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NOTES AND CORRESPONDENCE

Virus Effect on Marine *Synechococcus* Spp. Loss in Subtropical Western Pacific Coastal Waters During Winter

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ABSTRACT

Little is known about microbial processes and the effect that viruses have on *Synechococcus* spp. in aquatic environments. This study investigated diel variations in the *Synechococcus* spp. abundance in three size-fractionated water samples (200, 10, and 2 μm fractions). Experiments diluting *Synechococcus* spp. with virus-free water (30 kDa filtrate) in winter months were performed. We found *Synechococcus* spp. to be more abundant in virus-diluted water than in the other fractions during night time. These results suggest that protozoan grazing did not contribute importantly to a reduction in *Synechococcus* spp. abundance but that viral lysis more likely caused *Synechococcus* spp. mortality in these marine coastal waters during winter.

Key words: Diel variations, *Synechococcus* spp., Size-fractionated, Viruses

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1. INTRODUCTION

The importance of picoplanktonic cyanobacteria in oceanic phytoplankton communities is well established (Agawin et al. 2000). Intensive studies on *Synechococcus* spp. abundance have reported consistent seasonal patterns in variation, characterized by low abundance in winter and peak abundance in summer (Li 1998; Tsai et al. 2005, 2008). A positive correlation has also been found between the *Synechococcus* spp. growth rate and *in situ* temperature (Agawin et al. 1998; Tsai et al. 2005). In addition, *Synechococcus* spp. have been found to display distinct diel changes in abundance, with higher division rates at dusk (Dolan and Šimek 1999; Vaulot and Marie 1999; Christaki et al. 2002; Tsai et al. 2009) and maximum abundances at night (Christaki et al. 2002; Tsai et al. 2009).

Diel patterns in abundance are likely to result from imbalances between growth and loss processes. Examples of loss processes affecting *Synechococcus* spp. abundance include nanoflagellate grazing (Dolan and Šimek 1999; Christaki et al. 2002) and viral lysis (Suttle and Chan 1994; Suttle 2000). It has been reported that heterotrophic nanoflagellate (HNF) ingestion of *Synechococcus* spp. is highest after *Synechococcus* spp. cell division (Dolan and Šimek 1999; Christaki et al. 2002) and that pigmented nano flagellates (PNF) regulate diel variations in *Synechococcus* spp. in the subtropical western Pacific coastal waters during summer (Tsai et al. 2009). These findings show that HNF and PNF exert great control over *Synechococcus* spp in summer. No study has investigated the grazing-mediated mortality effect of *Synechococcus* spp. in winter.

Another potential source of cyanobacterial mortality is viral lysis (Suttle 2000), but only one study has investigated the significance of virally mediated mortality for *Synechococcus* spp. in subtropical western Pacific coastal waters, and it was performed during summer (Tsai et al. 2012). In that study, Tsai et al. (2012) found that, while nanoflagellate grazing was a significant cause of *Synechococcus* spp. mortality, viral lysis was also an important cause of mortality, especially at night time. However, in the cold season in that area of the western Pacific, dial changes in *Synechococcus* spp. abundance are small and nanoflagellates are too low in abundance (< 500 cells mL^{-1}) to be a major factor in the *Synechococcus* spp. loss factor. Thus, in this study,

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we hypothesized that viruses might be the major cause of *Synechococcus* spp. mortality during winter. We used size-fractionation and dilution approaches to vary the abundance of grazers and viruses to examine the effect of their grazing on *Synechococcus* spp. in winter.

2. MATERIAL AND METHODS

2.1 Study Site, Sample Collection, and Enumeration

Samples were collected from the surface waters at an established coastal station (25°09.4'N, 121°46.3'E) along a rocky shore in northeastern Taiwan. On an annual scale, salinity ranges from 33.1 - 34.3 with lower data probably reflecting the influence of rainfall runoff. Monthly average nitrate concentrations are highest between November and May, when they may reach 12 μmol L^{-1}. The nitrate concentration decreases to 1 μmol L^{-1} between June and October (Tsai et al. 2005). The chlorophyll *a* concentrations in this study area range from 0.31 - 2.41 mg m^{-3} (Tsai et al. 2013). We conducted two 24-h diel variation studies of plankton abundance in February and March 2014. Water temperature was measured immediately after the bucket was cast. All samples were brought to the laboratory within 30 min.

