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Title: High contribution of *Synechococcus* to phytoplankton biomass in the aphotic hypolimnion in a deep freshwater lake (Lake Biwa, Japan)

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Running title: *Synechococcus* in the hypolimnion of Lake Biwa.

Key words: *Synechococcus*, vertical export, hypolimnion, Lake Biwa

Abstract

The effective transport of picophytoplankton to the mesopelagic layer in the ocean by cell aggregation and attachment to large particles has been reported. Those findings suggest that picophytoplankton play important roles in ecological processes in the deep ocean. In contrast, there is no information about vertical transportation of picophytoplankton cells from epilimnion in lakes, though the presence of picophytoplankton cells in hypolimnion have been reported. The present study demonstrated the possible importance of *Synechococcus* (Cyanobacteria) in ecological processes of the hypolimnion in the deep mesotrophic Lake Biwa, Japan. The chlorophyll *a* concentration in the 0.2–2.0-μm fraction, which is mainly derived from *Synechococcus*, accounted for a large portion (up to 28.8%) of the total chlorophyll *a* concentration in the hypolimnion during the thermal stratification period. We found a significant positive correlation between *Synechococcus* abundances in the epilimnion and hypolimnion during the stratification period. In addition, our incubation experiment revealed that *Synechococcus* did not show remarkable growth during the first 2 days in dark conditions. These results suggest the recent delivery of a significant fraction of *Synechococcus* cells from the epilimnion to the hypolimnion. Our results indicate that the abundance of *Synechococcus* makes a greater contribution to ecological processes in the hypolimnion of Lake Biwa than previously hypothesized, and this may also be the case for other deep lakes.

Introduction

In pelagic ecosystems, a considerable amount of phytoplankton production is lost through respiration, the release of extracellular organic matter, grazing and lysis mortalities, sedimentation, and physiological death (Bidle & Falkowski 2004, Reynolds 2006). It has been suggested that the mechanisms of production and loss differ between large and small phytoplankton species (Kiørboe 1993), and thus phytoplankton species play different ecological roles in food webs and/or matter cycling (Reynolds 2006). In general, large phytoplankton species (>20 μm) are less vulnerable to zooplankton grazing, and sink faster, than small phytoplankton species (Reynolds 2006). Consequently, large phytoplankton is thought to play important roles in organic matter transportation from the surface to deep aphotic layers in pelagic ecosystems. In contrast, small phytoplankton species (<20 μm) are vulnerable to zooplankton grazing, and previous studies have concluded that most of their production is readily removed from surface layers (Nagata 1988, Nagata et al. 1994, Hirose et al. 2008, Scanlan 2012).

The genus *Synechococcus* (Cyanobacteria) is widely distributed in surface oceans (Stockner 1988). Their cell length is 0.9 μm, on average, and they are one of the groups with the smallest size in phytoplankton communities (Kirchman 2008). *Synechococcus* included picophytoplankton sink so slowly (no faster than 0.01–0.02 μm s^{-1}) that the motion of the water is believed to keep them in suspension (Reynolds 2006). Thus, they are too small to sink to the mesopelagic layer of the ocean. However, a recent study using inverse modelling and network analyses suggested that of picophytoplankton carbon biomass, including *Synechococcus*, is transported from the surface to mesopelagic layers (Richardson & Jackson 2007). Recently, the effective transport of *Synechococcus* cells to the mesopelagic layer by cell aggregation (Lomas & Moran 2011) and attachment to large particles (Sohrin et al. 2011) was reported. Those findings suggest that *Synechococcus* play

72 an important role in ecological processes in the mesopelagic layer of the ocean.

73 *Synechococcus* are also distributed in freshwater lakes worldwide (Callieri et al. 2012).
74 In deep freshwater lakes, the hypolimnion is separated from the epilimnion by the thermic
75 barrier of thermocline. Stockner (1991) pointed out the possibility of transportation of
76 picocyanobacteria from the epilimnion to the hypolimnion. However, unlike studies on
77 vertical transportation of picocyanobacteria in oceans, there is no information about
78 vertical transportation of *Synechococcus* cells to the hypolimnion in lakes. In our previous
79 study, we found picophytoplankton cells in water samples from the hypolimnion (70 m) in
80 deep, mesotrophic Lake Biwa, Japan, during the stratification period (Takasu et al. 2012).
81 Callieri & Pinolini (1995) also reported that picophytoplankton cells were present in the
82 hypolimnion (deeper than 100 m) of the deep Lake Maggiore, Northern Italy. Therefore,
83 vertical transportation of *Synechococcus* from the epilimnion to the hypolimnion in lakes is
84 also possible, similar to vertical transportation in oceans.

85 In the present study, we hypothesized that *Synechococcus* produced in the epilimnion
86 of Lake Biwa are transported to the hypolimnion. To address this, we assessed the vertical
87 distribution of, and seasonal changes in, *Synechococcus* abundance throughout the water
88 column of Lake Biwa. To verify the major source of the *Synechococcus* population in the
89 hypolimnion of the lake, we also evaluated the growth potential of *Synechococcus* in dark
90 conditions. The results of the present study suggest that a significant fraction of
91 *Synechococcus* cells are transported from the epilimnion to the hypolimnion.

92

93 **Materials and methods**

94 *Sampling*

95 Lake Biwa is a large (surface area: 674 km^2), deep (maximum depth: 104 m), monomictic,
96 and mesotrophic lake located in the central part of Honshu Island, Japan. We collected
97 water samples at station Ie-1 (35° 12’ 58’’ N, 135° 59’ 55’’ E; *ca*. 75 m) in the north basin

of the lake.

Samples for assessment of the *Synechococcus* distribution were collected from April through August 2011. Vertical profiles of water temperature were determined using a CTD probe (SBE 911 plus; Sea Bird Electronics, Bellevue, WA, USA). In April, light intensity was measured using an LI-192 underwater quantum meter connected to an LI-1400 data logger (Li-Cor Inc., Lincoln, NE, USA). Secchi disk depth was measured throughout the study period. Samples for chlorophyll *a* analysis were collected at 0, 5, 10, 15, 30, and 70 m using Niskin X bottles and then poured into 500-mL polycarbonate bottles washed with 1.2 M HCl. Samples for *Synechococcus* enumeration were collected at 0, 5, 10, 20, 50, and 70 m, then poured into 100-mL polypropylene bottles and fixed immediately with glutaraldehyde (Wako Pure Chemical Co., Tokyo, Japan) to a final concentration of 1%. In April, samples for *Synechococcus* enumeration were collected at 5 and 70 m. In July, a sample for the incubation experiment was also collected at 5 m using a 10-L acrylic water sampler. For the dilution experiment, approximately 10 L of lake water were poured into acid-washed 10-L polyethylene bags.

