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

Kenji Tsuchiya[1], Noriko Tomioka[1], Kazuhiro Komatsu[1], Tomoharu Sano[1], Ayato Kohzu[1], Akio Imai[1], Kazuhide Hayakawa[2], Takamaru Nagata[3], Takahiro Okamoto[3], and Tomoyuki Ohara[3]

[1]National Institute for Environmental Studies
[2]Lake Biwa Environmental Research Institute, Otsu, Shiga 520-0022, Japan
[3]Lake Biwa Environmental Research Institute

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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 (32-52 mgC m-2 d-1) than inshore stations (43-110 mgC m-2 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.

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6   **Tomoyuki Ohara[2]**

7   [1] National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

8   [2] Lake Biwa Environmental Research Institute, 5-34 Yanagasaki, Otsu, Shiga 520-0022, Japan

9

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

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12   **Key Points:**

13   • Horizontal heterogeneity of bacterial production (BP) was pronounced in a large lake,
14   showing different regulation factors among seasons

15   • BP varied along with a gradient of water temperature in winter and phosphorus in the
16   other seasons

17   • Depth-integrated BP in inshore area significantly contributed to the whole-lake BP
18   despite the lower water depths than offshore area

19

20

21   **Abstract**

22   To clarify horizontal variability and regulation of bacterial production (BP), we investigated BP

23   and environmental variables along three east-west transects (Lines 12, 15, and 17) covering

24   inshore/offshore stations in Lake Biwa, Japan, during four seasons. In winter, surface BP along

25   Line 12 (southern transect) was higher than Lines 15 and 17 (central and northern transects) and

26   reflected the water-temperature distribution. Additionally, any nutrients and dissolved organic

27   carbon did not correlate to BP, suggesting water temperature regulated BP in winter. In spring,

28   BP was higher at eastern inshore stations, near large agricultural fields, and was correlated with

29   phosphorus concentration rather than water temperature, suggesting that the limitation shifted to

30   nutrient availability. As well as spring, surface BP in autumn was correlated with phosphorus.

31   Additionally, a negative correlation with water temperature in autumn suggested that nutrient

32   loadings through river and groundwater contributed to enhancing BP. In summer, surface BP at

33   offshore stations along Lines 15 and 17 was notably lower than the other stations. Summer BP

34   was correlated with phosphorus concentration, suggesting that allochthonous nutrient loading

35   determines horizontal BP variations. Moreover, summer depth-integrated BP (DBP) at offshore

36   stations was lower (32–52 mgC m^{-2} d^{-1}) than inshore stations (43–110 mgC m^{-2} d^{-1}) regardless

37   of water depth. The average DBP at inshore stations in summer was 2.1 times that offshore

38   stations, and the inshore/offshore DBP ratio was higher than the other seasons (0.58–1.0). The

39   results suggest that inshore BP significantly contributes to whole-lake BP in productive seasons.

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41   **Plain Language Summary**

42   Lakes receive large amounts of external nutrient and organic matter, affecting bacterial

43   production (BP) and spatial distributions. To estimate a whole-lake carbon budget, we need to

44   understand how BP varies spatially within the lake ecosystem and what factors regulate it. We

45   conducted whole-lake samplings to quantify water quality and BP and examine their

46   relationships in Lake Biwa, Japan, during four seasons. In winter, surface BP at southern stations

47   was higher than northern stations, and there was a positive relationship between the BP and

48   water temperature. Additionally, nutrients and organic matter did not correlate to BP, suggesting

49   water temperature regulated BP in winter. In the other seasons, surface BP was relatively high at

50   inshore stations and correlated with phosphorus concentration, suggesting that nutrient loadings

51 determined horizontal BP variations. The BP integrated with the entire water column in summer

52 at offshore stations was lower than inshore stations, despite the higher water depth at offshore

53 stations, whereas similar situations were not observed in the other seasons. The results suggest

54 that inshore BP significantly contributes to whole-lake BP in productive seasons. This study

55 provides essential information for estimating material cycles in lake ecosystems, contributing to

56 lake management and future predictions associated with climate change.

57

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 ± 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 ± 2.0	12	Reichert and Simon (1996)
Neusiedl	3	Year-round	2.3-19.5	6.8 ± 4.8	22	Reitner et al. (1999)
Hallwil	3	Year-round	2.0-2.5	2.3 ± 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 ± 3.0	4	This study

Lake Biwa is the largest lake in Japan. The major part of this lake (670.3 km^2) is the north basin, with 618 km^2 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.
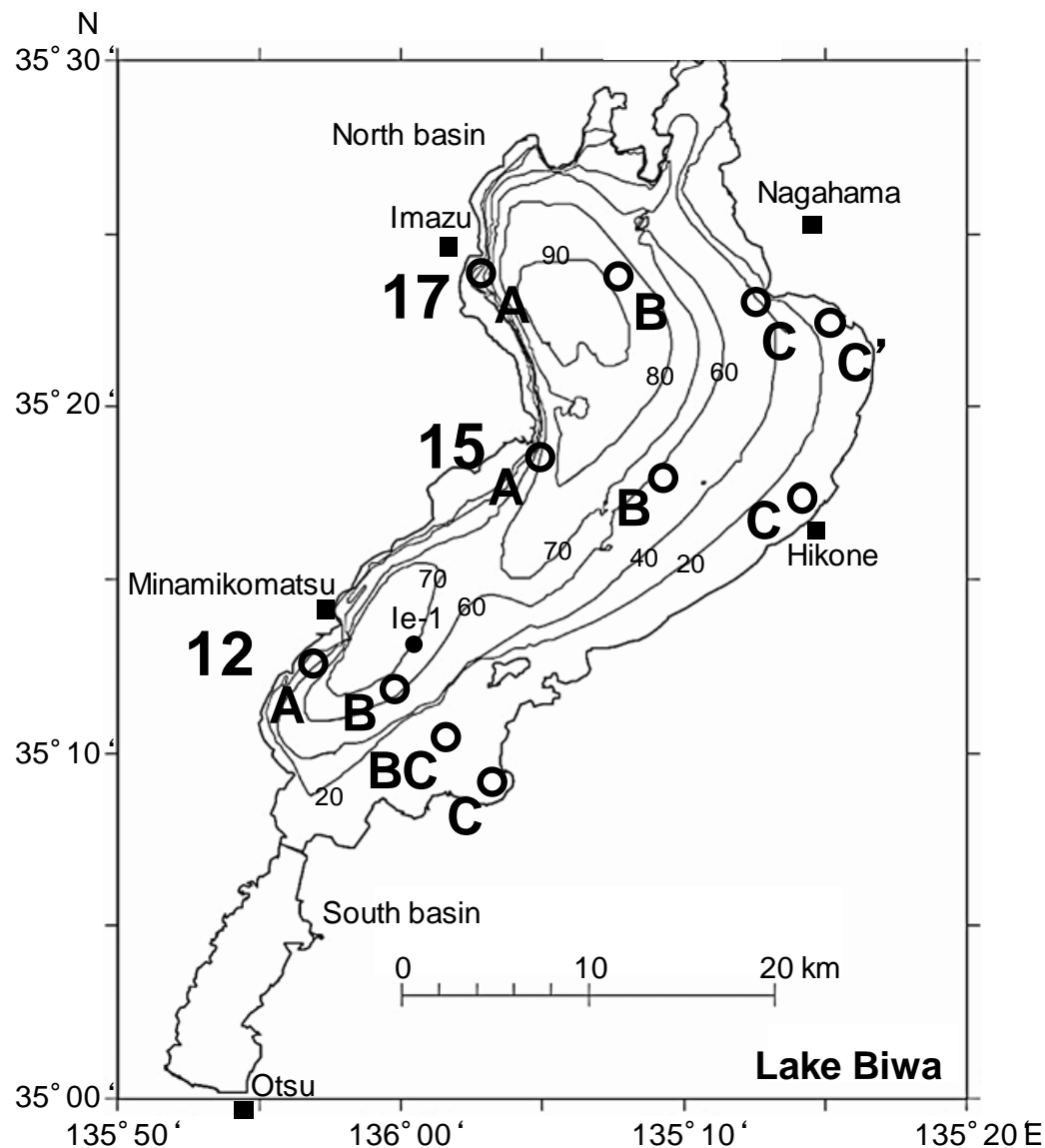
Fig. 1 Sampling stations in the north basin of Lake Biwa, Japan, along three transects (Lines 12, 15, and 17) with 3 or 4 stations each. Depth contours are in m. Square symbols represent weather observation stations around Lake Biwa, Japan.

