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Impact of short-term warming on seasonal variations in bacterial growth, grazing, and viral lysis in coastal waters of Taiwan

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ABSTRACT: Although temperature is a key parameter controlling the activity and growth of all microorganisms, information about how water temperature may affect the trophic interactions and carbon flow patterns of microbial food webs is not consistent. We investigated the response in bacterial gross growth, grazing rates, and viral lysis to small temperature changes (3°C above *in situ* values) in coastal waters of Taiwan using a modified dilution method approach over a 1 yr period from September 2013 to September 2014. Warming increased the bacterial gross growth rates and bacterial losses to grazing, demonstrating clear seasonality. The warming conditions led to a 5 to 200% increase in bacterial gross growth rates. The increase ratios of bacterial gross growth rates were low (5 to 25%) at higher ambient temperatures (>25°C) but increased exponentially at lower ambient temperatures (<25°C) with warming. Grazing increased in parallel with seasonal bacterial growth, while viral lysis did not. These results could prove useful in forming a testable hypothesis about the possible directions of change of microbial carbon fluxes that may accompany the warming of the coastal waters of Taiwan.

KEY WORDS: Climate change · Nanoflagellates · Microbial loop · Viruses · Carbon flux

INTRODUCTION

The functioning of microbial food webs in the euphotic zones of the ocean depends mainly on the availability of inorganic nutrients for phytoplankton growth (Zubkov et al. 2000), on the accessibility of carbon and nutrients for bacterial growth, and on their interactions with protists (nanoflagellates and ciliates) and viruses, interactions which are responsible for carbon flux and dissolved organic and inorganic nutrient recycling in the water column (Fuhrman 1999). Temperature is a potentially limiting factor in biogeochemical processes (Sarmiento et al. 2004), and water temperature changes affect microbial growth, respiratory rates, and organic carbon assimilation (Holding et al. 2013). Thus, with global temperatures rising at unprecedented rates (IPCC 2007), global warming may be a driver of many of the changes occurring in the structure of marine food webs (O'Connor et al. 2009, Adams et al. 2010) and oceanic biogeochemical cycles (Sarmiento et al. 2004).

Studies of the relationships between temperature and bacterial growth in natural bacterial assemblages have shown that temperature is closely related to bacterial production (Wiebe et al. 1992, Shiah & Ducklow 1994, Murrell 2003, McManus et al. 2004, Tsai et al. 2008). Experiments have shown that temperature increases cause significant changes in prokaryotic growth rates (Kirchman et al. 2009, Vázquez-Domínguez et al. 2012). For example, in the waters of the NW Mediterranean Sea, maximum differences between the bacterial gross growth rates in warmer and ambient temperatures were observed during

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winter, when temperatures *in situ* were the lowest of the year (Vázquez-Domínguez et al. 2012). In addition, while changes in bacterial community composition might have been due to a direct impact of higher water temperatures and the development of better adapted bacterial taxa (von Scheibner et al. 2014), several studies that investigated the effects of temperature on the transfer of carbon between bacteria and protists have shown that temperature has a positive effect on bacterial grazing rates (Rose & Caron 2007, Tsai et al. 2008, Vaqué et al. 2009, Lara et al. 2013). Vázquez-Domínguez et al. (2012) found that loss rates of bacteria due to grazing increased by nearly 50% in the NW Mediterranean waters in periods of warming, and suggested that under warmer conditions, bacteria were mainly channeled to higher trophic levels via heterotrophic nanoflagellates. They also found that bacterial losses to grazing demonstrated clear seasonality, with increases being higher in winter and lower in summer. Studies of larger data sets have indicated that viral lysis can be a significant source of bacterial mortality sometimes comparable to bacterivory by protists (Fuhrman & Noble 1995, Pradeep Ram et al. 2005). Available data have generally revealed a tight coupling between viruses and bacteria, suggesting a close relationship between these 2 communities (Weinbauer & Peduzzi 1995, Weinbauer 2004). Concerning the viral standing stock and activity, few correlations with temperature have been reported. Jiang & Paul (1994) documented a significant positive correlation between viral abundance and temperature in Tampa Bay (USA). However, much less is known about the effect of warming on viral lysis in marine systems (Lara et al. 2013).

How global warming will affect microbial communities and bacterial carbon fluxes is under debate. In the present study, we hypothesized that microbial activity and carbon fluxes may change in response to changes in environmental temperature. Therefore, we performed a series of modified dilution experiments over a 1 yr period to test for a differential response between bacterial growth, nanoflagellate grazing, and viral lysis to short-term experimental warming in a subtropical marine system on the coast of Taiwan.

MATERIALS AND METHODS

Sampling

Experiments were carried out at an established coastal station (25° 09.4′ N, 121° 46.3′ E) along a rocky shore in northeastern Taiwan from September 2013 to September 2014. The environment at this site has been previously described using data gathered from 1999 to 2001 (Tsai et al. 2005). Samples were obtained between 08:00 and 09:00 h local time in 10 l polycarbonate bottles and processed within 0.5 h.