Chlorophyll a

To determine chlorophyll *a* concentrations, 100-mL water samples were filtered through 0.2- and 2.0-µm polycarbonate filters (Whatman International, Ltd., Maidstone, England) and analysed using the *N,N*-dimethylformamide (Wako Pure Chemical Co., Tokyo, Japan) method (Moran & Porath 1980) with a fluorescence spectrometer (RF-5300PC; Shimadzu, Kyoto, Japan). Chlorophyll *a* concentrations in the 0.2–2.0-µm fraction (hereafter "pico-sized fraction") were calculated according to the following equations:

Chlorophyll *a* in pico-sized fraction = chlorophyll *a* concentration from 0.2-µm filter - chlorophyll *a* concentration from 2.0-µm filter

Enumeration of Synechococcus cells

Fixed water samples of 15- to 25-mL were filtered through 0.2-μm-pore-size black polycarbonate filters (Advantec, Tokyo, Japan), and *Synechococcus* cells retained on the filters were counted by epifluorescence microscopy (BX51, Olympus, Japan) using both blue (460–490-nm excitation by U-MWB2, Olympus) and green (520–550-nm excitation by U-WIG2, Olympus) excitation filter sets. Eukaryotic picophytoplankton exhibited red fluorescence when excited by blue light and weak (red) or no fluorescence under green light (Maclsaac & Stockner 1993). Two types of *Synechococcus* pigments have been described, differing in terms of the phycoerythrin (PE) and phycocyanin (PC) content of phycobiliproteins. PE- and PC-rich *Synechococcus* respectively exhibited orange and dull red fluorescence when excited by blue light, and fluoresced orange and red under green light (Maclsaac & Stockner 1993). These fluorescence characteristics allowed us to separately enumerate the three types of picophytoplankton. We counted at least 300 cells or 100 fields to estimate cell abundance. Images of *Synechococcus* cells were captured at 1000× magnification under an epifluorescence microscope equipped with a digital-camera (EOS Kiss X5, Canon, Tokyo, Japan). Digital images were used to determine the length, width, and fluorescence intensity of each cell, and more than 100 *Synechococcus* cells were used for each sample. The image analysis software ImageJ (National Institutes of Health) was used for measurement. Cell volumes were calculated by assuming that the cells were spheres. The cell specific orange fluorescence intensity under green excitation was also determined. PE-rich *Synechococcus* often predominates in Lake Biwa during summer, and the isolated strains exclusively exhibited a strong emission peak of PE (577 nm) under green excitation (546 nm) (Maeda et al. 1992). So, we measured fluorescence intensity of orange cells under the green excitation (520–550-nm excitation) as an indicator of cell specific PE fluorescence.

In addition, we microscopically observed *Synechococcus* microcolonies (from 5 to 50 cells), an aggregation without a clear separation from the single-celled *Synechococcus* (Callieri 2010). So, we also measured the size of the microcolonies in the same manner as individual *Synechococcus* cells.

Incubation experiment in dark conditions

A water sample was gently filtered through 20-μm mesh to remove mesozooplankton. A 50-L portion of the filtrate was gravity filtered through 0.2-μm filter cartridges (PALL Acropak Supor membrane capsules, PALL, Co., MI, USA) and collected into tanks. The 0.2-μm filtrate was then passed through a 30-kDa tangential flow filtration system (PES membrane, Millipore, Co., MA, USA) to prepare a grazer-and-virus-free diluent. To reduce grazing and viral lysis pressure, the 20-μm filtrate was diluted in 30-kDa diluent to 20% in a 5-L polycarbonate bottle washed with 1.2 M HCl before use. The bottle was then incubated for 48 h at the *in situ* temperature, in the dark. At the beginning (0 h) and end of the incubation (48 h), 50-mL subsamples for the enumeration of *Synechococcus* cells were collected into polypropylene tubes and immediately fixed with glutaraldehyde at a final concentration of 1%. The growth rate (μ, d^{-1}) of *Synechococcus* was calculated from the cell numbers at the beginning and end of the incubation experiment, with the assumption that *Synechococcus* growth would follow an exponential model:

$$\mu = (1/t) \ln (N_t/N_0)$$

where t is the duration of the incubation (days), and N_0 and N_t are *Synechococcus* cells (cells L^{-1}) at the beginning and end of the incubation, respectively.

Statistical analysis

All statistical analyses and visualizing boxplots were performed using the free statistical environment R (R Development Core Team 2013).

Results

Hydrography

The thermal stratification gradually developed from April to August (Fig. 1(a)). The depth of the euphotic zone was estimated on 26 April 2011. Light intensity in the water attenuated exponentially with depth (Fig. 1(b)). The euphotic depth (Z1%), which received 1% of the surface light intensity, was 20 m in April (Fig. 1(b)). The relatively constant Secchi disk depth (5.4 ± 1.5 m, data not shown) indicated that the euphotic depth did not vary markedly during the study period.

Contribution of pico-sized chlorophyll a *to total chlorophyll* a

Subsurface (0 to 10 m) chlorophyll *a* concentrations of the total (> 0.2 μm) and > 2.0 μm fractions were relatively low in July and August (Fig. 2). A single peak of subsurface chlorophyll *a* maximum was detected at 10 or 15 m in the > 2.0-μm fraction, except in April. In contrast, two peaks of chlorophyll *a* concentration were found in the pico-sized fraction from April and May. The contribution of the pico-sized fraction to total chlorophyll *a* concentration varied in the euphotic layer (Fig. 3). Interestingly, in the hypolimnion, on average, 16.8% of chlorophyll *a* was due to the pico-sized fraction (Fig. 3).