2 Materials and Methods

2.1 Study site and samping

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).

113   Many studies have been conducted in Lake Biwa, where environmental factors have been

114   observed over 40 years in a whole lake (Shiga-Prefecture, 2017). Additionally, since the external

115   loadings of organic matter and nutrients have been estimated in this lake (Sato et al. 2016), BP's

116   contributions to the lake's metabolism concerning the external loadings can be quantified.

117   Therefore, Lake Biwa is an appropriate lake to clarify the horizontal BP distribution and its

118   relationship with environmental variables for predicting the whole-lake carbon balance. We

119   sampled at a total of 11 stations along three transects (Fig. 1): Lines 17 (Sts. 17A, 17B, 17C, and

120   17C'), 15 (Sts. 15A, 15B, and 15C), and 12 (Sts. 12A, 12B, 12BC, and 12C) on 22 March, 11

121   May, 26 July and 21 November 2018 aboard R/V *Biwakaze* of the Lake Biwa Environmental

122   Research Institute, Shiga Prefecture. The water sample was collected from 3 depths (0, 20, and

123   40 m) with a bucket or a Niskin water sampler. If the water depth was shallower than the desired

124   collection depth, the water sample was collected 1 m above the lake bottom. In the water

125   samples, we measured nutrients (total nitrogen TN, total phosphorus TP, total dissolved nitrogen

126   TDN, total dissolved phosphorus TDP, nitrate NO_3^-, nitrite NO_2^-, ammonium NH_4^+ and

127   phosphate PO_4^{3-}), dissolved organic carbon (DOC), and bacterial production and abundance.

128   Vertical profiles of water temperature, conductivity, and chlorophyll *a* concentration were

129   determined with a conductivity-temperature-depth profiler equipped with oxygen, pH, and

130   chlorophyll sensors (AAQ-RINKO, JFE Advantech, Hyogo, Japan). The conversion from

131   sensor-measured in situ chlorophyll *a* (chl *a*) to methanol-extracted chl *a* measured by

132   spectrophotometer was conducted using the following equation: [methanol-extracted chl *a*] =

133   1.08 × [sensor-measured chl *a*] + 0.40 ($n = 10$, $R^2 = 0.996$, $p < 0.001$).

134   Water samples for dissolved nutrients (TDN, TDP, NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-}) and

135   DOC were immediately filtered through precombusted (450°C for 4 h) GF/F glass-fiber filters

136   (Whatman) on the survey vessel, and the filtrate was frozen for later analysis of dissolved

137   nutrients and DOC. Unfortunately, DOC samples stored in glass bottles collected in March were

138   broken during the transport process, and 80% of DOC data in March was lost.

139   For measurements of bacterial production (BP), 40 mL of collected lake water was

140   poured into 50-mL centrifuge tubes and incubated with 50 nM of [$^{15}N_5$]-2′-deoxyadenosine (^{15}N-

141   dA, NLM-3895-PK, Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA) in the dark at

142   *in situ* temperature for 3–8 hours depending on water temperature. The incubation was quenched

143   by adding 99.5% ethanol (special grade, Wako, Osaka, Japan) to the sample (final concentration,

144   >20%). After quenching, each water sample was filtered onto a 0.2-µm PTFE membrane filter

145   (Omnipore, Millipore, Burlington, MA, USA), and the filter was stored at –80°C until further

146   analysis.

147

2.2 Sample analysis

148

149   Nutrients were analyzed in our laboratory using a continuous flow analyzer (SWAAT,

150   BLTEC) (Nojiri, 1987; Otsuki et al., 1993). DOC measurements were conducted as

151   nonpurgeable DOC with a TOC analyzer (TOC-V, Shimadzu) equipped with a Pt catalyst on

152   quartz wool. At least three measurements were made for each sample, and analytical precision

153   was typically less than 2%. Potassium hydrogen phthalate (Kanto Chemical) was used as a

154   standard. Additionally, molecular size distributions of DOC were measured by high-performance

155   size exclusion chromatography (HPSEC) with total organic carbon detectors, according to

156   Kawasaki et al., 2011; Shimotori et al., 2016. In the HPSEC analysis, there were two peaks of

157   weight-averaged molecular sizes (Mw) of $1.3 \pm 0.1 \times 10^5$ Da and $2.2 \pm 0.2 \times 10^3$ Da ($n = 117$),

158   and we defined them as high-molecular-weight DOC (HMW-DOC) and low-molecular-weight

159   DOC (LMW-DOC), respectively, in the present study.

160   For bacterial production (BP), bacterial DNA was extracted from the filter using the

161   Extrap Soil DNA Kit Plus ver. 2 (J-Bio21, NIPPON STEEL & SUMIKIN Eco-Tech Corp.,

162   Chiba, Japan). Bacterial cells were broken by glass beads in a Fast Prep FP120 Cell Disrupter

163   (speed, 6.0; time, 40 s; MP Biomedicals, Santa Ana, CA, USA), and the released DNA was

164   purified by using magnetic beads, according to the manufacturer's protocols. The DNA

165   extraction efficiency was considered as 100% (Tsuchiya et al., 2019). After the DNA extraction,

166   the samples were processed according to Tsuchiya et al., (2015) and Tsuchiya et al., (2020a) for

167   quantifying the ^{15}N-dA incorporation rates. Briefly. The DNA samples were hydrolyzed to

168   nucleosides by three enzymes (nuclease P1, Wako; phosphodiesterase I, Worthington

169   Biochemical Corp.; alkaline phosphatase, Promega Corp.). After the enzymatic hydrolysis, the

170   amount of ^{15}N-dA incorporated during the incubation was analyzed by LC-MS/MS using

171     $^{13}C_{10}{}^{15}N_5$-deoxyadenosine (CNLM-3896-CA, Cambridge Isotope Laboratories, Inc.) as a

172   surrogate (internal standard).

