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**Isoprene production in seawater of Funka Bay, Hokkaido,  
Japan**

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Short title: Isoprene production in Funka Bay

Key words: Volatile organic compound (VOC), phytoplankton, bloom, photosynthesis,  
dark production, C<sub>5</sub>H<sub>8</sub>, hydrocarbon

28 Abstract (less than 250 words)

29 We carried out shipboard observations in Funka Bay, Hokkaido, Japan, monthly or  
30 bimonthly from December 2015 to November 2016. We measured vertical profiles of  
31 isoprene, chlorophyll-a (chl-a), and other parameters from surface to bottom layer  
32 (about 95 m) near the center of the bay. We found substantial increases in isoprene  
33 concentration in the surface mixed layer from February to March during the peak of the  
34 spring diatom bloom, in the bottom layer from March to April after the peak of the  
35 bloom, and in the subsurface layer (below the surface mixed layer) in summer from July  
36 to August, where there were also substantial chl-a concentration maxima. We attribute  
37 the isoprene increases in the surface and subsurface layers to photosynthetic production  
38 of isoprene by the dominant phytoplankton in the spring bloom and in summer, and that  
39 in the bottom layer to dark production of isoprene by diatom aggregates that settled  
40 from the surface euphotic zone. We also measured isoprene production in laboratory  
41 incubation experiments. The in-situ production rates of isoprene per unit chl-a in the  
42 surface mixed layer in the spring bloom, in the dark bottom layer during the bloom, and  
43 in the subsurface layer in summer ( $0.82$ ,  $0.03\text{--}0.13$ , and  $7.38$   $\text{pmol} (\mu\text{g chl-a})^{-1} \text{d}^{-1}$ ,  
44 respectively) were consistent with our incubation results. We believe that this is the first  
45 report focused on dark production of isoprene by diatoms; the production rate of  
46 isoprene under the dark condition ranged from 4% up to 16% of that by photosynthesis.

47

## 48 **1. Introduction**

49 Isoprene (2-methyl-1,3-butadiene; C<sub>5</sub>H<sub>8</sub>) comprises about half of the total global  
50 biogenic volatile organic compound (BVOC) emissions of 1 Pg (Guenther et al. 2012).  
51 Isoprene influences the oxidation capacity of the air by reacting with hydroxyl radicals  
52 (OH<sup>-</sup>), nitrate radicals (NO<sub>3</sub>), and ozone (O<sub>3</sub>) (Lelieveld et al. 2008). Although  
53 terrestrial vegetation accounts for the largest emissions of isoprene, marine-derived  
54 isoprene can also influence the oxidation capacity of remote marine air because the  
55 lifetime of isoprene in marine air (several hours) is much less than the transport time  
56 (several days) from terrestrial sources to remote regions of the ocean (Broadgate et al.  
57 1997; Palmer and Shaw 2005).

58 Marine isoprene is thought to be directly emitted from marine phytoplankton  
59 (e.g. Dani et al. 2017) and macroalgae (e.g. Broadgate et al. 2004) in association with  
60 their photosynthesis. A depth profile of isoprene concentration in the Gulf Stream off  
61 the Florida coast is similar to that of biological productivity as indicated by chl-a  
62 fluorescence (Milne et al. 1995). A global map of isoprene distribution obtained from  
63 some snap-shot observations (Ooki et al. 2015) shows high concentrations of isoprene  
64 (75–165 pmol L<sup>-1</sup>) localized in subtropical–subarctic transitional waters of shelf and  
65 slope areas in the western northwest Pacific Ocean near Japan in high productive season,  
66 where there was a spring phytoplankton bloom and chl-a concentrations were notably  
67 high (4.1–10.3 μg L<sup>-1</sup>).

68 High correlations between marine isoprene and chl-a have been reported in  
69 many studies; for example in the northwest Pacific Ocean near Japan (Kurihara et al.  
70 2010), Sagami Bay, Japan (Kurihara et al. 2012) and the East China Sea (Li et al. 2018).  
71 Isoprene production rates per unit chlorophyll (mol [g chl-a]<sup>-1</sup> d<sup>-1</sup>) by phytoplankton  
72 were measured in a number of incubation experiments, as summarized in Table 2 of  
73 Shaw et al. (2010), ranging over two orders of magnitude, depending on phytoplankton  
74 species and light intensity.

75 Isoprene production rates have been previously calculated from the measured isoprene  
76 concentrations in the surface mixed layer of the ocean basins (southeastern Pacific and  
77 southern Indian oceans) by three snap-shot observations assuming a steady state for  
78 isoprene in the mixed layer (Booge et al. 2018). The spatial variation of isoprene  
79 production rates was influenced by temperature, light intensity, nutrient levels, and  
80 salinity, which are also important factors for determining the plankton community and  
81 productivity. The steady-state assumption would be appropriate for calculating the  
82 spatial distribution of isoprene production on a basin scale; however, the isoprene  
83 concentrations vary widely with time. For example, the isoprene concentrations in the  
84 surface mixed layer doubled in 10 days within an iron-fertilized patch, where the  
85 phytoplankton cell numbers showed 4- to 20-fold increases over the same period  
86 (Moore and Wang 2006). The isoprene concentrations obviously respond to  
87 phytoplankton growth within several days.

88         Arnold et al. (2009) have evaluated the global ocean as an isoprene source by  
89 combining isoprene production rates of several phytoplankton types measured in  
90 laboratory incubations, satellite datasets of ocean biology, field observation datasets,  
91 and a global atmospheric chemistry model. They recommended that the combination of  
92 laboratory-measured individual phytoplankton isoprene productivities should be  
93 compared with true community isoprene productivities obtained from field observations.  
94 Therefore, isoprene production rate by a natural biological community should be  
95 evaluated within an identical water mass by time-series observation conducted in a  
96 semi-closed ocean area, such as semi-closed bay.

97         Many of previous studies focused on photosynthetic production of isoprene by  
98 plants, however, it has been reported that isoprene can also be formed by higher plants  
99 via the breakdown of stored carbohydrates when photosynthesis is blocked (Lerdau et al.  
100 1997). It has been reported that the dark release of isoprene occurs in cells of all living  
101 organisms (Sanadze, 2004). A dark release of isoprene by marine organisms has not

102 been mentioned so far, it is possible that the dark releases by marine organisms  
103 including micro algae can increase the isoprene concentration in seawater. As for  
104 isoprene consumption, several studies have reported that the soil provides a sink for  
105 atmospheric isoprene due to consumptions by microorganisms in the soil (Cleveland  
106 and Yavitt, 1998), and that the ubiquitous marine hydrocarbon-degrader, *Alcanivorax*  
107 *borkumensis*, can degrade isoprene in seawater (Alvarez et al., 2009). The turnover time  
108 of isoprene by chemical loss, mainly reaction with OH, has been estimated to be 19  
109 days which is much shorter (< 10%) than timescale of the biological production rate  
110 (Palmer and Shaw, 2005). Therefore, we have to notice that the isoprene production in a  
111 natural biological community is a net production including biological and chemical  
112 degradations as well as biological productions.

113           The purpose of this study was to estimate in-situ production rates of isoprene in  
114 a natural biological community from time-series observations in Funka Bay, Hokkaido,  
115 Japan. The observed in-situ production rates were compared with the rates measured by  
116 phytoplankton incubation experiments. On the basis of the observation and incubation  
117 results, we propose a new isoprene production process—dark production of isoprene by  
118 phytoplankton—which is not directly associated with the photosynthetic production of  
119 isoprene.

120

## 121 **2. Methods**

### 122 **2.1 Shipboard observations and seawater sampling**

123 Funka Bay is separated from the northwestern North Pacific Ocean by an 80-m-deep sill.  
124 The bay water exchanges with the open-ocean water of the North Pacific twice a year,  
125 mainly in early spring and in autumn (Ohtani and Kido 1980). In most years, a massive  
126 phytoplankton bloom consisting mainly of diatoms occurs in the surface layer, covering  
127 most of the bay from early March to early April (Odate et al. 1993; Kudo et al. 2007).  
128 We conducted observations monthly or bimonthly from December 2015 to November

129 2016 using the Training Ship *Ushio-maru*, operated by the School of Fisheries Sciences,  
130 Hokkaido University. Seawater was sampled at the center of Funka Bay (Station [St.]  
131 30; 42°16.2 N, 140°36.0 E; bottom depth, 96 m; Fig. 1) on 15 December 2015, and 5  
132 February, 22 March, 13 April, 17 May, 29 July, 27 August, and 1 November 2016.  
133 Conductivity-temperature-depth (CTD; SBE-19plus, Sea-Bird Electronics, Inc.,  
134 Washington, USA) observations without water sampling were carried out at 24 stations  
135 in the bay on 7 March and other dates.

136 Our methods for Funka Bay observations and seawater measurements have  
137 been recently reported (Shimizu et al. 2017). Seawater samples were collected in 2-L  
138 Niskin bottles attached to a rosette multisampler along with a CTD probe (SBE-19plus)  
139 and a photosynthetically-active-radiation (PAR) sensor. PAR measurements in  
140 December 2015 and February 2016 were not well executed. Surface seawater was  
141 collected with a plastic bucket, and bottom water was collected approximately 1 m  
142 above the seafloor with a Van-Dorn sampling bottle. The sampling depths were 0, 5, 10,  
143 20, 30, 40, 50, 60, 65, 70, 75, 80, 85 and “bottom-minus-1” m (approximately 95 m). In  
144 December 2015 and February 2016, seawater samples were collected from only 6 and 4  
145 depths, respectively, using a Van-Dorn sampling bottle and a bucket. Seawater aliquots  
146 (125 mL) were collected into dark glass bottles, each of which was overflowed with  
147 approximately 250 mL of seawater.

148 Microbial activity was arrested by the addition of 50  $\mu$ L of saturated mercuric  
149 chloride ( $\text{HgCl}_2$ ) solution. The bottles were crimp-sealed with a headspace of 0.5 mL by  
150 using an aluminum cap and a septum lined with butyl rubber. The sample bottles were  
151 kept in the dark at 4°C until pretreatment, which was typically carried out within 2  
152 weeks of sampling.

153

## 154 **2.2 Isoprene analysis**

155 Dissolved isoprene was collected from seawater samples by the purge-and-trap method.

156 The total volume of seawater (125 mL) in each sample bottle was introduced into a  
157 purge vessel by means of a high-purity nitrogen flow at 20 mL min<sup>-1</sup> for 7 min, and the  
158 dissolved gases were then purged by bubbling with nitrogen at a flow rate of 65 mL  
159 min<sup>-1</sup> for 35 min at 60°C. The purged gases were collected in a cold trap containing  
160 Tenax TA (GL Science, Tokyo, Japan) adsorbent resin (10 mg) cooled to -90°C. The  
161 cold trap was then sealed with 1/16-inch screw nuts (Swagelok, solon, OH, USA) and  
162 stored in a freezer (-30°C) prior to analysis by gas chromatography-mass spectrometry  
163 (GC-MS), typically within two weeks. The purge efficiencies for isoprene were nearly  
164 100%.