Picophytoplankton abundance

Numbers of *Synechococcus* cells decreased drastically below 10 m in depth (Fig. 4). However, they increased markedly from April to July or August at both the epilimnion (5.7×10^2 to 5.1×10^5 cells mL^{-1}; average, 1.2×10^5 cells mL^{-1}) and the hypolimnion (7.0×10^2 to 2.4×10^4 cells mL^{-1}; average, 1.0×10^4 cells mL^{-1}) (Fig. 4). PE-rich *Synechococcus* dominated in the picophytoplankton communities throughout the water column during the

study period (5.7×10^2 to 4.4×10^5 cells mL^{-1}; average ± standard deviation, 91.3 ± 10.3%), while PC-rich *Synechococcus* was considerably less abundant (below detection to 7.7×10^4 cells mL^{-1}). In the hypolimnion, PE-rich *Synechococcus* was exclusively found within the picophytoplankton community (average ± standard deviation, 99% ± 2.3%). Eukaryotic picophytoplankton cells constituted less than 1% of all picophytoplankton throughout the water column (data not shown).

Picophytoplankton cell volumes and fluorescence intensities

In May and August, the specific cell volume of *Synechococcus* increased with water depth (Fig. 5). In July, the specific cell volume of *Synechococcus* increased with depth until the bottom of the euphotic zone, and then decreased gradually.

In July and August, PE-rich *Synechococcus* microcolonies were found throughout the water column (Fig. 6). The volume of the microcolonies of *Synechococcus* cells typically increased to their maxima (average ± standard deviation; July, 3.4 ± 0.9 μm, 7.2 ± 5.9 μm^3; August, 4.0 ± 1.7 μm, 17.4 ± 23.8 μm^3) at the bottom of the euphotic zone (20 m), and then decreased gradually with depth towards the bottom of the hypolimnion. The cell-specific orange fluorescence intensity showed a scattered distribution (Fig. 7). However, cell-specific orange fluorescence intensities clearly showed that *Synechococcus* in the hypolimnion contained PE in relatively high (May and July), or at least equivalent (April, June, and August), amounts relative to the epilimnion (Fig. 7).

Incubation experiment

During the 48-h incubation in dark conditions, *Synechococcus* abundance did not change significantly (Table 1); indeed, the initial cell abundance was maintained for at least 2 days. The cell-specific orange fluorescence intensity increased during the incubation.

228 **Discussion**

229 *The possibility of Synechococcus transportation from the epilimnion to the hypolimnion*

230 Chlorophyll *a* size fractionation method has been used to estimate picophytoplankton
231 contribution to phytoplankton biomass (e.g. Tremblay & Legendre 1994, Marañón et al.
232 2001). Tremblay & Legendre (1994) concluded that there was no significant differences
233 between the carbon to chlorophyll *a* ratio of small and large phytoplankton. It therefore is
234 appropriate to assume that the chlorophyll *a* distribution in different size classes accounts
235 for the biomass size structure of the phytoplankton assemblages. The most interesting
236 finding of the present study was the constant and relatively high contribution (16.8% on
237 average) of the pico-sized chlorophyll *a* to the total chlorophyll *a* in the hypolimnion
238 throughout the study period (Fig. 3). Although the information about the distribution of
239 *Synechococcus* in the hypolimnion is limited, several studies on the distribution of
240 *Synechococcus* in the hypolimnion of freshwater lakes have been conducted (Padisák et al.
241 1997, Winder 2009, Callieri et al. 2012). However, to our knowledge, the contribution of
242 *Synechococcus* to the hypolimnetic total phytoplankton biomass has not yet been reported
243 for a large freshwater lake. The present study is the first to report the unexpectedly high
244 contribution of *Synechococcus* biomass to the total phytoplankton biomass in the
245 hypolimnion during the thermal stratification period. The contributions of
246 picophytoplankton in freshwater systems are highly variable (Bell & Kalff 2001). The
247 average of 16.8% in the present study falls into the ranges previously reported from
248 epilimnetic phytoplankton communities (0.2 to 43%; Stockner 1988, Bell & Kalff 2001).
249 The highest contribution in the present study was 28.8% (Fig. 3), suggesting occasional
250 importance of *Synechococcus* biomass in the hypolimnion. In addition, because chlorophyll
251 *a* derived from microcolonies was included in the > 2.0-μm fraction, our estimation of the
252 *Synechococcus* contribution to phytoplankton biomass may be conservative.
253 *Synechococcus* may be a food source for hypolimnetic nanoflagellates. Therefore, we

believe that our results underscore the importance of *Synechococcus* in the food web and/or matter cycling of the hypolimnion.

Several experimental studies have revealed that cyanobacteria can grow on organic substrates in dark conditions (Rippka 1972, Mannan & Pakrasi 1993). However, our incubation experiment revealed that *Synechococcus* maintained the initial cell abundance for at least 2 days in dark conditions, without significant growth (Table 1). We did not estimate growth rate of *Synechococcus* under the light condition using same manner with the dark incubation experiment. Thus, growth of *Synechococcus* under light condition still remains unclear in the present study. However, we simultaneously measured *Synechococcus* growth rate in diluted lake water with 0.2-μm filtrate to 20% at the *in situ* light condition in shore of the lake, and *Synechococcus* had positive growth rate (0.22 d^{-1}) in the diluted lake water (our unpublished data). Thus, it is likely that *Synechococcus* in Lake Biwa do not proliferate in dark condition, though they have ability to grow at the *in situ* light condition. Some laboratory experiments have demonstrated *Synechococcus* growth on high concentrations of labile organic substrates under optimal-temperature conditions in dark (Rippka 1972, Mannan & Pakrasi 1993). However, in the hypolimnion of Lake Biwa, labile organic matter concentration is limited, and water temperatures are low (Maki et al. 2010). Thus, *Synechococcus* growth may be limited in the hypolimnion of the lake. Indeed, oceanic *Synechococcus* cannot grow in natural seawater under dark conditions (Sohrin et al. 2011, Timmermans et al. 2005), though they can maintain their populations at a certain level (Sohrin et al. 2011). Hence, *in situ* growth of *Synechococcus* has a minor contribution to changes in their abundance in the hypolimnion of the lake.