Dilution experiments

We estimated viral lysis, nanoflagellate grazing rates, and bacterial growth rates using a modified dilution technique. Filter holders and incubation bottles were acid-cleaned with 10% HCl and rigorously rinsed with Milli-Q water. To set up the experiment for estimating bacterial mortality, the natural sample was first passed through 10 µm mesh and then filtered through a 47 mm Nuclepore filter (type PC, pore size of 0.2 µm; Fig. 1). The size fractionation used for the grazers (<10 µm) was chosen based on previous studies of samples from this site to eliminate ciliates but not nanoflagellates (Tsai et al. 2011). Because the traditional dilution series diluting natural seawater with a 0.2 µm filtered sample allows microzooplankton grazing (Landry & Hassett 1982), we 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 (Evans et al. 2003, Kimmance et al. 2007, Tijdens et al. 2008, Personnic et al. 2009). The 2 fractions (0.2 µm and 30 kDa) were mixed in order to obtain percentages of the initial 10 µm filtered whole water of ca. 25, 50, 75, and 100% (Fig. 1). For each level of dilution, triplicates were prepared in acid-washed and water-rinsed 100 ml polycarbonate bottles. Immediately after preparation, the bottles were moved to outside a laboratory close to the sampling site and then incubated for 24 h under natural light in a thermo-controlled incubator set at the same temperature as the seawater at the time of sampling (Tsai et al. 2013). For each experiment, samples were incu-

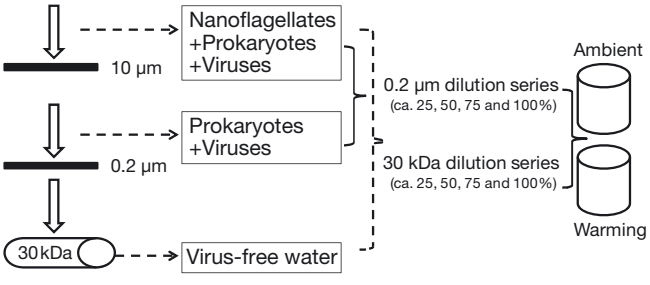

Fig. 1. Experimental design. For details, see 'Materials and methods'

bated at 2 temperatures: the temperature found *in situ* (Table 1), hereafter referred to as 'ambient,' and an experimental warming temperature set at 3°C above *in situ* values on average (Table 1). The temperature of the warming incubations was kept constant (±0.5°C) using thermo-controlled incubators under natural light. Incubations of both treatments started at 11:00 h local time.

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_{t_0} and N_t), assuming exponential growth (Landry & Hassett 1982):

$$k = \ln (N_t / N_{t_0})/(t - t_0) \quad (1)$$

where t_0 and t are the start and end of the experiment, respectively. Although usually interpreted as nanoflagellate grazing (G_b) (Tsai et al. 2013), the regression coefficient of apparent growth rate versus dilution factor for the 0.2 µm dilution series actually includes viral mortality (V_b), because most viruses pass through a 0.2 µm pore size filter (Evans et al. 2003). However, when virus-free seawater (30 kDa filtered) is used as a diluent, the regression reflects release from both grazing and viral mortality (G_b + V_b), and a direct estimate of V_b for bacteria can be obtained from the difference in slopes of the regression lines between the 2 dilution series. Specific growth rates (µ) were determined as the y-intercept value of the regression line obtained with the 30 kDa series.

Viral, bacterial, and nanoflagellate abundance counts

Viruses, bacteria, and nanoflagellates were counted using an epifluorescence microscope (Nikon Opti-

Table 1. Results from the parallel dilution experiments for bacteria under ambient and warming conditions over the experimental period. The significance (p) of the regression analyses (ANOVA) and the significance of the difference between the slopes of the regression of 0.2 µm and 30 kDa dilution series determined using an F-test are shown (*p < 0.05). G_b, V_b, and µ are nanoflagellate grazing, viral mortality, and bacterial growth rate, respectively

