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Variations of microbial loop carbon flux in western subtropical Pacific coastal water between warm and cold season

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ABSTRACT

Viral lysis and protist grazing differ in their impact on the aquatic food web. In this experimental study, an adapted version of the dilution technique was applied to simultaneously estimate the effect of grazing by protists (nanoflagellates and ciliates) and viral lysis on bacteria and carbon flow in western subtropical Pacific coastal water. Four separate experiments were carried out: two experiments in the warm season (>25 °C) and two in cooler seasons (<25 °C). In the warm season, grazing by protists (nanoflagellates and ciliates) removed about 69% of bacterial production. In the cooler seasons, ciliate grazing did not have a significant impact on bacteria, but viral lysis had a significant and major impact, removing 79% of the bacterial production. Grazing by protists and viral lysis play critical and seasonal roles in the functioning of marine microbial food webs.

1. Introduction

New methods of measuring bacterial production have shown that a large portion of the carbon assimilated through primary production passes through bacteria (Cole et al., 1988; Fuhrman and Azam, 1982; Pace and Cole, 1994). The realization that bacteria is a major pathway in the flux of energy in marine ecosystems has generated considerable interest in the structure and functioning of this part of the food web (Ducklow et al., 1986). Bacterial production is dependent on both abiotic and biotic factors. Abiotic factors (bottom-up control) include temperature, salinity and substrate availability (Almeida et al., 2001; Ameryk et al., 2005). The biotic factors (top-down control) are predation by protists (Pace, 1988) as well as viral infection and lysis (Taira et al., 2009; Wilhelm et al., 2002). The relative contribution of grazers and viruses to bacterial mortality has important consequences for the processing and transfer of organic matter in the ocean. When bacterial cells are grazed, energy is made available to higher trophic levels. When they are lysed, however, organic carbon and nutrients are dissolved and bacterial carbon is recycled as bacterial production in a viral loop (Bratbak et al., 1992; Middelboe et al., 1996; Wilhelm et al., 2002).

The microbial community of bacteria, nano- and microzooplankton plays an important role in coastal food webs (Pomeroy, 1974). The ciliate-mediated microheterotrophic pathway constitutes an important link between bacterial and nanoplankton carbon and upper trophic levels (Vargas and González, 2004; Vargas and Martínez, 2009; Vargas et al., 2008). Moreover, bacteria and bacteria-based food webs process a large fraction of local organic production, and the rates and temporal patterns of this processing are frequently regulated by temperature (Carlsson and Caron, 2001; Ducklow and Carlson, 1992; Rivkin et al., 1996). Shiah and Ducklow (1994) suggested that the relative importance of temperature and substrate supply might change seasonally. In summer, substrate supply regulates bacterial growth rates, while the low temperatures in nonsummer seasons probably constrain bacterial growth and microbial loop activity. However, the seasonal variations of the flux of organic carbon in the microbial loop are still poorly understood. Experimental tests of seasonal variations comparing the effect of grazing with that of viral lysis on bacteria are scarce in marine systems. Some previous studies showed that the importance of viral lysis has been shown to increase in environments where nanoflagellate grazing is reduced (Bettarel et al., 2004). Seasonally, viral lysis is reported to be the main cause of bacterial mortality during the winter (Tijdens et al., 2008; Wells and Deming, 2006). As mentioned above, thus, we hypothesize that the low-temperature suppresses bacterial growth and reduces microbial loop activity and that viral lysis may play a large role in determining the flux of organic carbon in the microbial loop, outweighing the impact of protists on bacteria in the cold seasons.

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2. Material and method

2.1. Sampling

Samples were collected at an established coastal station (25° 09.4′ N, 121° 46.3′ E) along a rocky shore in northeastern Taiwan with the seawater temperatures ranging from 16 to 27 °C over the sampling period. The environment at this site was previously described using data gathered from 1999 to 2001 (Tsai et al., 2005).

2.2. Dilution experiments

We estimated viral lysis, nanoflagellate and ciliate grazing rates using a modified dilution technique in parallel experiments. For each experiment there were three dilution series, two of them were diluted with 0.2 μm filtered water and one of them was diluted with 30 kDa filtered water. To set up the experiment, the natural sample was passed first through 10 μm mesh and then filtered through 47 mm Nuclepore filter (type PC, pore size of 0.2 μm). Filtered seawater sample (<10 μm) was then diluted with the 0.2 μm filtered seawater in a 4-point dilution series: 25, 50, 75, and 100% seawater (<10 μm). Filter holders and incubation bottles were acid-cleaned with 10% HCl and rigorously rinsed with Milli-Q water. The mixtures were incubated for 24 h in triplicate in 200-mL polycarbonate bottles under natural light in a water bath set at the same temperature that the seawater was at the time of sampling. The size fractionation used for the grazers (<10 μm) was chosen based on previous studies at this site to eliminate ciliates but not nanoflagellates (Tsai et al., 2011). The traditional dilution series involving diluting natural seawater with a 0.2 μm filtered sample allows microzooplankton grazing (Landry and Hassett, 1982). However, we also used 30 kDa (Kvick Start Cassettes) filtered seawater instead of 0.2 μm filtered water in the dilution series to modify both nanoflagellate grazing and viral mortalities. In this modified dilution experiment, a filtered seawater sample (<10 μm) was diluted with 30 kDa filtered water. In an additional dilution series to estimate the effect of ciliate and nanoflagellate grazing, the subsamples were passed through 100 μm mesh to remove larger zooplankton, and the remaining 0.2 μm diluents were immediately used to create the dilution series (25%, 50%, 75% and 100% of natural water).

