NOTES AND CORRESPONDENCE

Effects of bacteria-virus interaction on *Synechococcus* spp. growth in the coastal waters

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ABSTRACT

This study aims to investigate the relative importance of nanoflagellate grazing and viral lysis for heterotrophic bacteria and *Synechococcus* spp. mortality in coastal waters of the north-eastern Taiwan. The results showed that viral lysis and nanoflagellate grazing are the main factors responsible for the mortality of heterotrophic bacteria, however, the presence of viruses had a positive effect on the *Synechococcus* spp. growth. Moreover, this study observed different growth responses between heterotrophic bacteria and *Synechococcus* spp. population by using the modified dilution approach. The results also showed that there was lower net growth rate of *Synechococcus* spp. in 30 kDa dilution series than that observed in 0.2 μm series. Furthermore, based on the results obtained from modified dilution approach, we can estimate that about 12.8 ng N L⁻¹ h⁻¹ and 2.8 ng P L⁻¹ h⁻¹ was released by nanoflagellate grazing. In addition, this study estimated that, during the process of viral lysis, locally recycled nutrients released about 224 ng N L⁻¹ h⁻¹ and 24.9 ng P L⁻¹ h⁻¹ in this system. Our findings suggest that due to viral lysis, substantial portion of the recycled nutrients required for *Synechococcus* spp. growth was supplied sufficiently, emphasizing the importance of the viral shunt, especially in oligotrophic regions.

1. INTRODUCTION

Picophytoplankton (< 2 μm) contribute significantly to phytoplankton biomass and production in oligotrophic environments (Bell and Kalff 2001; Winder 2009; Grob et al. 2011). Among these small size picophytoplankton, *Synechococcus* spp. is virtually ubiquitous in marine environments and contributes to more than 50% of the primary production in oligotrophic oceans (Jardillier et al. 2010; Tsai et al. 2012). Compared to other larger group phytoplankton, *Synechococcus* spp. possesses high specific growth rates due to its small size, and has high specific affinity for nutrients (Agawin et al. 2000). Grazing and viral lysis are the two major causes of *Synechococcus* spp. mortality (Baudoux et al. 2008; Tsai et al. 2012). Grazing leads to the transfer of biomass to higher trophic levels, whereas viral lysis recycles carbon and nutrients within the microbial loop (Ayukai 1996).

The dilution approach, proposed by Landry and Hassett (1982), has been accepted as a standard method for estimating phytoplankton growth and grazing mortality rates (Ayukai 1996; Redden et al. 2002; Juhl and Murrell 2005; Anderson et al. 2018). Following this technique, Evans et al. (2003) estimated viral lysis and grazing effect on picoeukaryote, *Micromonas* simultaneously using a modified dilution method. One of the main assumptions of the dilution approach is that prey growth rate is independent of the dilution (Landry and Hassett 1982). However, in some nutrient limited waters, growth of *Synechococcus* spp. is lower in incubations that are diluted with virus-free diluents than those diluted with 0.2 μm diluents, containing viruses (Shelford et al. 2012; Tsai et al. 2018; Gong and Tsai 2019). Similarly, Tsai et al. (2018) found that *Synechococcus* spp. growth rate was clearly lower in treatments in which the

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viral abundance was reduced by using the modified dilution approach. These observations may violate the assumption associated with the dilution approach that prey growth rate is independent of dilution effect, but highlights that the presence of viruses had a positive effect on the growth of *Synechococcus* spp. Furthermore, Weinbauer et al. (2011) studied the interactions among *Synechococcus* spp., heterotrophic bacteria and viruses. Based on the results, they suggested that nutrient cycles that are controlled by viral lysis of heterotrophic bacteria may also control *Synechococcus* spp. growth. This is persuasive evidence denoting that viruses are important agents in controlling nutrient cycle in various aquatic environments.

A potential problem associated with reduced *Synechococcus* spp. growth in diluted water has been recognized in a recent study (Tsai et al. 2018). The study mentioned that *Synechococcus* spp. present in oligotrophic waters may depend on nutrients regenerated through trophic interactions between heterotrophic bacteria and grazers or viruses. However, initial experiments in this study of Tsai et al. (2018) were not performed to assess viral-induced mortality in heterotrophic bacteria, and therefore, it may not provide valuable insights into the source of nutrients that *Synechococcus* spp. primarily depends on. This study hypothesizes that there are different growth responses present between heterotrophic bacteria and *Synechococcus* spp. population, and tests the hypothesis by using the modified dilution approach. It aims to study how viral lysis and nanoflagellate grazing affect the mortality of heterotrophic bacteria and *Synechococcus* spp.

