**Title: Growth and mortality rates of prokaryotes in the hypolimnion of a deep freshwater lake (Lake Biwa, Japan)**

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**Abstract**

The presence of pico-sized cyanobacteria (genus *Synechococcus*) in hypolimnetic waters has been reported, and investigators have suggested that *Synechococcus* make a greater contribution to ecological processes in the hypolimnion than previously hypothesized. However, the ecological role of *Synechococcus* in food webs and/or matter cycling in the hypolimnion remains unknown. To address this issue, we assessed protistan grazing and the virus-mediated mortality of *Synechococcus* in the oxygenated hypolimnion of a large freshwater lake (Lake Biwa, Japan) during the stratification period. In addition, we compared the carbon flux through mortality of *Synechococcus* to that of heterotrophic bacteria in order to evaluate the role of *Synechococcus* in ecological processes within a hypolimnetic ecosystem. Our results suggest that the biomass of *Synechococcus* and heterotrophic bacteria in the hypolimnion was removed primarily by protistan grazing. The abundance of *Synechococcus* was highest in August, when the average *Synechococcus*:bacteria carbon biomass and daily grazing loss ratios were 10.8 and 11.0%, respectively. Thus, it is likely that the *Synechococcus* biomass is an important seasonal component of the carbon flux in the hypolimnetic microbial loop. Our results provide the first data on carbon flux through the mortality of both *Synechococcus* and bacteria in a hypolimnetic ecosystem.

## Introduction

In deep freshwater lakes, the hypolimnion, which comprises a large proportion of the water mass, is separated from the epilimnion by the thermocline. The hypolimnion receives photosynthetically derived organic matter from the epilimnion in particulate form after substantial decomposition by heterotrophs. This organic matter flux is an important food source for hypolimnetic vertebrate and invertebrate communities, and it sustains hypolimnetic ecosystems (Meyers and Ishiwatari 1993).

The widely distributed cyanobacterial genus *Synechococcus* is a major component of photosynthetic biomass in freshwater lakes (Sigee 2005). *Synechococcus*, which are among the smallest prokaryotes in phytoplankton communities, are vulnerable to microzooplankton grazing. Previous studies have suggested that most of their production is rapidly removed from the euphotic zone (Nagata 1988). Conversely, other researchers have reported the presence of pico-sized cyanobacteria in hypolimnetic waters (Callieri and Pinolini 1995, Takasu et al. 2015). Previously, we revealed that substantial numbers of intact *Synechococcus* cells were retained among larger organic particles that had sunk to the hypolimnion (Takasu et al. 2015). Thus, *Synechococcus* might be an important food source and/or item for hypolimnetic grazers. However, information about the fate of *Synechococcus* in a hypolimnetic ecosystem is limited, and the role of *Synechococcus* in food web and/or matter cycling remains unknown.

Protistan grazing and viral lysis are two important determinants of the fate of *Synechococcus* and heterotrophic bacteria (Sigee 2005). Protistan grazing transfers the prokaryotic biomass to organisms at higher trophic levels via the microbial loop, whereas viral lysis leads to the recycling of carbon and nutrients, each of which is derived from the lysed prokaryotic biomass and re-supplied to prokaryotes (Sigee 2005). Thus, it is important to characterize the relative contributions of grazing and lysis to *Synechococcus*

mortality in order to understand their role in ecological processes in the hypolimnion.

In the present study, we hypothesized that *Synechococcus* contributes to the food web and/or matter cycling in the oxygenated hypolimnion of Lake Biwa. To investigate this hypothesis, we assessed protistan grazing and virus-mediated *Synechococcus* mortality. To evaluate the role of *Synechococcus* within the hypolimnetic ecosystem, we compared the carbon flux through *Synechococcus* mortality to that of bacteria.

**Study site**

Lake Biwa is a large (surface area, 674 $km^2$; water volume, 27.3 $km^3$; watershed area, 3848 $km^2$), deep (maximum depth, 104 m), tectonic, freshwater (average concentrations of Cl, Na and Ca are 7.5, 5.2 and 10.4 mg $L^{-1}$、respectively; Fujinaga et al. 2005) lake in Japan. The mesotrophic and monomictic north basin of the lake has a water residence time of 5.5 years. We collected water samples at station Ie-1 (35° 12’ 58’’ N, 135° 59’ 55’’ E; *ca*. 75 m) in the north basin of the lake. The water column is vertically mixed from January to March and stratified during the rest of the year.