173   A cell-to-carbon conversion factor was obtained using the water sample collected at 5-m

174   depth of Sta. 12B in February 2018. Bacterial cells fixed with 1% glutaraldehyde were stained by

175   acridine orange, cell volumes were measured by epifluorescence microscopy (BX-40, Olympus,

176   Tokyo, Japan) with BX-FLA epifluorescence equipment, and the cell-to-carbon conversion

177   factor calculated using a conversion factor of 106 fgC μm^{-3} (Nagata, 1986), resulting in 11.8 fgC

178   cell^{-1}. The factor for converting ^{15}N-dA incorporation to cells produced was calculated as $1.83 \times$

179   10^6 cells (mol ^{15}N-dA)$^{-1}$ as determined by Tsuchiya et al., 2020b.

180   Bacterial abundance (BA) was estimated from measurements of 16S-rDNA

181   concentrations. The 16S-rDNA concentration was determined from the same extracted DNA

182   samples used for BP measurements. SYBR Green I PCR amplification was performed with a

183   LightCycler Instrument (Roche, Mannheim, Germany). For the real-time PCR assay of bacteria,

184   we used the primer set 357F (5′-CCTACGGGAGGCAGCAG-3′), 518R (5′-

185   GTATTACCGCGGCTGCTGG-3′). The 20-μL reaction mixture contained LightCycler 480

186   SYBR Green I Master (Roche), 4 pmol of each primer, and DNA extract. In the present study,

187   16S-rDNA concentration was converted to epifluorescence-enumerated bacterial cells (4′,6-

188   diamidino-2-phenylindole, DAPI) by using a conversion factor of 0.31 cells (16S-rDNA copy)$^{-1}$

189   as previously determined (Tsuchiya et al., 2020b).

190

3 Results

3.1. Bacterial production (BP)

193   Bacterial production (BP) in 2018 varied from 0.057 μgC L^{-1} d^{-1} at 40 m at Sta. 12B in

194   November to 18.2 μgC L^{-1} d^{-1} at 6 m at Sta. 12C in July (Fig. 2). BP was significantly positively

195   correlated with water temperature (Spearman's rank correlation test, $n = 104$, $p < 0.001$, Fig. 3a).

196   Variations of BP at certain water temperature levels (e.g., ca. 13–17°C and 28–32°C) were

197   relatively high for the surface samples (< 10 m). In March, BP in the surface samples along Line

198   12 (1.48 ± 0.15 μgC L^{-1} d^{-1}, $n = 3$) was 2.4 times that of Lines 15 and 17 (0.62 ± 0.12 μgC L^{-1}

199   d^{-1}, $n = 8$). There was no clear trend of horizontal BP variations in the middle and deeper

200   samples (> 10 m). In May, BP in the surface samples was relatively high from the eastern

201   stations of Lines 12 and 15 (12BC, 12C, and 15C; 5.27 ± 0.93 µgC L^{-1} d^{-1}, $n = 6$). BP in the

202   surface samples along Line 17 did not show a clear horizontal trend (2.69 ± 0.53 µgC L^{-1} d^{-1}, n

203   = 4). Among the middle and deeper samples, BP at 20 m at western stations (12A, 15B, and

204   17A) was relatively high. In July in the surface samples, BP was notably lower at the central

205   stations of Lines 15 and 17 (15B and 17B); in other words, BP was relatively high at the stations

206   closer to shore. At all stations, BP at 20 m was higher than in the deeper samples (> 20 m). As in

207   May, BP at 19 m at Sta. 17A was highest among middle and deep samples from all stations. In

208   November in the surface samples, BP was relatively high at the eastern stations of Lines 12 and

209   15 (12C and 15C; 4.10 ± 1.24 µgC L^{-1} d^{-1}, $n = 4$); as in May, there was no clear horizontal trend

210   of BP along Line 17 (2.59 ± 0.18 µgC L^{-1} d^{-1}, $n = 4$). BP at 20 m increased compared to the

211   other months, but there was no clear horizontal trend.

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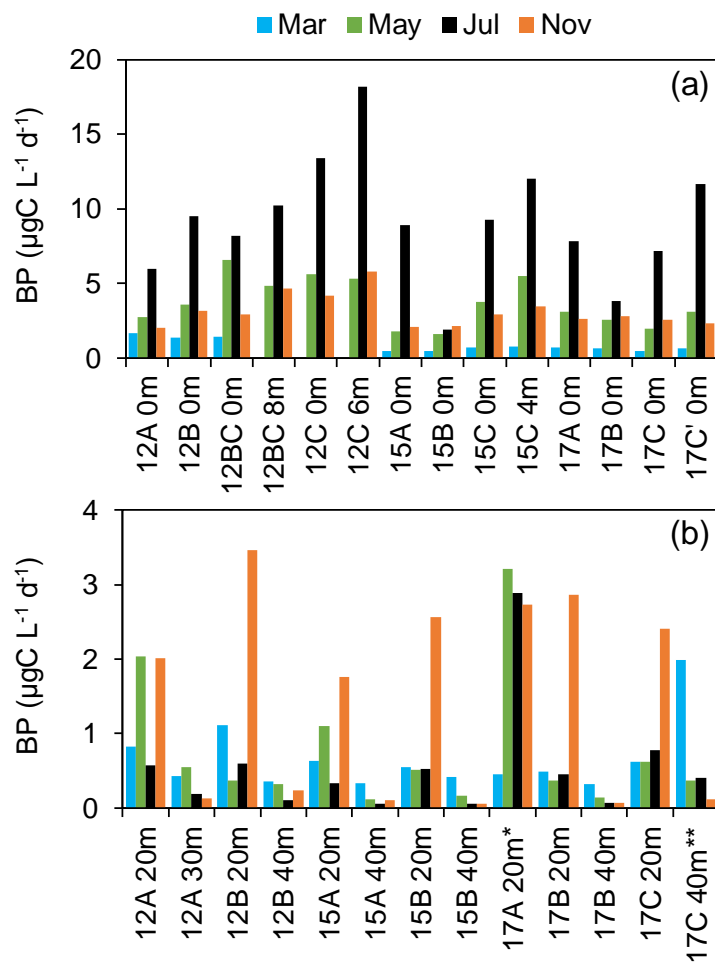
213   **Fig. 2** Bacterial production (BP) in the north basin of Lake Biwa. Sampling depths were < 10 m

214   (a) and > 10 m (b). Blue, green, black, and orange bars represent the samples collected in March,

215   May, July, and November 2018, respectively. * and ** indicate that sampling depths were 19 m

216   in July and 39 m in March, respectively.