2.3. Interpretation of results from dilution experiments

The net growth rate of bacteria (k, d^{-1}) was calculated for each sample based on microscopic cell counts at the start and the end of the experiment (N_{t0} and N_t), assuming exponential growth (Landry and Hassett, 1982):

$$k = \ln(N_t/N_{t0})/(t-t_o)$$

where t and t_0 are the start and end of the experiment. Although usually interpreted as nanoflagellate grazing (G_{bn}) (Landry and Hassett, 1982), the regression coefficient of apparent growth rate versus dilution factor for the 0.2 μm dilution series (K2) actually includes viral mortality (V_b), because most viruses pass through a 0.2 μm pore (Evans et al., 2003). However, when virus-free seawater (30 kDa filters) is used as a diluent (K1), the regression reflects release from both grazing and viral mortality ($G_{bn} + V_b$), and a direct estimate of viral mortality (V_b) for bacteria can be obtained from the difference in slopes of the regression lines between the two dilution series (K1 vs. K2) (Fig. 1).

Rates of bacterial mortality caused by ciliate grazing (G_{bc}) could then be estimated by calculating the difference in the net growth rate of bacteria (k, d^{-1}) between the 10 μm and 100 μm dilution series in 100% of whole water (K2 vs. K3) (Fig. 1):

$$K2-K3 = G_{bc}, \text{ while the } DF = 100\%.$$

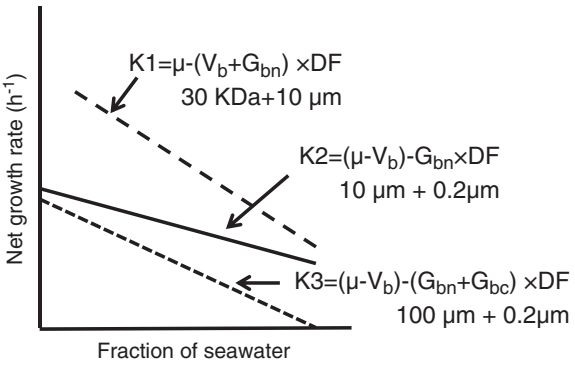

Fig. 1. Idealized dilution plots and analysis when filtered water containing viruses (standard protocol used to determine nanoflagellate and ciliate grazing, K2 and K3) and viral-free water are used to dilute sample water (K1) in the incubations that make up each dilution series. K = net growth rate of bacteria, μ = growth rate of bacteria, G_{bn} = bacterial mortality due to nanoflagellate grazing, G_{bc} = bacterial mortality due to ciliate grazing, V_b = bacterial mortality due to viral lysis, and DF = fraction of sample water.

2.4. Nanoflagellate growth and mortality assays

At the same time, the ciliate grazing rates on nanoflagellates (G_{nc}) was also measured using the dilution method. Briefly, the natural sample (<100 μm) was diluted in 0.2 μm filtered seawater at 4 levels (25, 50, 75, and 100% seawater) to obtain the ciliate grazing on nanoflagellates (Fig. 1). The regression coefficient of growth rate represents nanoflagellate losses due to ciliate grazing. Additionally, growth rates of nanoflagellates (μ_n) were determined as the y-intercept value of the regression line.

2.5. Trophic pathways of carbon cycling

A carbon budget was determined by combining the cellular carbon content estimates and data obtained from the dilution experiments. Carbon content for heterotrophic bacteria was based on values (20 fg C $cell^{-1}$) reported in Lee and Fuhrman (1987). Carbon production (BP in mg C m^{-3} d^{-1}), losses due to nanoflagellate and ciliate grazing ($G_{(bn)}$, $G_{(bc)}$, mg C m^{-3} d^{-1}), and viral lysis ($V_{(b)}$, mg C m^{-3} d^{-1}) were calculated using the following formulae, respectively: $BP = \mu_b \times BB$, $G_{(bn)} = G_{bn} \times BB$, $G_{(bc)} = G_{bc} \times BB$, and $V_{(b)} = V_b \times BB$, where μ_b is the dilution-based specific growth (y-intercept of the 30 kDa regression), G_{bn}, G_{bc} and V_b are the dilution-based nanoflagellate and ciliate grazing and viral lysis rates, and BB is the heterotrophic bacterial biomass (mg C m^{-3}) at the sampling time. Furthermore, nanoflagellate content was based on 220 fg C μm^{-3} (Børsheim and Bratbak, 1987) and ciliate content on 140 fg C μm^{-3} (Putt and Stoecker, 1989).

2.6. Viral, bacterial, nanoflagellate and ciliate abundance counts

Viruses, bacteria, and nanoflagellates were counted using an epifluorescence microscope (Nikon Optiphot-2) (1000×). Viruses were processed with a slight modification of a protocol described by Nobel and Fuhrman (1998). Briefly, samples from 0.5 to 1 mL were filtered on Anodisc filter (0.02 μm pore size, Whatman) backed by 0.45 μm pore size Millipore filter. The samples were then placed on drops of SYBR Green I (Molecular Probes) solution diluted at 1:400 in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and stained for 15 min in the dark. The membranes were placed on glass slides and added with 25 μL of 50% glycerol/50% PBS buffer (0.85% NaCl, 0.05 M NaH_2PO_4, Ph 7.5) containing 0.1% *p*-phenylenediamine as antifade and mounting agents. Subsamples of 1–2 mL or 20 mL were filtered onto 0.2 μm or 0.8 μm black Nuclepore filters for bacteria and nanoflagellates, respectively. Samples were stained with DAPI at a