**Methods**

Samples were collected on 19 May, 5 June, 11 August, and 27 August 2015 during the stratification period. Vertical profiles of water temperature and light intensity were determined using a CTD probe (SBE 911 Plus; Sea Bird Electronics, Bellevue, WA, USA). Water samples were collected using Niskin X bottles.

To determine chlorophyll *a* (chl *a*) concentrations, water samples of 285 ml collected at depths of 5 m (epilimnion) and 65 m (hypolimnion) were filtered through 0.2- and 2.0-μm polycarbonate filters (Whatman International, Ltd., Maidstone, England) and analyzed by fluorometry (Fluorometer 10-AU; Turner Designs, Sunnyvale, CA, USA) according to Welschmeyer (1994). Chl *a* concentrations in the 0.2–2.0-μm fraction

(hereafter, the "pico-sized fraction") were calculated according to Takasu et al. (2015).

Samples for microbial enumeration were collected at 65 m then fixed immediately with glutaraldehyde (Wako Pure Chemical Industries, Osaka, Japan; final concentration: 1%, vol/vol) and stored at 4°C in the dark until the preparation of microscope slides. For enumeration of *Synechococcus*, fixed water samples of 15 to 40 mL were filtered through 0.2-μm-pore-size black polycarbonate filters (Advantec, Tokyo, Japan). Phycoerythrin (PE)-rich *Synechococcus*, the most abundant picophytoplankton in the lake (> 99% of *Synechococcus* in the hypolimnion; Takasu et al. 2015), were counted using an optical setting for PE (U-MNIB2; Olympus, Tokyo, Japan). At least 300 cells or 100 fields were counted to estimate cell abundance.

From the fixed water sample, 1 mL was used for the enumeration of bacteria. Bacterial cells were stained with 4',6-diamidino-2-phenylindole (DAPI; Wako Pure Chemical Industries; final concentration: 10 μg $mL^{-1}$) for 10 min, filtered on black-stained 0.2-μm-pore-size black polycarbonate filters (Advantec), and counted under an epifluorescence microscope (BX61; Olympus) (Porter and Feig 1980) using an optical setting for DAPI (U-MWU2; Olympus). At least 300 bacterial cells were counted within a minimum of 20 randomly selected fields.

From the fixed water sample, 15 mL were used for the enumeration of heterotrophic nanoflagellates (HNF), and 0.1 mL (1 mL from samples diluted $10^9$ with 0.02-μm-filtered distilled water) was used for the enumeration of viral-like particles (VLP). HNF were double-stained with DAPI (final concentration: 10 μg $mL^{-1}$) and fluorescein isothiocyanate (Dojindo Molecular Technology, Inc., Rockville, MD, USA; final concentration: 10 μg $mL^{-1}$) for 10 min, collected on 0.8-μm-pore-size black polycarbonate filters (Whatman), and counted using epifluorescence microscopy under ultraviolet (UV; U-MWU2; Olympus) and blue (IB-NIB; Olympus) excitation according to Sherr and Sherr (1983). For HNF counting, a minimum of 100 randomly selected fields were inspected. VLP were counted

113 using epifluorescence microscopy under blue excitation by the SYBR Green I (Molecular
114 Probes Inc., Eugene, OR, USA; final concentration: $5 \times 10^{-5}$ dilution of commercial stock;
115 30 min of incubation) method (Patel et al. 2008) using 0.02-μm-pore-size Anodisc filters
116 (Whatman; GE Healthcare, Wauwatosa, WI, USA). More than 300 VLP were counted, and
117 a minimum of 10 randomly selected fields were examined.

118 The length and width of *Synechococcus*, bacteria, and HNF cells were measured in
119 each sample using image analysis software (ImageJ; National Institutes of Health, Bethesda,
120 MD, USA). Images were captured at a magnification of 1,000× with a charge-coupled
121 device camera (DP70; Olympus). The *Synechococcus*, bacteria and HNF cell volume was
122 calculated by assuming that the cells were spherical. The carbon biomass of *Synechococcus*,
123 bacteria, and HNF was determined by combining the cell volume data with a carbon
124 conversion factor estimated for both unicellular cyanobacteria and bacteria in this lake (106
125 fg C $\mu m^{-3}$; Nagata 1986) and HNF (71 fg C $\mu m^{-3}$; Fenchel and Finlay 1983). Equivalent
126 spherical diameters (ESDs) were calculated according to Hansen et al. (1994).