165 Concentrated isoprene in the cold trap was thermally desorbed (200°C) from  
166 the resin and transferred to an automated preconcentration GC-MS system (Agilent  
167 5973, 6890; Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary  
168 column (CP-Porabond Q, 0.32 mm × 50 m, Agilent Technologies).

169 Gravimetrically-prepared standard gas (Taiyo Nissan, Inc., Tokyo, Japan)  
170 containing isoprene at 10 parts per billion (ppb) was used to calibrate the GC-MS  
171 system. The standard gas measurements were carried out once a day. The analytical  
172 precision for isoprene of ±4% (=standard deviation/mean) was evaluated from the  
173 repetitive measurement ( $n = 6$ ) of a water-based standard solution. Details of the  
174 GC-MS analysis are described elsewhere (Ooki and Yokouchi 2011a, b, Ooki et al.,  
175 2015b).

176

### 177 **2.3 Chl-a and nutrient concentrations**

178 Chl-a concentrations in discrete seawater samples (100 mL) were measured by the  
179 Welschmeyer method (Welschmeyer 1994) using a fluorometer (10-AU-005, Turner  
180 Designs, San Jose, CA, USA). Concentrations of nitrogenous nutrients (NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>,  
181 and NH<sub>4</sub><sup>+</sup>) in discrete seawater samples were measured by the colorimetric method with  
182 a QuAAtro 2-HR system (BL-tec, Osaka, Japan; Seal Analytical, Norderstadt,

183 Germany). Analytical precisions were 0.5% for  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$  as determined  
184 by repetitive measurement ( $n = 5$ ) of reference seawater for nutrient standards (KANSO,  
185 standard Lot BT, Osaka, Japan). We used the combined concentrations of these  
186 compounds as the dissolved inorganic nitrogen (DIN) concentration.

187

## 188 **2.4 Incubation experiments**

189 Isoprene production was investigated in two phytoplankton incubation experiments.  
190 Culture medium seawater was collected from a coastal region near Hokkaido in the  
191 northern Japan Sea (salinity = 33.8) and was filtered through an acid-cleaned  $0.22 \mu\text{m}$   
192 cellulose membrane filter (Millipore). The filtered seawater was autoclaved for 20 min  
193 at  $121^\circ\text{C}$ . Subcultured strain of *Thalassiosira weissflogii* CCMP1336 was added to the  
194 culture medium with f/2 nutrient (Price et al., 1989). The initial cell density of  
195 *Thalassiosira weissflogii* in the medium was controlled to be approximately 1000 cells  
196  $\text{mL}^{-1}$ . One-hundred milliliters of culture medium was poured into a glass bottle (120  
197 mL), which was crimp-sealed with an aluminum cap and septum. These operations were  
198 done in a clean bench. Glass bottles were moved to an incubator at temperature of  $20^\circ\text{C}$ ,  
199 and light intensity of  $100 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  from an LED light with a light:dark cycle  
200 of 13-h:11-h. A culture without diatoms was incubated as a control. Fig 2 (upper panel)  
201 is a schematic diagram showing the culture conditions and the process for isoprene  
202 collection from the incubation bottles. Incubation lasted 85 days in experiment I and 53  
203 days in experiment II. Incubation bottle in experiment II was moved into continuous  
204 dark conditions on day 12 to investigate the dark production of isoprene. The number of  
205 experiment bottle and control bottle was one for each experiment.

206 Head-space gas in incubation bottles was collected every 2 or 3 days during the  
207 incubation periods (53 or 85 d). Pure air (20%  $\text{O}_2$  and 80%  $\text{N}_2$ ) mixed with  $\text{CO}_2$  (400  
208 ppm) was introduced into the bottle through an injection needle at a flow rate of  $50 \text{ mL}$   
209  $\text{min}^{-1}$  for 30 min while the culture medium was stirred by a magnetic stirrer. The

210 head-space gas was collected through another needle, and the water vapor in the gas  
211 was removed by using a Nafion drying tube (Perma Pure, Lakewood, NJ, USA). To  
212 concentrate isoprene, the sample gas was then introduced into a cold trap containing 10  
213 mg Tenax TA absorbent resin at  $-90^{\circ}\text{C}$  using a Free Piston Stirling Cooler (FPSC;  
214 Twinbird, Ltd., Tsubame-Sanjo, Japan). The cold traps were stored in a freezer at  $-25^{\circ}\text{C}$   
215 until GC-MS analysis. After the collection of isoprene, the incubation bottle was opened  
216 by removing the butyl rubber septum, and 250  $\mu\text{L}$  of culture medium was collected to  
217 measure the chl-a concentration and cell density. The bottle was then re-sealed with  
218 septum and aluminum cap and the incubation continued. The 250  $\mu\text{L}$  sample was diluted  
219 with 5.0 mL of filtered seawater. Cell density in the diluted sample was measured by  
220 triplicate cell counts by an optical microscope, and chl-a concentration in the diluted  
221 sample was measured by Welschmeyer method after filtration and DMF extraction.

222         The collection efficiency for isoprene in the incubation bottles using this  
223 method was 80% at room temperature (about  $20^{\circ}\text{C}$ ). We adjusted the isoprene  
224 production rates in the incubation bottles assuming that 20% of the isoprene remained in  
225 the bottle after each collection. Calculation method of isoprene production rate was  
226 drawn in a lower panel of Fig 2.

227         The mean concentration of isoprene in a series of control incubation  
228 bottles—with culture medium but no diatoms—was  $11.7 \pm 1.7 \text{ pmol L}^{-1}$  ( $n = 40$ ). This  
229 means that there was on average  $1.17 \pm 0.17 \text{ pmol}$  of isoprene contamination in the  
230 incubation bottles (water volume of 0.1 L and gas head-space of 0.02 L). Contamination  
231 likely occurred when the bottle was opened to collect water for chl-a analysis. Although  
232 we did not measure the isoprene concentration in the laboratory air, ambient room-air  
233 concentrations were reported as 1.4 ppb in China (Duan et al. 2016) and 3.6–7.2 ppb in  
234 the UK (Wang et al. 2017), which are several times higher than in outside air. The main  
235 source of isoprene in room air is human breath (Stonner et al. 2017). If the gas  
236 head-space in the incubation bottles (0.02 L) was replaced with room air having an

237 isoprene concentration of 1.4–7.2 ppb, the isoprene contamination would amount to  
238 1.3–6.4 pmol. We assumed the detection limit for the difference in isoprene  
239 concentrations between the incubation bottles and the control bottles ([Incubation bottle  
240 C<sub>5</sub>H<sub>8</sub>] – [Control C<sub>5</sub>H<sub>8</sub>]) to be two times the standard deviation ( $=2 \times 1.7 \text{ pmol L}^{-1}$ ) of  
241 the blank (control) measurement.

242 Isoprene concentrations in the incubation bottles were 76–607 pmol L<sup>-1</sup> under  
243 light-dark cycle conditions (hereinafter, light conditions) (28 samples in experiment (I)  
244 + 4 samples in experiment (II)), and 10.5–38.3 pmol L<sup>-1</sup> under dark conditions (15  
245 samples in experiment (II)). The concentration differences between incubation and  
246 control bottles were 69.3–570 pmol L<sup>-1</sup> in the light and –2.4 to 138.8 pmol L<sup>-1</sup> in the  
247 dark. The concentration differences for 5 samples (5 sets of incubation and control  
248 bottles) under dark conditions were below the detection limit ( $<3.4 \text{ pmol L}^{-1}$ ), whereas  
249 those for 10 samples under the dark were above the detection limit ( $13.7 \pm 10.1 \text{ pmol L}^{-1}$ ,  
250 mean  $\pm$  standard deviation,  $n = 10$ ).

251

### 252 **3. Results and discussion**

#### 253 **3.1 Hydrographic features**

254 Funka Bay is influenced by two main water masses that enter from the open ocean:  
255 Tsugaru (T) water that originates from the subtropical North Pacific Ocean and Oyashio  
256 (O) water that originates from the subarctic North Pacific. In addition, there are two  
257 water masses formed by changes in the bay: winter (W) water is formed by winter  
258 cooling of T water, and summer (S) water is formed by summer warming and salinity  
259 lowering of O water. In this study, we classified the water masses in the bay according  
260 to the temperature–salinity ranges listed in Table 1, which are modified from Ohtani  
261 and Kido (1980) because the temperature range of O water was expanded for this study.

262 The temperature–salinity ranges for classification of the water masses are  
263 plotted in Figure 3. In addition to the four main water masses—T, W, O, and S—we

264 defined transition water types: mixing types ‘WO’, ‘WOT’, and ‘ST’, and temperature  
265 changing types ‘TW’ and ‘OS’. These transition waters filled in areas of the  
266 temperature–salinity ranges not occupied by the four main water masses (Fig. 3). For  
267 example, WO water is a transition water between W and O waters.

268 We produced vertical profiles of temperature and salinity at the sampling  
269 station (St. 30) in Funka Bay (Fig. 4). We used the temperature–salinity ranges of the  
270 six water masses to show the temporal variation of the water-mass structure at St. 30 in  
271 the bay (Fig. 5).

272 In December, Funka Bay was occupied by high-salinity T water at all depths. This  
273 water cooled in winter, transitioning to TW water in February and W water in March.  
274 Low salinity O water was found at the surface in the mouth of Funka Bay on 7 March  
275 (not shown in Fig. 5), and this water flowed to the sampling station (St. 30), changing  
276 the water there from W water to WO water on 22 March (0–10 m), to O and WO water  
277 (0–35 m) on 13 April, and to O and WO water (0–75 m) on 17 May (Fig. 5). The W  
278 water remained in the bottom layer (75–95 m) from March to May. The O water was  
279 warmed by solar radiation in summer, changing to S water in July (0–18 m). In July and  
280 August, high-salinity T water flowed in the middle to deep layers of the bay from the  
281 open ocean, changing the S water to ST or WOT waters (18–95 m) in July, to T water  
282 (72–95 m) in August, and to T water throughout the water column (0–95 m) in  
283 November.

284

### 285 **3.2 Seasonal variation in vertical profiles of biochemical parameters and light**

286 We generated separate plots of the vertical distributions of isoprene ( $C_5H_8$ ), apparent  
287 oxygen utilization (AOU), DIN, and chl-a for December–April (Fig. 6a) and May–Nov  
288 (Fig. 6b). Vertical profiles of PAR for March, April, May, July, and August are  
289 presented separately (Fig. 7). We also determined the depth at which PAR was 0.5% of

290 surface PAR, and the mixed-layer depth, defined as the depth at which the potential  
291 density is higher by  $0.05 \text{ kg m}^{-3}$  than that at the reference depth of 5 m (Fig. 6a, b).