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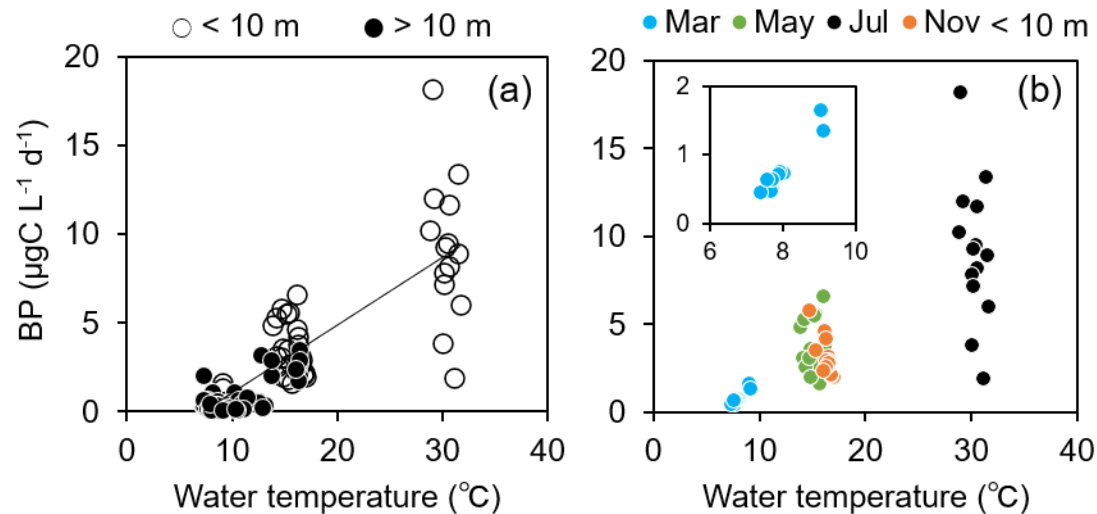
219   **Fig. 3** Bacterial production (BP) versus water temperature in the north basin of Lake Biwa,

220   Japan. (a) Open and filled circles represent < 10 m and > 10 m depths, respectively. (b) Blue,

221   green, black, and orange circles represent the samples collected in March, May, July, and

222   November 2018, respectively, in the surface waters (< 10-m depth).

223

224   **3.2 Relationships between bacterial production and environmental variables at the surface**

225   In the surface samples in March, BP was significantly positively correlated with water

226   temperature ($p < 0.01$, Fig. 3b, Table 2), whereas BP was negatively correlated to water

227   temperature in November. In other seasons, there were no significant relationships between BP

228   and water temperature. As to DOC, LMW-DOC, and HMW-DOC, BP was significantly

229   positively correlated to them in year-round (Fig. 4a-c, Table 2). However, BP in each season did

230   not correlate to them significantly. In the surface samples, BP was significantly correlated with

231   TP in May, July, and November (Fig. 4d, Table 2). Although BP did not significantly correlate to

232   TP in year-round, there was a significant relationship between BP and TP when winter data were

233   excluded (Year-round without winter, Table 2). BP was correlated to TN in May and July (Fig.

234   4e, Table 2). BP was correlated to Chl *a* in March, May, and July (Fig. 4f, Table 2).

235   There were significantly positive regressions between BA and BP in each season's

236   surface samples (Fig. 5). The slopes of the linear regressions were steepest in March (0.81),

237   almost the same in May and November (0.30 and 0.29, respectively), and gentlest in July (0.12).

238

239   **Table 2** Summary of spearman's rank correlation coefficients (rs) between bacterial production

240   (BP) and environmental variables in the epilimnion (< 10-m depth). WT; water temperature,

241   DOC; dissolved organic carbon, LMW-DOC; low-molecular-weight DOC, HMW-DOC; high-

242   molecular-weight DOC, TP; total phosphorus, TN; total nitrogen, Chl *a*: chlorophyll *a*, n.s.; not

243   significant

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

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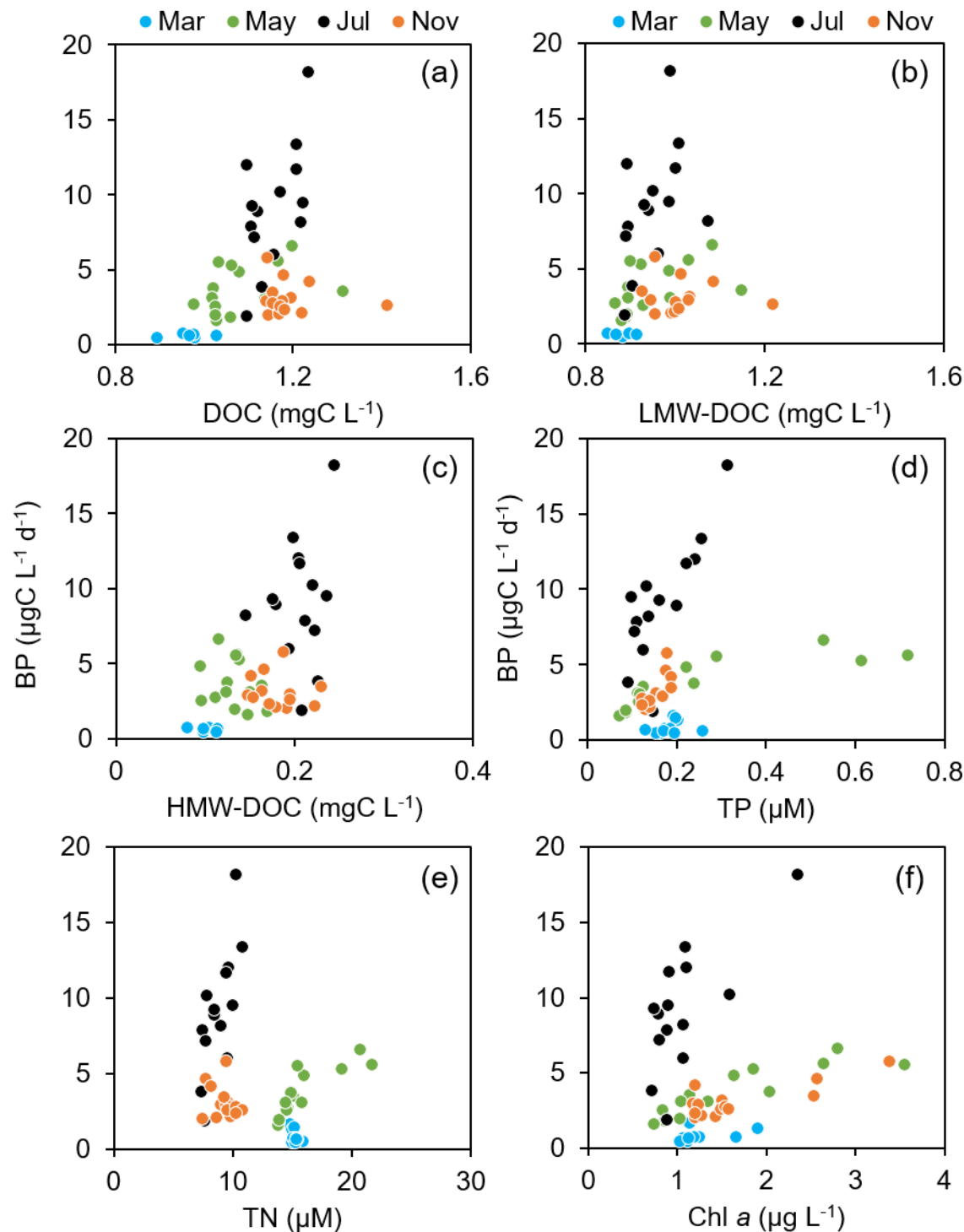
Fig. 4 Bacterial production (BP) versus (a) dissolved organic carbon (DOC) concentration, (b) low-molecular-weight DOC (LMW-DOC) concentration, (c) high-molecular-weight DOC (HMW-DOC) concentration, (d) total phosphorus (TP) concentration, (e) total nitrogen (TN) concentration, and (f) chlorophyll *a*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