Month/ Year	Treatment	0.2 µm dilution series			30 kDa dilution series			Ratio of grazing to total mortality
		Slope (d^{-1}) (G_b)	y-intercept (d^{-1})	p	Slope (d^{-1}) (G_b + V_b)	y-intercept (d^{-1}) (µ)	p	$G_b/(G_b + V_b)$
Sep-13	Ambient (28°C)	0.72	2.40	<0.05	2.40*	3.84	<0.05	0.30
	Warming	1.92	3.84	<0.05	2.64*	4.32	<0.01	0.73
Oct-13	Ambient (25°C)	1.92	2.40	<0.05	2.88*	3.12	<0.01	0.67
	Warming	3.36	3.60	<0.01	4.32*	4.32	<0.05	0.78
Dec-13	Ambient (18°C)	0.24	0.48	0.89	0.48*	0.72	<0.05	~0
	Warming	1.44	1.20	<0.01	1.92*	2.16	<0.05	0.75
Jan-14	Ambient (19°C)	0.24	0.12	0.81	0.48*	0.48	<0.05	~0
	Warming	0.48	0.48	<0.05	1.20*	1.44	<0.05	0.40
Feb-14	Ambient (18°C)	0.48	1.44	<0.05	1.92*	1.92	<0.01	0.25
	Warming	1.44	1.92	<0.01	3.12*	3.84	<0.01	0.46
Mar-14	Ambient (22°C)	0.72	0.96	<0.05	2.16*	2.16	<0.01	0.33
	Warming	2.40	2.88	<0.01	3.84*	4.56	<0.01	0.63
Apr-14	Ambient (23°C)	0.72	0.24	<0.05	2.16*	2.16	<0.05	0.33
	Warming	1.20	2.64	<0.05	2.40*	3.84	<0.05	0.50
May-14	Ambient (24.5°C)	2.88	2.4	<0.01	3.12	3.36	<0.05	~1.00
	Warming	3.36	3.12	<0.01	3.84	4.08	<0.05	~1.00
Jun-14	Ambient (27°C)	4.08	3.12	<0.01	5.52*	4.32	<0.01	0.74
	Warming	4.56	3.60	<0.01	5.76*	4.80	<0.01	0.79
Jul-14	Ambient (30°C)	2.40	2.16	<0.05	3.12*	3.12	<0.01	0.77
	Warming	3.12	2.88	<0.05	4.08*	3.36	<0.01	0.76
Aug-14	Ambient (31°C)	2.64	3.36	<0.05	3.60*	4.32	<0.01	0.73
	Warming	2.88	3.60	<0.01	4.08*	5.04	<0.01	0.71
Sep-14	Ambient (29.5°C)	3.12	3.60	<0.01	3.84*	4.08	<0.01	0.81
	Warming	3.84	3.60	<0.05	4.32	4.32	<0.05	~1.00

phot-2; 1000×). Viruses were processed with a slight modification of a protocol described by Noble & Fuhrman (1998). Briefly, samples from 0.5 to 1 ml were filtered on Anodisc filters (0.02 µm pore size, Whatman) backed by 0.45 µm pore size Millipore filters. 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 to which we added 25 µl of 50% glycerol/50% phosphate-buffered saline (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 to 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 final concentration of 1 µg ml^{-1} (Porter & Feig 1980) to count bacteria and heterotrophic nanoflagellates (HNF). Pigmented nanoflagellates (PNF) and HNF were counted based on the absence or presence of chlorophyll autofluorescence using a separate filter set optimized for chlorophyll or DAPI under a 1000× epifluorescence microscope (Nikon-Optiphot-2). Bacteria and HNF were identified by their blue fluorescence under UV illumination. PNF were identified by their orange and red autofluorescence under blue excitation light. To obtain reliable estimates of abundance, we counted 30, 30, and 50 fields of view for viruses, bacteria, and nanoflagellates, respectively.

Statistical analysis

Least-square regression analysis was performed to analyze the relationship between net bacterial growth rate and fraction of 30 kDa and 0.2 µm dilution series (Fig. 2). 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 an *F*-test. If the regression slopes of 30 kDa and 0.2 µm dilution series were significantly different, we calculated the magnitude of V_b. Data analyses were performed comparing ambient and warmer conditions with a nonparametric Mann-Whitney test. STATISTICA 7.0 software was used for all statistical operations. A probability value of <0.05 was considered significant.

RESULTS

Bacterial growth rates

Surface water temperatures during the study period showed strong seasonality, with maximum values recorded during the summer period (33°C in September 2014) and minimum values during winter (18°C in February 2014; Table 1). Regression analysis was applied to the dilution experiments, allowing us to determine both the growth and mortality coefficient in each of the parallel dilution series (Table 1). The