Water samples were immediately filtered through 200-μm mesh after collection. Sub-samples (1000 mL) were filtered through 47 mm Nuclepore filters (type PC), which had a pore size of 10 μm. Other sub-samples (2000 mL) were filtered through 2-μm pore size Nuclepore filters under low pressure (< 50 mm Hg). Based on previous studies at this site, the size fractionation for gazers should be < 10 μm so that ciliates but not nanoflagellates are eliminated (Tsai et al. 2011). One thousand mL experimental water (< 200 μm) was kept as an unfiltered treatment sample with micro- and nanoplankton grazers present. We assumed that the filtrates from the 2 μm filters contained picoplankton and viruses, those from the 10 μm filters contained nanoflagellates, picoplankton, and viruses, and those from the 200 μm mesh plankton net contained ciliates, nanoflagellates, picoplankton, and viruses. An additional dilution experiment was performed to examine the virus impact on *Synechococcus* spp. abundance. Water was filtered in series through 2 and 0.2 μm pore-size, 47 mm diameter polycarbonate filters (AMD Manufacturing), with the first filter removing nano flagellate grazers, and the second concentrating picoplankton (Wilhelm et al. 2002). A transfer pipette was used to keep the *Synechococcus* spp. in suspension above the 0.2 μm filter. Viruses were removed using a Prep Scale-TFF Cartridge (Millipore) with a 30 kDa molecular weight cut-off (virus-free water). Dilution was subsequently performed by adding 20 mL of *Synechococcus* spp. concentrate to 230 mL of virus-free water. Each 250 mL of fractionated water was incubated in a 500-mL polycarbonate bottle under natural light in thermo-controlled incubators set at *in situ* temperature, the temperature of the seawater at the time of sampling for 24 h. The incubated temperatures were 15 and 18°C in February and March, respectively. Sub-samples were taken from each in triplicate at 2 h intervals after the experiments were set-up. During each sampling period, *in situ* surface water was also collected every 2 h. Net growth rates (b) of *Synechococcus* spp. were calculated using $b = \frac{\ln(C/C_0)}{\tau}$, where C_0 and C are the *Synechococcus* spp. abundance at the beginning and end of the time interval τ, respectively.

Viruses and *Synechococcus* spp. were counted using an epifluorescence microscope (Nikon Optiphot-2) at 1000× magnification. Viruses were processed using a slight modification of a procedures described by Noble and Fuhrman (1998). Briefly, samples from 0.5 - 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 then placed on glass slides and treated with 25 μL of 50% glycerol and 50% PBS buffer (0.85% NaCl, 0.05 M NaH2PO4, pH 7.5) containing 0.1% *p*-phenylenediamine as antifade and mounting agents. Ten mL sub-samples were filtered onto 0.2 μm black Nuclepore filters to count *Synechococcus* spp. *Synechococcus* spp. cells were identified by their orange autofluorescence under blue excitation light. *Synechococcus* spp. dividing cells were also counted and the frequency of dividing cells (FDC) was examined. The FDC was calculated by dividing the number of cells in division by the total number of cells counted. To obtain reliable abundance estimates we counted 20 and 30 fields of view for viruses and *Synechococcus* spp., respectively.

3. RESULTS AND DISCUSSION

The two diel cycles were sampled in the winter of 2014 (February and March). The water temperature ranged between 14.5 and 15°C in February and between 17.5 - 18°C in March. There was a significantly lower viral abundance in the virus reduced treatment than the other treatments at the beginning of the experiments in February and March, respectively (about 90% of decrease, *ANOVA*, $p < 0.05$) (Fig. 1). Dilution with a 30 kDa filtrate successfully reduced the abundance of viruses. At the beginning of these experiments the *Synechococcus* spp. average abundance in the initial water samples in February and March were $4.8 \pm 0.5 \times 10^3$ and $8.8 \pm 0.7 \times 10^3$ cells mL^{-1}, respectively. *Synechococcus* spp. abundances did not differ significantly in any of the three size-fractionated or diluted samples (*ANOVA*, $p > 0.05$) (Figs. 2a and b).