250   the north basin of Lake Biwa, Japan. Blue, green, black, and orange circles represent the samples

251   collected in March, May, July, and November 2018, respectively.

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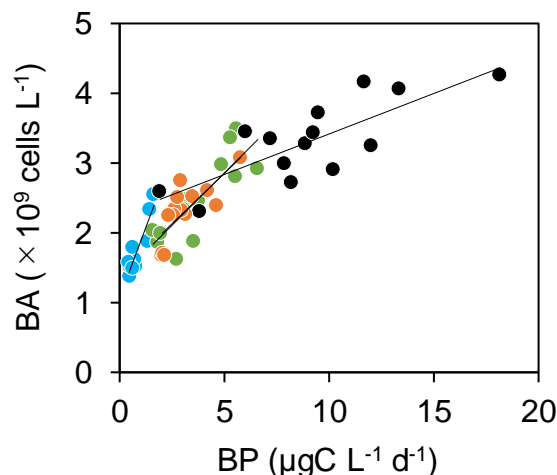
Fig. 5 Bacterial abundance (BA) versus bacterial production (BP) in surface waters of the north

255   basin of Lake Biwa, Japan, in March, May, July and November 2018 (blue, green, black and

256   orange circles, respectively). Regression lines for March: $[BA] = 0.81 \times [BP] + 1.1$ ($n = 10$, $R^2 =$

257   0.84, $p < 0.001$); May: $[BA] = 0.30 \times [BP] + 1.4$ ($n = 14$, $R^2 = 0.68$, $p < 0.001$); July: $[BA] =$

258    $0.12 \times [BP] + 2.3$ ($n = 14$, $R^2 = 0.62$, $p < 0.001$); and November: $[BA] = 0.29 \times [BP] + 1.4$ ($n =$

259   14, $R^2 = 0.60$, $p < 0.01$). The regression lines for May and November are almost

260   indistinguishable.

261

3.3 Depth-integrated BP

263   Depth-integrated BP (DBP) ranged from 2.0 mgC m^{-2} d^{-1} at Sta. 17C' in March to 110

264   mgC m^{-2} d^{-1} at Sta. 12B in July (Table 3). In March and November, DBP significantly increased

265   with maximum water depth (Fig. 6a, d). Although the regression of DBP on maximum depth for

266   May was not significant, when the three transects were analyzed together, the regressions were

267   significant when the Line 12 data were analyzed separately from those for Lines 15 and 17 (Fig.

268   6b). For July, there was no significant regression (Fig. 6c). No clear trends were found in the

269   relationship between DBP and distance from shore in March, May, and November (Fig. 6e, f, h).

270   In July, DBP at Sts. 15B and 17B (> 4 km offshore) were relatively low compared to the stations

271   closer to shore (Table 3, Fig. 6g). The inshore/offshore ratio of DBP was lowest in November

272   (0.58) and highest in July (2.1) (Table 3).

273

274   **Table 3** Depth-integrated bacterial production (BP) in Lake Biwa in 2018. Stations 15B and 17B

275   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

276   ND: No data
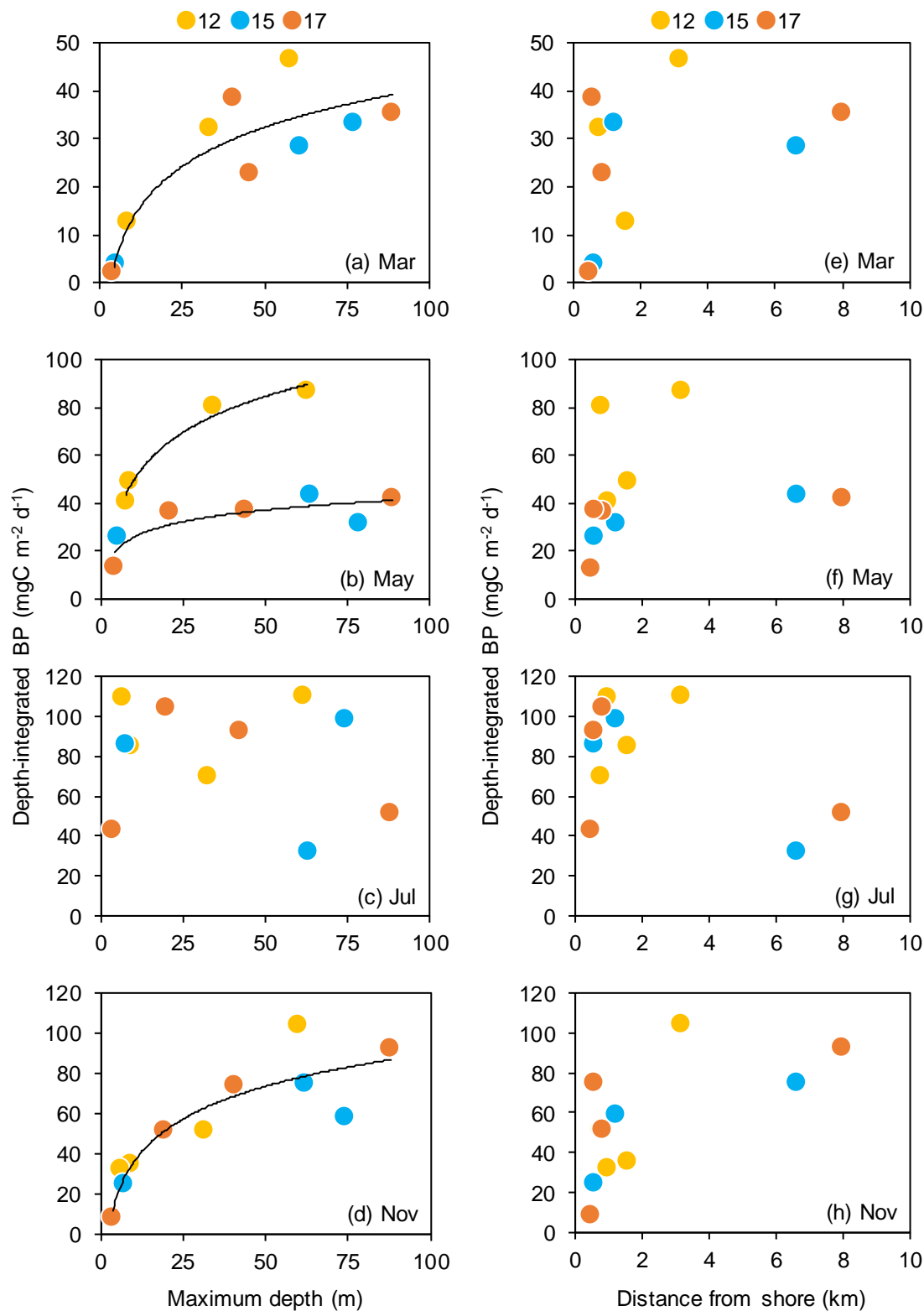
Fig. 6 Depth-integrated bacterial production (DBP) in each season versus maximum depth (left) or distance from shore (right) in the north basin of Lake Biwa, Japan. Yellow, blue and orange