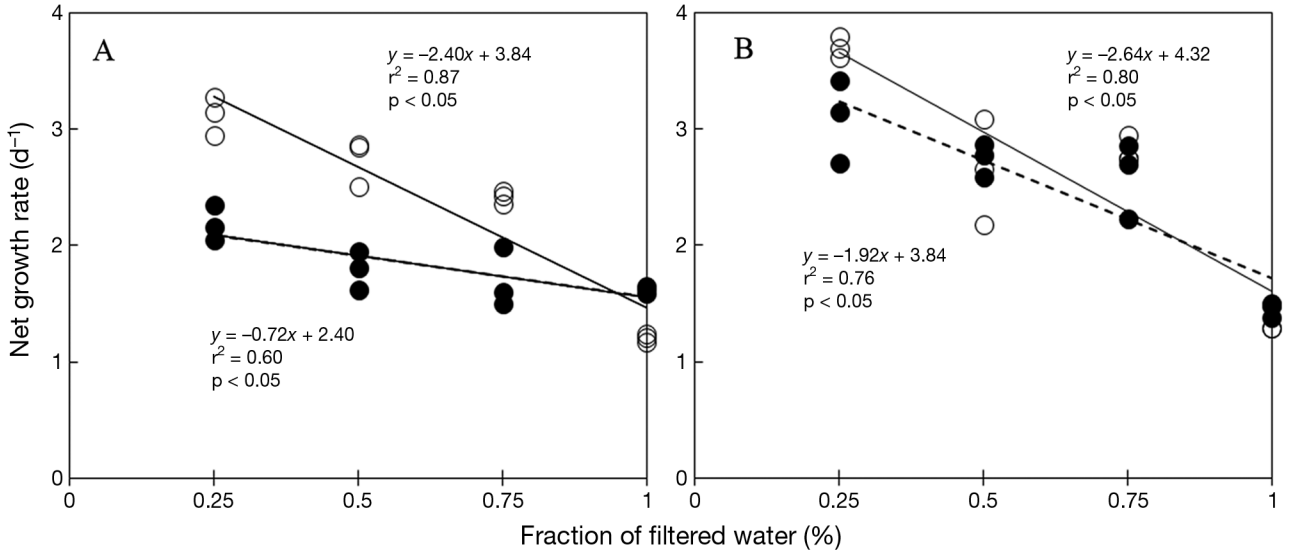

Fig. 2. Bacterial net growth rate vs. fraction of sample water in the dilution series experiments incubated in September (A) at ambient temperature and (B) at warming temperature. ○: seawater samples (<10 µm) diluted with 30 kDa filtered seawater; ●: seawater samples (<10 µm) diluted with 0.2 µm filtered seawater

y-intercepts of these regression lines for the 30 kDa series represent the gross growth rates of bacteria in the absence of lytic and grazing pressure (Table 1, Fig. 3). During incubation at ambient temperatures, seasonal variations of bacterial growth ranged between 0.48 and 4.32 d^{-1} (Table 1, Fig. 3A), and a clear seasonal pattern was found. During the incubation at warmer temperature, the gross growth rates of bacteria varied between 1.44 d^{-1} in January 2014 and 5.04 d^{-1} in August 2014 (Fig. 3A). The warmer conditions produced a significant increase in bacterial growth rates throughout the study period (Mann-Whitney test, $p < 0.05$; Table 1, Fig. 3A). The warming conditions led to a 5 to 200% increase in bacterial growth rates (Fig. 3B). The maximum differences (up to 200%) between the gross growth rates of bacteria in the warmer and ambient temperatures were found in winter, when temperatures *in situ* were the lowest of the year (Fig. 3B). Furthermore, we found the increase ratios to be low (5–25%) at higher ambient temperatures (>25°C), but these ratios increased exponentially at lower ambient temperatures (<25°C) with warming (Fig. 4).

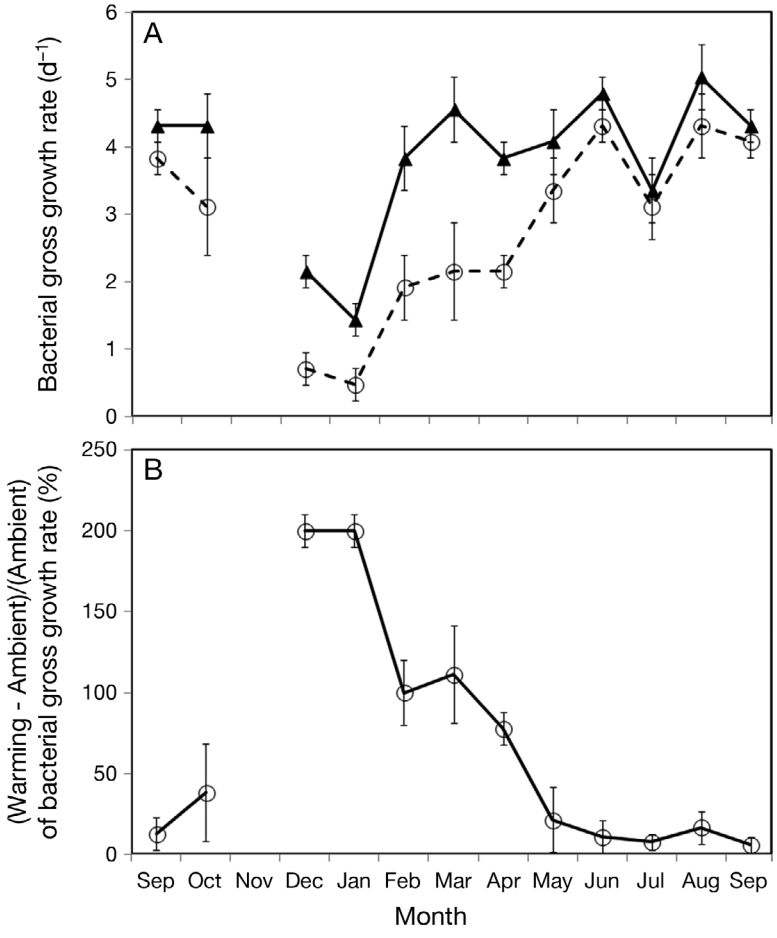

Fig. 3. (A) Gross growth rate of bacteria under ambient (O) and warming (▲) conditions. (B) Ratio of the difference between gross growth rates measured under the warming conditions and those measured under the ambient conditions to the ambient situation