127 For the dilution experiments, approximately 5 L of lake water were collected at a
128 depth of 65 m then were gently filtered through a 1.2 M HCl-washed 20-μm nylon mesh to
129 remove mesozooplankton. In Lake Biwa, it has been reported that the ciliates are not
130 important grazers of prokaryotes, and that the main grazers of prokaryotes are HNF
131 (Nakano et al. 1998, Sekino et al. 2007). Thus, we used 20-μm mesh for pre-filtration,
132 though this filtration step may remove ciliates. A 1-L portion of the filtrate was passed
133 through a 0.2-μm-pore-size polyether sulphone ultrafiltration membrane (Vivaflow200;
134 Sartorius, Göttingen, Germany) equipped with a peristaltic pump (Masterflex Tubing Pump
135 System L/S; Masterflex, Gelsenkirchen, Germany) and collected into 1-L polycarbonate
136 bottles washed with 1.2 M HCl before use. After the filtration, half of the 0.2-μm filtrate
137 was passed through a 30-kDa polyether sulphone ultrafiltration membrane (Vivaflow200;
138 Sartorius) to prepare a grazer-and-virus-free diluent. The ultrafiltration membranes were

cleaned before use with 0.5 mM NaOCl/0.5 M NaOH.

The 20-µm filtrate was diluted in 0.2-µm or 30-kDa diluent to dilution levels of 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 in 250-mL polycarbonate bottles washed with 1.2 M HCl before use. The dilution level of 0.1 was not prepared for May and June. The bottles were then incubated for 36–48 h at *in situ* temperatures in the dark. Subsamples for the enumeration of *Synechococcus* were collected at the beginning (0 h) and end of the incubations, fixed immediately with glutaraldehyde (final concentration: 1%, vol/vol), and stored at 4°C in the dark until the preparation of microscope slides. During sample collection and handling, gloves were worn and care was taken to minimize contamination.

The apparent growth rates ($\mu_{app}$, $d^{-1}$) of bacteria and *Synechococcus* were calculated from their cell abundances at the beginning and end of the incubation experiment, with the assumption that bacterial and *Synechococcus* growth would follow an exponential model (Landry and Hassett 1982):

$$\mu_{app} = (1/t) \ln (N_t/N_0), \quad (1)$$

where $t$ is the duration of incubation (days), and $N_0$ and $N_t$ are the abundances of *Synechococcus* or bacteria (cells $mL^{-1}$) at the beginning and end of the incubation, respectively. Two dilution series were prepared: a 30-kDa dilution series to estimate the combined effects of the protistan grazing and viral lysis rates ($g+v$, $d^{-1}$) and a 0.2-µm dilution series to determine the effect of the protistan grazing rate ($g$, $d^{-1}$) on *Synechococcus* and bacteria. The slope of the regression lines from the 0.2-µm dilution series represents the grazing rate. The difference between the slopes of the regression lines represents the bacterial mortality rate due to viral lysis ($v$, $d^{-1}$); this difference was tested using an analysis of covariance (ANCOVA). The intercept of the 30-kDa dilution series provides the instantaneous growth rate ($\mu$, $d^{-1}$) of *Synechococcus* and bacteria in the absence of grazing

or viral lysis (Evans et al. 2003).

The carbon flux through mortality of *Synechococcus* and bacteria was estimated by combining data from the dilution experiments with carbon conversion factors estimated for *Synechococcus* and bacteria (106 fg C $\mu m^{-1}$; Nagata 1986). The carbon production (*CP*; $\mu g\ C\ L^{-1}\ d^{-1}$) and losses to grazing (*GL*; $\mu g\ C\ L^{-1}\ d^{-1}$) were calculated using the following formulas:

$$CP = \mu \times P_0, \quad (2)$$

$$GL = CP \times (g/\mu), \quad (3)$$

where $\mu$ ($d^{-1}$) is the dilution-based specific growth (y-intercept of the 0.2-μm regression, see Results), $g$ is the dilution-based grazing rate (in $d^{-1}$), and $P_0$ (in $\mu g\ C\ L^{-1}$) is the initial carbon biomass of *Synechococcus* or bacteria.