292

### 293 **3.2.1 Chl-a, DIN, and oxygen concentrations**

294 In February, chl-a concentrations were uniform for the depth range of 0–85 m with an  
295 mean of  $0.84 \mu\text{g L}^{-1}$ . The chl-a concentrations increased substantially from February to  
296 March at all depths. The mean on 22 March was  $9.3 \mu\text{g L}^{-1}$ , and maxima were found at  
297 5 m ( $16.8 \mu\text{g L}^{-1}$ ) and 95 m ( $11.7 \mu\text{g L}^{-1}$ ). These concentrations are consistent with  
298 previously reported maximum values ( $5\text{--}20 \mu\text{g L}^{-1}$ ) over a period from February to  
299 April during the spring diatom bloom at the same location in Funka Bay (Odate et al.  
300 1993; Kudo et al. 2007). We suggest that we observed the peak of the spring diatom  
301 bloom on 22 March. The concentration maximum of chl-a in the deepest water on 22  
302 March ( $9.3 \mu\text{g L}^{-1}$ ) suggested that large amounts of diatoms were suspended near the  
303 bottom during the peak of (or just after the peak) diatom bloom. The data suggest that  
304 large amounts of diatom aggregates produced in the surface euphotic zone had been  
305 sinking from the surface to the bottom.

306 Chl-a concentrations decreased from March to April at all depths. Nevertheless,  
307 there were still high concentrations of chl-a ( $3.0\text{--}5.7 \mu\text{g L}^{-1}$ ) in April at 30–50 m, which  
308 was below the mixed-layer depth of 11 m. The depth of 0.5% of surface PAR, which is  
309 thought to be the compensation depth (photosynthesis = respiration), was 15 m in  
310 March, and the PAR became zero in the middle layer shallower than 60 m in March and  
311 April. Presumably the large amounts of phytoplankton below 60 m in March and April  
312 could not photosynthesize in the dark. Negative values for AOU found at depths of 0–  
313 40 m in March and 0–30 m in April indicated that there was net production  
314 (photosynthesis > respiration) in those layers. The decrease of the DIN concentrations in  
315 the surface mixed layer from February ( $8.1\text{--}12.7 \mu\text{mol L}^{-1}$  at 0–95 m) to March ( $<0.05\text{--}$   
316  $3.1 \mu\text{mol L}^{-1}$  at 0–35 m) indicated that nutrients in the surface mixed layer of the bay

317 had been consumed by the massive diatom bloom in March. Despite DIN depletion in  
318 the surface mixed layer ( $<0.05 \mu\text{mol L}^{-1}$  at 0–11 m) in April, there were moderate chl-a  
319 concentrations ( $0.7\text{--}2.7 \mu\text{g L}^{-1}$ ) at the surface. It is possible that the inflow of O water in  
320 April supplied nutrients to the surface of the bay and maintained new production in the  
321 surface waters.

322         There was a clear chl-a maximum at the surface (0 m) on 29 July ( $0.71 \mu\text{g L}^{-1}$ ),  
323 despite DIN depletion ( $<0.05 \mu\text{mol L}^{-1}$ ) in the mixed layer. We suggest that new  
324 production at the surface was supported by a temporary supply of nutrients through  
325 freshwater inputs (river and rain) before 29 July because the salinity decreased by 0.9 at  
326 5-m depth from 17 May to 29 July. On 27 August, there was a distinct chl-a maximum  
327 (approximately  $0.31 \mu\text{g L}^{-1}$ ) at 20–50 m depths, just below the surface mixed layer (17  
328 m), and within the euphotic zone with sufficient DIN ( $0.52\text{--}8.2 \mu\text{mol L}^{-1}$ ) to support  
329 primary production. This type of chlorophyll maximum is commonly observed in  
330 summer in the open ocean and is referred to as a “subsurface chlorophyll maximum”.  
331 The subsurface chl-a maximum layer shallower than 30 m was oligotrophic condition  
332 with low nutrient level (DIN =  $0.52 - 0.70 \mu\text{mol L}^{-1}$ ). In November, there were  
333 moderate chl-a concentrations ( $0.87\text{--}1.04 \mu\text{g L}^{-1}$ ) in the surface mixed layer (0–57 m)  
334 with high concentrations of DIN ( $3.50\text{--}3.80 \mu\text{mol L}^{-1}$ ).

335

### 336 **3.2.2 Isoprene ( $\text{C}_5\text{H}_8$ )**

337 In December and February, isoprene concentrations were vertically uniform from winter  
338 deep mixing, averaging  $21.8$  and  $22.8 \text{ pmol L}^{-1}$ , respectively. These mean values were  
339 half of the annual mean of  $42.8 \text{ pmol L}^{-1}$  during the observation period and consistent  
340 with the open-ocean mean of  $19.7 \text{ pmol L}^{-1}$  in the northwest Pacific near Japan in April  
341 (Kurihara et al. 2010).

342         The concentrations increased from 5 February to 22 March over the depth  
343 range of 0–50 m. We suggest that there was photosynthetic production of isoprene

344 during the massive diatom bloom in March. High isoprene concentrations in the mixed  
345 layer (0–35 m) on 22 March were vertically uniform within the narrow range of 56.8–  
346 67.8 pmol L<sup>-1</sup>. The concentrations in this depth range decreased slightly from 22 March  
347 to 13 April. Note that the isoprene concentrations in the bottom layer (80–95 m)  
348 increased substantially from 22 March (mean, 20.7 pmol L<sup>-1</sup>) to 13 April (28.1 pmol L<sup>-1</sup>),  
349 where W water persisted during this period. We present calculated isoprene  
350 production rates in the surface mixed layer (0–35 m) in March and the bottom layer  
351 (80–95 m) from March to April in section 3.3.

352 From May to August, there was a substantial isoprene concentration maximum  
353 in the subsurface O or ST water at depths of 20–40 m, just below the surface mixed  
354 layer (subsurface layer). The isoprene concentrations in this subsurface water, which  
355 was shallower than the depth of 0.5% surface PAR, substantially increased from 17  
356 May (mean, 42.4 pmol L<sup>-1</sup>) to 29 July (86.4 pmol L<sup>-1</sup>) and 27 August (124.9 pmol L<sup>-1</sup>).  
357 We suggest that these increases are due to photosynthetic production of isoprene and the  
358 fact that isoprene produced below the surface mixed layer was prevented from sea–air  
359 out-gassing.

360

### 361 **3.3 Isoprene production rates**

362 We estimated in-situ production rate of isoprene in seawater of Funka bay from an  
363 increment of isoprene during a period between two observation dates. The in-situ  
364 production means net-production including biogenic production of isoprene mainly by  
365 phytoplankton, bacterial degradation, and chemical degradation. We supposed that the  
366 biological production is about ten times of the total degradations based on results of  
367 global isoprene flux model by Palmer and Shaw (2005). To minimize the effect of  
368 horizontal transport of isoprene, we selected periods and depths where the water masses  
369 had been stayed within the bay as shown in Fig 5. However, the effect of horizontal  
370 transportation of isoprene in the same water mass within the bay can't be still ruled out.

371 We additionally conducted a shipboard observation in Funka bay at the station of 30  
372 (center of the basin) and 23 (back of the basin, 14 km away from station 30) in May of  
373 2018. The difference of isoprene concentrations between the two stations in the depth  
374 range of 20 – 30 m, which was below the mixed layer within the euphotic layer, was  
375 11%. We notice that the following estimations of isoprene production rate have  
376 uncertainty of 11% or more owing to possible horizontal transportation effect.

377

### 378 **3.3.1 In-situ isoprene production rates in the surface mixed layer during the spring** 379 **diatom bloom**

380 Isoprene concentrations increased by 42.4 pmol L<sup>-1</sup> in the surface mixed layer (0–35 m)  
381 between 5 February and 22 March during a massive diatom bloom. In January - March  
382 2016, diatom aggregates were collected by a NORPAC net with a 100-μm mesh by the  
383 Training Ship *Ushio-maru*, which was collecting zooplankton at the same station in  
384 Funka Bay (no chl-a data were collected). The volume of aggregates in the NORPAC  
385 samples started to increase since 25 February. Eight days mean of surface chlorophyll-a  
386 concentration in 5 – 12 March was much higher than that in 26 February to 4 March.  
387 (MODIS aqua, Sea-viewing Wide Field-of-view Sensor (SeaWiFS) Ocean Color Data,  
388 NASA OB.DAAC, Greenbelt, MD, USA.  
389 [http://doi.org/10.5067/ORBVIEW-2/SEAWIFS\\_OC.2014.0](http://doi.org/10.5067/ORBVIEW-2/SEAWIFS_OC.2014.0).) We assumed that the  
390 spring diatom bloom had started around 25 February and that the massive diatom bloom  
391 that we observed in Funka Bay on 22 March had persisted for the previous 26 days  
392 ( $=T_{\text{bloom}}$ ). The isoprene production rate ( $P_{\text{Mar}(0-35 \text{ m})}$ ) was estimated by the following  
393 equation.