Bacterial loss rates

The regression coefficients (slopes) for the 0.2 µm fractionated series, which represent bacterial grazing mortality (G_b), ranged from undetermined to 4.08 d^{-1} at ambient temperature (Table 1, Fig. 5A). In December 2013 and January 2014, ambient experiments did not show a significant increase in bacterial growth upon dilution of 0.2 µm fractionated water ($p > 0.05$; Table 1). This result indicates that no impact on bacterial mortality was evident for nanoflagellate grazing (Table 1, Fig. 5A). Furthermore, the bacterial losses to grazers showed the same seasonality as the bacterial gross growth rates, with maximum values reached in the warm seasons (Fig. 5A). Experimental warming led to 12–250% increases in bacterial grazing rates, with higher values found in samples collected in February and March 2014 (Fig. 5B).

The regression slopes of the 0.2 µm and 30 kDa dilution series did not differ significantly from each other in May 2014 ambient and warmer experiments

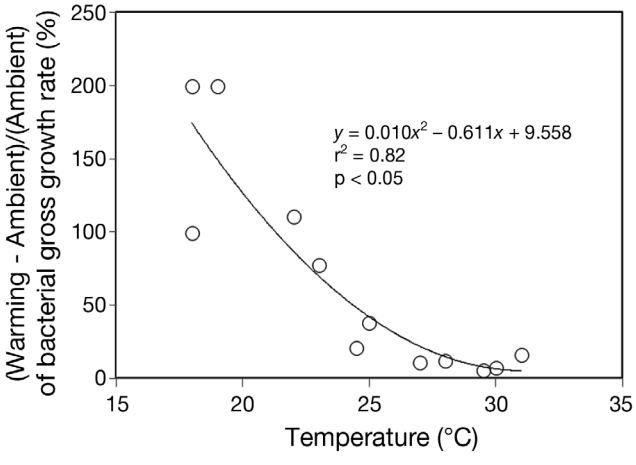

Fig. 4. Relationship between temperature and ratio of the difference between growth rates measured under the warming conditions and those measured under the ambient conditions to the ambient situation

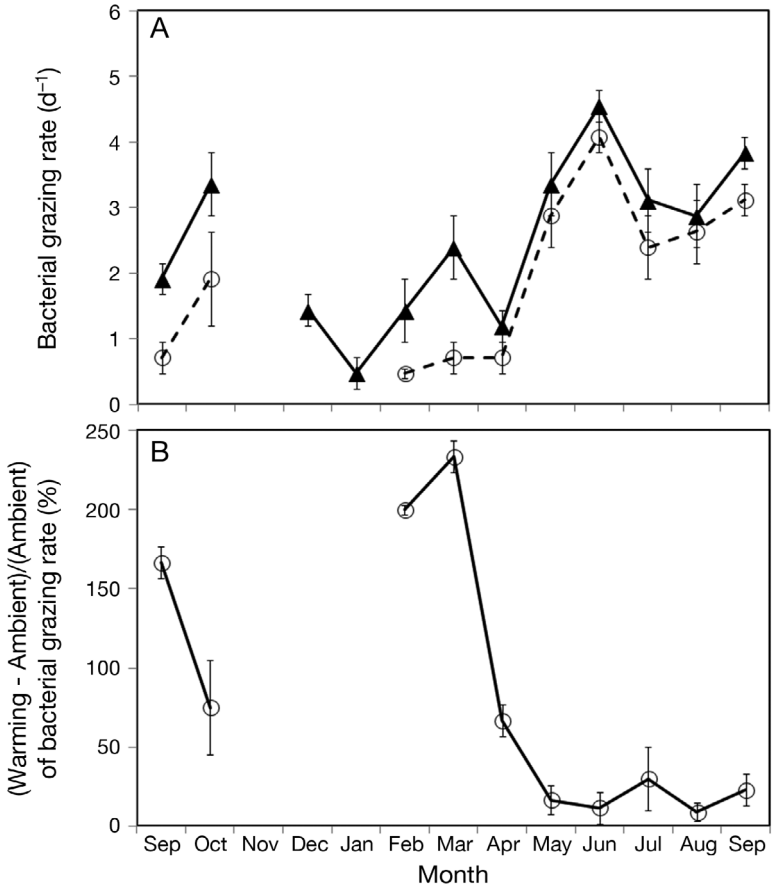

Fig. 5. (A) Grazing rate of bacteria under ambient (O) and warming (▲) conditions. (B) Ratio of the difference between grazing rates measured under the warming conditions and those measured under the ambient conditions to the ambient situation