All statistical analyses were performed using the free statistical environment R (R Development Core Team 2015).

## Results

The euphotic depth ($Z_{1\%}$) did not exceed 25 m throughout the study period, indicating that below this depth was the aphotic layer (Table 1). The contributions of the pico-sized fraction to the total chl *a* concentration at 5 and 65 m were 42.3 ± 11% (average ± SD) and 14.3 ± 3.3%, respectively (Table 1). The cellular abundance of *Synechococcus* at a depth of 65 m increased markedly from June ($0.47 \times 10^3$ cells $mL^{-1}$) to August ($2.83 \times 10^3$ cells $mL^{-1}$; Table 2). In contrast, the abundances of bacteria, HNF, and VLP were relatively constant throughout the study period (Table 2). The ESD of *Synechococcus* (1.33–1.62 μm) was about three times larger than that of bacteria (0.46–0.60 μm; Table 2)

throughout the study period. The ESD of HNF was 4.77–5.24 μm (Table 2).

In three out of the four experiments, there was a significant relationship between the apparent growth rate of *Synechococcus* or bacteria and the level of dilution in both the 0.2-μm and 30-kDa dilution series (Table 3). However, there was no significant difference (ANCOVA, $P > 0.1$) between the regression slopes of the 0.2-μm and 30-kDa dilution series in any experiment (Table 3). Owing to these results, the growth rate could not be determined from the 30-kDa and virus-mediated mortality rates.

The growth rate ($\mu$) of *Synechococcus* and bacteria in the absence of protistan grazing ranged from -0.200 (±SE; 0.045) to -0.007 (±SE; 0.115) and from 0.053 (±SE; 0.080) to 0.502 (±SE; 0.846), respectively (Table 3). The grazing mortality rates ($g$) of *Synechococcus* and bacteria varied from 0.382 (±SE; 0.078) to 0.616 (±SE; 0.174) and from 0.305 (±SE; 0.131) to 0.846 (±SE; 0.293), respectively (Table 3). High rates of grazing mortality among bacteria tended to be accompanied by a high bacterial growth rate (Table 3).

The *Synechococcus* carbon biomass and loss to protistan grazing were 0.06–0.66 μg C $L^{-1}$ and 0.070–0.22 μg C $L^{-1}$ $day^{-1}$, respectively (Table 4). Higher estimates were obtained from samples taken during the period of high *Synechococcus* abundance (August; Fig. 1). Daily carbon losses from grazing accounted for 33.4–61.6% (average ± SD: 44.3 ± 15.1%) of the *Synechococcus* biomass. The bacterial carbon biomass and loss to protistan grazing were higher than those of *Synechococcus*, ranging from 3.10 to 9.78 μg C $L^{-1}$ versus 0.94 to 3.98 μg C $L^{-1}$ $day^{-1}$, respectively (Table 4).

## Discussion

We applied the modified dilution technique to estimate the growth and mortality rates of prokaryotes in the hypolimnion of a lake. We did not find significant differences

between the 0.2-μm and 30-kDa regressions (Table 3). It has been suggested that viral lysis rates < 0.1 $d^{-1}$ are difficult to detect using the modified dilution method (Kimmance and Brussaard 2010). Thus, our failure to detect viral lysis rates suggests that they were < 0.1 $d^{-1}$. Indeed, Pradeep Ram et al. (2010) found a low frequency of bacterial cells infected by viruses in the hypolimnion of Lake Biwa, suggesting that this is the norm. In any case, the effects of viral lysis on the growth rates of *Synechococcus* and heterotrophic bacteria may be negligible in the present study, though we could not estimate growth rates from the y-intercepts of the 30-kDa regressions.

In the present study, we detected high grazing mortality rates of *Synechococcus* and bacteria, whereas the viral lysis rates were negligible (Table 3). This finding suggests that protistan grazing plays a key role in the removal of prokaryotic cells from the hypolimnion of Lake Biwa.