394

$$395 \quad P_{\text{Mar}(0-35 \text{ m})} = (\Delta C_5\text{H}_8 + F_{\text{sea-air}})/[\text{chl-a}]_{(0-35 \text{ m})}/T_{\text{bloom}} , \quad (1)$$

396

397 where  $\Delta C_5H_8$  is the increase in the amount of isoprene ( $=1.5015 \times 10^6$  pmol m<sup>-2</sup>) in the  
398 surface mixed layer (0–35 m) between the observation dates on 5 February and 22  
399 March (gray-shaded areas in Fig. 8), and  $[chl-a]_{(0-35m)}$  is the time-integrated average of  
400 chl-a ( $=1.4 \times 10^5$   $\mu$ g m<sup>-2</sup>) amount from 25 February to 22 March in the same layer. We  
401 used chl-a fluorescence surface monitoring data (Thermosalinograph XR-420CTX;  
402 Richard Brancker Research Ltd. Ottawa, ON Canada; minimum detectable level: 0.02  
403  $\mu$ g L<sup>-1</sup>) measured at the same station on 5 and 25 February and 8 and 22 March  
404 (Yamaoka et al., *submitted*) to interpolate the surface chl-a concentration from 25  
405 February to 22 March. The calculation method is shown in Fig. 9. We obtained  
406 exponential curve ( $y = 0.521 e^{0.0567x}$ ) of chl-a increase in the surface during the period.  
407 The time-integrated average of chl-a amount in the depth range of 0 – 35 m from 25  
408 February to 22 March was calculated using the same exponential increase rate.  $F_{sea-air}$  is  
409 the time-integrated sea-to-air flux of isoprene during the diatom bloom from 25  
410 February to 22 March. The fluxes were calculated according to Ooki et al. (2015) using  
411 hourly wind speeds at the Muroran meteorological weather station on the coast of Funka  
412 Bay (Fig. 1), and a Henry's law constant of isoprene in seawater (Ooki and Yokouchi,  
413 2011b). Mean isoprene concentration (45.0 pmol L<sup>-1</sup>) in the surface water on 5 February  
414 and 22 March was used for the flux calculation. Atmospheric isoprene concentration  
415 was assumed to be 80 patm, which is half of the mean value observed over a forest in  
416 Hokkaido (Ieda et al. 2006), based on an atmospheric lifetime of 1–2 h and a transport  
417 time of about 1–2 h from the surrounding forest to the center of Funka Bay. The  
418 sea-to-air flux was estimated to be  $5.36 \times 10^4$  pmol m<sup>-2</sup> d<sup>-1</sup>. The time-integrated flux,  
419  $F_{sea-air}$ , was  $1.45 \times 10^6$  pmol m<sup>-2</sup>. The settings of production rate calculation were  
420 summarized in Table 2. The  $P_{Mar(0-35m)}$  was estimated to be  $0.82$  pmol ( $\mu$ g chl-a)<sup>-1</sup> d<sup>-1</sup>.  
421 Note that estimated production means net production of isoprene including both  
422 productions and consumptions in seawater, and we assumed that the seawater in the  
423 surface layer (0 – 35 m) was not vertically mixed with lower layer.

424

### 425 **3.3.2 In-situ production rate of isoprene in the subsurface layer in July–August**

426 The isoprene concentrations in ST water at 20–40 m showed a substantial increase of  
427 38.5 pmol L<sup>-1</sup> (=ΔC<sub>C<sub>5</sub>H<sub>8</sub></sub>) from 29 July (mean, 86.4 pmol L<sup>-1</sup>) to 27 August (124.9 pmol  
428 L<sup>-1</sup>). This depth range was just below the surface mixed layer (17 m in July and 18 m in  
429 August) and shallower than the depths of 0.5% of surface PAR (45 m in July and 51 m  
430 in August). A distinct chl-a maximum was found within that depth range in August  
431 (mean, 0.29 μg L<sup>-1</sup> = [chl-a]<sub>(20-40m Aug)</sub>). We suggest that the isoprene increase from July  
432 to August was due to photosynthetic production of isoprene in the euphotic zone. Note  
433 that effects of high salinity T water inflows were obvious in July in that depth range  
434 compared with the lower salinity profile in May, and the salinity profile was found to  
435 become vertically smooth in August (Fig. 4). We assumed that the water in that depth  
436 range (20 – 40 m) had been stayed in the bay since 29 July until 27 August, on ground  
437 that the basin-wide anticyclonic circulation formed in the surface of the bay in summer  
438 (Miyake et al., 1998), which would restrict the water exchange between the basin of the  
439 bay and open ocean, was found to be strengthened in July - August 2016 analyzed by  
440 Acoustic Doppler Current Profiler (ADCP) observation data at the same station  
441 (Onishi et al., 2017).

442 The isoprene production rate per unit chl-a (=P<sub>Aug(20-40m)</sub>) was calculated by the  
443 following equation using the mean [chl-a]<sub>(20-40m July-Aug)</sub> (0.18 μg L<sup>-1</sup>) and the time  
444 interval of 29 d between the observation dates in July and August. Note that the  
445 sea-to-air flux can be omitted for the calculation below the surface mixed layer, and we  
446 assumed that the seawater in the subsurface layer (20 – 40 m) was not vertically mixed  
447 with upper and lower layers.

448

$$449 \quad P_{\text{Aug}(20-40\text{m})} = \Delta C_{\text{C}_5\text{H}_8} / [\text{chl-a}]_{(20-40\text{m})} / 29 \text{ d} \quad (2)$$

450

451  $P_{\text{Aug}(20-40\text{m})}$  was calculated as  $7.38 \text{ pmol } (\mu\text{g chl-a})^{-1} \text{ d}^{-1}$ , which is much higher than the  
452 rate during the spring diatom bloom in March ( $0.82 \text{ pmol } [\mu\text{g chl-a}]^{-1} \text{ d}^{-1}$ ).

453

### 454 **3.3.3 Comparison between isoprene production in-situ and in diatom incubation** 455 **experiments under light conditions**

456 We followed cell densities, isoprene production, and chl-a concentrations in both  
457 experiments I and II (Figs. 10 and 11, respectively). Chl-a concentrations in the  
458 incubation bottles increased exponentially from  $3.4$  to  $301 \mu\text{g L}^{-1}$  in the first 13 days of  
459 experiment I, and from  $4.5$  to  $165 \mu\text{g L}^{-1}$  in the first 8 days of experiment II. These  
460 intervals are considered the exponential growth phase. The chl-a concentrations  
461 gradually increased to around  $307 \mu\text{g L}^{-1}$  over the next 7 days of experiment I, and to  
462  $187 \mu\text{g L}^{-1}$  over the next 6 days of experiment II. These intervals are referred to as the  
463 “steady phase”. The incubation bottles in experiment II were moved into continuous  
464 dark conditions after the steady phase. The chl-a concentrations in experiment I  
465 decreased from  $307 \mu\text{g L}^{-1}$  on day 20 to  $66 \mu\text{g L}^{-1}$  on day 48. This interval is called the  
466 “decline phase”. After the decline phase, the concentrations varied within a narrow  
467 range ( $53\text{--}59 \mu\text{g L}^{-1}$ ) for 24 days. This interval is called the “stagnation phase”.

468 We calculated the chl-a content per cell (Figs. 10 and 11, middle panels). The  
469 chl-a content was  $2\text{--}4.6$  (mean,  $3.1$ )  $\text{pg chl-a cell}^{-1}$  in the exponential growth and steady  
470 phases of experiment I, and was the highest ( $1.9\text{--}6.0$ ; mean,  $4.7 \text{ pg chl-a cell}^{-1}$ ) in the  
471 stagnation phase.

472 The production rates per unit chl-a in the exponential growth phase were  $0.55\text{--}$   
473  $1.6$  (mean,  $1.1$ )  $\text{pmol } (\mu\text{g chl-a})^{-1} \text{ d}^{-1}$  for experiment I and  $1.8\text{--}2.4$  (mean,  $2.2$ )  $\text{pmol } (\mu\text{g}$   
474  $\text{chl-a})^{-1} \text{ d}^{-1}$  for experiment II. The isoprene production rates normalized to chl-a  
475 concentration and cell density were the highest in the exponential growth phase and the  
476 lowest in the steady and decline phases (mean,  $0.35 \text{ pmol } [\mu\text{g chl-a}]^{-1} \text{ d}^{-1}$ ) in experiment  
477 I. Our incubation results are consistent with previous diatom incubation results of  $0.65\text{--}$

478 1.26 pmol ( $\mu\text{g chl-a}$ )<sup>-1</sup> d<sup>-1</sup> (*Chaetoceros debilis*, *C. neogracilis*) under PAR of about 100  
479  $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$  (Bonsang et al. 2010), which is the same PAR level as in our  
480 experiment, and 2.66 pmol ( $\mu\text{g chl-a}$ )<sup>-1</sup> d<sup>-1</sup> (*Thalassiosira weissflogii*) with a high PAR  
481 of 300  $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$  (Exton et al. 2013).

482 The in-situ isoprene production rate during the spring diatom bloom ( $P_{\text{Mar}(0-35\text{m})}$ ;  
483 0.82 pmol [ $\mu\text{g chl-a}$ ]<sup>-1</sup> d<sup>-1</sup>) is consistent with our diatom incubation results in  
484 steady and decline phases (means; 0.35 – 2.2 pmol [ $\mu\text{g chl-a}$ ]<sup>-1</sup> d<sup>-1</sup>). In contrast, the  
485 in-situ production rate in summer (July–August) ( $P_{\text{Aug}(20-40\text{m})}$ ; 7.38 pmol [ $\mu\text{g chl-a}$ ]<sup>-1</sup> d<sup>-1</sup>)  
486 was much higher than the diatom incubation results and in-situ  $P_{\text{Mar}(0-35\text{m})}$ . The  
487 difference between  $P_{\text{Mar}(0-35\text{m})}$  and  $P_{\text{Aug}(20-40\text{m})}$  was likely due to the difference in  
488 dominant phytoplankton species in each season. The main phytoplankton in Funka Bay  
489 during the spring bloom are large diatoms (cell size > 10  $\mu\text{m}$ ) (Odate 1989). In the early  
490 phase of the diatom bloom in February–March, *Thalassiosira spp.* are the most  
491 abundant diatoms, and toward the end of the bloom, *Chaetoceros spp.* become the  
492 dominant diatoms (Shinada et al. 1999). During the summer, cell density of  
493 phycoerythrin-rich cyanobacteria, such as *Synechococcus sp.*, rapidly increased from  
494 spring ( $10^5$  cells/L) to summer ( $10^8$  cells/L) in the upper stratified low nutrient layer,  
495 predominating the cell density of pico-phytoplankton less than 2  $\mu\text{m}$  (Odate 1989).  
496 *Synechococcus* occupied approximately 10 % of depth-integrated Chl-a concentration  
497 within euphotic zone (0 – 39 m; 1 % of surface PAR) in Funka bay in August 2010  
498 (Isada et al., 2017), while it occupied approximately 30 % of Chl-a in the oligotrophic  
499 surface water (Isada et al., 2015). The subsurface layer (20 – 40 m) in August 2010 at  
500 the same station was rich in nutrients ( $\text{NO}_2^- + \text{NO}_3^- = 0.9 - 3.4 \mu\text{mol L}^{-1}$ ) and low  
501 temperature (5 – 10 °C) (Hioki et al., 2015), while that in July and August 2016 was  
502 oligotrophic ( $\text{NO}_2^- + \text{NO}_3^- = 0.02 - 0.28 \mu\text{mol L}^{-1}$  and  $\text{NO}_2^- + \text{NO}_3^- = 0.02 - 1.42 \mu\text{mol}$   
503  $\text{L}^{-1}$ , respectively) and high temperature (12 – 20 °C) (this study). Since picoplankton,  
504 such as *Synechococcus* prevails in high temperature oligotrophic water in the

505 Mediterranean Sea ( $\text{NO}_2^- + \text{NO}_3^- < 1 \mu\text{mol L}^{-1}$ ) (Agawin et al., 2000), we supposed that  
506 phycoerythrin-rich cyanobacteria, such as *Synechococcus* sp., prevailed in the  
507 subsurface layer of Funka bay in July – August 2016.