Most studies reported *Synechococcus* spp. abundance to increase during nighttime, reaching a maximum abundance at midnight (Agawin and Agustí 1997; Christaki et al. 2002; Tsai et al. 2005, 2009). Diel variations in *Synechococcus* spp. abundance in the current study followed a similar

pattern in the three size-fractionated samples throughout the incubation periods on all sampling dates (Figs. 2a and b). However, in the nighttime 2-μm sample treated with diluted viruses (2 μm + 30 kDa), we found a higher abundance of *Synechococcus* spp. than in the other fractions after 22 h in February and after 20 h in March (*ANOVA*, $p < 0.05$) (Figs. 2a and b), indicating that *Synechococcus* spp. abundance remained high during the nighttime under virus-reduced conditions. In contrast, a previous study on the western Gulf of Mexico during summer reported that the presence of viruses had a positive effect on *Synechococcus* spp. growth (Weinbauer et al. 2011). The results from that study suggested that heterotrophic bacteria viral lysis could have released enough nutrients to sustain *Synechococcus* spp. growth in the presence of viruses. In the current study, *Synechococcus* spp. growth was not dependent on virus-mediated nutrient cycling by bacteria in waters where nutrient concentrations were previously reported to be high during winter (Tsai et al. 2005).

In a previous study at the same site, Tsai et al. (2009) reported bacterivory by pigmented nanoflagellates to be the probable underlying biological factor regulating diel variations in *Synechococcus* spp. during summer. In the present study on diel variation in winter, variations in *Synechococcus* spp. abundance remained similar in three fractions (200, 10, and 2 μm fractions) throughout the incubation period. This result suggests that protozoan grazing did not play an important biological role in *Synechococcus* spp. abundance loss, supporting our hypothesis that viruses are the major factor contributing to *Synechococcus* spp. mortality in these marine coastal waters in winter. This study found diel variations of *in situ* viral abundance during the study periods, and we expected that the abundance of viruses would increase when *Synechococcus* spp. decreased at nighttime (Fig. 3a). However, we found, no pronounced diel pattern of viral abundance in February and we observed only a general increase in viral abundance at nighttime in March (Fig. 3a). Our study did not directly address the *Synechococcus* spp.-viral abundance relationship, since almost all of the viruses we studied are bacteriophages (Wommack and Colwell 2000).

One important dynamic feature of *Synechococcus* spp. in marine environments is that they have higher division rates at dusk or in the afternoon (Agawin and Agustí 1997; Christaki et al. 2002; Tsai et al. 2005, 2009). However, Ayukai (1996) reported the nutrient-limitation effects to be different from those postulated by Landry and Hassett (1982). Ayukai (1996) found *Synechococcus* spp. growth to be reduced in the most of their diluted treatments. Our results showed that the growth rate did not vary within the dilution. FDC did not vary significantly between undiluted (2 μm) and diluted (2 μm + 30 kDa) waters (*t*-test, $p < 0.05$) (Figs. 2c and d). Nor did they vary between the beginning and end of the experiments. Average nitrate concentrations at the surface were high (> 10 μmol L^{-1}) when temperatures fell below 20°C at our study site (Tsai et al. 2005). Thus, the nutrient supply may not be limited in winter months at this site. Other factors, including temperature, may have a more dominant effect on *Synechococcus* spp. growth in the cold season. The importance of temperature as a positive regulator of marine *Synechococcus* spp. growth is well recognized (Agawin et al. 1998), and it has been suggested that *Synechococcus* spp. abundance is directly related to temperatures during the cold seasons (Li 1998). *Synechococcus* spp. growth and abundance varied with temperature in this study, as the average abundance in March (16.5×10^3 cells mL^{-1}) was higher than it was in February (8×10^3 cells mL^{-1}) (Figs. 2a and b).