### 2. MATERIALS AND METHODS

#### 2.1 Sampling

Samples were collected on 14 and 18 July 2019, between 8 am and 9 am (local time) from the surface water of the coastal area of north-eastern Taiwan (25°09.4’N, 121°46.3’E). Previous long-term sampling at this study site has revealed significant seasonal variability in temperature, nutrients and picoplankton abundance (Tsai et al. 2005, 2008, 2012, 2013). Water temperature was measured immediately after the sampling bucket was cast. All samples were transported to the laboratory within 30 min.

#### 2.2 Modified Dilution Approach

In order to measure the contribution of grazers and viruses in association with heterotrophic bacteria and *Synechococcus* spp. mortality, modified dilution method (Evans et al. 2003) was conducted. Sequential dilution of the natural community managed with two different types of seawater filters was used to reduce predator-prey (0.2 μm) and viral-host (30 kDa tangential flow filtration system) encounter rates. Filtered seawater sample (< 10 μm) was then diluted with the 0.2 μm and 30 kDa filtered seawater in a 4-point dilution series: 25, 50, 75, and 100% seawater (< 10 μm). The size fractionation used for grazers (< 10 mm) was chosen based on previous studies at this site to eliminate ciliates but not nanoflagellates (Tsai et al. 2012). Two modified dilution experiments were performed during the warm season (July), following the details of experimental set-up and incubation conditions described in Tsai et al. (2018). All treatments were incubated for 12 h in triplicate in 200 mL polycarbonate bottles under natural light in a water bath set at the in situ temperature of the seawater at the time of sampling. In the present study, nutrients were not added to the experimental bottles due to the following reasons: (1) to determine bacterial and *Synechococcus* spp. Growth and the effect of grazing and viral lysis without involving the bias of nutrient addition; (2) to highlight the importance of the effects of recycling nutrients which are required for *Synechococcus* spp. growth.

Bacteria and *Synechococcus* spp. were counted using an epifluorescence microscope (Nikon Optiphot-2; 1000x) and abundance count was also conducted as described in Tsai et al. (2005). Significance of the regression lines was tested using analysis of variance (ANOVA). Moreover, the significance between the slopes of the 30 kDa and 0.2 μm dilution series was determined using the *F*-test. If the regression slopes of 30 kDa and 0.2 μm dilution series were significantly different, we then calculated the magnitude of viral lysis. STATISTICA 7.0 software was used to perform all statistical operations. A probability value of < 0.05 was considered significant.

### 3. RESULTS AND DISCUSSION

Both sampling procedures maintained the same temperature range (28.5 - 29°C). Bacterial abundance measured in the collected seawater (14 and 28 July) was $6 \times 10^7$ and $8 \times 10^6$ cells mL$^{-1}$, respectively. Furthermore, abundance of *Synechococcus* spp. was $5 \times 10^4$ and $7 \times 10^4$ cells mL$^{-1}$ on 14 and 28 July, respectively. A good linearity with negative slope was found while measuring bacterial net growth rate in both dilution series (Figs. 1a and b). The regression coefficients (slopes) were 0.056 and 0.065 h$^{-1}$ for the 0.2 μm fractionated series, and 0.10 and 0.172 h$^{-1}$ for the 30 kDa fractionated series in the two experiments (Figs. 1a and b). Additionally, there were significant differences noted between the two slopes of regression lines as seen in both experiments (*F*-test, $p < 0.05$) (Figs. 1a and b). The difference between the two slope values were 0.044 and 0.107 h$^{-1}$, indicating that bacterial mortality was caused mainly due to lysis of viruses. In the present study, we observed higher net growth rate of bacteria in the 30 kDa series than that in the 0.2 μm series (Figs. 1a and b), which suggests that under these conditions lytic pressure is relatively high (Baudoux et al. 2008). Furthermore, the *y*-intercepts of these regression lines were 0.09...
and 0.153 h\(^{-1}\), respectively for 30 kDa fractionated series in both experiments (Figs. 1a and b). These values represent the bacterial growth rate in the absence of grazing pressure and viral lysis.