508 The diatom used in our incubations, *Thalassiosira weissflogii*, had isoprene  
509 production rates of  $0.27\text{--}2.4 \text{ pmol } (\mu\text{g chl-a})^{-1} \text{ d}^{-1}$  (this study). Bonsang et al. (2010)  
510 reported that the cyanobacterium *Synechococcus* sp. had an isoprene production rate of  
511  $4.97 \text{ pmol } (\mu\text{g chl-a})^{-1} \text{ d}^{-1}$ , the highest among the nine species they tested. This  
512 production rate is consistent with our results for in-situ  $P_{\text{Aug}(20\text{--}40\text{m})}$ . Furthermore, the  
513 isoprene production rates for diatom (Meskhidze et al., 2015; Exton et al., 2013) and  
514 cyanobacteria (Shaw et al., 2003; Exton et al., 2013) rose with temperature increase.

515 We concluded that the relatively low value for in-situ  $P_{\text{Mar}(0\text{--}35\text{m})}$  during the  
516 spring bloom and the high value for  $P_{\text{Aug}(20\text{--}40\text{m})}$  in summer were attributable to isoprene  
517 production by large diatoms such as *Thalassiosira* spp. and phycoerythrin containing  
518 organisms such as *Synechococcus* spp., respectively. The high value for  $P_{\text{Aug}(20\text{--}40\text{m})}$   
519 would be also attributable to higher isoprene productions by diatom and cyanobacteria  
520 at higher temperature.

521

### 522 **3.3.4 Isoprene production in the dark bottom layer of Funka Bay**

523 The isoprene concentration in the bottom layer (80–95 m) of the bay increased by  $7.4$   
524  $\text{pmol L}^{-1}$  ( $=\Delta C_{\text{C}_5\text{H}_8}$ ) from 22 March to 13 April in the W water, where there were no  
525 temperature or salinity changes. There was a high concentration of chl-a ( $9.86 \mu\text{g L}^{-1}$ ) in  
526 the bottom layer ( $[\text{chl-a}]_{\text{bottom}}$ ) on 22 March, whereas it was low ( $0.22\text{--}0.65 \mu\text{g L}^{-1}$ ) on  
527 13 April. The large numbers of diatoms produced in the spring bloom in March were  
528 likely sinking from the surface euphotic zone to the bottom layer during this period,  
529 blocking the sunlight from the bottom layer. On the basis of these observations, we  
530 hypothesize that isoprene is produced by phytoplankton in the dark. The in-situ

531 production rate of isoprene in the dark bottom layer,  $P_{\text{Mar}(80-95\text{m})}$ , was calculated using  
532 the following equation.

533

$$534 \quad P_{\text{Mar}(80-95\text{m})} = \Delta C_{\text{C}_5\text{H}_8} / [\text{chl-a}]_{\text{bottom}} / T_{\text{dark}} , \quad (3)$$

535

536 where  $T_{\text{dark}}$  (days) is the time-period for isoprene production in the dark by diatom cells  
537 suspended in the bottom layer between 22 March and 13 April. We assumed  $T_{\text{dark}}$  to be  
538 6–22 days. This means that the high concentrations of chl-a in the bottom layer could  
539 have persisted for more than 6 days after 22 March. The minimum time of 6 days was  
540 roughly calculated from the sinking time of diatom aggregates from the surface to the  
541 bottom layer (95 m) at a sinking velocity of 14.4 m d<sup>-1</sup> (=1 cm min<sup>-1</sup>). The sinking  
542 velocity was roughly measured in a simple experiment where we observed diatom  
543 aggregates collected in a bottle. Seebah et al. (2014) have reported the sinking velocity  
544 of *Thalassiosira weissflogii* aggregates of a size that could be trapped by the mesh in  
545 our NORPAC net (>0.5 mm) ranging between 8 and 110 m d<sup>-1</sup>. The in-situ  $P_{\text{Mar}(80-95\text{m})}$   
546 in the dark was estimated at 0.03–0.13 pmol (μg chl-a)<sup>-1</sup> d<sup>-1</sup>, which is 4–16% of in-situ  
547  $P_{\text{Mar}(0-35\text{m})}$  in the light.

548 We tested for the dark production of isoprene in incubation experiment II (Fig.  
549 11). On day 12 of the incubation, during the steady phase, the incubation bottle was  
550 moved into continuous dark conditions. In the dark, chl-a concentrations decreased  
551 sharply from 187 μg L<sup>-1</sup> on day 14 to 9.9 μg L<sup>-1</sup> on day 35, and they remained low (8.3–  
552 11.7 μg L<sup>-1</sup>) for 14 days until the end of the incubation (day 53). The mean production  
553 rates were 0.19 pmol (μg chl-a)<sup>-1</sup> d<sup>-1</sup> (=  $P_{\text{dark}}$ ) or  $2.9 \times 10^{-7}$  pmol cell<sup>-1</sup> d<sup>-1</sup>, which are  
554 9% and 6%, respectively, of those in the exponential growth phase in the light. This  
555 production rate during the incubation was somewhat higher than the in-situ  $P_{\text{Mar}(80-95\text{m})}$   
556 in the dark (0.03–0.13 pmol [μg chl-a]<sup>-1</sup> d<sup>-1</sup>).

557 Many of previous incubation studies (e.g. Bonsang et al., 2010, Exton et al.,  
558 2013, Dani et al., 2017) have not noticed isoprene production in the dark; however, the  
559 results of some of these studies seem to show that small amounts of isoprene were  
560 emitted in the dark. In a plot of isoprene production rate vs. PAR intensity (Fig. 2 in  
561 Bonsang et al. 2010), it looks like the phytoplankton produced isoprene at 0–0.2 pmol  
562 ( $\mu\text{g chl-a}^{-1} \text{d}^{-1}$ ) in the dark (PAR = 0), which is about 0–15% of their production rate  
563 under light conditions. Incubation results from another study show that the isoprene  
564 production rate per cell by the cyanobacterium *Prochlorococcus* sp. dropped sharply in  
565 the dark, and the rate after 48 h in the dark was reported to be 16% of the rate in  
566 exponential growth phase in the light (Fig. 4c in Shaw et al. 2003). A recent incubation  
567 study reported values of isoprene production rate in the dark by diatom, *Thalassiosira*  
568 *weissflogii*, to be 0.48 pmol ( $\mu\text{g chl-a}^{-1} \text{h}^{-1}$ ) at 18 °C, which was 14 % of that under the  
569 light with irradiance intensity of 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Meskhidze et al., 2015).s As for  
570 isoprene production by macroalgae, the green macroalgae *Ulva intestinalis* produced  
571 isoprene in the dark at 0.6 pmol ( $\text{g dry-wt}^{-1} \text{h}^{-1}$ ), which was 16% of that in the light  
572 (Broadgate et al. 2004). Apparently isoprene can also be formed by higher plants via the  
573 breakdown of stored carbohydrates when photosynthesis is blocked (Lerdau et al. 1997).  
574 Broadgate et al. (2004) have inferred a similar pathway for isoprene production by  
575 marine macroalgae in the dark.

576 We conclude that isoprene production rate by diatom in the dark ranged from  
577 4% to 16% of that in the light associated with their photosynthesis. The contribution  
578 from dark production would be important in a dim subsurface layer below the  
579 compensation depth (around 0.5% of surface PAR) where the chl-a concentration  
580 maximum is found, and at night. Note that our values for in-situ  $P_{\text{Mar}(0-35\text{m})}$  and  $P_{\text{Aug}(20-40\text{m})}$   
581 include nighttime production ( $P_{\text{dark}}$ ).

582

### 583 3.3.5 Isoprene emission factor (EF)

584 In a recent study, the isoprene production rate per unit chlorophyll ( $P_{\text{chl}}$ ; pmol [ $\mu\text{g}$   
585 chl-a] $^{-1}$  h $^{-1}$ ) was calculated as a function of PAR ( $I$ ;  $\mu\text{mol photon m}^{-2}$  s $^{-1}$ ) using the  
586 following equation (Gantt et al. 2009; Booge et al. 2018):

587

$$588 \quad P_{\text{chl}} = \text{EF} \times (\ln I)^2, \quad (4)$$

589

590 where EF is the emission factor of isoprene measured by incubation experiment for  
591 specific phytoplankton species. This equation has been used for the global isoprene  
592 production estimate (Gantt et al. 2009) and basin-scale isoprene budgets (Booge et al.  
593 2018).

594 In this study, the in-situ isoprene EF in Funka Bay was calculated from our  
595 results for production rates ( $P_{\text{Mar}(0-35\text{m})}$  and  $P_{\text{Aug}(20-40\text{m})}$ ) and observed PAR. The values  
596 for  $P_{\text{Mar}(0-35\text{m})}$  and  $P_{\text{Aug}(20-40\text{m})}$  were converted to 0.0680 pmol ( $\mu\text{g chl-a}$ ) $^{-1}$  h $^{-1}$  and 0.527  
597 pmol ( $\mu\text{g chl-a}$ ) $^{-1}$  h $^{-1}$ , respectively, by dividing by the daytime hours (12 hours in March  
598 and 14 hours in August). The surface PAR at noon in March was 1200  $\mu\text{mol photon m}^{-2}$   
599 s $^{-1}$ , and that in August was 2000  $\mu\text{mol photon m}^{-2}$  s $^{-1}$ . The amount of solar radiation  
600 (300–2000 nm) on a clear day in March at the Muroran weather station was 2.7 MJ m $^{-2}$   
601 and that in August was 3.2 MJ m $^{-2}$ . We converted the solar radiation measured at the  
602 weather station to the surface PAR at St. 30 by multiplying by a factor of 440  
603 (=1200/2.7) in March and 620 (=2000/3.2) in August. The depth profile of PAR was  
604 determined using the measured PAR attenuation profiles (Fig. 7).

605 The mean values of  $I$  and  $(\ln I)^2$  from 0 to 35 m depth in daytime (0700–1900  
606 local time) from 25 February to 22 March were 46.2  $\mu\text{mol photon m}^{-2}$  s $^{-1}$  and 6.0,  
607 respectively, and those from 20 to 40 m depth in daytime (0600–2000 local time) from  
608 29 July to 27 August were 26.9  $\mu\text{mol photon m}^{-2}$  s $^{-1}$  and 8.3. The in-situ  $\text{EF}_{\text{Mar}(0-35\text{m})}$   
609 and  $\text{EF}_{\text{Aug}(20-40\text{m})}$  were calculated as 0.011 and 0.063, respectively. The in-situ  $\text{EF}_{\text{Mar}(0-35\text{m})}$

610 35m) during a massive diatom bloom was somewhat higher than the EF for diatoms  
611 (0.0064) reported by Booge et al. (2018).

