanding is right, it depends on only significant correlation between temperature and bacterial growth rate. I am not sure whether purpose of this analysis is to find an effective predictor of bacterial growth rate or to extract more important factor. If the purpose is the later, authors should present relationships between variation of bacterial growth rate and other related factors such as primary productivity. Many of studies pointed pot the importance of resource availability for bacterial growth.** Without the presentation relationship between temperature and other potentially important factors, readers can't decide the importance of temperature.

Thank you. In the revised version, we redrew figures 4 to compare temperature and Chl *a* with bacterial growth rates. We made necessary adjustments in p. 9, lines 8-12, which now read: Moreover, our study shows that temperature can plan an important role in controlling bacterial growth, as we found there to be positive

relationships between the growth rates and temperature (growth rate (h^{-1}) = 0.019 temp−0.29, r^2 = 0.43, $p < 0.05$) (Fig. 4A), and be a better degree of explanation than Chl a (Fig. 4B).

Comment: The third, **authors should discuss about grazing by other organisms such as gelatinous plankton as potential removal process of bacteria because they also are potentially important grazer of picoplankton in subtropical area** (for example, Bedo et al., (1993) Bull. Mar. Sci. 53: 2–14). **Additionally I can't understand the logic why authors decided that contribution of ciliate grazing is not so high.** Because selective feeding of ciliates has been reported by many of studies, grazing on Synechococcus could not be applicable for grazing on bacteria.

Thank you very much. We have made necessary adjustments and reworded this paragraph in the discussion section (p. 15, line 7 to p. 16, line 3).

Comment: **I feel somehow mismatch for using "total mortatlity" as sum of nanoflagellate grazing rate and viral lysis rate (mg+mv) while they discuss other bacterial removal processes.**

Thank you. We avoided these sentences about total mortality misunderstand as much as possible; we deleted this sentences in the Discussion section and change to **"the ratio of seasonal variations of grazing effect (mg) to these two sources of bacterial mortality (mg+mv) changed from 21 to 76% (Table 2)".** I hope this is acceptable.

Comment: Moreover authors should refer values or trends in literature with detail of location or characteristic of environments and experimental design. Without the information readers can't decide whether the comparison is appropriate or not. One example is "This result similar to other studies (Jacquet et al., 2005; Tijdens et al., 2008), which showed viral lysis to be the main cause of bacterial mortality during cold season experiments. Jacquet al. (2005) also observed that during the January experiment viral lysis removed up to 100% of the potential bacterial production." Readers must read the paper to know whether this cold season is productive season or not. **Furthermore authors compare their results with previous studies in freshwater environments without any explanation (If needed and appropriate, comparison with freshwater environment should be discussed).** Because the present study was conducted in a particular environment in which water temperature in winter is not so cold, authors should refer carefully results of previous studies for comparison with this study.

Thank you. In the revised version, we can't find enough data to compare these results with previous studies in freshwater environments (Jacquet et al., 2005; Tijdens et al., 2008). Thus, we deleted this paragraph and reworded this. We made necessary adjustments in p. 17, lines 17-25, which now read: **These results imply that the carbon and nutrients released upon viral lysis of bacteria were recycled within the microbial loop (Noble and Fuhrman, 2000). The role of viral lysis and nanoflagellate grazing in bacterial mortality may change spatially and temporally. The importance of viral lysis has been shown to increase in situations where nanoflagellate grazing is reduced (Bettarel et al., 2004). Furthermore, the loss of bacterial biomass was caused by viral lysis in the cold seasons (September to December) of this study, possibly due to UV inactivation of viruses in the surface water samples in the summer (Suttle and Chen, 1992; Wilhelm et al., 1998; Hofer and Sommaruga, 2001).**

Specific points: 1. Page 17238 Lines 8-11: “Our hypothesis was: : :” Is “production” “bacterial abundance”? **If authors’ hypothesis is to examine whether viruses and nanoflagellates play a significant role in controlling “bacterial production”, authors should compare BP DOC release rate both by viruses and by nanoflagellate grazing.**

Thank you for your careful consideration of our paper. We sorry for not thinking this through clearly. To us, it is not easy to compare DOC release rate both by viruses and by nanoflagellate grazing with dilution method. For this reason, we have made necessary adjustments in final paragraph of the Abstract section in p. 4, lines 15-21. It now reads: **Until now, studies in the coastal ecosystem of subtropical western Pacific environment have mainly focused on bacterial mortality due to nanoflagellate grazing (Tsai et al., 2005; 2008; 2011). The contribution of viruses to bacterial mortality is still poorly understood. In this study, our main goals were to use the modified dilution approach to estimate grazing and viral mortality of bacteria and compared their relative contributions of both on bacterial mortality for about 2 years in the coastal ecosystem of subtropical western Pacific.**

Comment: **In Methods: 20 fg C cell^-1 is used for as carbon content for heterotrophic bacteria. Although this value may not important analysis in the present discussion, this value is higher typical value in subtropical environments (Fukuda et al., 1998: Applied and Environmental Microbiology 64: 3352-3358.**

Very helpful comment. In the revised version, we have made necessary adjustments in p. 6, line 12 and carbon content for heterotrophic bacteria was based on values reported in Caron et al. (1995) (15 fg C $cell^{-1}$). Furthermore, we redrew Table 2 for this change.

Comment: **In Method: Authors present the number of fields of view for counting microbes. Because error can be estimated from numbers of counted cell, authors should show them. Number of cells in a field of view depends on abundance of microbes and filtered volume.**

We sorry for not thinking this through clearly. We have made necessary adjustments and redrew Figure 2.

Comment: **Before submitting revised version, authors should correct typos in the manuscript including figures.**

This is our fault, in the revised version, we We have made necessary adjustments.