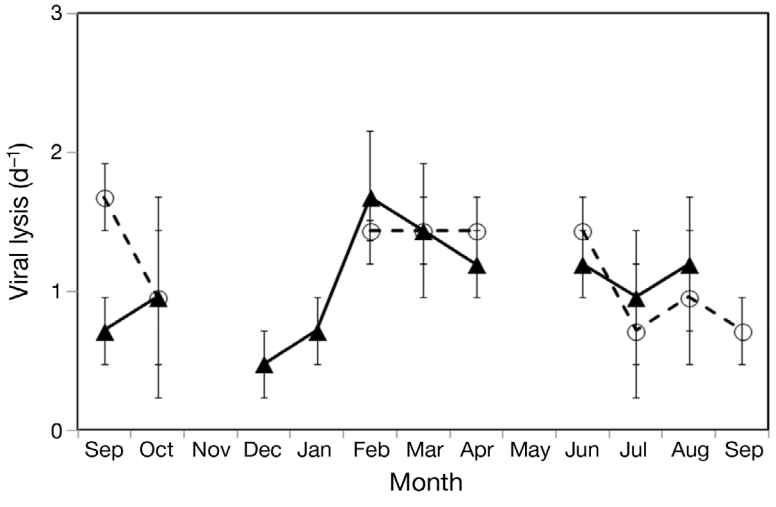

Fig. 6. Seasonal variations in viral lysis of bacteria under ambient (O) and warming (▲) conditions

and in the September 2014 warmer experiment ($p > 0.05$), indicating that viral infection (viral lysis) did not have a detectable impact on bacterial mortality (Table 1, Fig. 6). During the study period, viral lysis accounted for 0.48 to 1.68 d^{-1} and did not show a clear seasonal trend (Fig. 6). Warming the samples did not produce a significant change in viral lysis on bacteria (Mann-Whitney test, $p > 0.05$; Fig. 6).

Overall, both viruses and nanoflagellates as bacterial mortality factors varied between months in this study (Fig. 7). Viral-induced bacterial mortality ranged between undetected and 100% of total mortality (Table 1, Fig. 7). We found that virus-induced mortality was significantly higher than mortality due to nanoflagellates at *in situ* temperatures in the colder season between December 2013 and April 2014 (Mann-Whitney test, $p < 0.05$; Fig. 7). However, the loss rates of bacteria due to grazing increased with experimental warming in the colder seasons (Fig. 7).

DISCUSSION

Experimental approaches have been shown to be extremely useful for investigating the effects of environmental changes on the structure and dynamics of microbial communities (Jacquet et al. 2007, Pradeep Ram & Sime-Ngando 2008). In particular, the dilution method is considered to involve the least disturbance to the trophic webs (Landry & Hassett 1982). A major objective of the present study was to understand the effects of temperature on bacterial growth and mortality caused by grazing and viruses in the oligotrophic surface waters over the course of a year. We found that warming increased the gross growth rates of bacteria with a clear seasonality and caused the loss rates of bacteria due to grazing to increase by nearly 12 to 250% with warming, a finding similar to that reported by a study on bacterial production and grazing rates under warming conditions in Antarctic waters (Vaqué et al. 2009). Our results were also similar to findings reported for the NW Mediterranean (Vázquez-Domínguez et al. 2012), where warming changed the function of the microbial food web

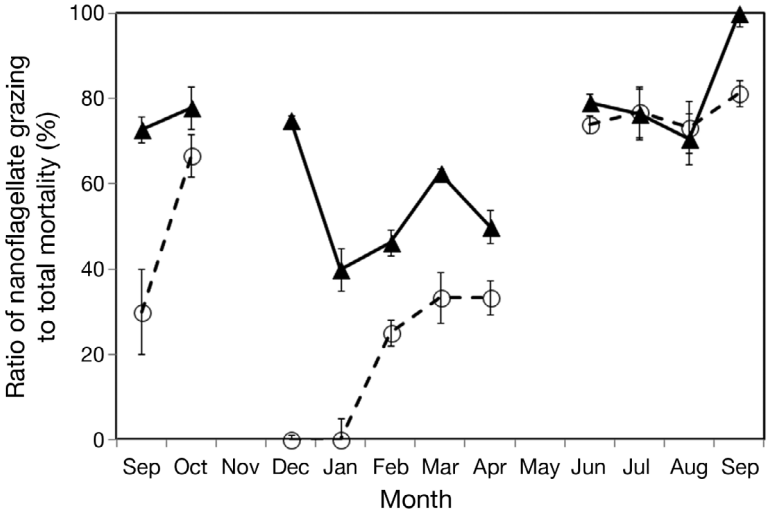

Fig. 7. Seasonal variations in ratios of nanoflagellate grazing to total mortality over the experimental period under ambient (○) and warming (▲) conditions

by increasing the rates of bacterial production and the bacterial carbon flux to predators.