280   plots represent Line 12, 15 and 17 transects, respectively. The regression curves represent (a)

281   [DBP] = 11.7 × ln [depth] – 13.4 ($n = 10$, $R^2 = 0.81$, $p < 0.001$); (b) [DBP_Line12] = 21.7 × ln

282   [depth] + 0.0885 ($n = 4$, $R^2 = 0.98$, $p < 0.05$) and [DPB_Lines15&17] = 7.02 × ln [depth] + 9.78

283   ($n = 7$, $R^2 = 0.71$, $p < 0.05$); and (d) [DBP] = 23.4 × ln [depth] – 18.1 ($n = 11$, $R^2 = 0.82$, $p <$

284   0.001).

285

4 Discussion

287   The present study examined spatial and seasonal variations of BP and environmental

288 variables in the Lake Biwa and found a significantly positive relationship between BP and water

289 temperature in year-round data, suggesting that water temperature was a primary BP regulation

290 factor. This relationship agrees with previous studies (Goosen et al., 1997; Pomeroy & Wiebe,

291 2001). However, the coefficient of variance (CV) of BP (0.35-0.49) was much larger than that of

292 water temperature (0.030-0.075) at the surface in each season; in other words, the BP variation

293 was more considerable than that of water temperature. The large BP variations in each season

294 were attributed to the horizontal distributions. The DOC, LMW-DOC, and HMW-DOC

295 concentrations correlated to BP in year-round data, whereas there were no significant

296 relationships in each season (Table 2), suggesting that DOC could not be an explanatory factor

297 for horizontal BP variations. The other environmental variables showed significant correlations

298 to BP in each season and can be explanatory factors for BP horizontal variations.

299   In March, BP in the surface samples sensitively responded to variations in water

300 temperature (Fig. 4a, Table 2); however, BP was correlated with neither TP nor TN

301 concentrations. The chl *a* concentration was correlated to water temperature ($rs = 0.79$, $n = 10$, p

302 < 0.05) as well as BP, suggesting that water temperature could be the primary limiting factor for

303 both bacterioplankton and phytoplankton in winter. The importance of water temperature

304 affecting bacterial growth, especially during winter, was also emphasized in estuarine and marine

305 environments (e.g. Shiah & Ducklow, 1994a, b). The slope of the linear regression of BA on BP

306 was highest in March (Fig. 5). The relationship between BA and BP has been used to evaluate

307 bottom-up and top-down regulation (Billen et al., 1990; Pace & Cole, 1994). The regressions of

308 BA as a function of BP should have a steep slope if BA is strongly controlled by bottom-up

309 factors such as resources and water temperature. Alternatively, when other factors such as

310 mortality are most important in regulating BA, there should be no relationships or shallower

311 slopes between them. The ingestion rates of bacteria by heterotrophic nanoflagellates, *Daphnia*

312 *galeata,* and *Bosmina longirostris* in Lake Biwa were relatively low in March (Nakano et al.,

313 1998). Therefore, in March, bacteria should have been regulated by bottom-up control, especially

314 by water temperature.

315   The latitudinal difference of water temperature (Line 12 > Lines 15 and 17) could be

316 attributed to Lake Biwa's climate conditions. Air temperatures in 20–23 March 2018, including

317 sampling days, at weather observation stations of the Japan Meteorological Agency around Lake

318 Biwa (https://www.jma.go.jp/jma/, last accessed on 6 March 2020) varied along a latitudinal

319 gradient, i.e., Line 12 > Line 15 > Line 17 in this period (see Supporting Information, Fig. A1).

320 The results suggest that climate conditions such as air temperature clearly linked to the

321 horizontal distribution of water temperature and consequently affected biological productivity

322 within the lake in winter.

323   In May, BP was significantly positively correlated with TP, TN, and chl *a* concentrations

324 (Fig. 4, Table 2). These results suggest that the effect of resource limitation on BP became more

325 pronounced. The spatial distribution of the loading of nutrients, organic matter, or both affects

326 the horizontal BP variation. It is related to higher BP at eastern stations of Lines 12 and 15 in the

327 surface samples. Nutrient discharges into Lake Biwa by rivers flowing into the eastern area of

328 the lake were larger than those in the western area, which is attributed to the high population

329 densities in the urbanized areas and many agricultural fields east of the lake (Taniguchi & Tase,

330 1999). Since TP and TN concentrations included bacterial biomass as a particulate form and

331 there was a significantly positive relationship between BP and BA (Fig. 5), the correlations with

332 TP and TN did not necessarily indicate limiting factors for BP. The relationship between BP and

333 various forms of N and P (dissolved/particulate and inorganic/organic) was significant only with

334 particulate nitrogen for N, but with dissolved total/organic phosphorus, PO_4^{3-}, and particulate

335 phosphorus for P (Table A1). The results suggest that the horizontal variations of BP could have

336 been regulated by phosphorus as their resource.