In the present study, bacterial growth and grazing mortality rates were positively correlated ($r$ = 0.961, $P$ < 0.05), suggesting that bacterial grazing mortality depends on bacterial production in the hypolimnion. On the other hand, *Synechococcus* did not proliferate and showed different grazing rates among experiments (Table 3). This result suggests that the grazing mortality rate of hypolimnetic *Synechococcus* is independent of the growth rate, though several previous studies using the conventional dilution technique found a positive correlation between cyanobacterial growth and grazing mortality rates (Nagata 1988). One well-supported hypothesis is the "size-selective grazing" of prey by predators (Gonzalez et al. 1992). Hansen et al. (1994) reported that the size ratio between HNF and their optimal prey was 3:1 (ESD:ESD). In the present study, the size ratio between HNF and *Synechococcus* was 3.3 ± 0.43 (average ± SD), suggesting that *Synechococcus* would be an optimal food size for HNF. Conversely, the size ratio between HNF and bacteria was 10.0 ± 0.43 (average ± SD). Because the observed HNF:prey size ratios in the literature range from 2:1 to 8:1 (Hansen et al. 1994), bacteria may be

243 inappropriate food particles for HNF in the hypolimnion. Thus, it is likely that the principal
244 factor controlling the *Synechococcus* biomass differs from that of bacteria in the
245 hypolimnion.

246 Positive relationships between viral lysis and host growth have been reported for
247 both bacteria (Weinbauer et al. 2003) and *Synechococcus* (Pasulka et al. 2015). We also
248 found the positive relationships between viral lysis and bacterial growth in the epilimnion
249 of Lake Biwa (Takasu et al. 2014). In the present study, however, viral lysis remained low
250 in the hypolimnion. Personnic et al. (2009) suggested that viruses could have a long latent
251 period (more than 48 h) when bacterial activity is low during cold winter season (4.2 to
252 11.8 °C) in three peri-alpine lakes. Because the hypolimnion of Lake Biwa has a constant
253 cold temperature (8°C) throughout the stratification period, the latent period of
254 hypolimnetic viruses may be longer than the duration of our incubation experiments (36–48
255 h). In addition, it is likely that high oxygen concentration in the hypolimnion of Lake Biwa
256 does not inhibit HNF grazing activity (Pradeep Ram et al. 2010), and most of bacterial cells
257 were consumed by HNF grazing before lysed by viruses.

258 In addition, the low viral lytic pressure on *Synechococcus* in the present study
259 might be attributable to a state of inactivity or dormancy among *Synechococcus* in the
260 hypolimnion. Although viral lysis rate of *Synechococcus* in the epilimnion is not available,
261 a previous study suggested that the cyanophages are not important components of viral
262 communities in Lake Biwa (Pradeep Ram et al. 2010). Thus, viral lysis is likely to be minor
263 as a mortality source for *Synechococcus* throughout water column of Lake Biwa.

264 Despite low viral lytic pressure on prokaryotes, the range of virus-to-prokaryote
265 abundance ratios (VPRs) were 11.9–28.5 in the hypolimnion, falling within the range of the
266 epilimnion (Takasu et al. 2014). In addition to host abundance and growth rate, factors that
267 decrease the viral population may also account for the observed VPR, since viral
268 populations are determined by both viral production and decay. Previous studies

demonstrated that several processes are involved in the removal of viruses from water columns in the surface layer, including extracellular proteases and high UV radiation (Sigee 2005). The low extracellular protease activity and absence of UV radiation in the hypolimnion (Kim et al. 2007) may allow VPRs similar to those in the epilimnion, owing to the relatively low rate of viral decay.

In the present study, the contribution of the pico-sized fraction to the total chl *a* concentration in the hypolimnion (average ± SD: 14.3 ± 3.3%; Table 1) reinforces the importance of *Synechococcus* as an organic matter transporter in Lake Biwa (Takasu et al. 2015). The *Synechococcus*-to-bacteria carbon biomass ratio ($Syn_{CB}/Bac_{CB}$) and daily grazing loss ratio ($Syn_{GL}/Bac_{GL}$) were high in August (10.8 ± 0.3 and 11.0 ± 4.0%, respectively; Table 5). Thus, it is likely that the *Synechococcus* biomass is an important seasonal component of the carbon flux in the hypolimnetic microbial loop (Fig. 1). However, the highest *Synechococcus* abundance in the present study (maximum: $2.8 \times 10^3$ cells $mL^{-1}$) was lower than that observed in our hypolimnion monthly monitoring efforts in 2011 (maximum: $2.4 \times 10^4$ cells $mL^{-1}$; Takasu et al. 2015) and 2010 (maximum: $4.4 \times 10^4$ cells $mL^{-1}$; author's unpublished data). Our estimates of $Syn_{CB}/Bac_{CB}$ and $Syn_{GL}/Bac_{GL}$ in the present study may be conservative with respect to the contribution of *Synechococcus* to the carbon flux in the hypolimnion of Lake Biwa. Although we do not know the reason why *Synechococcus* abundance was low during the present study period, transportation of the epilimnetic *Synechococcus* abundance may largely affects the abundance of the hypolimnteic *Synechococcus*.

