

Horizontal variability and regulation of bacterial production in a large, temperate lake

Kenji Tsuchiya¹, Noriko Tomioka¹, Kazuhiro Komatsu¹, Tomoharu Sano¹, Ayato Kohzu¹, Akio Imai¹, Kazuhide Hayakawa², Takamaru Nagata², Takahiro Okamoto², and Tomoyuki Ohara²

¹ National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

² Lake Biwa Environmental Research Institute, 5-34 Yanagasaki, Otsu, Shiga 520-0022, Japan

Corresponding author: Kenji Tsuchiya (ktsuchiya@soka.gr.jp)

Key Points:

- Horizontal heterogeneity of bacterial production (BP) was pronounced in a large lake, showing different regulation factors among seasons
- BP varied along with a gradient of water temperature in winter and phosphorus in the other seasons
- Depth-integrated BP in inshore area significantly contributed to the whole-lake BP despite the lower water depths than offshore area

Abstract

To clarify horizontal variability and regulation of bacterial production (BP), we investigated BP and environmental variables along three east-west transects (Lines 12, 15, and 17) covering inshore/offshore stations in Lake Biwa, Japan, during four seasons. In winter, surface BP along Line 12 (southern transect) was higher than Lines 15 and 17 (central and northern transects) and reflected the water-temperature distribution. Additionally, any nutrients and dissolved organic carbon did not correlate to BP, suggesting water temperature regulated BP in winter. In spring, BP was higher at eastern inshore stations, near large agricultural fields, and was correlated with phosphorus concentration rather than water temperature, suggesting that the limitation shifted to nutrient availability. As well as spring, surface BP in autumn was correlated with phosphorus. Additionally, a negative correlation with water temperature in autumn suggested that nutrient loadings through river and groundwater contributed to enhancing BP. In summer, surface BP at offshore stations along Lines 15 and 17 was notably lower than the other stations. Summer BP was correlated with phosphorus concentration, suggesting that allochthonous nutrient loading determines horizontal BP variations. Moreover, summer depth-integrated BP (DBP) at offshore stations was lower ($32\text{--}52\text{ mgC m}^{-2}\text{ d}^{-1}$) than inshore stations ($43\text{--}110\text{ mgC m}^{-2}\text{ d}^{-1}$) regardless of water depth. The average DBP at inshore stations in summer was 2.1 times that offshore stations, and the inshore/offshore DBP ratio was higher than the other seasons (0.58–1.0). The results suggest that inshore BP significantly contributes to whole-lake BP in productive seasons.

Plain Language Summary

Lakes receive large amounts of external nutrient and organic matter, affecting bacterial production (BP) and spatial distributions. To estimate a whole-lake carbon budget, we need to understand how BP varies spatially within the lake ecosystem and what factors regulate it. We conducted whole-lake samplings to quantify water quality and BP and examine their relationships in Lake Biwa, Japan, during four seasons. In winter, surface BP at southern stations was higher than northern stations, and there was a positive relationship between the BP and water temperature. Additionally, nutrients and organic matter did not correlate to BP, suggesting water temperature regulated BP in winter. In the other seasons, surface BP was relatively high at inshore stations and correlated with phosphorus concentration, suggesting that nutrient loadings

determined horizontal BP variations. The BP integrated with the entire water column in summer at offshore stations was lower than inshore stations, despite the higher water depth at offshore stations, whereas similar situations were not observed in the other seasons. The results suggest that inshore BP significantly contributes to whole-lake BP in productive seasons. This study provides essential information for estimating material cycles in lake ecosystems, contributing to lake management and future predictions associated with climate change.

1 Introduction

Heterotrophic bacteria play an essential role in material and energy cycling in aquatic ecosystems through the degradation and remineralization of organic matter (Azam, 1998). Bacteria produce their biomass (= bacterial production; BP) using dissolved and particulate organic matter; this production links to microbial and grazing food webs (Wylie & Currie, 1991). Lake ecosystems receive large inputs of allochthonous organic matter: notably, bacterial use of terrestrially-derived resources in some lake food webs can be as high as 40% to 94% (Tanentzap et al., 2017). Substantial allochthonous organic matter and nutrients from surrounding ecosystems can enter via rivers, groundwater and runoff. The relative intensity of external loading impact on ecosystems varies spatially, especially in larger lakes. Thus, horizontal variability of bacterial dynamics and related environmental variables should be investigated to estimate the carbon balance of a whole lake and understand the fate of allochthonous inputs.

Horizontal variability of BP has been studied in Lakes Michigan (Scavia & Laird, 1987), Constance (Güde, 1990; Reichart & Simon, 1996), Nuesiedl (Reitner et al., 1999), Hallwil (Filippini et al., 2008), and Mangueira (They & Marques, 2019). The maximum to minimum BP ratios within a horizontal sampling, defined as the horizontal variability in lake ecosystems, vary among seasons and lakes (1.6-19.5, Table 1). The BP varied along with inshore/offshore gradients (allochthonous substrate dependent) (Scavia & Laird, 1987; Güde, 1990; Filippini et al., 2008), similar gradients of primary production (autochthonous substrate dependent) (Güde, 1990; Reitner et al., 1999) and no clear gradients (Reichart & Simon, 1996; They & Marques, 2019). Although the horizontal variability of BP should have reflected their regulation factors, there is little study available in which quantitative relationships between BP and environmental variables have been shown in lacustrine ecosystems (e.g. a relationship between BP and PP in Güde, 1990).

Table 1 Ratios of the maximum (max) over the minimum (min) bacterial production in the epilimnion (< 10-m depth) in horizontal variability. N represents the number of field investigations.

Lake	Stations	Season	max/min ratio	mean \pm SD	N	Reference
Michigan	3-5	Summer	1.6-3.3	2.5	2	Scavia and Laird (1987)
Constance	5	ND	11.1	11.1	1	Güde (1990)
	9	Spring, Summer	2.0-8.4	3.8 \pm 2.0	12	Reichert and Simon (1996)
Neusiedl	3	Year-round	2.3-19.5	6.8 \pm 4.8	22	Reitner et al. (1999)
Hallwil	3	Year-round	2.0-2.5	2.3 \pm 0.3	4	Filippini et al. (2008)
Mangueira	7	Spring	1.9	1.9	1	They and Marques (2019)
Biwa	10-11	Year-round	2.9-9.5	5.2 \pm 3.0	4	This study

Lake Biwa is the largest lake in Japan. The major part of this lake (670.3 km²) is the north basin, with 618 km² surface area and 46.5 m mean depth (104 m maximum depth; Fig. 1). In the north basin, heterotrophic bacteria were severely limited by phosphorus (Gurung & Urabe, 1999). Thus, it is predictable that BP varies horizontally along a gradient of phosphorus. However, since measurements of BP in this lake have been conducted only at fixed stations: Ie-1 (maximum depth approx. 73 m, Fig. 1; Nagata, 1987; Gurung et al., 2002; Ram et al., 2010) or 12B (maximum depth approx. 60 m, Fig. 1; Tsuchiya et al., 2020b), horizontal variability of BP has not been clarified in this lake. Demonstrating the BP horizontal variability along with gradients of phosphorus or the other environmental variables, the knowledge should contribute to large-scale predictions of bacterial metabolism and understanding the biogeochemical cycling of a whole lake.

We investigated the horizontal variabilities of BP and environmental variables in the north basin of Lake Biwa in 2018 in four seasons to elucidate the spatial and temporal heterogeneity in BP and the relationships between BP and regulation factors. Spatial distribution patterns were examined along three transects covering the inshore and offshore stations of the lake (Fig. 1). Its relations to environmental variables were analyzed in each of the four seasons.