612 The in-situ  $EF_{Aug(20-40m)}$  of 0.063 was higher than the EFs (0.0053–0.0176) of  
613 seven phytoplankton species as summarized by Booge et al. (2018). The high in-situ  
614  $EF_{Aug(20-40m)}$  in the subsurface layer was likely due to the photosynthetic characteristics  
615 of the prevailing planktons having pigments other than chl-a (e.g. phycoerythrin), such  
616 as *Synechococcus* spp., in summer in Funka Bay, and to higher emission rate at higher  
617 temperature. Barlow and Alberte (1985) reported that *Synechococcus* spp., having the  
618 photosynthetic pigment phycoerythrin, photosynthesize maximally at the very low  
619 photon flux densities ( $10-50 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) found below the surface mixed layer.  
620 In contrast, diatoms generally show optimum photosynthesis at high photon flux  
621 densities. The estimated high in-situ  $EF_{Aug(20-40m)}$  under relatively low light conditions  
622 (mean,  $26.9 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) are likely due to low light-adapted phycoerythrin  
623 containing planktons, such as *Synechococcus*, with high efficiency photosynthesis and  
624 isoprene emission below the surface mixed layer of Funka Bay.

625

#### 626 **4. Conclusions**

627 From the shipboard observations in Funka Bay, Hokkaido, Japan, vertical  
628 profiles of isoprene, chl-a, dissolved oxygen, nutrients, and physical parameters  
629 (temperature, salinity, and PAR) from the surface to the bottom layer (1 m above the sea  
630 floor at 96 m depth) were obtained monthly or bimonthly from December 2015 to  
631 November 2016.

632 On 22 March, high concentrations of chl-a (mean;  $9.3 \mu\text{g L}^{-1}$ ) were found in the  
633 total depths with two distinct maxima of  $16.8 \mu\text{g L}^{-1}$  at the surface (5 m) and  $11.7 \mu\text{g L}^{-1}$   
634 at the bottom water (95 m), indicating the massive diatom spring bloom and subsequent  
635 settling of diatom cells from the surface to the bottom. We found increases in  
636 concentrations of isoprene in the surface mixed layer (0 – 35 m) from 5 February to 22

637 March, and the bottom layer (80 – 95 m) from 22 March to 13 April, during the diatom  
638 bloom. We calculated the isoprene production rates per unit chlorophyll amount to be  
639 0.82 pmol ( $\mu\text{g chl-a}$ )<sup>-1</sup> d<sup>-1</sup> in the surface mixed layer and 0.03 – 0.13 pmol pmol ( $\mu\text{g}$   
640 chl-a)<sup>-1</sup> d<sup>-1</sup> in the bottom layer. On 27 August, concentration maximum of chl-a was  
641 found between the mixed layer depth (18 m) and the compensation depth (51 m) with a  
642 mean concentration of 0.29  $\mu\text{g L}^{-1}$ . We found the concentration increase of isoprene  
643 from 29 July to 27 August in the depth range of 20 – 40 m, forming a distinct maximum  
644 of isoprene on 27 August. We calculated the isoprene production rate per unit  
645 chlorophyll amount in this depth range to be 7.38 pmol ( $\mu\text{g chl-a}$ )<sup>-1</sup> d<sup>-1</sup> from July to  
646 August.

647 We could evaluate in-situ productions of isoprene associated with  
648 photosynthesis in the surface mixed layer during the early stage of massive spring  
649 diatom bloom (February - March) and below the surface mixed layer (subsurface layer)  
650 in summer (August), and dark production in the bottom layer during the last stage of the  
651 bloom (March – April).

652 We considered that the difference in photosynthetic production rates in spring  
653 (bloom) and summer (post-bloom) is attributed to the respective dominant  
654 phytoplankton in the bay: large diatoms in spring such as *Thalassiosira* spp. and  
655 *Chaetoceros* spp., and most likely the small cyanobacterium *Synechococcus* spp. in  
656 summer. The in-situ isoprene production rate per unit chl-a in the subsurface layer in  
657 summer was more than ten times that in the surface mixed layer in the spring diatom  
658 bloom. We attributed the calculated high emission factor (EF) as a function of PAR in  
659 summer to low light-adapted *Synechococcus* spp., which can efficiently photosynthesize  
660 in the dim subsurface layer. High temperature would also contributed to the high  
661 isoprene emission in summer. The estimated in-situ isoprene production rates and  
662 in-situ EFs were largely consistent with our incubation results and previous incubation  
663 studies. It must be noted, however, that the isoprene production properties in the

664 subsurface layer, where there are high isoprene emitters adapted to low light conditions,  
665 are different from the results of previous incubations carried out under intense light  
666 conditions.

667           We considered that the isoprene production in the bottom layer during the last  
668 stage of diatom bloom (March – April) is attributed to the dark production by diatoms  
669 settled from the surface to the bottom. We confirmed the dark production of isoprene  
670 through diatom incubation experiments, and we found that the isoprene production rate  
671 per unit chl-a in the dark ranged from 4% up to 16% of photosynthetic isoprene  
672 production rate in the light. This is the first study to clearly document isoprene  
673 production by phytoplankton in the dark.

674

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683 16H02929).

684

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847 appendicularians in Funka Bay before and after intrusion of Coastal Oyashio Water  
848 Assemblage of copepods and appendicularians. *Fisheries Science*.

849 Table 1 Temperature and salinity ranges of  
850 uniform water masses in Funka Bay, Japan

	Temp. (°C)	Salinity
Tsugaru (T) water	8–15 °C	33.6–34.2
Winter (W) water	–1 to 5 °C	33.25–34.2
Oyashio (O) water	–1 to 10 °C <sup>a</sup>	32.6–33.0
Summer (S) water	10–25 °C	30.0–32.25

851 <sup>a</sup>Temperature range of Oyashio water was expanded from  
852 the original definition (–1 to 2.5 °C; Ohtani and Kido 1980).

853

854

855 Table 2 Settings of C<sub>5</sub>H<sub>8</sub> production rate calculation

	$\Delta C_{C_5H_8}$	$F_{sea-air}$	[chl-a]	$T$
$P_{Mar(0-35m)}$	$[C_{C_5H_8}]_{(0-35\ m)} \text{ on 22 Mar}$ - $[C_{C_5H_8}]_{(0-35\ m)} \text{ on 5 Feb}$ (*)	Time-integrated sea-air flux (**)	Time-integrated average of [chl-a] <sub>(0-35m)</sub> amount from 25 Feb to 22 Mar (*)	26days; from 25 Feb to 22 Mar
$P_{Aug(20-40m)}$	$[C_{C_5H_8}]_{(20-40\ m)} \text{ on 29 Jul}$ - $[C_{C_5H_8}]_{(20-40\ m)} \text{ on 27 Aug}$		Mean of [chl-a] <sub>(20-40m)</sub> on 29 Jul and 27 Aug	29days; from 29 Jul to 27 Aug
$P_{Mar(80-95m)}$	$[C_{C_5H_8}]_{(80-95\ m)} \text{ on 13 Apr}$ - $[C_{C_5H_8}]_{(80-95\ m)} \text{ on 22 Mar}$		Mean of [chl-a] <sub>(80-95m)</sub> on 22 Mar	6 – 21 days (***)

856 (\*) Values of concentration change of C<sub>5</sub>H<sub>8</sub> and chl-a amount were multiplied by the depth range of 35 m in

857 accordance with the unit of  $F_{sea-air}$ .

858 (\*\*\*)  $K \cdot ([C_5H_8] - p C_5H_{8air} \cdot H)$ ;  $K = 0.31 \times u^2 (Sc/660)^{-0.5}$  (Wanninkhof, 1992), where u is hourly wind speed obtained  
 859 from the Muroran meteorological weather station, and Sc is Schmitt number. H: Henry's law constant of C<sub>5</sub>H<sub>8</sub> in  
 860 seawater (Ooki and Yokouchi, 2011), [C<sub>5</sub>H<sub>8</sub>]: mean concentration of C<sub>5</sub>H<sub>8</sub> in the surface water on 5 February and 22  
 861 March,  $pC_5H_{8air}$ : partial pressure of C<sub>5</sub>H<sub>8</sub> (80 patm). Mean flux obtained from the hourly flux was used for the  
 862 production rate calculation.

863 (\*\*\*) 6 days from 22 March to 27 March, and 22 days from 22 March to 13 April.

864 Figure captions

865

866 Fig. 1 Map showing the location of observation stations 30 and 23 in Funka Bay,  
867 Hokkaido, Japan, and Muroran, site of weather observations. Observation at the station  
868 23 was additionally conducted in May 2018.

869

870 Fig. 2 Schematic diagram of incubation experiments and sample gas collection (upper  
871 panel), and calculation method of isoprene production rate (lower panel).

872

873 Fig. 3 Temperature–salinity ranges of water masses in Funka Bay, Japan

874

875 Fig. 4 Vertical profiles of temperature and salinity at station 30 in Funka Bay, Japan.  
876 Upper panels are December 2015 and February 2016, middle panels are March–May  
877 2016, and lower panels are July–November 2016. The light-gray profiles in the middle  
878 and lower panels are the last profiles from the upper and middle panels, respectively,  
879 copied for comparison. W, winter water; O, Oyashio water

880

881 Fig. 5 Seasonal trend of water-mass structure at station 30 in Funka Bay, Japan. The  
882 four main water masses are Tsugaru water (T), winter water (W), Oyashio water (O),  
883 and summer water (S). Transition waters are WO, WOT, ST, TW and OS. Depth ranges  
884 and periods for estimations of isoprene production rates ( $P_{\text{Mar}(0-35\text{m})}$ ,  $P_{\text{Bottom}}$ , and  
885  $P_{\text{Aug}(20-40\text{m})}$ ) were enclosed by bold broken line.

886

887 Fig. 6a Vertical profiles of isoprene ( $\text{C}_5\text{H}_8$ ), apparent oxygen utilization (AOU),  
888 dissolved inorganic nitrogen (DIN), and chlorophyll-a (chl-a) in December 2015–  
889 February 2016 (upper panels) and March–April 2016 (lower panels) at station 30 in  
890 Funka Bay, Japan. The x-axis of chl-a in March and April was decupled with the

891 labeled value.