Although high protistan grazing pressure on *Synechococcus* has been reported in natural aquatic systems (Christaki et al. 2001), previous laboratory studies suggested that *Synechococcus* is a low-quality component of the protistan diet (Caron et al. 1991). Apple et al. (2011) evaluated *Synechococcus* as a food source for different protist grazers. They found that the suitability of *Synechococcus* varied among protistan taxa, and that

*Synechoccus* may be a viable food source for small protists such as colorless cryptomonads (6–8 μm in diameter). Thus, the biomass of *Synechococcus* in the hypolimnion may contribute to the hypolimnetic food web via the microbial loop. Additional grazing experiments conducted using major HNF taxa in the hypolimnion (e.g., kinetoplastids; Mukherjee et al. 2015) and *Synechococcus* will enhance our understanding of the role of *Synechococcus* in the carbon flux of the hypolimnion.

Our current understanding of the fate of prokaryotes is based mainly on research conducted in the surface layer. The incorporation of hypolimnetic microbial processes into ecological and biogeochemical models of freshwater lakes has been largely hampered by limitations to our knowledge regarding the fate of prokaryotes in the hypolimnion. The present study is the first to provide data regarding carbon flux through the mortality of prokaryotes (*Synechococcus* and bacteria) in a hypolimnetic ecosystem.

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## Table and Figures

**Table 1.** Temperature, euphotic depth ($Z_{1\%}$) and chlorophyll *a* (Chl *a*) in the epi- and hypolimnion of Lake Biwa during the stratification period.

**Table 2.** *Synechococcus*, bacteria, heterotrophic nanoflagellates (HNF) and viral-like particles (VLPs) at 65 m during the stratification period.

**Table 3.** Summary of growth ($\mu$), grazing mortality ($g$), lysis mortality ($v$), and total mortality ($m+v$) of *Synechococcus* (A) and bacteria (B) from the dilution experiments.

**Table 4.** Carbon biomass (CB) of *Synechococcus*, bacteria and heterotrophic nanoflagellates (HNF), and daily production (CP) and grazing loss (GL) of *Synechococcus* and bacteria.

**Table 5.** *Synechococcus*-to-bacteria carbon biomass ratio ($Syn_{CB}/Bac_{CB}$) and grazing loss ratio ($Syn_{GL}/Bac_{GL}$).

**Fig. 1** Carbon flow in May and June (A) and August (B). S, B, H, V denote *Synechococcus*, bacteria, heterotrophic nanoflagellates, and viruses, respectively. Numerical numbers indicate carbon biomass or carbon flow through grazing mortality. n.a., not available.

**Table 1**

| Date (day/mo/yr) | $Z_{1\%}$ (m) | Depth (m) | Water temp. (°C) | Chl *a* (μg $L^{-1}$) | Pico-Chl *a*[a] (μg $L^{-1}$) | Pico-Chl *a*[b] (%) |
|---|---|---|---|---|---|---|
| 19/05/2015 | 25.0 | 5 | 16.7 | 2.20 | 1.08 | 49.1 |
| | | 65 | 7.2 | 0.16 | 0.02 | 12.5 |
| 05/06/2015 | 21.8 | 5 | 19.8 | 2.17 | 0.69 | 31.7 |
| | | 65 | 7.5 | 0.11 | 0.02 | 18.2 |
| 11/08/2015 | 19.0 | 5 | 29.5 | 1.30 | 0.70 | 53.8 |
| | | 65 | 7.4 | 0.13 | 0.02 | 12.3 |
| 27/08/2015 | 16.5 | 5 | 26.6 | 2.77 | 0.93 | 33.6 |
| | | 65 | 7.5 | 0.08 | n.d. | n.a. |

[a]Pico-sized fraction chl *a* (see Methods)

[b]Contribution of pico-sized fraction to total chl *a*

n.d., not determined; n.a., not available.