337   In July, the BP variations in the surface samples were larger than that in other seasons

338   (Fig. 3). A similar larger BP variation was observed in another lake (Lake Kasumigaura, the

339   second largest lake in Japan) at higher water temperatures; the variations of BP in Lake

340   Kasumigaura were attributed to autochthonous substrate supply of dissolved organic carbon from

341   primary production (Tsuchiya et al., 2019). In the present study, BP was significantly correlated

342   not to DOC, but rather to TP and TN concentration, suggesting that the large BP variations

343   should be attributed to phosphorus and/or nitrogen limitations. Since PO_4^{3-} concentrations were

344   below the detection limit in this season except for Sts. 12BC and 12C, we examined whether

345   particulate or dissolved P was the dominant source for BP (Fig. 7). Particulate P explained 64%

346   of the BP variations, although particulate P partially comprises bacteria, while dissolved P

347   explained 31% of the variation. However, we found obvious outliers (the two lowest BP values)

348   in the relationship between BP and dissolved P at Sts. 15B and 17B. When the two outliers were

349   excluded from the regression analysis, dissolved P also explained BP's variations (69%). The

350   results for these outliers suggest that particulate P should be a dominant P source for BP at the

351   surface of these offshore stations (15B and 17B). Besides, dissolved P could be transformed into

352   refractory substrates by photochemical and/or biological reactions. Exposure of surface-water

353   dissolved organic matter (DOM) to sunlight led to a 75% reduction of BP in marine ecosystems,

354   suggesting that the photomineralization of bioreactive DOM likely contributed to the decrease in

355   BP (Benner & Biddanda, 1998). Further study is needed to clarify the relationship between BP

356   and refractory dissolved organic P and material cycling in such strongly P-limited environments.

357   We uniformly observed relatively high BP in inshore stations, which we attribute to nutrient

358   loading via some combination of river inflow, groundwater seepage, and elution from sediments.

359   As for nitrogen, there were no significant relationships between BP and any dissolved forms of N

360   other than particulate nitrogen as well as the case in spring (Table A1), suggesting that N was not

361   likely to be a limiting factor for BP. The result is consistent with a previous study demonstrating

362   that bacterial growth at the surface in Lake Biwa was severely limited by phosphorus, but not

363   nitrogen and organic carbon (Gurung & Urabe, 1999).

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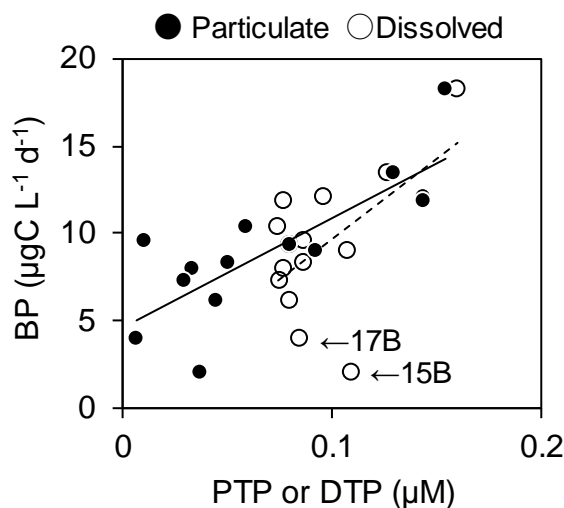
Fig. 7 Bacterial production (BP) versus particulate total phosphorus (PTP) and dissolved total phosphorus (DTP) concentrations in the surface waters of the north basin of Lake Biwa, Japan, in July 2018. The regression lines represent $[BP] = 62 \times [PTP] + 4.6$ ($n = 14$, $R^2 = 0.64$, $p < 0.001$) 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.
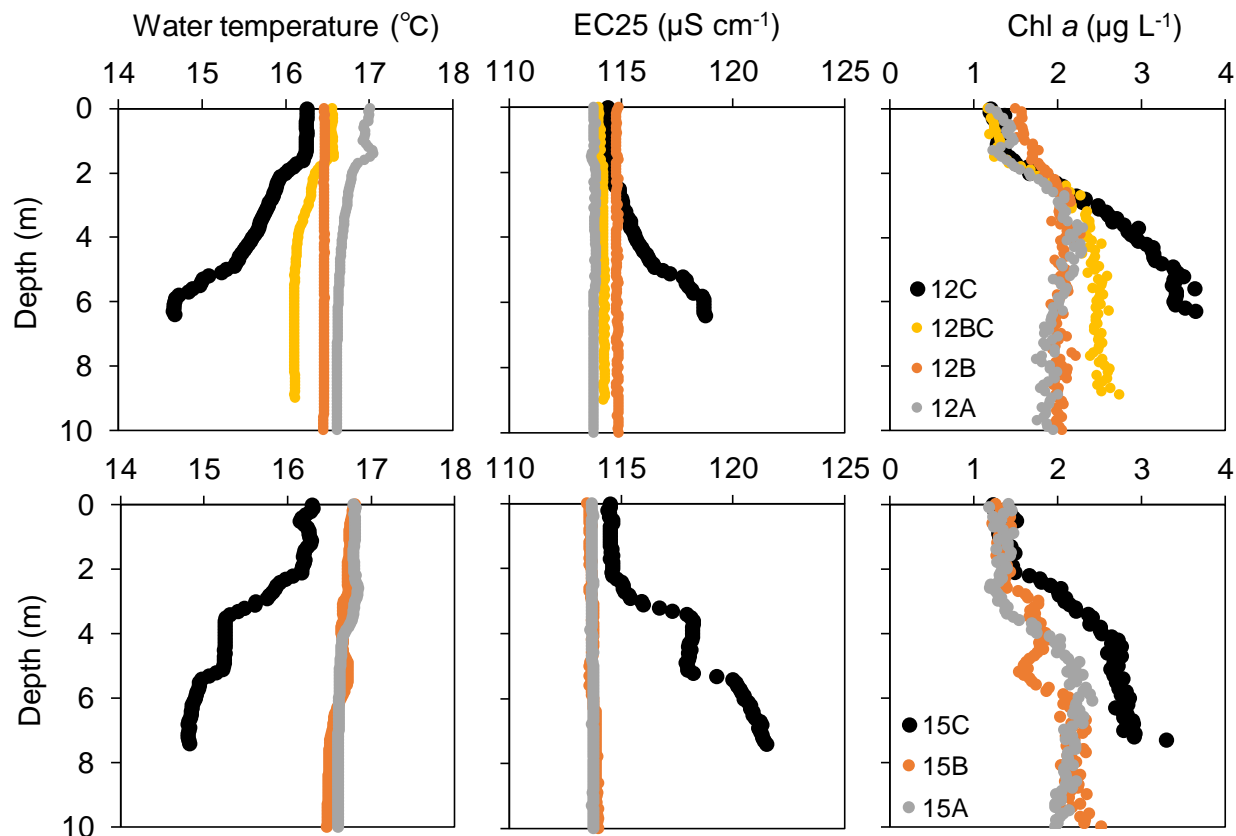
Fig. 8 Vertical distributions of water temperature, EC25, and chlorophyll *a* concentration (Chl *a*) in the north basin of Lake Biwa, Japan, in November 2018. Upper and lower figures represent Line 12 and 15 transects, respectively. Gray, orange, yellow and black circles represent stations A, B, BC, 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

397   ratios for horizontal variation (~9.5) seem larger than the range of diel variation shown above.