892

893 Fig. 6b Vertical profiles of isoprene ( $C_5H_8$ ), apparent oxygen utilization (AOU),  
894 dissolved inorganic nitrogen (DIN), and chlorophyll-a (chl-a) in May 2016 (upper  
895 panels) and July–November 2016 (lower panels) at station 30 in Funka Bay, Japan

896

897 Fig. 7 Vertical profiles of photosynthetically active radiation (PAR) in 2016 at station  
898 30 in Funka Bay, Japan. The depths of 0.5% of surface PAR are marked by arrows. The  
899 data for April were measured on 14 April at another station in Funka Bay. Weather  
900 conditions on 14 April were cloudy

901

902 Fig. 8 Changes in isoprene concentrations in the surface mixed layer from 5 February to  
903 22 March 2016

904

905 Fig.9 Interpolation of surface chl-a concentrations on 25 February and 8 March from the  
906 fluorescence monitoring data. ‘Obs.’ is the chl-a concentration measured by  
907 Welshmeyer method (this study), ‘Fluor.’ is the chl-a concentration measured by  
908 fluorometer (surface monitoring data by Yamaoka et al. (*submitted*)), and ‘Cal.’ is the  
909 interpolated calibration data of chl-a concentration.

910

911 Fig. 10 Results of incubation experiment I. Shaded and white areas indicate the  
912 exponential growth phase (i), steady phase (ii), decline phase (iii), and stagnation phase  
913 (iv)

914

915 Fig. 11 Results of incubation experiment II. Shaded and white areas indicate the  
916 exponential growth phase (i), steady phase (ii), decline phase (iii), and stagnation phase  
917 (iv). Cultures were moved from 13-h:11-h (L:D) into continuous darkness on day 12

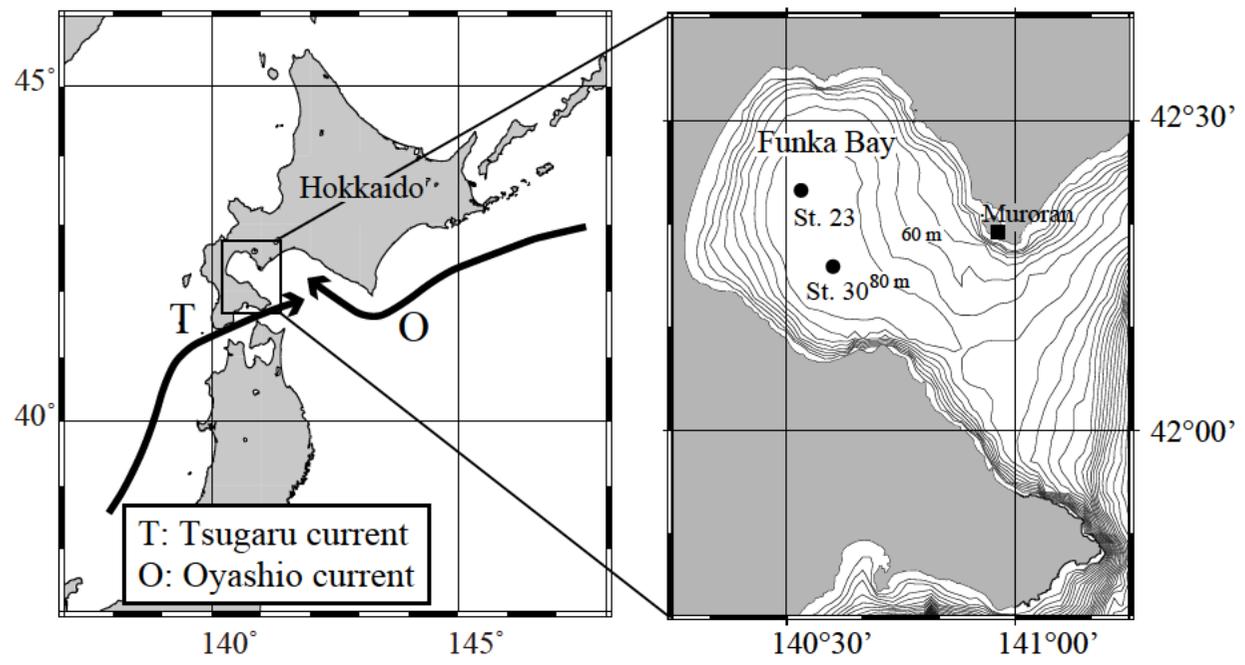
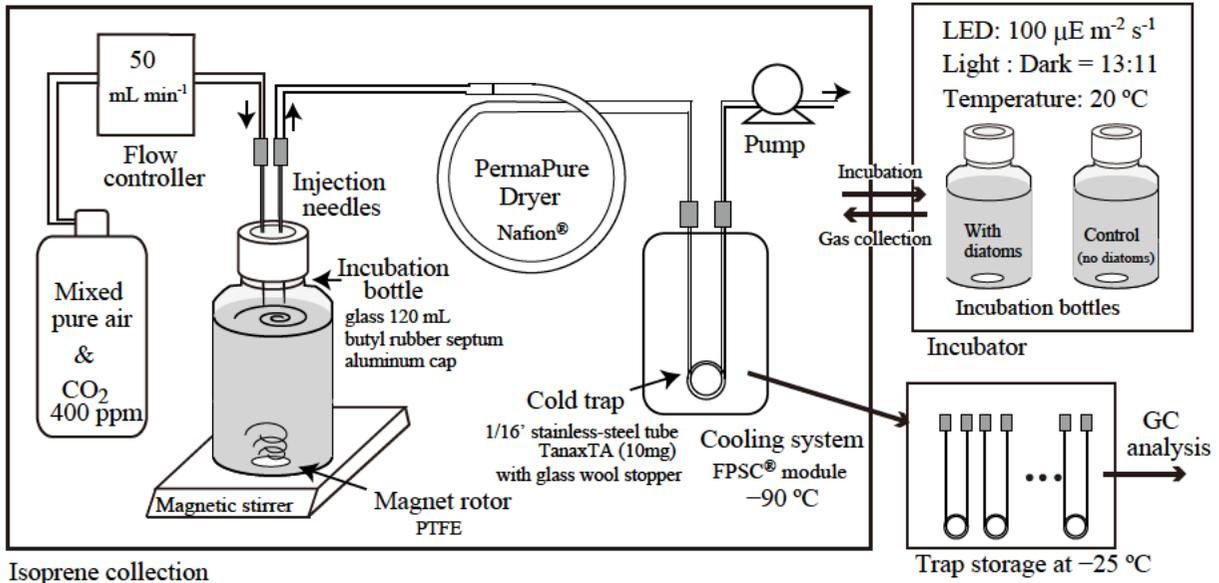
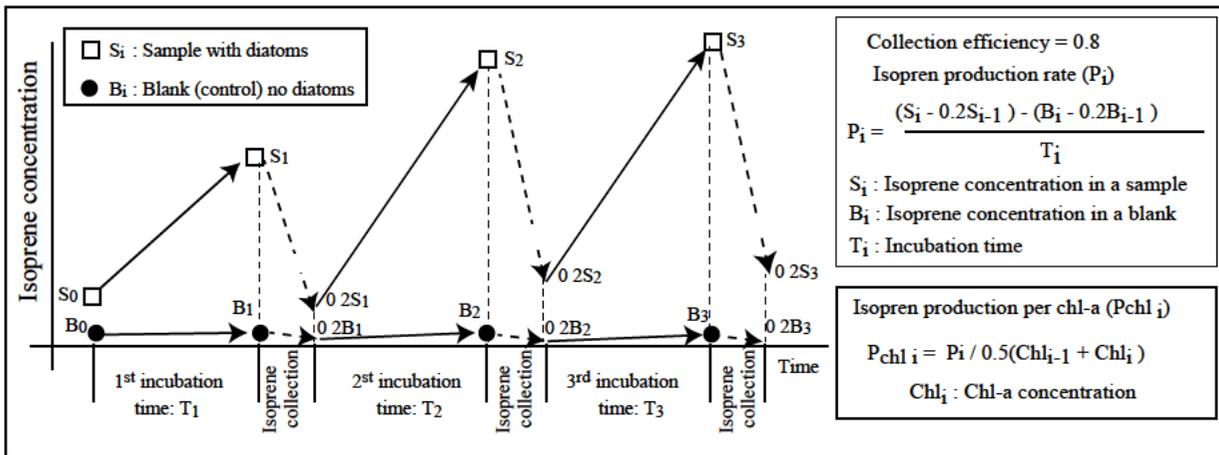