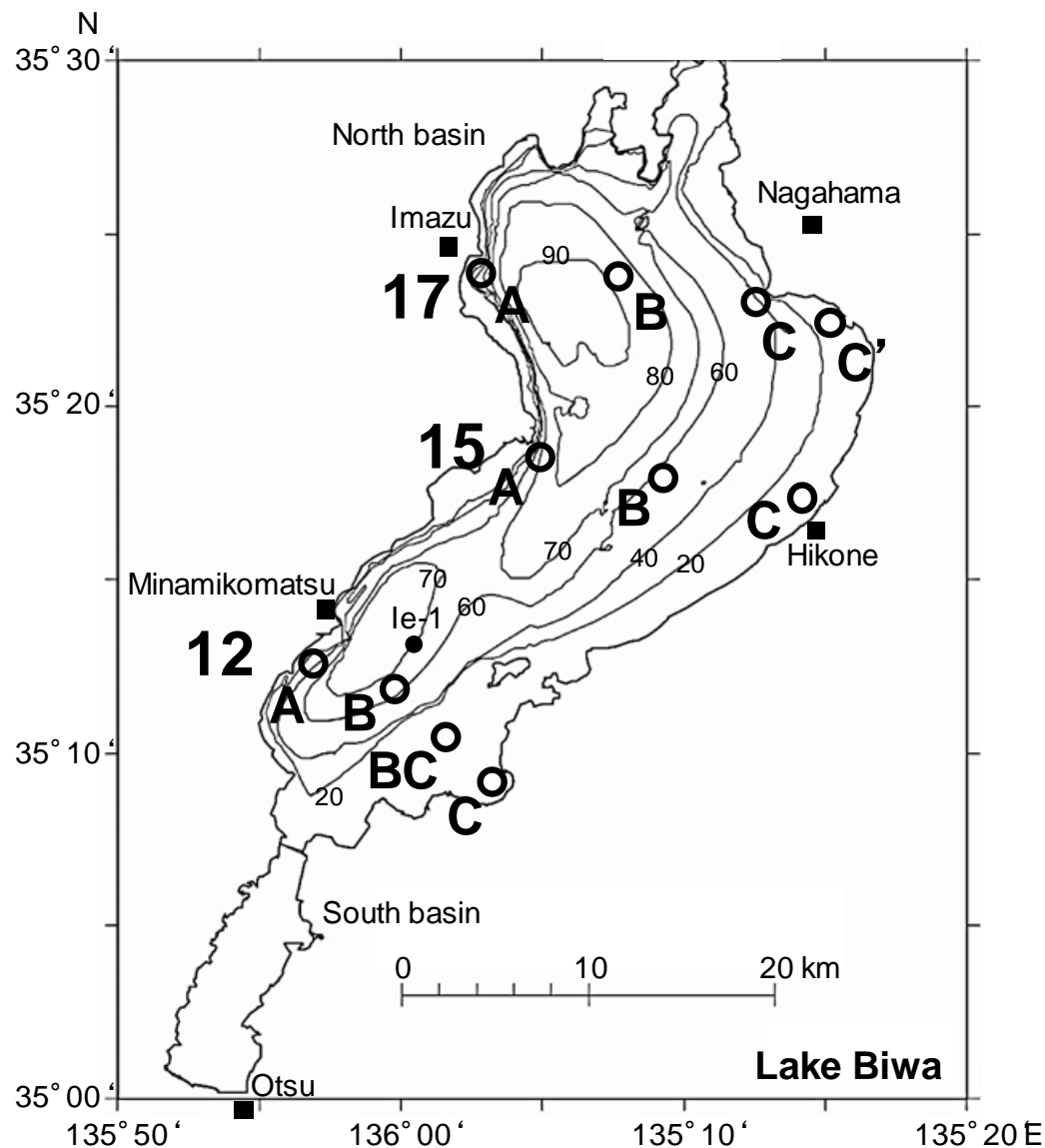


Fig. 1 Sampling stations in the north basin of Lake Biwa, Japan, along three transects (Lines 12, 15, and 17) with 3 or 4 stations each. Depth contours are in m. Square symbols represent weather observation stations around Lake Biwa, Japan.

2 Materials and Methods

2.1 Study site and sampling

The north basin of Lake Biwa is mesotrophic and monomictic, stratified from May to November (seasonal thermocline) and well mixed from January to March (Hayakawa, 2004).

Many studies have been conducted in Lake Biwa, where environmental factors have been observed over 40 years in a whole lake (Shiga-Prefecture, 2017). Additionally, since the external loadings of organic matter and nutrients have been estimated in this lake (Sato et al. 2016), BP's contributions to the lake's metabolism concerning the external loadings can be quantified. Therefore, Lake Biwa is an appropriate lake to clarify the horizontal BP distribution and its relationship with environmental variables for predicting the whole-lake carbon balance. We sampled at a total of 11 stations along three transects (Fig. 1): Lines 17 (Sts. 17A, 17B, 17C, and 17C'), 15 (Sts. 15A, 15B, and 15C), and 12 (Sts. 12A, 12B, 12BC, and 12C) on 22 March, 11 May, 26 July and 21 November 2018 aboard R/V *Biwakaze* of the Lake Biwa Environmental Research Institute, Shiga Prefecture. The water sample was collected from 3 depths (0, 20, and 40 m) with a bucket or a Niskin water sampler. If the water depth was shallower than the desired collection depth, the water sample was collected 1 m above the lake bottom. In the water samples, we measured nutrients (total nitrogen TN, total phosphorus TP, total dissolved nitrogen TDN, total dissolved phosphorus TDP, nitrate NO_3^- , nitrite NO_2^- , ammonium NH_4^+ and phosphate PO_4^{3-}), dissolved organic carbon (DOC), and bacterial production and abundance. Vertical profiles of water temperature, conductivity, and chlorophyll *a* concentration were determined with a conductivity-temperature-depth profiler equipped with oxygen, pH, and chlorophyll sensors (AAQ-RINKO, JFE Advantech, Hyogo, Japan). The conversion from sensor-measured in situ chlorophyll *a* (chl *a*) to methanol-extracted chl *a* measured by spectrophotometer was conducted using the following equation: [methanol-extracted chl *a*] = $1.08 \times [\text{sensor-measured chl } a] + 0.40$ ($n = 10$, $R^2 = 0.996$, $p < 0.001$).

Water samples for dissolved nutrients (TDN, TDP, NO_3^- , NO_2^- , NH_4^+ and PO_4^{3-}) and DOC were immediately filtered through precombusted (450°C for 4 h) GF/F glass-fiber filters (Whatman) on the survey vessel, and the filtrate was frozen for later analysis of dissolved nutrients and DOC. Unfortunately, DOC samples stored in glass bottles collected in March were broken during the transport process, and 80% of DOC data in March was lost.

For measurements of bacterial production (BP), 40 mL of collected lake water was poured into 50-mL centrifuge tubes and incubated with 50 nM of [$^{15}\text{N}_5$]-2'-deoxyadenosine (^{15}N -dA, NLM-3895-PK, Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA) in the dark at *in situ* temperature for 3–8 hours depending on water temperature. The incubation was quenched

by adding 99.5% ethanol (special grade, Wako, Osaka, Japan) to the sample (final concentration, >20%). After quenching, each water sample was filtered onto a 0.2- μ m PTFE membrane filter (Omnipore, Millipore, Burlington, MA, USA), and the filter was stored at -80°C until further analysis.

2.2 Sample analysis

Nutrients were analyzed in our laboratory using a continuous flow analyzer (SWAAT, BLTEC) (Nojiri, 1987; Otsuki et al., 1993). DOC measurements were conducted as nonpurgeable DOC with a TOC analyzer (TOC-V, Shimadzu) equipped with a Pt catalyst on quartz wool. At least three measurements were made for each sample, and analytical precision was typically less than 2%. Potassium hydrogen phthalate (Kanto Chemical) was used as a standard. Additionally, molecular size distributions of DOC were measured by high-performance size exclusion chromatography (HPSEC) with total organic carbon detectors, according to Kawasaki et al., 2011; Shimotori et al., 2016. In the HPSEC analysis, there were two peaks of weight-averaged molecular sizes (M_w) of $1.3 \pm 0.1 \times 10^5$ Da and $2.2 \pm 0.2 \times 10^3$ Da ($n = 117$), and we defined them as high-molecular-weight DOC (HMW-DOC) and low-molecular-weight DOC (LMW-DOC), respectively, in the present study.