We investigated the role of Synechococcus spp. in association with growth incubation experiments, and the results confirmed a positive relationship between net growth rate of Synechococcus spp. and dilution factor, implying that the assumption of the dilution method was not met (Figs. 1c and d). Some modified dilution experiments have been tested to estimate viral lysis rates of phytoplankton (Evans et al. 2003; Tsai et al. 2018). This study did not estimate the effect of viral lysis on Synechococcus spp., as it was suspected that Synechococcus spp. might not be top-down controlled by viral lysis or nanoflagellate grazing. In this study, it was evident that Synechococcus spp. growth was bottom-up controlled with nutrient limitation being an important factor. This study also confirmed that different growth responses exist between heterotrophic bacteria and Synechococcus spp. population by using the modified dilution approach. Interestingly, our results reported that the net growth rate of Synechococcus spp. in 30 kDa dilution series was lower than that in 0.2 μm series (Figs. 1c and d). Similar patterns have been observed in other studies, namely reduced net growth rate detected at higher dilution levels; this suggests possible nutrient limitation during their research (Baudoux et al. 2007; Shelford et al. 2012; Tsai et al. 2018). Concerning the different patterns of heterotrophic bacteria and Synechococcus spp. in response to dilution experiments, the explanation for reduced Synechococcus spp. growth rate in 30 kDa dilution series is still unclear. This finding is consistent with previous studies (Weinbauer et al. 2011; Shelford et al. 2012), which pointed out that viruses enhance the growth rate of Synechococcus spp. These results suggested an indirect effect of viral lysis of heterotrophic bacteria. Over the past decades, there has been an increased focus to study the importance of nutrients regeneration in marine environments, and the role of viruses in this process (Ayukai 1996; Weinbauer et al. 2011; Shelford et al. 2012; Gong and Tsai 2019).

This study found that nanoflagellate grazing and viral lysis both are the major factors for mortality of bacteria in the experiments, with an average of 0.06 and 0.08 h\(^{-1}\), respectively (Fig. 2). Many studies on the degree of viral lysis and grazing have focused on heterotrophic bacteria (Jacquet et al. 2005; Fischer et al. 2006; Wells and Deming 2006; Tsai et al. 2013), and reported that the impact of viral lysis and grazing varies according to divergent factors such as different seasons, environments and host organisms. Moreover, loss of bacterial cells either through grazing or due to viral lysis can be expected to have different biogeochemical and ecological consequences. It is observed that grazing leads

Fig. 1. Net growth rate vs. fraction of 10 μm filtered water for the parallel dilution experiments for bacteria (a) (b) and Synechococcus spp. were conducted on 14th and 28th July. Experiments were performed in 30 kDa ultra filtrate (■) and 0.2 μm filtered seawater (○).
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Concerning the role of nanoflagellate grazing, besides their contribution in promoting carbon flux, they may also contribute to the release of nutrients and directly influence nitrogen (N) or phosphorus (P) cycle in water (Chase and Price 1997). Hence, rough calculation can be made from the experiments conducted in this study to assess nitrogen and phosphorus levels, as they are potentially released by the process of nanoflagellate grazing on bacteria. In this study, a carbon content of 20 fg C cell\(^{-1}\) (Lee and Fuhrman 1987) and C:N:P ratio of 45:9:1, respectively for bacteria (Goldman et al. 1987) was used to estimate the concentration of nitrogen and phosphorus released by grazing or viral lysis. Additionally, based on the average heterotrophic bacterial biomass (14 μg C L\(^{-1}\)) and grazing rate (0.06 h\(^{-1}\)), we estimated that about 12.8 ng N L\(^{-1}\) h\(^{-1}\) and 2.8 ng P L\(^{-1}\) h\(^{-1}\) was released by nanoflagellate grazing, which highlights that nanoflagellate can release a significant fraction (10% of N and 20% of P) of ingested prey organic matter (Caron et al. 1990; Ferrier-Pagès et al. 1998) (Fig. 2).

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![Fig. 2. Picture of nanoflagellate grazing and viral lysis of bacteria, their contribution in balancing carbon flux, and maintaining the release of nutrients for bacterial and Synechococcus spp. growth.](image-url)
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