# Viral Infection and Mortality of Bacteria in the Northern Lake Biwa in Japan

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**Keywords:** Northern Lake Biwa; viral infection; bacterial mortality; transmission electron microscopy (TEM)

## Introduction

Viruses are the most abundant biological entities in the aquatic environment, infecting and lysing the bacterial cells. The consequence of the interaction of bacteria and viruses hampers the flow of carbon, nitrogen or other nutrients to higher trophic levels and shunt the flux to the pool of dissolved organic matter (Fuhrman 1999, Suttle 2007). Bacterial mortality by viral infection in Lake Biwa, however, is still unclear, that allows the biological modeling to lack the viral shunting process. In this research, we investigated the bacterial mortality in the largest freshwater lake (Lake Biwa, Japan).

## Material and Methods

Deep water samples were collected at the depth of 60 m in the northern Lake Biwa (35° 23' 41" N, 136° 07' 57" E) using vandorn water sampler from July through December in 2016. Surface water samples were collected at the depth of 0.5 m using stainless bucket. Glutaraldehyde (final concentration, 1% v/v) was used for fixing collected water samples immediately on the ship.

For bacterial and viral enumeration, the methods described in Patel (2007) and Nobel & Fuhrman (1998) are conducted. The microscope slides were observed under a fluorescence microscopy (BZ-9000, KEYENCE).

For evaluating bacterial mortality, frequency of visibly infected cell (FVIC) were observed using transmission electron microscopy (TEM). Bacterial cells in the fixed samples (~1 L) were collected on TEM grids (400-mesh, carbon coated Formvar film) by ultracentrifugation (Himac CS 100GXII, Hitachi; S52ST Swing-Out-Rotor at 70,000 × *g* for 20 min at 4 °C) according to Pradeep Ram (2010). Triplicate grids were prepared for each sample. Each grid was stained at room temperature (25 °C) for 30 min with EM stainer (electron staining; 1.0-fold; Nisshin EM Co., Ltd). The stained grids were rinsed with ultra-pure water for five times to remove excess stains and air-dried for overnight. The samples were observed under a TEM (H-7650, Hitachi) operated at 80 kV at a magnification of 3,000 to 50,000×. Infected bacterial cell was defined when three and more intracellular viruses were examined. More than 500 bacterial cells were examined per grid to determine FVIC. Frequency of infected cells (FIC) was converted from FVIC using the equation: FIC = 9.524 FVIC – 3.256 according to Weinbauer *et al.* (2002). FIC was converted to frequency of bacterial mortality due to viral lysis (FVML) using the equation: FVML = (FIC + 0.6 FIC2)/(1 – 1.2

FIC) (Binder 1999).

## Results and Conclusions

Viral and bacterial abundances in the surface layer (viral abundance 6.0 ± 2.4 × $10^7$ $mL^{-1}$, bacterial abundance 2.0 ± 1.0 × $10^6$ $mL^{-1}$; mean ± SD) were significantly greater than those in deep layer (viral abundance 2.2 ± 0.8 × $10^7$ $mL^{-1}$, bacterial abundance 0.7 ± 0.2 × $10^6$ $mL^{-1}$) during the investigation period (Figure 1.1a, b; $p < 0.05$).

FVIC in the surface layer and the deep layer were 1.8 ± 0.6% and 2.2 ± 0.6% during the investigation period, respectively (Figure 1.1c). FMVL, bacterial mortality converted from FVIC, were 19.7 ± 9.9% in the surface water and 27.4 ± 11.1% in the deeper layer. This means that approximately 20 to 30% of bacterial production are lysed by viral infection and return to the pool of dissolved organic matter (DOM), assuming that bacterial mortality and production are equal.

This research provided that the interaction of virus and bacteria in Lake Biwa significantly influences on the flux of microbial loop, which means bacterial production, supposed to be flowed through the higher trophic levels, is recycled back in DOM pool. Further investigations are required to clarify the carbon patterns in microbial food web, virus-bacteria-HNF (grazer) systems in Lake Biwa.

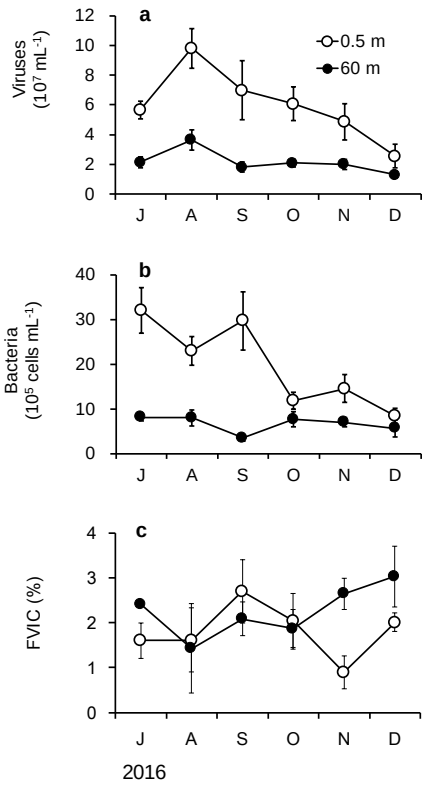


**Figure 1.1** Seasonal variation of (a) viral and (b) bacterial abundance, and (c) frequency of visibly infected cells (FVIC) at the depth of 0.5 and 60 m at an offing site of the northern Lake Biwa. Error bars indicate SD (N = 10 in (a)

## References

Binder B. (1999): Reconsidering the Relationship between Virally Induced Bacterial Mortality and Frequency of 3Infected Cells, *Aquatic Microbial Ecology*, **18**, 207–215.

Fuhrman J.A. (1999): Marine Viruses and their Biogeochemical and Ecological Effects, *Nature*, **399**, 541–548.

Noble R.T. and Fuhrman J.A. (1998): Use of SYBR Green I for Rapid Epifluorescence Counts of Marine Viruses and Bacteria, *Aquatic Microbial Ecology*, **14**, 113-118.

Patel A., Noble R.T., Steele J.A., Schwalbach M.S., Hewson I. and Fuhrman J.A. (2007): Virus and Prokaryote Enumeration from Planktonic Aquatic Environments by Epifluorescence Microscopy with SYBR Green I, *Nature Protocols*, **2** (2), 269-276.

Pradeep Ram A.S., Nishimura Y., Tomaru Y., Nagasaki K. and Nagata T. (2010): Seasonal Variation in Viral-induced Mortality of Bacterioplankton in the Water Column of a Large Mesotrophic Lake (Lake Biwa, Japan), *Aquatic Microbial Ecology*, **58**, 249-259.

Suttle C.A. (2007): Marine Viruses – Major Players in the Global Ecosystem, *Nature Reviews Microbiology*, **5**, 801-812.

Weinbauer M.G., Winter C., Höfle M.G. (2002): Reconsidering Transmission Electron Microscopy based Estimates of Viral Infection of Bacterioplankton using Conversion Factors derived from Natural Communities, *Aquatic Microbial Ecology*, **27**, 103–110.