We found that virus dilution resulted in higher *Synechococcus* spp. abundance between 22 and 6 h (local time) and lower loss rates than other fractions (*ANOVA*, $p < 0.05$) (Table 1), suggesting that *Synechococcus* spp. loss during the nighttime may be due to viral lysis. To the best of our knowledge, no previous study has directly measured viral lysis on *Synechococccus* spp. in subtropical western Pacific coastal waters during winter. In a previous study at the same site during summer, Tsai et al. (2013) showed that nanoflagellate grazing might play a key role in controlling bacteria biomass and might exceed the impact of viral lysis because of the higher abundance of nanoflagellates at that time. In the current study the abundance of nanoflagellates was relatively low in winter (Fig. 3b), leading us to believe that nano flagellate grazing did not play an important biological role in *Synechococcus* spp. abundance loss. On the other hand, the abundances of *Synechococcus* spp. was significantly increased in samples treated with 30 kDa filtrate, most likely the result of a reduced viral abundance in the filtrate during the cold months. These results suggest that the carbon and nutrients released during picoplankton viral lysis were recycled within the microbial loop instead of being transferred to higher trophic levels in the winter.

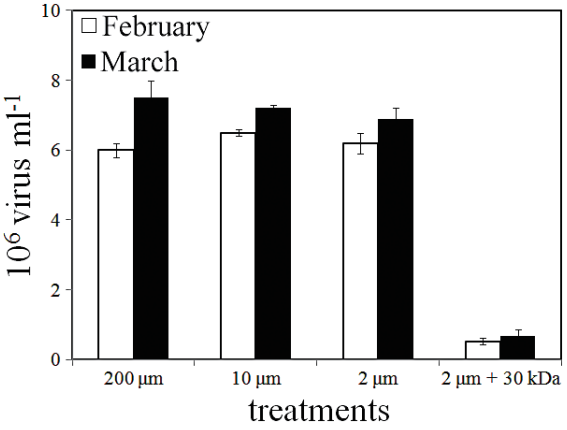

Fig. 1. Virus abundance in each treatment at the experiment beginning in February and March, respectively.

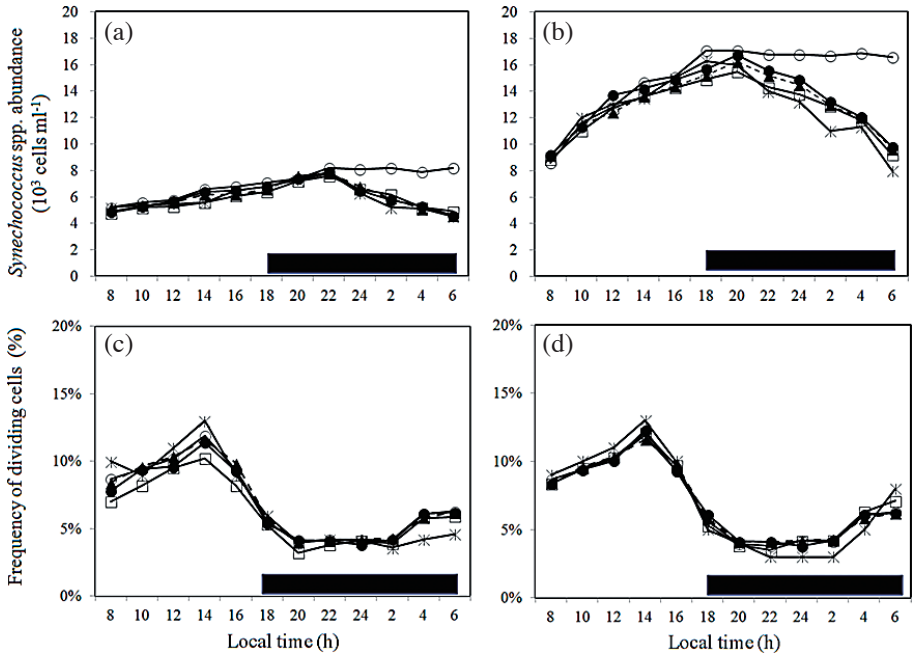

Fig. 2. *Synechococcus* spp. abundance time-series (a) February, (b) March, and frequency of dividing cells, (c) February, (d) March. (∗: *in situ*, □: 200 μm, ▲: 10 μm, ●: 2 μm, and ○: 2 μm + 30 kDa). The filled black bar represents the dark period. All values of standard deviation were < 1 in each sample.