For bacterial production (BP), bacterial DNA was extracted from the filter using the Extrap Soil DNA Kit Plus ver. 2 (J-Bio21, NIPPON STEEL & SUMIKIN Eco-Tech Corp., Chiba, Japan). Bacterial cells were broken by glass beads in a Fast Prep FP120 Cell Disrupter (speed, 6.0; time, 40 s; MP Biomedicals, Santa Ana, CA, USA), and the released DNA was purified by using magnetic beads, according to the manufacturer's protocols. The DNA extraction efficiency was considered as 100% (Tsuchiya et al., 2019). After the DNA extraction, the samples were processed according to Tsuchiya et al., (2015) and Tsuchiya et al., (2020a) for quantifying the ^{15}N -dA incorporation rates. Briefly. The DNA samples were hydrolyzed to nucleosides by three enzymes (nuclease P1, Wako; phosphodiesterase I, Worthington Biochemical Corp.; alkaline phosphatase, Promega Corp.). After the enzymatic hydrolysis, the amount of ^{15}N -dA incorporated during the incubation was analyzed by LC-MS/MS using

$^{13}\text{C}_{10}^{15}\text{N}_5$ -deoxyadenosine (CNLM-3896-CA, Cambridge Isotope Laboratories, Inc.) as a surrogate (internal standard).

A cell-to-carbon conversion factor was obtained using the water sample collected at 5-m depth of Sta. 12B in February 2018. Bacterial cells fixed with 1% glutaraldehyde were stained by acridine orange, cell volumes were measured by epifluorescence microscopy (BX-40, Olympus, Tokyo, Japan) with BX-FLA epifluorescence equipment, and the cell-to-carbon conversion factor calculated using a conversion factor of $106 \text{ fgC } \mu\text{m}^{-3}$ (Nagata, 1986), resulting in $11.8 \text{ fgC cell}^{-1}$. The factor for converting ^{15}N -dA incorporation to cells produced was calculated as $1.83 \times 10^6 \text{ cells (mol } ^{15}\text{N-dA})^{-1}$ as determined by Tsuchiya et al., 2020b).

Bacterial abundance (BA) was estimated from measurements of 16S-rDNA concentrations. The 16S-rDNA concentration was determined from the same extracted DNA samples used for BP measurements. SYBR Green I PCR amplification was performed with a LightCycler Instrument (Roche, Mannheim, Germany). For the real-time PCR assay of bacteria, we used the primer set 357F (5'-CCTACGGGAGGCAGCAG-3'), 518R (5'-GTATTACCGCGGCTGCTGG-3'). The 20- μL reaction mixture contained LightCycler 480 SYBR Green I Master (Roche), 4 pmol of each primer, and DNA extract. In the present study, 16S-rDNA concentration was converted to epifluorescence-enumerated bacterial cells (4',6-diamidino-2-phenylindole, DAPI) by using a conversion factor of $0.31 \text{ cells (16S-rDNA copy)}^{-1}$ as previously determined (Tsuchiya et al., 2020b).

3 Results

3.1. Bacterial production (BP)

Bacterial production (BP) in 2018 varied from $0.057 \text{ } \mu\text{gC L}^{-1} \text{ d}^{-1}$ at 40 m at Sta. 12B in November to $18.2 \text{ } \mu\text{gC L}^{-1} \text{ d}^{-1}$ at 6 m at Sta. 12C in July (Fig. 2). BP was significantly positively correlated with water temperature (Spearman's rank correlation test, $n = 104$, $p < 0.001$, Fig. 3a). Variations of BP at certain water temperature levels (e.g., ca. $13\text{--}17^\circ\text{C}$ and $28\text{--}32^\circ\text{C}$) were relatively high for the surface samples ($< 10 \text{ m}$). In March, BP in the surface samples along Line 12 ($1.48 \pm 0.15 \text{ } \mu\text{gC L}^{-1} \text{ d}^{-1}$, $n = 3$) was 2.4 times that of Lines 15 and 17 ($0.62 \pm 0.12 \text{ } \mu\text{gC L}^{-1}$

d^{-1} , $n = 8$). There was no clear trend of horizontal BP variations in the middle and deeper samples (> 10 m). In May, BP in the surface samples was relatively high from the eastern stations of Lines 12 and 15 (12BC, 12C, and 15C; $5.27 \pm 0.93 \mu\text{gC L}^{-1} \text{d}^{-1}$, $n = 6$). BP in the surface samples along Line 17 did not show a clear horizontal trend ($2.69 \pm 0.53 \mu\text{gC L}^{-1} \text{d}^{-1}$, $n = 4$). Among the middle and deeper samples, BP at 20 m at western stations (12A, 15B, and 17A) was relatively high. In July in the surface samples, BP was notably lower at the central stations of Lines 15 and 17 (15B and 17B); in other words, BP was relatively high at the stations closer to shore. At all stations, BP at 20 m was higher than in the deeper samples (> 20 m). As in May, BP at 19 m at Sta. 17A was highest among middle and deep samples from all stations. In November in the surface samples, BP was relatively high at the eastern stations of Lines 12 and 15 (12C and 15C; $4.10 \pm 1.24 \mu\text{gC L}^{-1} \text{d}^{-1}$, $n = 4$); as in May, there was no clear horizontal trend of BP along Line 17 ($2.59 \pm 0.18 \mu\text{gC L}^{-1} \text{d}^{-1}$, $n = 4$). BP at 20 m increased compared to the other months, but there was no clear horizontal trend.

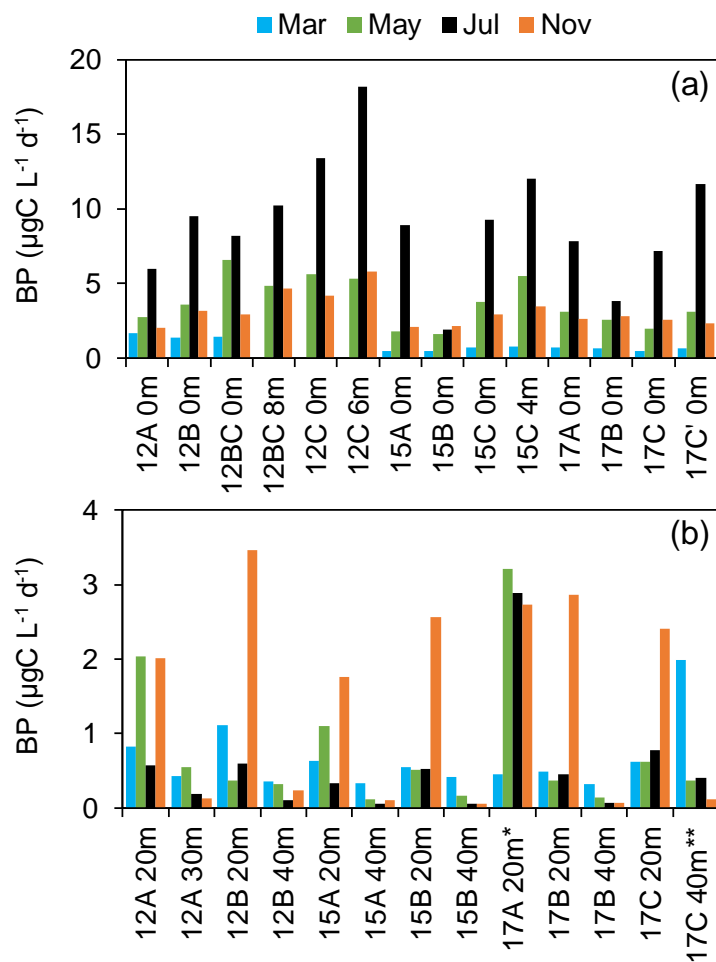


Fig. 2 Bacterial production (BP) in the north basin of Lake Biwa. Sampling depths were < 10 m (a) and > 10 m (b). Blue, green, black, and orange bars represent the samples collected in March, May, July, and November 2018, respectively. * and ** indicate that sampling depths were 19 m in July and 39 m in March, respectively.