The present study is the first to demonstrate the distribution of *Synechococcus* in the entire water column of Lake Biwa. A significant positive correlation between *Synechococcus* abundance in the euphotic zone (10 m) and in the bottom of the hypolimnion (70 m) was found during the stratification period ($r = 0.94$, $p = 0.019$; Fig. 8).

In addition, the incubation experiment suggests that *Synechococcus* cannot grow in dark conditions (Table 1). These results suggest the recent delivery of a significant fraction of *Synechococcus* cells from the epilimnion to the hypolimnion. Thus, the *Synechococcus* population in the hypolimnion may be supplied primarily by the sinking of populations of their epilimnetic counterparts during the stratification period. In deep lakes, PE-rich *Synechococcus* typically dominate at the bottom of euphotic zone and form a deep chlorophyll maximum (Callieri 2007). The deep chlorophyll maximum is quite unstable and suddenly disappear, depending on both abiotic and biotic interactions (Callieri 2012). Thus, PE-rich *Synechococcus* cells may be supplied from deep chlorophyll maximum to hypolimnion of deep lakes, though fate of *Synechococcus* in the deep chlorophyll maximum has not yet been clarified.

It has previously been demonstrated that *Synechococcus* forms aggregates with sinking particles (Waite *et al*., 2000), zooplankton faecal pellets (Waite et al. 2000, Stukel et al. 2013), and other *Synechococcus* cells (Waite et al. 2000, Lomas & Moran 2011), all of which are thought to be major processes that accelerate the sinking flux of *Synechococcus* in the ocean (Richardson & Jackson 2007, Lomas & Moran 2011, Sohrin et al. 2011, Stukel et al. 2013). In contrast, in freshwater lakes, co-aggregation of *Synechococcus* with large particles (Klut & Stockner 1991), zooplankton faecal pellets (Callieri 2007) and other *Synechococcus* cells (Callieri 2010, Callieri et al. 2012) has been reported, but no association between *Synechococcus*-containing aggregates and the vertical transportation of *Synechococcus* cells to the hypolimnion in lakes has been reported. In addition, there is no information about the hypolimnetic distribution of *Synechococcus*. Because nanoflagellates are the major consumers of *Synechococcus* cells in the epilimnion of Lake Biwa (Nagata 1988), zooplankton faecal pellets are likely not the major transportation carrier of *Synechococcus* in the lake. The formation of microcolonies may accelerate their sinking velocity (Fig. 6). The decrease in microcolony volume from the lower layers of the

306 euphotic zone to that of the hypolimnion suggests the supply of *Synechococcus* cells
307 removed from the microcolonies in the hypolimnion (Fig. 6). Further study is required to
308 elucidate the mechanisms of *Synechococcus* transportation from the epilimnion to the
309 hypolimnion.
310
311 *The physiological state of Synechococcus in the hypolimnion*
312 In the present study, the contribution of PE-rich cells to total *Synechococcus* cells in the
313 hypolimnion (average, 98.7%) was higher than that of the epilimnion (average, 86.4%;
314 $p<0.0001$). Previous physiological studies have reported that PE-rich cells have
315 advantageous at low light conditions (Callieri et al. 2012), and the result in the present
316 study supports those in the previous studies. The relatively high fluorescence intensity of
317 *Synechococcus* cells in the hypolimnion than in the epilimnion ($p<0.0001$, Fig. 7) was
318 consistent well with the result of dark incubation experiment (Table 1). It has been reported
319 that cyanobacteria accumulate PE in dark, and their cells immediately initiate
320 photosynthesis when transferred to the light condition (Allen 1984). Thus, increase in
321 cell-specific fluorescence intensity of *Synechococcus* in the hypolimnion may be their
322 natural response to darkness.
323 Catabolism of cellular stores of endogenous carbon sources has been hypothesized as
324 the mechanism of prolonged cyanobacterial survival in the dark (Montechiaro et al., 2006;
325 Jiao et al., 2014). In the present study, a marked decrease in cell volume below the
326 thermocline was observed in April (Fig. 5), possibly due to the consumption of stored
327 carbon sources to facilitate long-term survival in the dark (Montechiaro et al., 2006).
328 Proteins (amino acids) comprise about half of the carbon in a *Synechococcus* cell (Kaiser &
329 Benner 2008), and phycobiliproteins are the most abundant proteins in the cyanobacterial
330 cells (Allen 1984). Thus, accumulation and catabolism of PE may be one of the reasons of
331 their prolonged survival in the hypolimnion. However, no such decrease in cell volume was

observed in other months (Fig. 5). Therefore, our results do not support the notion that catabolism of cellular stores of endogenous carbon sources is a major survival mechanism in the hypolimnion. Another hypothesis is the direct uptake of dissolved organic matter by *Synechococcus* in the hypolimnion. It is well known that most of the dissolved organic matter in the deep sea is refractory (Hansell 2013). So, it is unlikely that cyanobacteria utilize the dissolved organic matter in the deep ocean (Jiao et al., 2014). In contrast, semi-labile organic matter is supplied to the hypolimnion of Lake Biwa during the mixing period, although a large fraction of the dissolved organic matter in the hypolimnion is also refractory (Maki et al. 2010). Thus, one possible explanation for the survival of *Synechococcus* in the hypolimnion of Lake Biwa is its utilization of semi-labile dissolved organic matter. The permanent oxygenated hypolimnion may also support relatively high abundance of *Synechococuus* at the hypolimnion in Lake Biwa. In general, aerobic catabolism of organic matter results in higher energy gain (Søballe & Pool 1999), though some cyanobacteria can utilize organic matter and survive in dark under anaerobic conditions (Richardson & Castenholz 1987).

Synechococcus abundances below 20 m were nearly constant (Fig. 4), although the cell volume tended to increase with depth, except in April (Fig. 5). This result supports the hypothesis that *Synechococcus* would utilize dissolved organic matter to maintain their cell abundance without significant cell division during the sinking process. Similar results were also reported from oceanic studies (Albertano et al. 1997, Sohrin et al. 2011). In addition, bacterial cell volume in the hypolimnion was larger than that in the epilimnion of Lake Biwa (Takasu et al. 2013). The slow growth rate likely favours the enlargement of *Synechococcus* cells in the absence of cell division (Albertano et al. 1997). Further analyses of *in situ* organic matter utilization ability and the carbon and nitrogen contents of hypolimnetic *Synechococcus* will enhance our understanding of their trophic status and survival mechanisms in the hypolimnion of Lake Biwa.