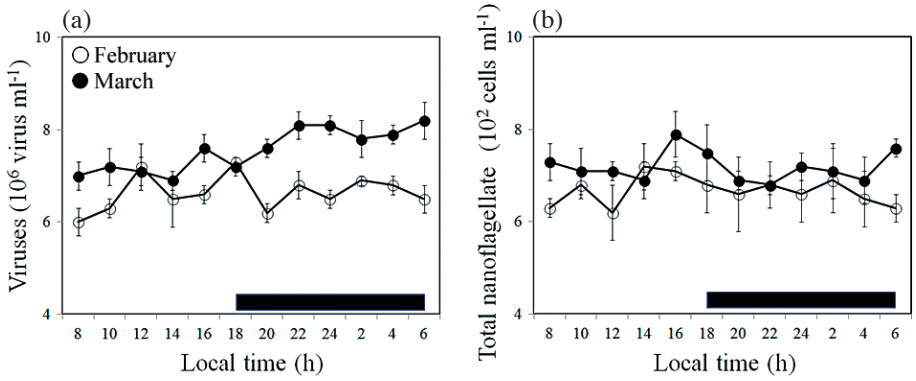

Fig. 3. *In situ* viral (a) time-series and total nanoflagellate abundance (b) in February and March, respectively. The filled black bar represents the dark period.

Table 1. Mean values (±SD) of *Synechococcus* spp. net growth rates during the decreased abundance period in each experimental treatment.

	Experimental treatment	**Decrease period of *Synechococcus* spp. (h)**	**Net Growth rate (b) (h^{-1})**
February	200 μm	22 - 06	-0.065 ± 0.003
	10 μm		-0.066 ± 0.002
	2 μm		-0.068 ± 0.002
	2 μm + 30 kDa		0
March	200 μm	20 - 06	-0.052 ± 0.004
	10 μm		-0.053 ± 0.002
	2 μm		-0.054 ± 0.003
	2 μm + 30 kDa		-0.003 ± 0.001

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REFERENCES

Agawin, N. S. R. and S. Agustí, 1997: Abundance, frequency of dividing cells and growth rates of *Synechococcus* sp. (cyanobacteria) in the stratified Northwest Mediterranean Sea. *J. Plankton Res.*, **19**, 1599-1615, doi: 10.1093/plankt/19.11.1599. [Link]

Agawin, N. S. R., C. M. Duarte, and S. Agustí, 1998: Growth and abundance of *Synechococcus* sp. in a Mediterranean Bay: Seasonality and relationship with temperature. *Mar. Ecol. Prog. Ser.*, **170**, 45-53, doi: 10.3354/meps170045. [Link]

Agawin, N. S. R., C. M. Duarte, and S. Agustí, 2000: Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. *Limnol. Oceanogr.*, **45**, 591-600, doi: 10.4319/lo.2000.45.3.0591. [Link]

Ayukai, T., 1996: Possible limitation of the dilution technique for estimating growth and grazing mortality rates of picoplanktonic cyanobacteria in oligotrophic tropical waters. *J. Exp. Mar. Biol. Ecol.*, **198**, 101-111, doi: 10.1016/0022-0981(95)00208-1. [Link]

Christaki, U., C. Courties, H. Karayanni, A. Giannakourou, C. Maravelias, K. Ar. Kormas, and P. Lebaron, 2002: Dynamic characteristics of *Prochlorococcus* and *Synechococcus* consumption by bacterivorous nanoflagellates. *Microb. Ecol.*, **43**, 341-352, doi: 10.1007/s00248-002-2002-3. [Link]

Dolan, J. R. and K. Šimek, 1999: Diel periodicity in *Synechococcus* populations and grazing by heterotrophic nanoflagellates: Analysis of food vacuole contents. *Limnol. Oceanogr.*, **44**, 1565-1570, doi: 10.4319/lo.1999.44.6.1565. [Link]

Landry, M. R. and R. P. Hassett, 1982: Estimating the grazing impact of marine micro-zooplankton. *Mar. Biol.*, **67**, 283-288, doi: 10.1007/BF00397668. [Link]