**Table 2**

| Date (day/mo/yr) | Depth (m) | *Synechococcus* Cell number ($10^3$ cells $mL^{-1}$) | *Synechococcus* Cell volume ($\mu m^3$; Mean ± SD) | *Synechococcus* ESD (μm; Mean ± SD) | Bacteria Cell number ($10^5$ cells $mL^{-1}$) | Bacteria Cell volume ($\mu m^3$; Mean ± SD) | Bacteria ESD (μm; Mean ± SD | HNF Cell number ($10^2$ cells $mL^{-1}$) | HNF Cell volume ($\mu m^3$; Mean ± SD) | HNF ESD (μm; Mean ± SD | VLPs Particle number ($10^7$ particles $mL^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 19/05/2015 | 65 | 0.47 | 1.26 ± 1.81 | 1.33 ± 0.51 | 8.9 | 0.050 ± 0.122 | 0.46 ± 0.24 | 1.5 | 77.2 ± 288 | 5.20 ± 2.56 | 1.6 |
| 05/06/2015 | 65 | 0.47 | 2.28 ± 1.30 | 1.63 ± 0.31 | 8.4 | 0.109 ± 0.162 | 0.60 ± 0.24 | 1.1 | 79.6 ± 68.3 | 5.24 ± 1.48 | 1.5 |
| 11/08/2015 | 65 | 2.83 | 2.18 ± 2.39 | 1.60± 0.48 | 9.2 | 0.064 ± 0.505 | 0.50 ± 0.28 | 1.8 | 59.6 ± 42.0 | 4.77 ± 1.26 | 1.1 |
| 27/08/2015 | 65 | 1.44 | 2.24 ± 1.73 | 1.62± 0.43 | 5.6 | 0.052 ± 0.137 | 0.47 ± 0.23 | 1.2 | 66.2 ± 56.2 | 4.94 ± 1.21 | 1.6 |

ESD, Equivalent spherical diameter

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426 **Table 3**

(A) *Synechococcus*

| Date (day/mo/yr) | Diluent | Dilution level | Linear fit $r^2$ | Linear fit $p$ | Regresion slopes $p$ | $\mu$ | ±SE | $g$ | ±SE | $v$ | $g+v$ | ±SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19/05/2015 | 0.2 µm | 5 | 0.611 | n.s. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 0.992 | 0.109 |
| | 30 kDa | | 0.965 | <0.01 | | 0.075 | 0.072 | | | | | |
| 05/06/2015 | 0.2 µm | 5 | 0.808 | <0.05 | n.s. | **-0.007** | 0.115 | **0.616** | 0.174 | -0.02 | 0.596 | 0.098 |
| | 30 kDa | | 0.925 | <0.01 | | 0.028 | 0.065 | | | | | |
| 11/08/2015 | 0.2 µm | 6 | 0.761 | <0.05 | n.s. | **-0.062** | 0.056 | **0.329** | 0.092 | 0.01 | 0.339 | 0.100 |
| | 30 kDa | | 0.740 | <0.05 | | -0.035 | 0.061 | | | | | |
| 27/08/2015 | 0.2 µm | 6 | 0.869 | <0.01 | n.s. | **-0.200** | 0.045 | **0.382** | 0.078 | 0.00 | 0.382 | 0.078 |
| | 30 kDa | | 0.856 | <0.01 | | -0.222 | 0.047 | | | | | |

(B) Bacteria

| Date (day/mo/yr) | Diluent | Dilution level | Linear fit $r^2$ | Linear fit $p$ | Regresion slopes $p$ | $\mu$ | ±SE | $g$ | ±SE | $v$ | $g+v$ | ±SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19/05/2015 | 0.2 µm | 5 | 0.736 | <0.1 | n.s. | **0.502** | 0.194 | **0.846** | 0.293 | -0.30 | 0.546 | 0.165 |
| | 30 kDa | | 0.797 | <0.05 | | 0.546 | 0.165 | | | | | |
| 05/06/2015 | 0.2 µm | 5 | 0.957 | <0.001 | n.s. | **0.313** | 0.042 | **0.522** | 0.064 | 0.06 | 0.586 | 0.091 |
| | 30 kDa | | 0.933 | <0.001 | | 0.387 | 0.060 | | | | | |
| 11/08/2015 | 0.2 µm | 6 | 0.873 | <0.001 | n.s. | **0.094** | 0.050 | **0.429** | 0.082 | 0.31 | 0.736 | 0.152 |
| | 30 kDa | | 0.854 | <0.001 | | 0.239 | 0.092 | | | | | |
| 27/08/2015 | 0.2 µm | 6 | 0.575 | <0.1 | n.a. | **0.053** | 0.080 | **0.305** | 0.131 | n.a. | n.a. | n.a. |
| | 30 kDa | | 0.241 | n.s. | | n.a. | n.a. | | | | | |

427 Statistically significant values are shown in bold.

428 n.s., not significant; n.a., not available.