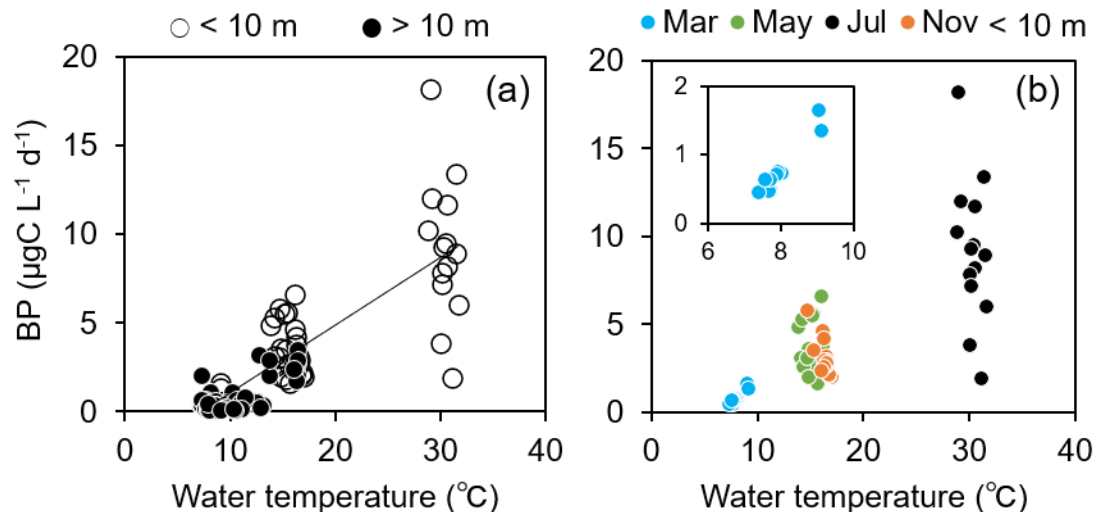


Fig. 3 Bacterial production (BP) versus water temperature in the north basin of Lake Biwa, Japan. (a) Open and filled circles represent < 10 m and > 10 m depths, respectively. (b) Blue, green, black, and orange circles represent the samples collected in March, May, July, and November 2018, respectively, in the surface waters (< 10-m depth).

3.2 Relationships between bacterial production and environmental variables at the surface

In the surface samples in March, BP was significantly positively correlated with water temperature ($p < 0.01$, Fig. 3b, Table 2), whereas BP was negatively correlated to water temperature in November. In other seasons, there were no significant relationships between BP and water temperature. As to DOC, LMW-DOC, and HMW-DOC, BP was significantly positively correlated to them in year-round (Fig. 4a-c, Table 2). However, BP in each season did not correlate to them significantly. In the surface samples, BP was significantly correlated with TP in May, July, and November (Fig. 4d, Table 2). Although BP did not significantly correlate to

TP in year-round, there was a significant relationship between BP and TP when winter data were excluded (Year-round without winter, Table 2). BP was correlated to TN in May and July (Fig. 4e, Table 2). BP was correlated to Chl *a* in March, May, and July (Fig. 4f, Table 2).

There were significantly positive regressions between BA and BP in each season's surface samples (Fig. 5). The slopes of the linear regressions were steepest in March (0.81), almost the same in May and November (0.30 and 0.29, respectively), and gentlest in July (0.12).

Table 2 Summary of spearman's rank correlation coefficients (*rs*) between bacterial production (BP) and environmental variables in the epilimnion (< 10-m depth). WT; water temperature, DOC; dissolved organic carbon, LMW-DOC; low-molecular-weight DOC, HMW-DOC; high-molecular-weight DOC, TP; total phosphorus, TN; total nitrogen, Chl *a*: chlorophyll *a*, n.s.; not significant

Season		WT	DOC	LMW-DOC	HMW-DOC	TP	TN	Chl <i>a</i>
Winter (March)	<i>rs</i>	0.939	0.143	0.314	-0.200	0.318	-0.518	0.794
	<i>n</i>	10	6	6	6	11	11	10
	<i>p</i>	0.0048	n.s.	n.s.	n.s.	n.s.	n.s.	0.0172
Spring (May)	<i>rs</i>	0.015	0.486	0.530	0.068	0.930	0.925	0.925
	<i>n</i>	14	14	14	14	14	14	14
	<i>p</i>	n.s.	n.s.	n.s.	n.s.	<0.001	<0.001	<0.001
Summer (July)	<i>rs</i>	-0.305	0.486	0.530	0.068	0.710	0.758	0.675
	<i>n</i>	14	14	14	14	14	14	14
	<i>p</i>	n.s.	n.s.	n.s.	n.s.	0.0105	0.0063	0.0150
Autumn (November)	<i>rs</i>	-0.727	-0.054	0.110	-0.214	0.811	-0.182	0.464
	<i>n</i>	14	14	13	13	14	14	14
	<i>p</i>	0.0087	n.s.	n.s.	n.s.	0.0035	n.s.	n.s.
Year-round	<i>rs</i>	0.689	0.498	0.394	0.509	0.176	-0.382	0.067
	<i>n</i>	52	48	47	47	53	53	52
	<i>p</i>	<0.001	<0.001	0.0076	<0.001	n.s.	0.0059	n.s.
Year-round without winter	<i>rs</i>	0.412	0.268	0.164	0.302	0.477	-0.098	0.078
	<i>n</i>	42	42	41	41	42	42	42
	<i>p</i>	0.0083	n.s.	n.s.	n.s.	0.0022	n.s.	n.s.