Li, W. K. W., 1998: Annual average abundance of heterotrophic bacteria and *Synechococcus* in surface ocean waters. *Limnol. Oceanogr.*, **43**, 1746-1753, doi: 10.4319/lo.1998.43.7.1746. [Link]

Noble, R. T. and J. A. Fuhrman, 1998: Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquat. Microb. Ecol.*, **14**, 113-118, doi: 10.3354/ame014113. [Link]

Suttle, C. A. 2000: Cyanophages and their role in the ecology of cyanobacteria. In: Whitton, B. A. and M. Potts (Eds.), The Ecology of Cyanobacteria: Their Diversity in Time and Space, Kluwer Academic Publishers, Boston, MA, 563-589, doi: 10.1007/0-306-46855-7_20. [Link]

Suttle, C. A. and A. M. Chan, 1994: Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. *Appl. Environ. Microbiol.*, **60**, 3167-3174.

Tsai, A. Y., K. P. Chiang, J. Chang, and G. C. Gong, 2005: Seasonal diel variations of picoplankton and nanoplankton in a subtropical western Pacific coastal ecosystem. *Limnol. Oceanogr.*, **50**, 1221-1231, doi: 10.4319/lo.2005.50.4.1221. [Link]

Tsai, A. Y., K. P. Chiang, J. Chang, and G. C. Gong, 2008: Seasonal variations in trophic dynamics of nanoflagellates and picoplankton in coastal waters of the western subtropical Pacific Ocean. *Aquat. Microb. Ecol.*, **51**, 263-274, doi: 10.3354/ame01196. [Link]

Tsai, A. Y., W. M. Chin, and K. P. Chiang, 2009: Diel patterns of grazing by pigmented nanoflagellates on *Synechococcus* spp. in the coastal ecosystem of subtropical western Pacific. *Hydrobiologia*, **636**, 249-256, doi: 10.1007/s10750-009-9954-y. [Link]

Tsai, A. Y., G. C. Gong, R. W. Sanders, W. H. Chen, C. F. Chao, and K. P. Chiang, 2011: Importance of bacterivory by pigmented and heterotrophic nanoflagellates during the warm season in a subtropical western Pacific coastal ecosystem. *Aquat. Microb. Ecol.*, **63**, 9-18, doi: 10.3354/ame01470. [Link]

Tsai, A. Y., G. C. Gong, R. W. Sanders, K. P. Chiang, J. K. Huang, and Y. F. Chan, 2012: Viral lysis and nanoflagellate grazing as factors controlling diel variations of *Synechococcus* spp. summer abundance in coastal waters of Taiwan. *Aquat. Microb. Ecol.*, **66**, 159-167, doi: 10.3354/ame01566. [Link]

Tsai, A. Y., G. C. Gong, and J. Hung, 2013: Seasonal variations of virus- and nanoflagellate-mediated mortality of heterotrophic bacteria in the coastal ecosystem of subtropical western Pacific. *Biogeosciences*, **10**, 3055-3065, doi: 10.5194/bg-10-3055-2013. [Link]

Vaulot, D. and D. Marie, 1999: Diel variability of photosynthetic picoplankton in the equatorial Pacific. *J. Geophys. Res.*, **104**, 3297-3310, doi: 10.1029/98JC01333. [Link]

Weinbauer, M. G., O. Bonilla-Findji, A. M. Chan, J. R. Dolan, S. M. Short, K. Šimek, S. W. Wilhelm, and C. A. Suttle, 2011: *Synechococcus* growth in the ocean may depend on the lysis of heterotrophic bacteria. *J. Plankton Res.*, **33**, 1465-1476, doi: 10.1093/plankt/fbr041. [Link]

Wilhelm, S. W., S. M. Brigden, and C. A. Suttle, 2002: A dilution technique for the direct measurement of viral production: A comparison in stratified and tidally mixed coastal waters. *Microb. Ecol.*, **43**, 168-173.

Wommack, K. E. and R. R. Colwell, 2000: Virioplankton: Viruses in aquatic ecosystems. *Microbiol. Mol. Biol. Rev.*, **64**, 69-114, doi: 10.1128/MMBR.64.1.69-114.2000. [Link]