358

359 *Conclusion*

360 The biomass of *Synechococcus* significantly contributed to that of the total phytoplankton
361 community in the hypolimnion of Lake Biwa during the thermal stratification period,
362 accounting for up to 28.8% (16.8% on average) of the total phytoplankton biomass. The
363 roles of *Synechococcus* in food web and/or matter cycling of the hypolimnion may be more
364 important than previously hypothesized.

365

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Table 1. Growth rates and cell-specific fluorescence intensity of *Synechococcus* in dark conditions.

Parameter	PE-rich	PC-rich	Total
Growth rate (day^{-1})	-0.04	-0.07	-0.04
Cell-specific orange fluorescence intensity at the end of the incubation (relative to initial, %)	119	nd	nd

Nd, not determin

Figure legends

Fig. 1. Depth profiles of (a) water temperature and (b) light intensity. The depth of the euphotic zone was estimated on 26 April 2011.

Fig. 2. Depth profiles of chlorophyll *a* concentrations in the (a) > 0.2-, (b) > 2.0-, and (c) 0.2−2.0-μm fractions.

Fig. 3. Contribution of the 0.2−2.0-μm fraction to the total chlorophyll *a* concentration (> 0.2 μm) shown by a box plot. Boxes and bars indicate the quartile (Q1 and 3) and median values, respectively. Whisker represents the value of the most extreme data point that is no more than 1.5 times the inter-quartile. Outliers are shown by open circles. Data are compiled over the study period.

Fig. 4. Depth profiles of (a) total, (b) phycoerythrin-rich, and (c) phycocyanin-rich *Synechococcus* cells.

Fig. 5. Depth profile of *Synechococcus* cell volumes shown by boxplots. Boxes and bars indicate the quartile (Q1 and 3) and median values, respectively. Whisker represents the value of the most extreme data point that is no more than 1.5 times the inter-quartile. Outliers are shown by open circles.

Fig. 6. Vertical size distribution of phycoerythrin-rich *Synechococcus* microcolonies shown by boxplots. Boxes and bars indicate the quartile (Q1 and 3) and median values, respectively. Whisker represents the value of the most extreme data point that is no more than 1.5 times the inter-quartile. Outliers are shown by open circles. The inserts represent phycoerythrin-rich *Synechococcus* microcolonies under green excitation of epifluorescence microscope. The sample was taken at 70 m in August 2011.

Fig. 7. Depth profile of cell-specific orange fluorescence (phycoerythrin) intensity shown by boxplots. Boxes and bars indicate the quartile (Q1 and 3) and median values, respectively. Whisker represents the value of the most extreme data point that is no more than 1.5 times the inter-quartile. Outliers are shown by open circles.

512 **Fig. 8.** Relationship between *Synechococcus* cell abundances at 10 and 70 m in depth. The

513 *Synechococcus* abundance of April used the data from 5 m rather than 10 m.

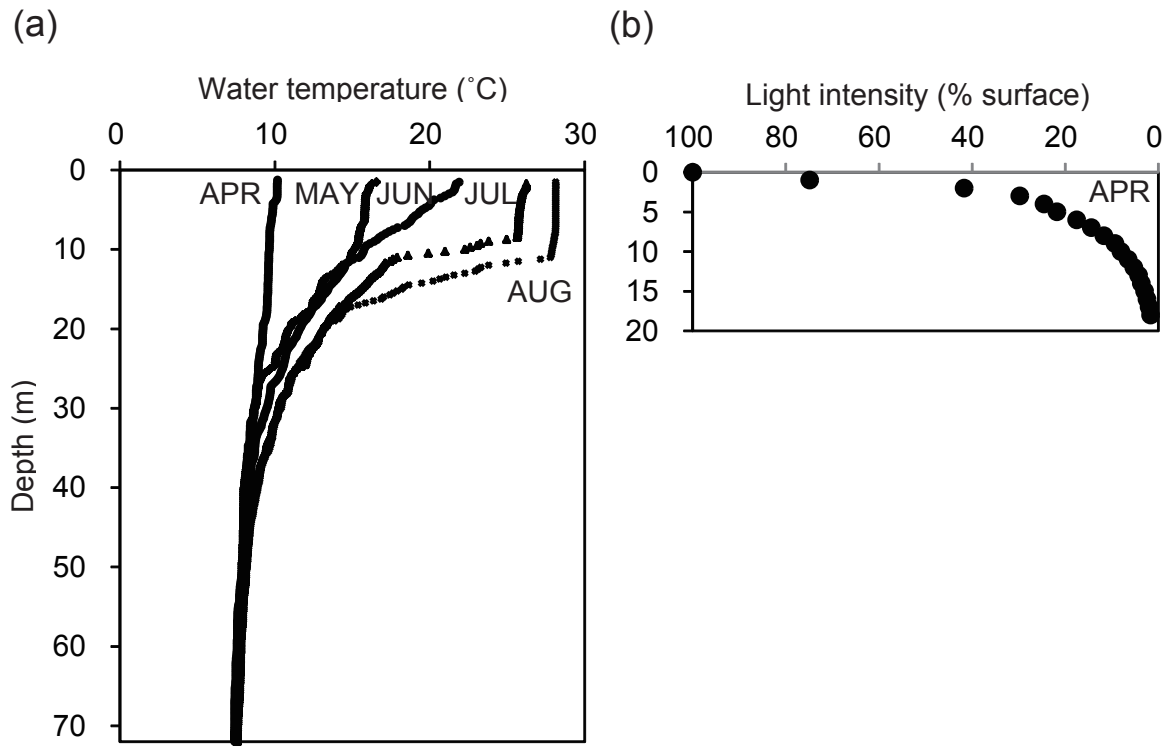

Fig.1.Takasu *et al*.