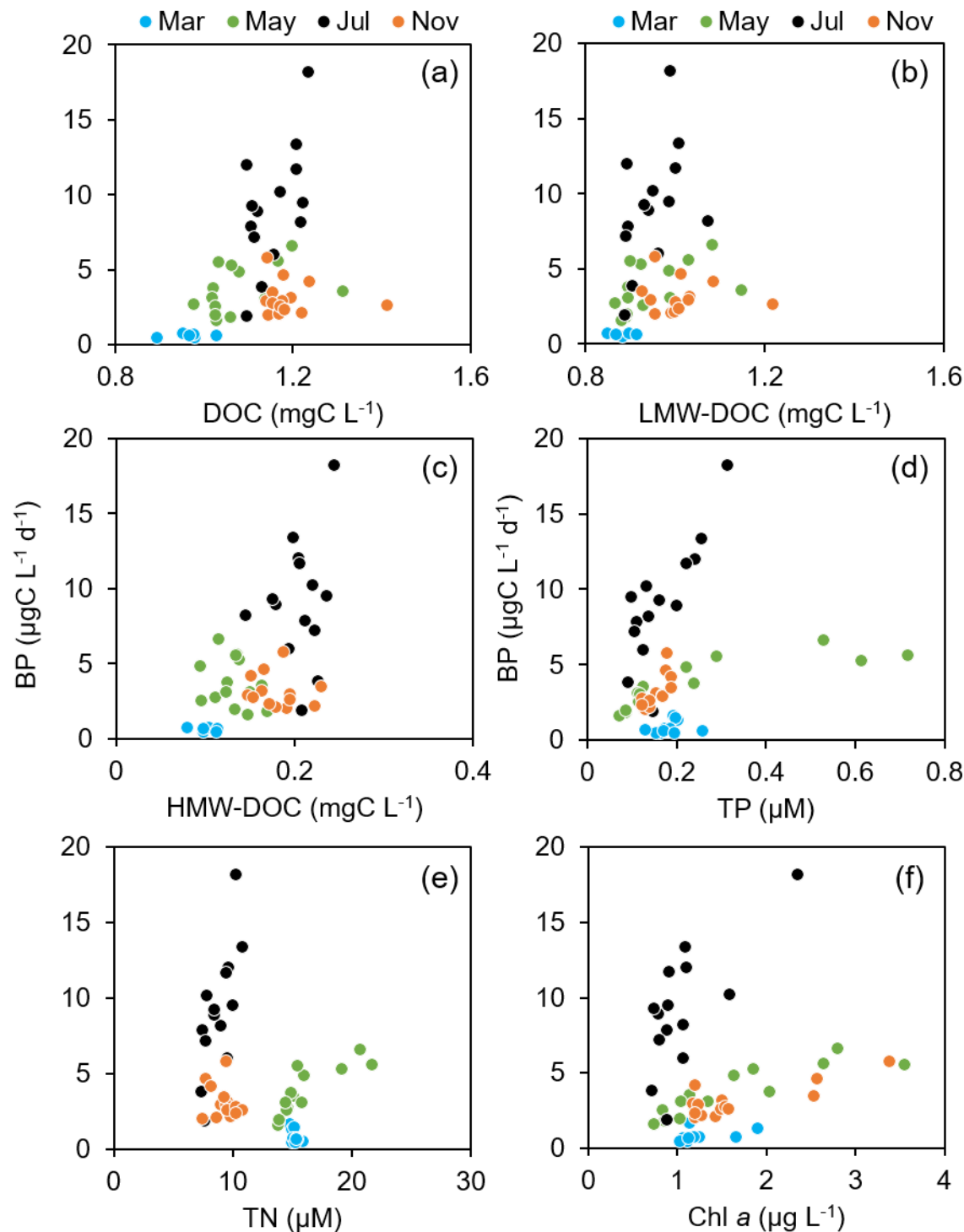


Fig. 4 Bacterial production (BP) versus (a) dissolved organic carbon (DOC) concentration, (b) low-molecular-weight DOC (LMW-DOC) concentration, (c) high-molecular-weight DOC (HMW-DOC) concentration, (d) total phosphorus (TP) concentration, (e) total nitrogen (TN) concentration, and (f) chlorophyll *a* concentration (Chl *a*) in the surface waters (< 10-m depth) in

the north basin of Lake Biwa, Japan. Blue, green, black, and orange circles represent the samples collected in March, May, July, and November 2018, respectively.

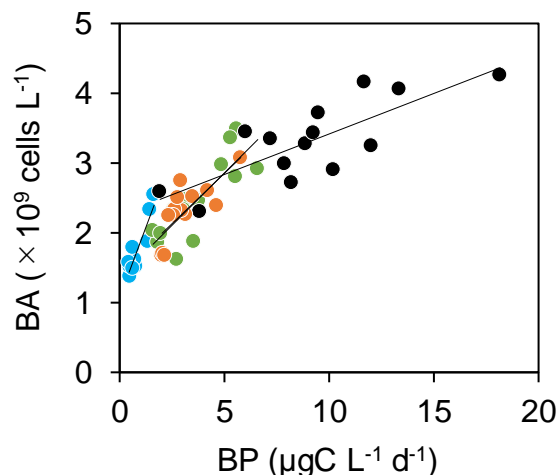


Fig. 5 Bacterial abundance (BA) versus bacterial production (BP) in surface waters of the north basin of Lake Biwa, Japan, in March, May, July and November 2018 (blue, green, black and orange circles, respectively). Regression lines for March: $[BA] = 0.81 \times [BP] + 1.1$ ($n = 10$, $R^2 = 0.84$, $p < 0.001$); May: $[BA] = 0.30 \times [BP] + 1.4$ ($n = 14$, $R^2 = 0.68$, $p < 0.001$); July: $[BA] = 0.12 \times [BP] + 2.3$ ($n = 14$, $R^2 = 0.62$, $p < 0.001$); and November: $[BA] = 0.29 \times [BP] + 1.4$ ($n = 14$, $R^2 = 0.60$, $p < 0.01$). The regression lines for May and November are almost indistinguishable.

3.3 Depth-integrated BP

Depth-integrated BP (DBP) ranged from $2.0 \text{ mgC m}^{-2} \text{ d}^{-1}$ at Sta. 17C' in March to $110 \text{ mgC m}^{-2} \text{ d}^{-1}$ at Sta. 12B in July (Table 3). In March and November, DBP significantly increased with maximum water depth (Fig. 6a, d). Although the regression of DBP on maximum depth for May was not significant, when the three transects were analyzed together, the regressions were significant when the Line 12 data were analyzed separately from those for Lines 15 and 17 (Fig. 6b). For July, there was no significant regression (Fig. 6c). No clear trends were found in the relationship between DBP and distance from shore in March, May, and November (Fig. 6e, f, h). In July, DBP at Sts. 15B and 17B ($> 4 \text{ km}$ offshore) were relatively low compared to the stations closer to shore (Table 3, Fig. 6g). The inshore/offshore ratio of DBP was lowest in November

(0.58) and highest in July (2.1) (Table 3).

Table 3 Depth-integrated bacterial production (BP) in Lake Biwa in 2018. Stations 15B and 17B are classified as offshore (> 4 km from shore) and the others as inshore.

Station	Average depth (m)	Distance from shore (km)	Depth-integrated BP (mgC m ⁻² d ⁻¹)			
			Mar	May	Jul	Nov
12A	33.1	0.8	32.3	80.5	70.0	51.1
12B	60.5	3.2	46.5	86.7	110.4	103.7
12BC	8.9	1.6	12.8	49.1	84.7	35.0
12C	6.9	1.0	ND	40.6	109.1	32.2
15A	76.0	1.3	33.4	31.5	98.2	58.4
15B	62.5	6.7	28.6	43.4	31.8	75.0
15C	6.4	0.6	3.9	25.7	85.7	24.7
17A	26.3	0.9	23.0	36.7	104.0	51.5
17B	88.4	8.0	35.4	41.8	51.6	92.0
17C	41.9	0.6	38.6	37.3	92.3	74.3
17C'	3.9	0.5	2.0	12.9	43.2	8.2
Offshore (15B & 17B) average			32.0	42.6	41.7	83.5
Inshore average			24.1	44.6	88.6	48.8
Inshore/Offshore ratio			0.75	1.0	2.1	0.58