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**Table 4**

| Date (day/mo/yr) | Microbes | CB (μg C $L^{-1}$) | CP (μg C $L^{-1}d^{-1}$) | GL (μg C $L^{-1}d^{-1}$) |
|---|---|---|---|---|
| 19/05/2015 | *Synechococcus* | 0.06 | n.a. | n.a. |
| | Bacteria | 4.71 | 2.36 | 3.98 |
| | HNF | 0.81 | n.d. | n.d. |
| 05/06/2015 | *Synechococcus* | 0.11 | -0.001 | 0.07 |
| | Bacteria | 9.78 | 3.06 | 5.10 |
| | HNF | 0.64 | n.d. | n.d. |
| 11/08/2015 | *Synechococcus* | 0.66 | -0.04 | 0.22 |
| | Bacteria | 6.22 | 0.59 | 2.67 |
| | HNF | 0.75 | n.d. | n.d. |
| 27/08/2015 | *Synechococcus* | 0.34 | -0.07 | 0.13 |
| | Bacteria | 3.10 | 0.16 | 0.94 |
| | HNF | 0.87 | n.d. | n.d. |

CB, carbon biomass; CP, carbon production; GL, grazing loss; n.d., not determined; n.a., not available.

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437 **Table 5**

| Date (day/mo/yr) | $Syn_{CB}/Bac_{CB}$ (%) | $Syn_{GL}/Bac_{GL}$ (%) |
|---|---|---|
| 19/05/2015 | 1.3 | n.a. |
| 05/06/2015 | 1.2 | 1.4 |
| 11/08/2015 | 10.6 | 8.2 |
| 27/08/2015 | 11.0 | 13.8 |

438 n.a., not available.

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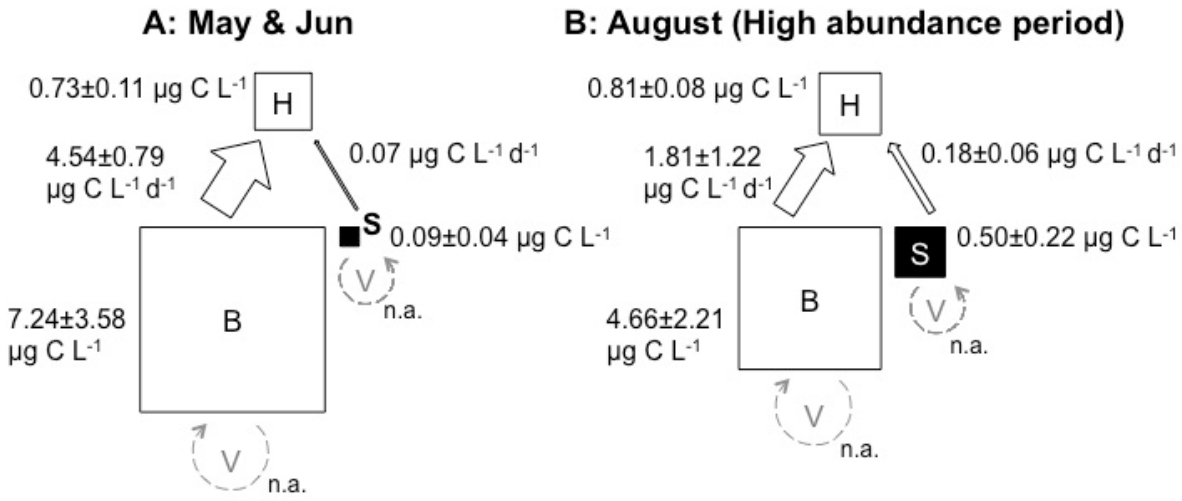


**Figure 1. Takasu & Nakano**

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