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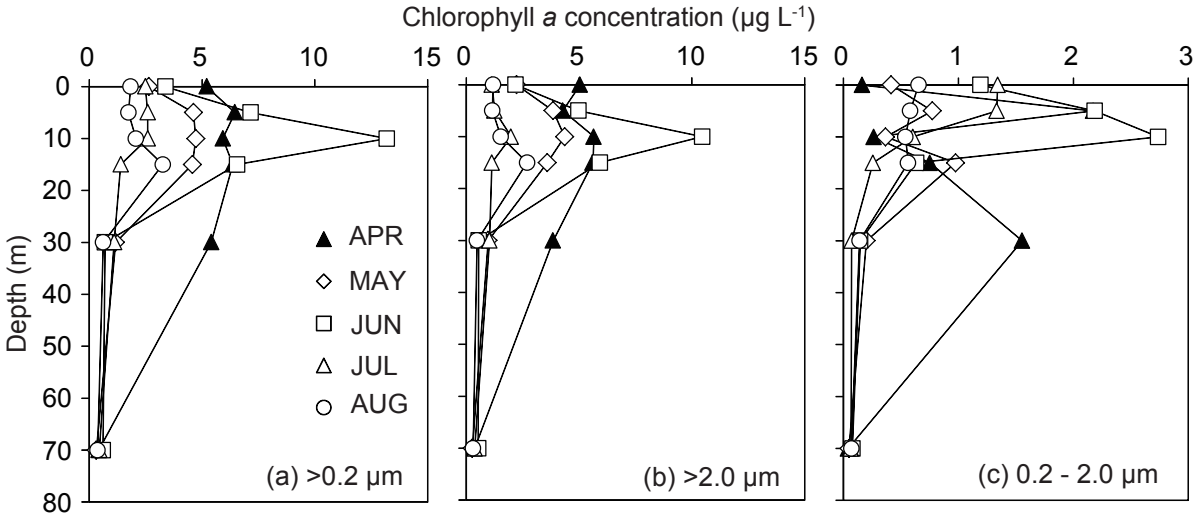

Fig.2.Takasu *et al*.

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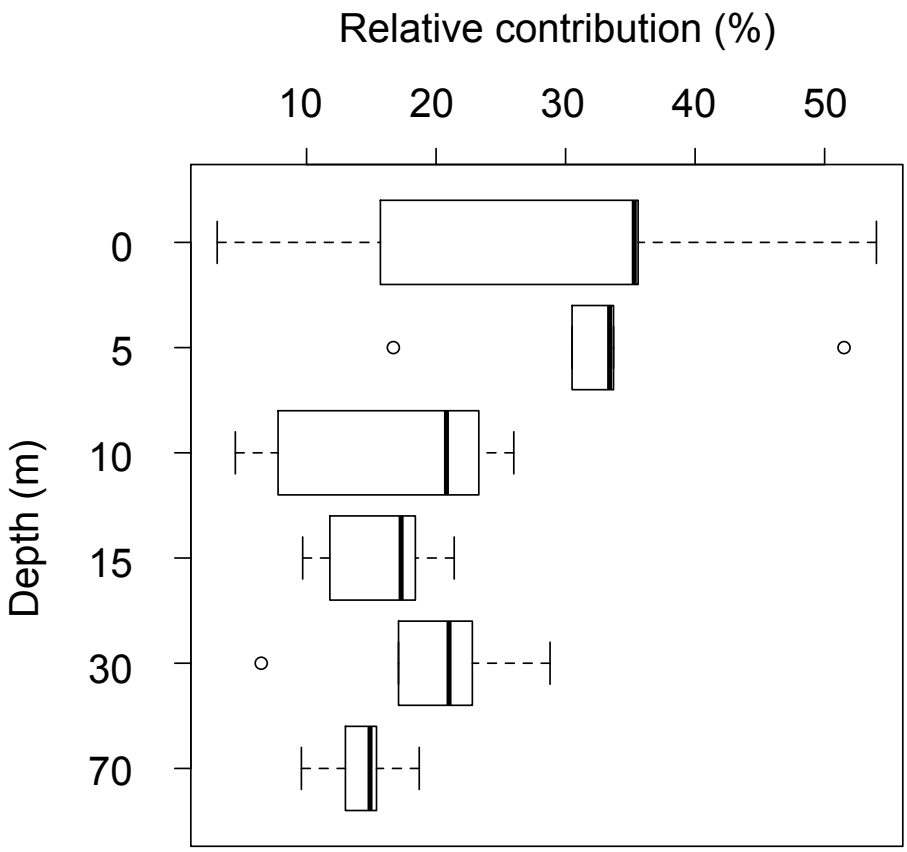

Fig. 3. Takasu *et al.*

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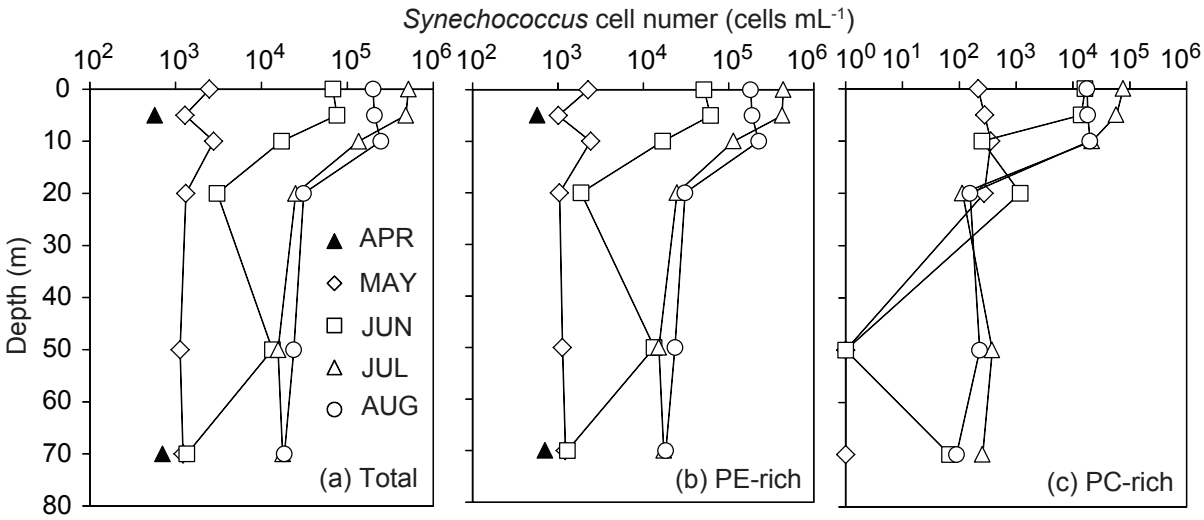

Fig.4.Takasu *et al*.