ND: No data

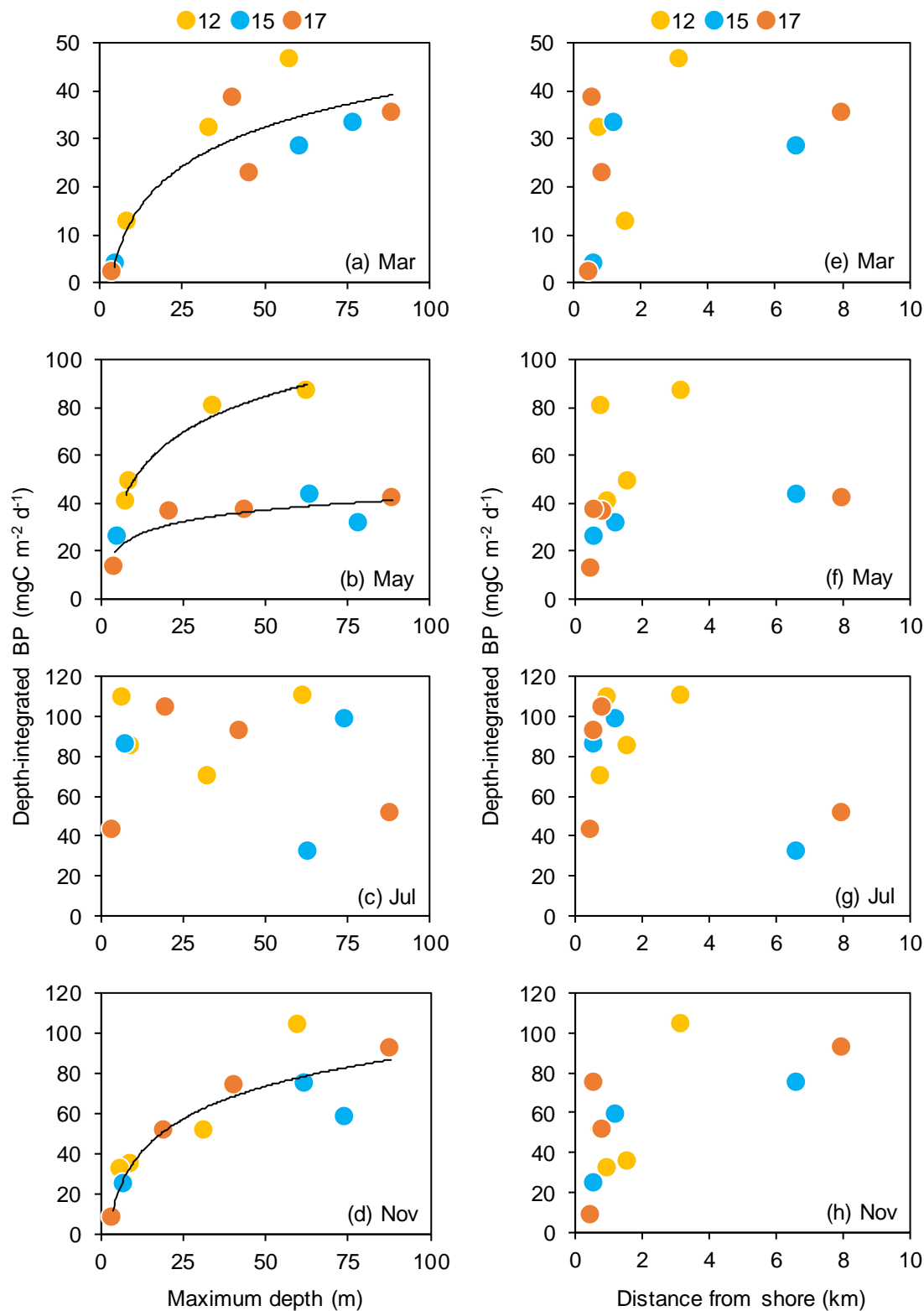


Fig. 6 Depth-integrated bacterial production (DBP) in each season versus maximum depth (left) or distance from shore (right) in the north basin of Lake Biwa, Japan. Yellow, blue and orange

plots represent Line 12, 15 and 17 transects, respectively. The regression curves represent (a) $[DBP] = 11.7 \times \ln [\text{depth}] - 13.4$ ($n = 10$, $R^2 = 0.81$, $p < 0.001$); (b) $[DBP_Line12] = 21.7 \times \ln [\text{depth}] + 0.0885$ ($n = 4$, $R^2 = 0.98$, $p < 0.05$) and $[DPB_Lines15\&17] = 7.02 \times \ln [\text{depth}] + 9.78$ ($n = 7$, $R^2 = 0.71$, $p < 0.05$); and (d) $[DBP] = 23.4 \times \ln [\text{depth}] - 18.1$ ($n = 11$, $R^2 = 0.82$, $p < 0.001$).

4 Discussion

The present study examined spatial and seasonal variations of BP and environmental variables in the Lake Biwa and found a significantly positive relationship between BP and water temperature in year-round data, suggesting that water temperature was a primary BP regulation factor. This relationship agrees with previous studies (Goosen et al., 1997; Pomeroy & Wiebe, 2001). However, the coefficient of variance (CV) of BP (0.35-0.49) was much larger than that of water temperature (0.030-0.075) at the surface in each season; in other words, the BP variation was more considerable than that of water temperature. The large BP variations in each season were attributed to the horizontal distributions. The DOC, LMW-DOC, and HMW-DOC concentrations correlated to BP in year-round data, whereas there were no significant relationships in each season (Table 2), suggesting that DOC could not be an explanatory factor for horizontal BP variations. The other environmental variables showed significant correlations to BP in each season and can be explanatory factors for BP horizontal variations.

In March, BP in the surface samples sensitively responded to variations in water temperature (Fig. 4a, Table 2); however, BP was correlated with neither TP nor TN concentrations. The chl *a* concentration was correlated to water temperature ($rs = 0.79$, $n = 10$, $p < 0.05$) as well as BP, suggesting that water temperature could be the primary limiting factor for both bacterioplankton and phytoplankton in winter. The importance of water temperature affecting bacterial growth, especially during winter, was also emphasized in estuarine and marine environments (e.g. Shiah & Ducklow, 1994a, b). The slope of the linear regression of BA on BP was highest in March (Fig. 5). The relationship between BA and BP has been used to evaluate bottom-up and top-down regulation (Billen et al., 1990; Pace & Cole, 1994). The regressions of BA as a function of BP should have a steep slope if BA is strongly controlled by bottom-up

factors such as resources and water temperature. Alternatively, when other factors such as mortality are most important in regulating BA, there should be no relationships or shallower slopes between them. The ingestion rates of bacteria by heterotrophic nanoflagellates, *Daphnia galeata*, and *Bosmina longirostris* in Lake Biwa were relatively low in March (Nakano et al., 1998). Therefore, in March, bacteria should have been regulated by bottom-up control, especially by water temperature.

The latitudinal difference of water temperature (Line 12 > Lines 15 and 17) could be attributed to Lake Biwa's climate conditions. Air temperatures in 20–23 March 2018, including sampling days, at weather observation stations of the Japan Meteorological Agency around Lake Biwa (<https://www.jma.go.jp/jma/>, last accessed on 6 March 2020) varied along a latitudinal gradient, i.e., Line 12 > Line 15 > Line 17 in this period (see Supporting Information, Fig. A1). The results suggest that climate conditions such as air temperature clearly linked to the horizontal distribution of water temperature and consequently affected biological productivity within the lake in winter.

In May, BP was significantly positively correlated with TP, TN, and chl *a* concentrations (Fig. 4, Table 2). These results suggest that the effect of resource limitation on BP became more pronounced. The spatial distribution of the loading of nutrients, organic matter, or both affects the horizontal BP variation. It is related to higher BP at eastern stations of Lines 12 and 15 in the surface samples. Nutrient discharges into Lake Biwa by rivers flowing into the eastern area of the lake were larger than those in the western area, which is attributed to the high population densities in the urbanized areas and many agricultural fields east of the lake (Taniguchi & Tase, 1999). Since TP and TN concentrations included bacterial biomass as a particulate form and there was a significantly positive relationship between BP and BA (Fig. 5), the correlations with TP and TN did not necessarily indicate limiting factors for BP. The relationship between BP and various forms of N and P (dissolved/particulate and inorganic/organic) was significant only with particulate nitrogen for N, but with dissolved total/organic phosphorus, PO_4^{3-} , and particulate phosphorus for P (Table A1). The results suggest that the horizontal variations of BP could have been regulated by phosphorus as their resource.

