

Interactive comment on “Seasonal variations of viral- and nanoflagellate-mediated mortality of heterotrophic bacteria in the coastal ecosystem of subtropical Western Pacific” by A.-Y. Tsai et al.

Anonymous Referee #2

Received and published: 22 February 2013

General

The present paper addresses effects of nanoflagellate grazing and viral lysis on seasonal dynamics of bacteria in coastal ecosystem of subtropical western Pacific. Seasonal variation of a relationship between grazing rate of nanoflagellates and lysis rate in a subtropical coastal environment will be fit for interests of BGD readers, because knowledge about this relationship in the subtropical Pacific is limited. I, however, have some major concerns regarding the data presentation as describe below. Thus I can't recommend publishing the present version of the manuscript without major revision.

At first the most serious problem is to direct comparison between variables which have

C8472

different dimensions. 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). If authors use the present comparison, the results of comparison using BP must be shown. Still more there is a similar problem in a comparison $\text{mg} + \text{mv} / \text{BP}$ (%) 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 nanoflagellates and viruses (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. Anyway authors should revise these analysis and related discussion.

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.

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

C8473

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. I guess that authors could emphasize the importance of nanoflagellate grazing and viral lysis by careful comparing differences between G+V and BP and between bacterial abundance among two months without excluding ciliate grazing. I feel somehow mismatch for using "total mortality" as sum of nanoflagellate grazing rate and viral lysis rate (mg+mv) while they discuss other bacterial removal processes. If purpose of using this "total" is to examine the relative importance of viral lysis rate to nanoflagellate grazing rate, simple mv/mg ratio could be useful for the discussion.

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.

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

C8474

should compare BP DOC release rate both by viruses and by nanoflagellate grazing.

2. 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).

3. 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.

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

Interactive comment on Biogeosciences Discuss., 9, 17235, 2012.

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