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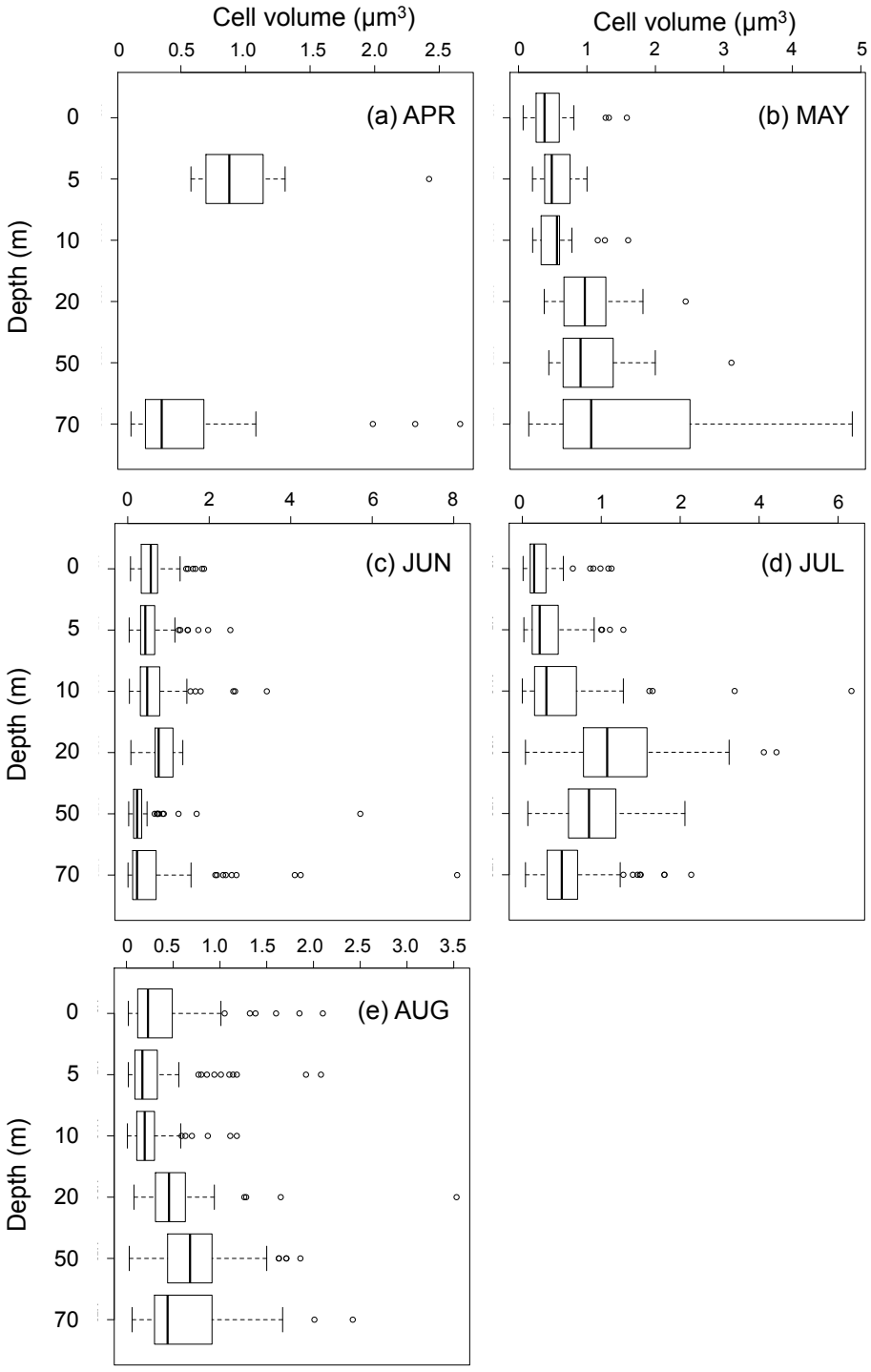