In July, the BP variations in the surface samples were larger than that in other seasons (Fig. 3). A similar larger BP variation was observed in another lake (Lake Kasumigaura, the second largest lake in Japan) at higher water temperatures; the variations of BP in Lake Kasumigaura were attributed to autochthonous substrate supply of dissolved organic carbon from primary production (Tsuchiya et al., 2019). In the present study, BP was significantly correlated not to DOC, but rather to TP and TN concentration, suggesting that the large BP variations should be attributed to phosphorus and/or nitrogen limitations. Since PO_4^{3-} concentrations were below the detection limit in this season except for Sts. 12BC and 12C, we examined whether particulate or dissolved P was the dominant source for BP (Fig. 7). Particulate P explained 64% of the BP variations, although particulate P partially comprises bacteria, while dissolved P explained 31% of the variation. However, we found obvious outliers (the two lowest BP values) in the relationship between BP and dissolved P at Sts. 15B and 17B. When the two outliers were excluded from the regression analysis, dissolved P also explained BP's variations (69%). The results for these outliers suggest that particulate P should be a dominant P source for BP at the surface of these offshore stations (15B and 17B). Besides, dissolved P could be transformed into refractory substrates by photochemical and/or biological reactions. Exposure of surface-water dissolved organic matter (DOM) to sunlight led to a 75% reduction of BP in marine ecosystems, suggesting that the photomineralization of bioreactive DOM likely contributed to the decrease in BP (Benner & Biddanda, 1998). Further study is needed to clarify the relationship between BP and refractory dissolved organic P and material cycling in such strongly P-limited environments. We uniformly observed relatively high BP in inshore stations, which we attribute to nutrient loading via some combination of river inflow, groundwater seepage, and elution from sediments. As for nitrogen, there were no significant relationships between BP and any dissolved forms of N other than particulate nitrogen as well as the case in spring (Table A1), suggesting that N was not likely to be a limiting factor for BP. The result is consistent with a previous study demonstrating that bacterial growth at the surface in Lake Biwa was severely limited by phosphorus, but not nitrogen and organic carbon (Gurung & Urabe, 1999).

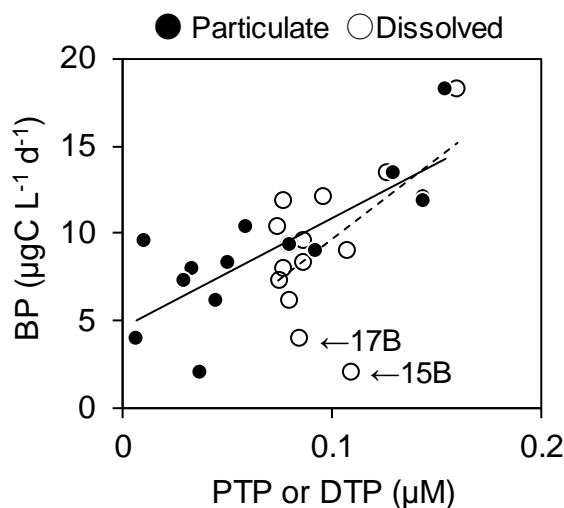


Fig. 7 Bacterial production (BP) versus particulate total phosphorus (PTP) and dissolved total phosphorus (DTP) concentrations in the surface waters of the north basin of Lake Biwa, Japan, in July 2018. The regression lines represent $[BP] = 62 \times [PTP] + 4.6$ ($n = 14$, $R^2 = 0.64$, $p < 0.001$) and $[BP] = 92 \times [DTP] + 0.44$ ($n = 14$, $R^2 = 0.31$, $p < 0.05$).

In November, BP was positively correlated with TP and chl *a* concentrations (Fig. 4). Besides, BP was relatively high at eastern stations such as 12C and 15C (Fig. 3a). Compared to central and western stations along Lines 12 and 15, water temperatures at the eastern Sts. 12C and 15C were lower at depth, while specific conductivity (EC25) and chl *a* were relatively high (Fig. 8). EC25 can be used as a proxy for inflows of different water masses such as river water and groundwater (Bischof et al., 2019). The concentrations of nutrients and dissolved organic carbon are higher in river water and groundwater in the Lake Biwa watershed than in lake water (Taniguchi & Tase, 1999; Mostofa et al., 2007). Thus, the results suggest that allochthonous nutrients and organic matter entering predominantly from the eastern shore enhanced bacterial activity directly, indirectly via phytoplankton activity, or both.

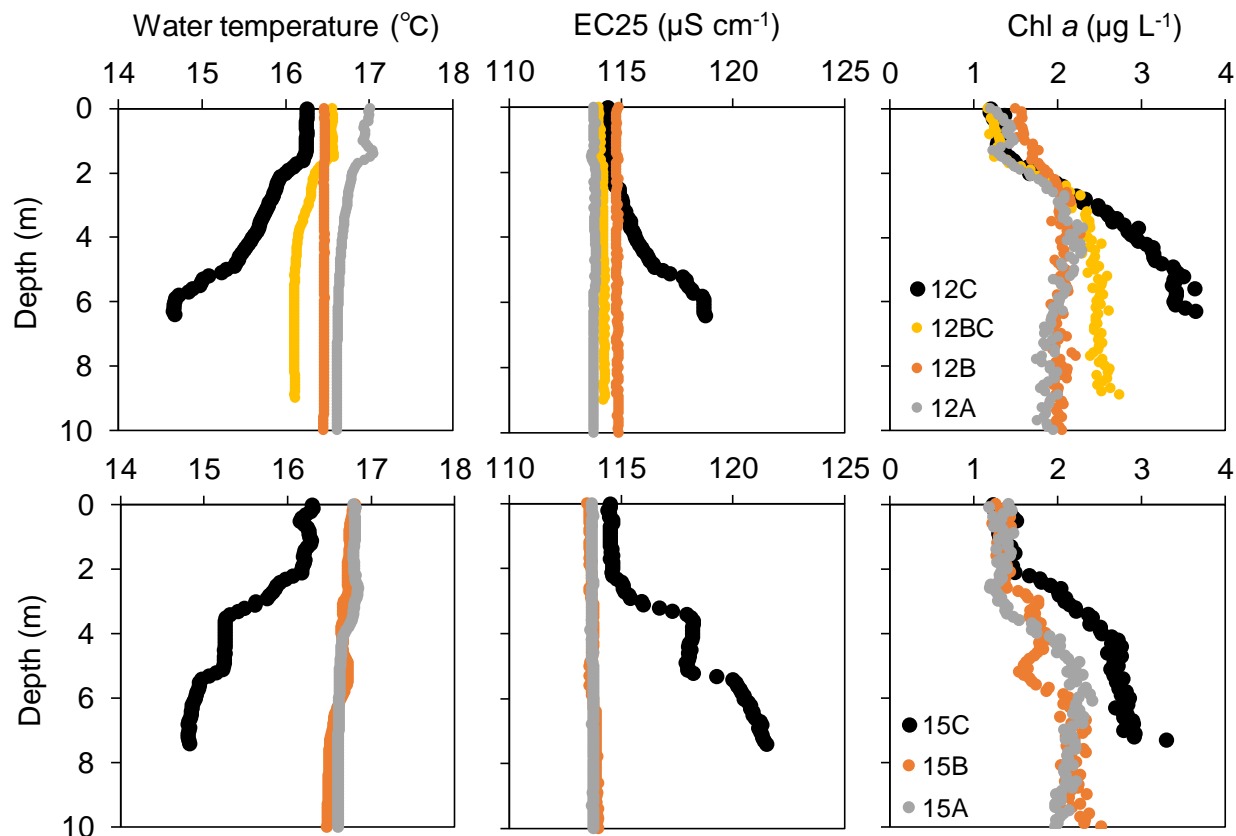


Fig. 8 Vertical distributions of water temperature, EC25, and chlorophyll *a* concentration (Chl *a*) in the north basin of Lake Biwa, Japan, in November 2018. Upper and lower figures represent Line 12 and 15 transects, respectively. Gray, orange, yellow and black circles represent stations A, B, BC, and C, respectively, on each transect.