The number of studies which have applied the modified dilution method to estimate the relative importance of the effects of viral lysis and grazing on prokaryote mortality has increased in recent years (Kimmance et al. 2007, Baudoux et al. 2008, Tijdens et al. 2008, Personnic et al. 2009, Taira et al. 2009, Tsai et al. 2012, 2013). One advantage of the modified dilution method is that it provides insight into the quantitative significance of both viral lysis and grazing without the need of conversion factors (Jacquet et al. 2005). However, there are potential disadvantages to the technique in which the treatments with 30 kDa fractions were used to produce virus-free water from virus-replete 0.2 µm filtered water. This could not only remove viruses but could also change the organic matter field and thus the bioavailability of dissolved organic matter (Bonilla-Findji et al. 2009), although the enrichment of dissolved organic matter and nutrients through filtration (0.2 µm and ultrafiltration) was reported to be normal (with 10% increase from the original) in some experiments (Taira et al. 2009). However, the extra concentrations of dissolved organic matter and nutrients did not change the results during the cold seasons in this study, because *in situ* nutrients were high (NO_3 > 10 µmol l^{-1}) in winter (Tsai et al. 2005). On the other hand, because viruses increase nutrient recycling (Wilhelm & Suttle 1999), by adding virus-free filtrate to the 10 µm filtered water, the nutrient status within the 30 kDa dilution series may be different from that of the 0.2 µm system. Thus, during the incubation period, bacterial growth rates may be suppressed in the 30 kDa dilution series. Therefore, although there are some drawbacks of the modified dilution approach and there remains a need for further testing in natural waters, this approach can potentially be used to partition bacterial mortality into grazing and virus-induced fractions.

The values and ranges of seasonal variations in bacterial growth rates found in this study approximate those reported by Tsai et al. (2013) for the same study site. However, to our knowledge, our study is the first to assess the differential response between bacterial growth, nanoflagellate grazing, and viral lysis to experimental warming in a subtropical marine system. The main bottom-up control factors of bacterioplankton dynamics in aquatic environments are availability of dissolved organic matter and temperature (Kelley et al. 1998, Murrell 2003, McManus et al. 2004, Rose et al. 2009). Raising the water temperature experimentally usually stimulates bacterial growth (Shiah & Ducklow 1994, Kirchman et al. 1995, Kirchman & Rich 1997, Vázquez-Domínguez et al. 2012). In the present study, warming produced an effect on bacterial gross growth rates, with a clear seasonality. This result is similar to that observed by Vázquez-Domínguez et al. (2012) in the NW Mediterranean. In that study, the maximum differences between the gross growth rates in warmer and ambient temperatures were found in winter. Nevertheless, experimental warming produced a small increase in bacterial growth rates (8 to 22%) in samples collected during the warmer seasons (>25°C). One of the factors that can modulate the response of bacterial growth to warming is resource availability (Shiah & Ducklow 1994). Li (1998) suggested that over the course of a year, temperature is the dominant factor that affects bacterial growth in colder waters. Other factors, such as substrate supply, may be important in warmer waters. A similar result has been observed in Chesapeake Bay (USA), where temperature regulates bacterial production rates below 20°C, and nutrients are more important when *in situ* temperatures are above this threshold (Shiah & Ducklow 1994). In our case, during the warmer periods (>25°C), experimental warming produced a small increase in bacterial growth rates. The substrates and nutrients could be exhausted by fast-growing bacteria, which would then begin to limit bacterial growth.

In previous experiments by Tsai et al. (2013), the ratio of seasonal variations of grazing effect to grazing and viral lysis of bacterial mortality changed from 21 to 76%, suggesting that nanoflagellate grazing could play a key role in controlling bacterial biomass and might exceed the impact of viral lysis during the summer period (Tsai et al. 2013). Our results substantiate earlier studies suggesting that nanoflagellates play an important role in bacterial carbon transfer to higher levels of the food web at this study site during summer (Fig. 7). Furthermore, compared with ambient and warmer conditions, bacterial losses due to nanoflagellate grazing also exhibited a clear seasonality, with higher increases in winter and lower in summer (Fig. 5B). Thus, we should expect that a small increase in temperature would lead to an increment of the top-down control of bacterial abundance. Protist grazing is closely linked with bacterial abundance and activity, and is affected by any change in the bacterial metabolic state and abundance. We had expected that grazing would increase in parallel with bacterial growth, and our expectation was confirmed by the response in our microbial community (Fig. 8). Preference of nanoflagellates for larger and actively dividing bacteria (Chrzanowski & Šimek 1990) might contribute to the growth rates of bacteria in this study. Furthermore, because grazing losses varied simultaneously with the growth rates, bacterivory appeared to be the main contributing factor to control bacterial abundance during our study period. However, we did not find a significant regression difference between ambient and warming treatments (*F*-test, $p > 0.05$; Fig. 8). This suggests that environmental warming will not directly result in an increase in grazing rates of bacteria, but rather, nanoflagellate grazing would primarily be affected by bacterial activity, as exhibited by higher bacterial growth. However, we did not observe a change in the microbial top predators, i.e. ciliate abundance. Vidussi et al. (2011) showed a rapid response of ciliates to warming, which caused a greater abundance of nanoflagellates in the Thau Lagoon (France). If warming increased the grazing rates on nanoflagellates by ciliates, this would indicate that temperature exerts a positive effect on carbon fluxes in the microbial loop.