398   Moreover, the variations of BP in each season were related to the variations of environmental

399   variables (Figs. 4, 7, and 8). Especially in Lake Biwa, BP is severely limited by phosphorus, but

400   not organic carbon (Gurung & Urabe, 1999). The results suggest that nutrient supply processes

401   strongly influence the horizontal variability of BP in this lake.

402   The average DBP of inshore stations was 2.1 times that of offshore stations in July, and

403   the inshore/offshore ratio was higher than those in the other seasons (0.58–1.0; Table 3). To

404   estimate the relative contribution of inshore BP to the whole north basin of Lake Biwa (about

405   450 km^2 for inshore and 168 km^2 for offshore), the average DBP for inshore and offshore

406   stations was multiplied by the respective areas. In July, the integrated inshore and offshore BP

407   values were 39.9 tC d^{-1} and 7.0 tC d^{-1}, respectively, suggesting that the inshore BP accounted for

408   85% of whole-lake BP (46.9 tC d^{-1}). The inshore BP in March, May, and November accounted

409   for 66.9%, 73.8%, and 61.1% of the whole-lake BP, respectively; for these months, the

410   percentage contributions of inshore waters were equivalent to or lower than the percentage of the

411   inshore area (72.9% = 450 km^2/618 km^2) and disproportionate contributions of inshore BP was

412   not confirmed. The result agrees with the results shown in Fig. 6: DBP in March, May, and

413   November were better explained not by the distance from shore but rather by the maximum

414   depth. The results demonstrate that inshore BP disproportionately contributed to whole-lake BP

415   in the most productive season (July).

416   In conclusion, we quantified horizontal heterogeneity of BP in each season in Lake Biwa:

417   BP was regulated by primarily water temperature and secondarily phosphorus availability.

418   Dissolved organic carbon, including LMW- and HMW-DOC, did not link to horizontal

419   distributions of BP in the surface waters. The horizontal BP heterogeneity in the surface waters

420   was largest in summer (max/min ratio ~9.5), and minimum values were measured at offshore

421   stations. Moreover, mean DBP at inshore stations was 2.1 times that of offshore stations,

422   clarifying the substantial contribution of inshore to whole-lake BP, especially in the most

423   productive season.

424

425   **Acknowledgments**

432

433   **References**

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544 **Table A1** Summary of spearman's rank correlation coefficients (rs) between bacterial production

545 (BP) and environmental variables at the surface samples (< 10 m). PN; particulate nitrogen,

546 DTN; dissolved total nitrogen, DON; dissolved organic nitrogen, PP; particulate phosphorus,

547 DTP; dissolved total phosphorus, DOP; dissolved organic phosphorus, n.s.; not significant, ND:

548 not determined

Season		PN	DTN	NO_{2+3}	NH_4	DON	PP	DTP	PO_4	DOP
Spring	rs	**0.864**	0.4945	0.4593	-0.1077	0.4989	**0.947**	**0.692**	**0.679**	**0.684**
(May)	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	rs	**0.6689**	-0.1209	-0.4158	0.2967	-0.0945	**0.7846**	0.3055	ND	0.3275
(July)	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.

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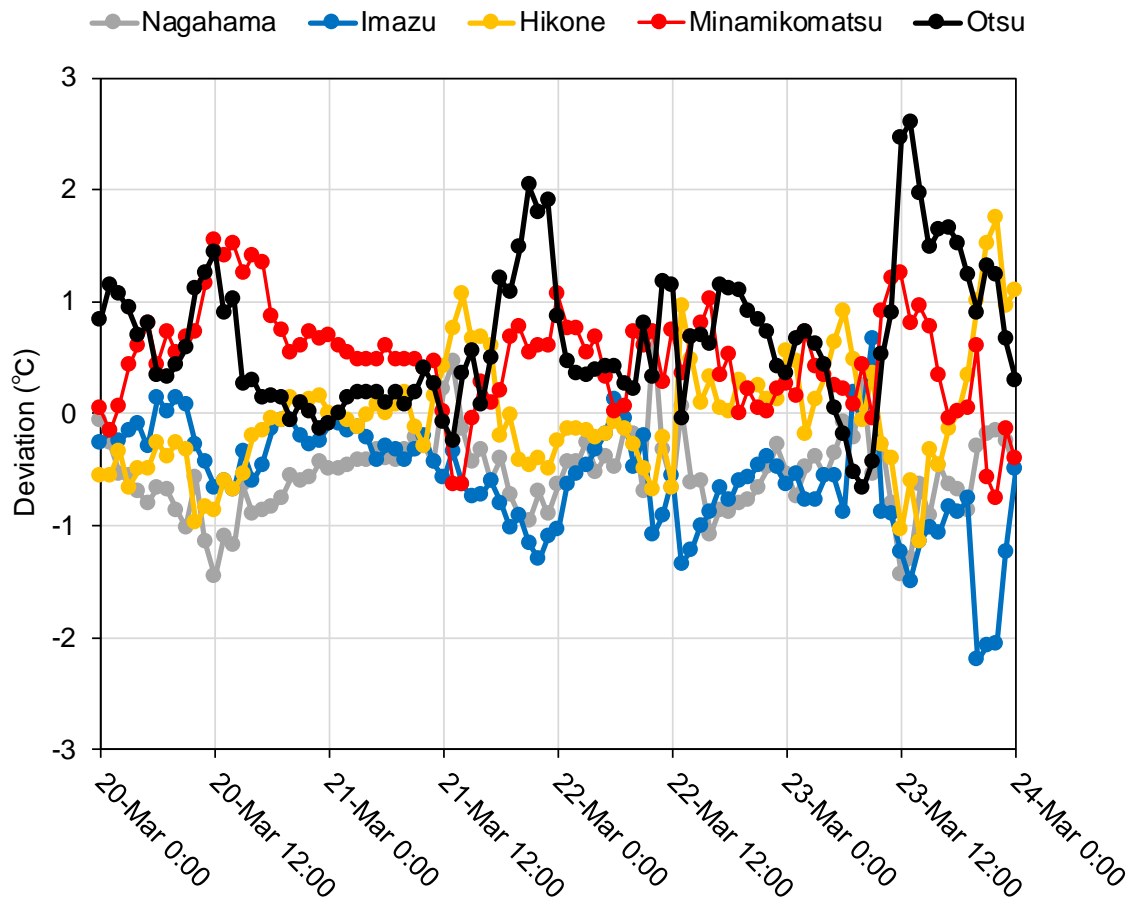
552 **Fig. A1.** Deviations from the mean of air temperature from 0:00 on 20 March to 0:00 on 24

553 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).