Our observation of a horizontal gradient of BP from the shore to the central waters, especially in summer, is consistent with results from Lakes Michigan (Scavia & Laird, 1987) and Constance (Güde, 1990). Ratios of maximum (max) over minimum (min) BP in horizontal variability ranged from 1.6 to 19.5 (Table 1). Although the max/min ratio in Lake Biwa was within this range (5.2 ± 3.0 , $n = 4$, Table 1), there were relatively high values of 4.1 in spring and winter and 9.5 in summer. Reichart and Simon argued that the spatial variability is in the same range as that of the diurnal range of bacterial growth dynamics (Reichart & Simon, 1996). The ranges of BP diel variations were 1.8–3.8 (2.6 ± 0.8 , $n = 4$) in Lake Constance (Simon, 1994), 1.3–5.0 (2.8 ± 1.3 , $n = 8$) in the Mediterranean Sea (Gasol et al., 1998), 1.7–2.5 ($n = 2$) in Lake Michigan (Reichart & Simon, 1996) and 2.9 ($n = 1$) in Lake Hallwil (Filippini et al., 2008). Although the diel BP variation was not assessed in the present study, the relatively high max/min

ratios for horizontal variation (~ 9.5) seem larger than the range of diel variation shown above. Moreover, the variations of BP in each season were related to the variations of environmental variables (Figs. 4, 7, and 8). Especially in Lake Biwa, BP is severely limited by phosphorus, but not organic carbon (Gurung & Urabe, 1999). The results suggest that nutrient supply processes strongly influence the horizontal variability of BP in this lake.

The average DBP of inshore stations was 2.1 times that of offshore stations in July, and the inshore/offshore ratio was higher than those in the other seasons (0.58–1.0; Table 3). To estimate the relative contribution of inshore BP to the whole north basin of Lake Biwa (about 450 km² for inshore and 168 km² for offshore), the average DBP for inshore and offshore stations was multiplied by the respective areas. In July, the integrated inshore and offshore BP values were 39.9 tC d⁻¹ and 7.0 tC d⁻¹, respectively, suggesting that the inshore BP accounted for 85% of whole-lake BP (46.9 tC d⁻¹). The inshore BP in March, May, and November accounted for 66.9%, 73.8%, and 61.1% of the whole-lake BP, respectively; for these months, the percentage contributions of inshore waters were equivalent to or lower than the percentage of the inshore area ($72.9\% = 450 \text{ km}^2 / 618 \text{ km}^2$) and disproportionate contributions of inshore BP was not confirmed. The result agrees with the results shown in Fig. 6: DBP in March, May, and November were better explained not by the distance from shore but rather by the maximum depth. The results demonstrate that inshore BP disproportionately contributed to whole-lake BP in the most productive season (July).

In conclusion, we quantified horizontal heterogeneity of BP in each season in Lake Biwa: BP was regulated by primarily water temperature and secondarily phosphorus availability. Dissolved organic carbon, including LMW- and HMW-DOC, did not link to horizontal distributions of BP in the surface waters. The horizontal BP heterogeneity in the surface waters was largest in summer (max/min ratio ~ 9.5), and minimum values were measured at offshore stations. Moreover, mean DBP at inshore stations was 2.1 times that of offshore stations, clarifying the substantial contribution of inshore to whole-lake BP, especially in the most productive season.

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Table A1 Summary of spearman's rank correlation coefficients (r_s) between bacterial production (BP) and environmental variables at the surface samples (< 10 m). PN; particulate nitrogen, DTN; dissolved total nitrogen, DON; dissolved organic nitrogen, PP; particulate phosphorus, DTP; dissolved total phosphorus, DOP; dissolved organic phosphorus, n.s.; not significant, ND: not determined

Season		PN	DTN	NO ₂₊₃	NH ₄	DON	PP	DTP	PO ₄	DOP
Spring (May)	r_s	0.864	0.4945	0.4593	-0.1077	0.4989	0.947	0.692	0.679	0.684
	n	14	14	14	14	14	14	14	14	14
	p	<0.001	n.s.	n.s.	n.s.	n.s.	<0.001	0.0061	0.0075	0.0070
Summer (July)	r_s	0.6689	-0.1209	-0.4158	0.2967	-0.0945	0.7846	0.3055	ND	0.3275
	n	14	14	14	14	14	14	14		14
	p	0.0089	n.s.	n.s.	n.s.	n.s.	0.0009	n.s.		n.s.

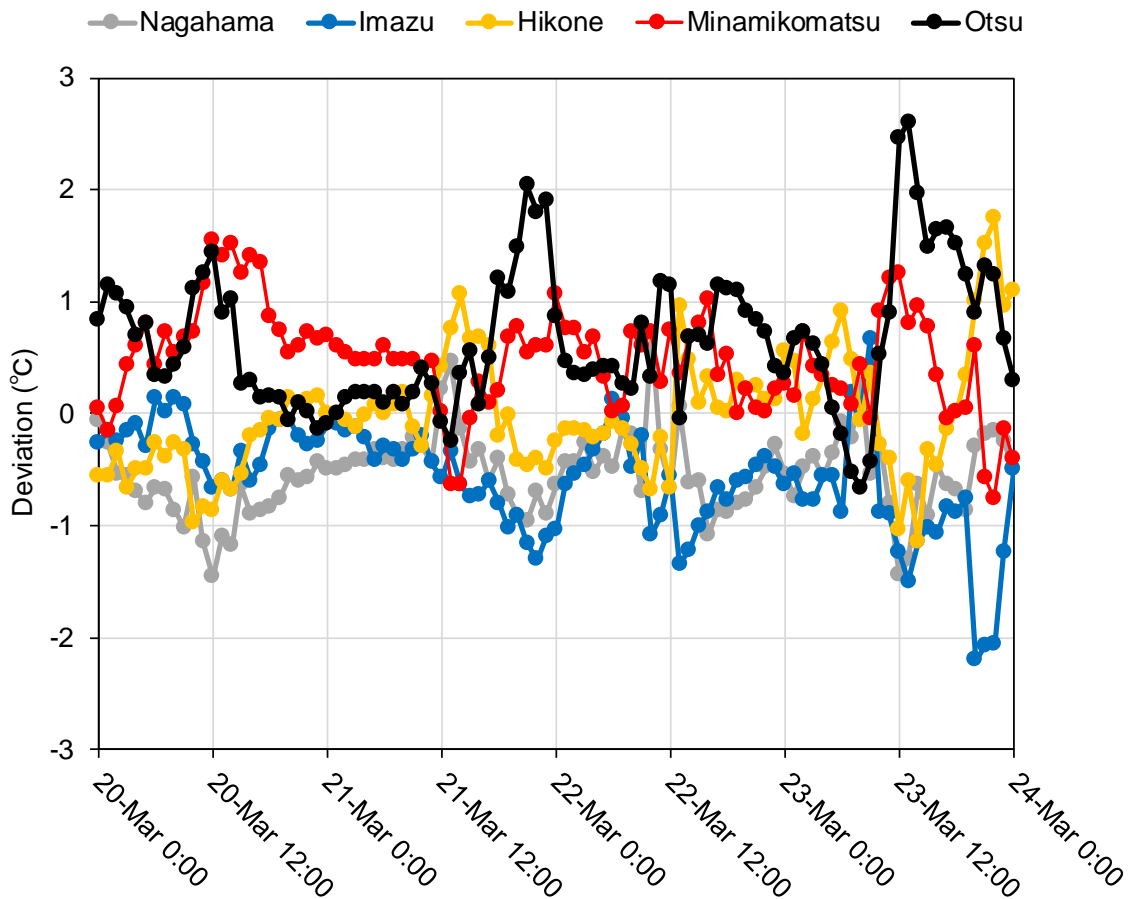


Fig. A1. Deviations from the mean of air temperature from 0:00 on 20 March to 0:00 on 24 March in 2018 observed at five weather observation stations around Lake Biwa, Japan: Imazu,

554 Nagahama (near Line 17), Hikone (near Line 15), Minamikomatsu (near Line 12) and Otsu (near
555 south basin). The altitudes above sea level of these stations were similar (86–95 m). There were
556 significant differences between the deviations among the stations: Nagahama = Imazu < Hikone
557 < Minamikomatsu = Otsu (Tukey-Kramer’s multi comparison test; total $n = 485$, $p < 0.01$). The
558 data were obtained from the Japan Meteorological Agency (<https://www.jma.go.jp/jma/>, last
559 accessed on 6 March 2020).