Fig.5. Takasu *et al.*

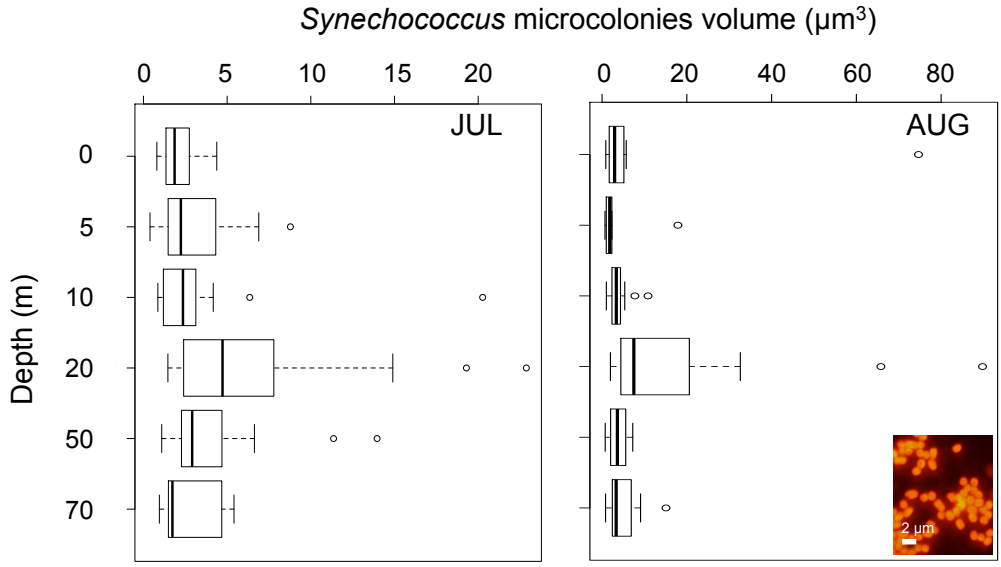

Fig. 6. Takasu *et al.*

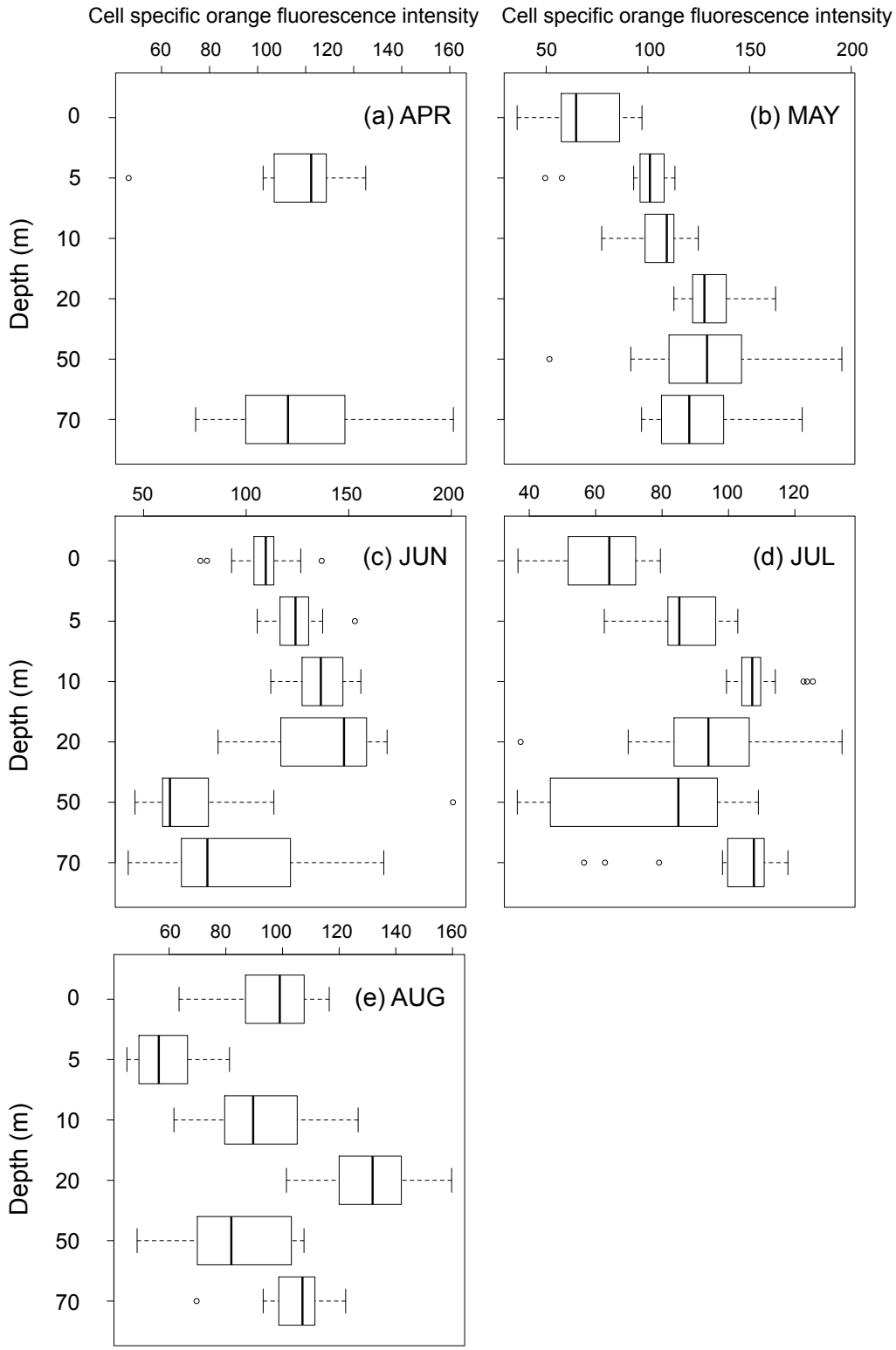

Fig. 7. Takasu *et al.*

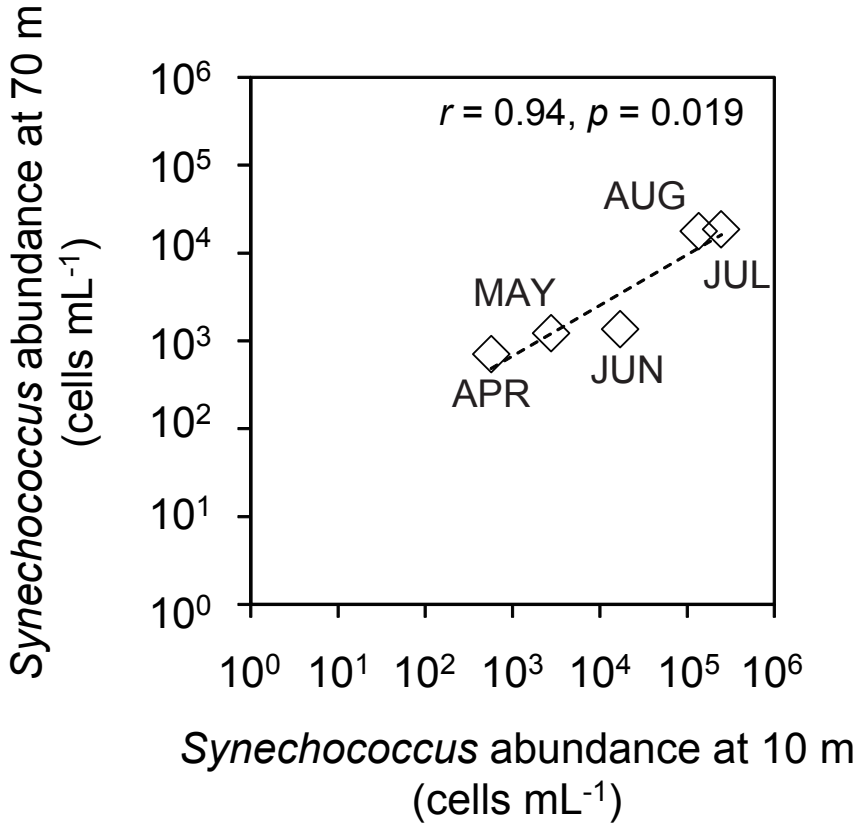

Fig. 8. Takasu *et al.*