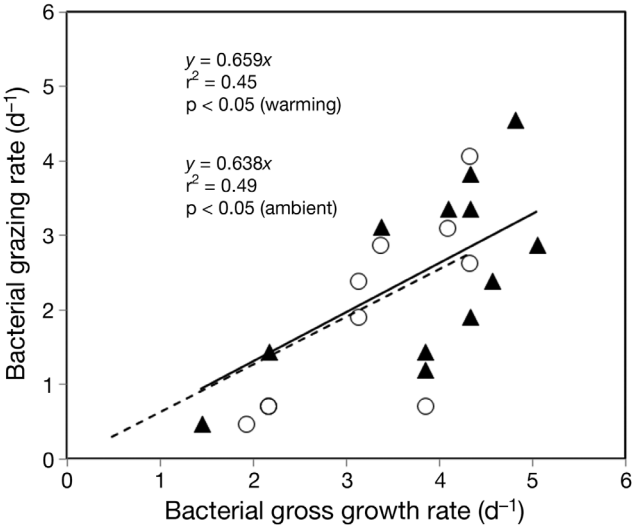

Fig. 8. Relationship between bacterial grazing rates and bacterial gross growth rates over the experimental period under ambient (○) and warming (▲) conditions

Several methods have been used to estimate viral mortality in the microbial food web. These include tracking changes in viral abundance, production, and decay rate (Heldal & Bratbak 1991, Wilhelm et al. 2002) as well as measuring the frequency of virus-infected bacterial cells (Proctor et al. 1993, Binder 1999). These methods are based on several assumptions about latent periods of bacterial infection and/or burst sizes for viruses. Viral lysis was identified as the main cause of bacterial mortality between December and April (Fig. 7). Our results confirm our previous study showing that the loss of bacterial biomass was caused by viral lysis in the cold seasons (Tsai et al. 2013). Moreover, increases in temperature are likely to influence the interactions between viruses and their host cells. If prokaryotic growth rates increase with temperature, the length of the lytic cycles will decrease and the burst size will increase, promoting viral production (Danovaro et al. 2011). However, contrary to our expectations, viral lysis did not increase with warming (Fig. 6). It would be prudent to remember that host specificity is a possible cause which should be taken into consideration when interpreting results from our experiments. Another limitation of this study may be that our experiment time was too short to detect changes in microbial community structure, although Gattuso et al. (2002) reported that the bacterial community structure of a lake changed over a 24 h incubation. Likewise, few correlations have been documented between virioplankton activity and temperature (Jiang & Paul 1994, Pradeep Ram et al. 2005). Jiang & Paul (1994) reported a significant positive correlation between the abundance of viruses and temperature in Tampa Bay. A positive correlation has also been found between infectious cyanophages and temperature in the Gulf of Mexico (Suttle & Chan 1993), while Garza & Suttle (1998) reported that increasing temperatures had a negative effect on cyanophage survival. In addition, Suttle & Chen (1992) noted that

increased temperatures affected the decay of virus infectivity. The interactions between viruses and grazers on bacteria are probably very complex (Miki & Jacquet 2008) and may involve various synergistic effects. A plausible explanation for why viral lysis did not increase with warming is that grazers may preferentially graze on virus-infected bacteria, which might change viral mortality responses (Kimmance et al. 2007). We did not assess preferential grazing, so it is uncertain whether it actually occurred during our dilution experiments and, if so, whether it would cause an underestimation of viral mortality rates. To our knowledge, no previous studies have characterized viral infection of marine bacteria at our study site, so the time period between their infection and lysis of bacteria is unknown. If the period to full cell lysis of bacteria was longer than the 24 h incubation period, then lysis rates may have been underestimated and, therefore, viral lysis was not detected in our study.

In conclusion, the impact of simulated climatic factors as shown in our short-term experiment mainly affected the mediated trophic biological interactions in our study, especially the grazing of nanoflagellates on bacteria. These results could prove to be useful in testing our hypotheses about the possible pathways of change of microbial carbon fluxes to be (1) release by viruses or (2) grazing by nanoflagellates in the warming coastal waters off Taiwan. However, with regard to global warming, either the increasing bacterial production rates due to warming or the increasing bacterial loss due to grazing would result in increasing the return of CO_2 to the ecosystem through respiration processes. In nature, the adaptation and replacement of microbial communities would take place over a time scale much longer than we were able to reproduce in this experiment. Although this study may not offer a solution to our problem, our results do support our hypothesis of the effects of warming on microbial food webs.

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