Figure 1



Isoprene collection



Calculation method of isoprene production rate

Figure 2

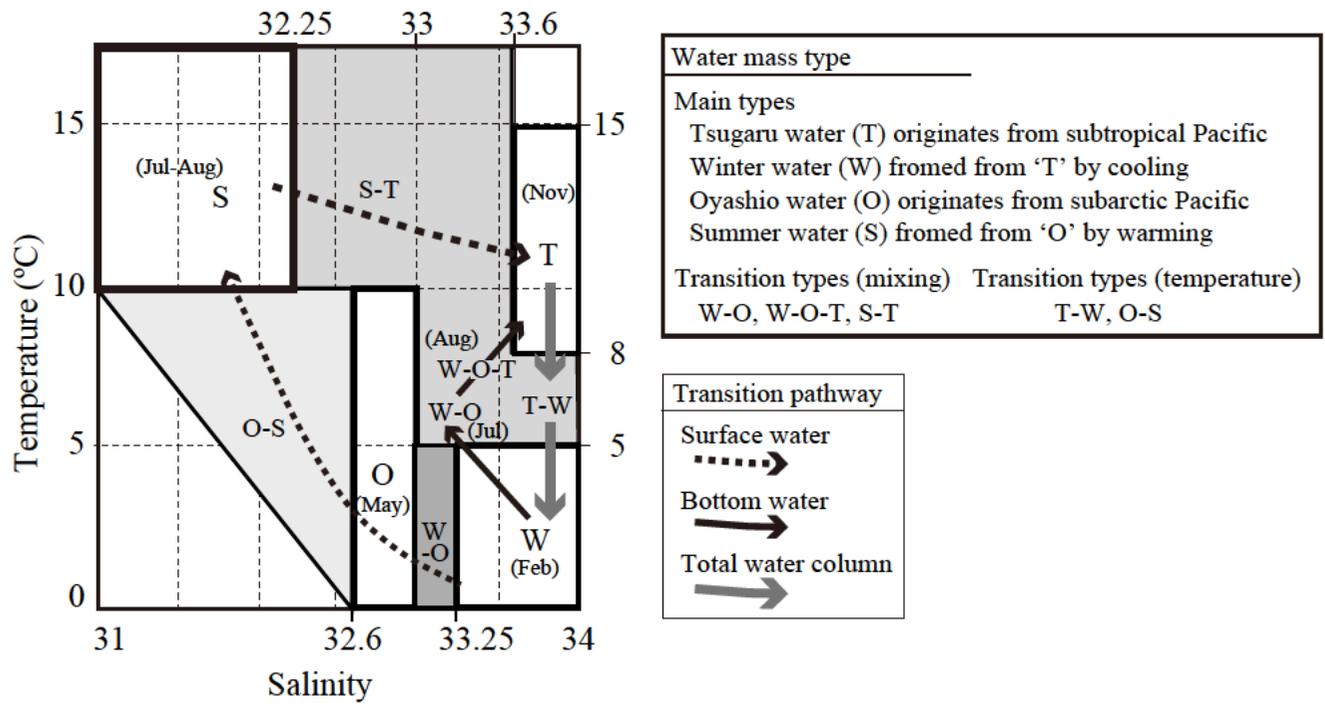


Figure 3

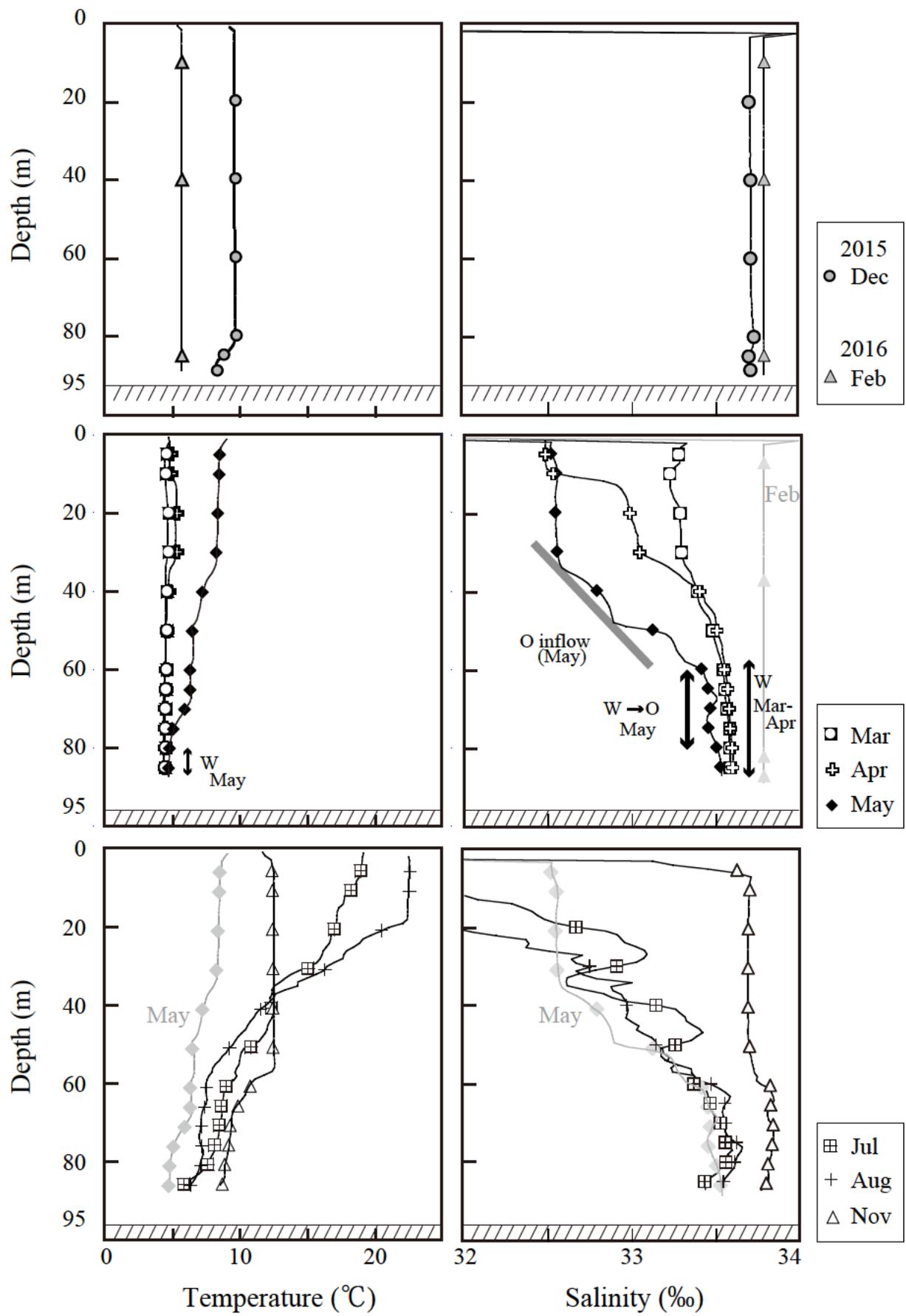


Figure 4

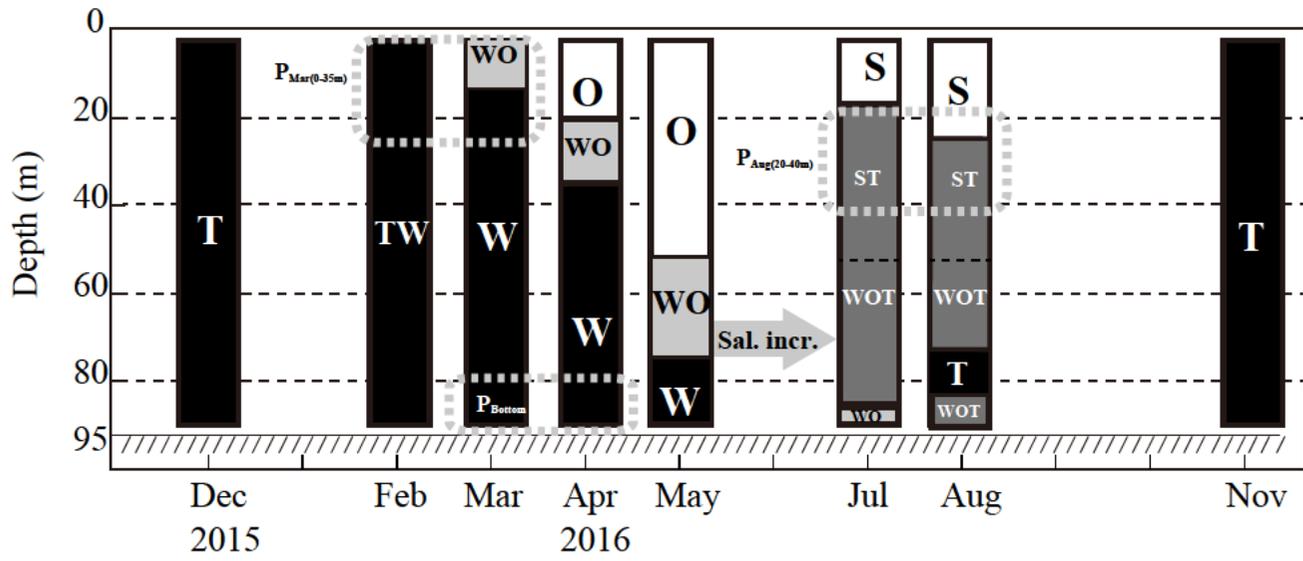


Figure 5

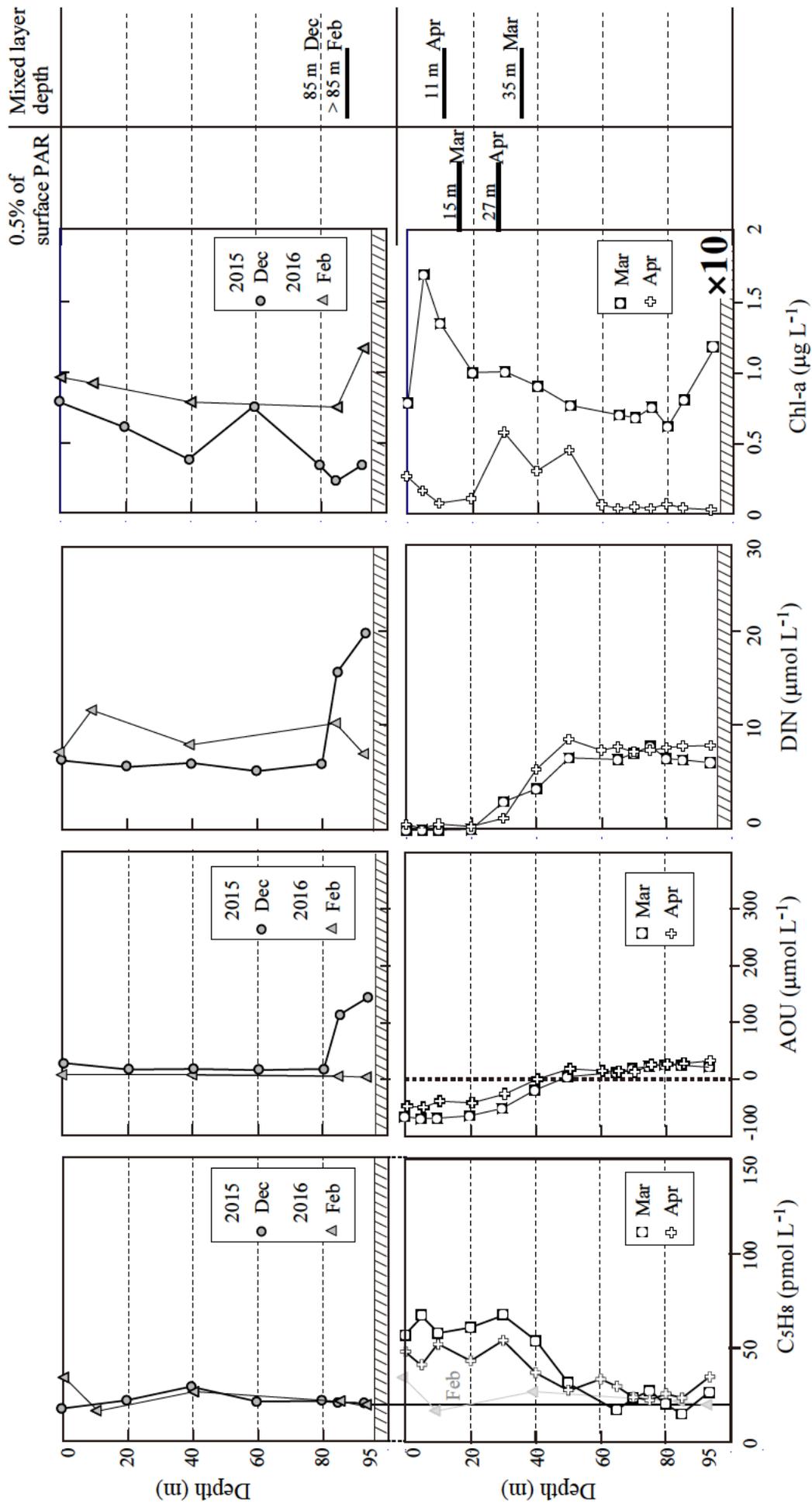


Figure 6a

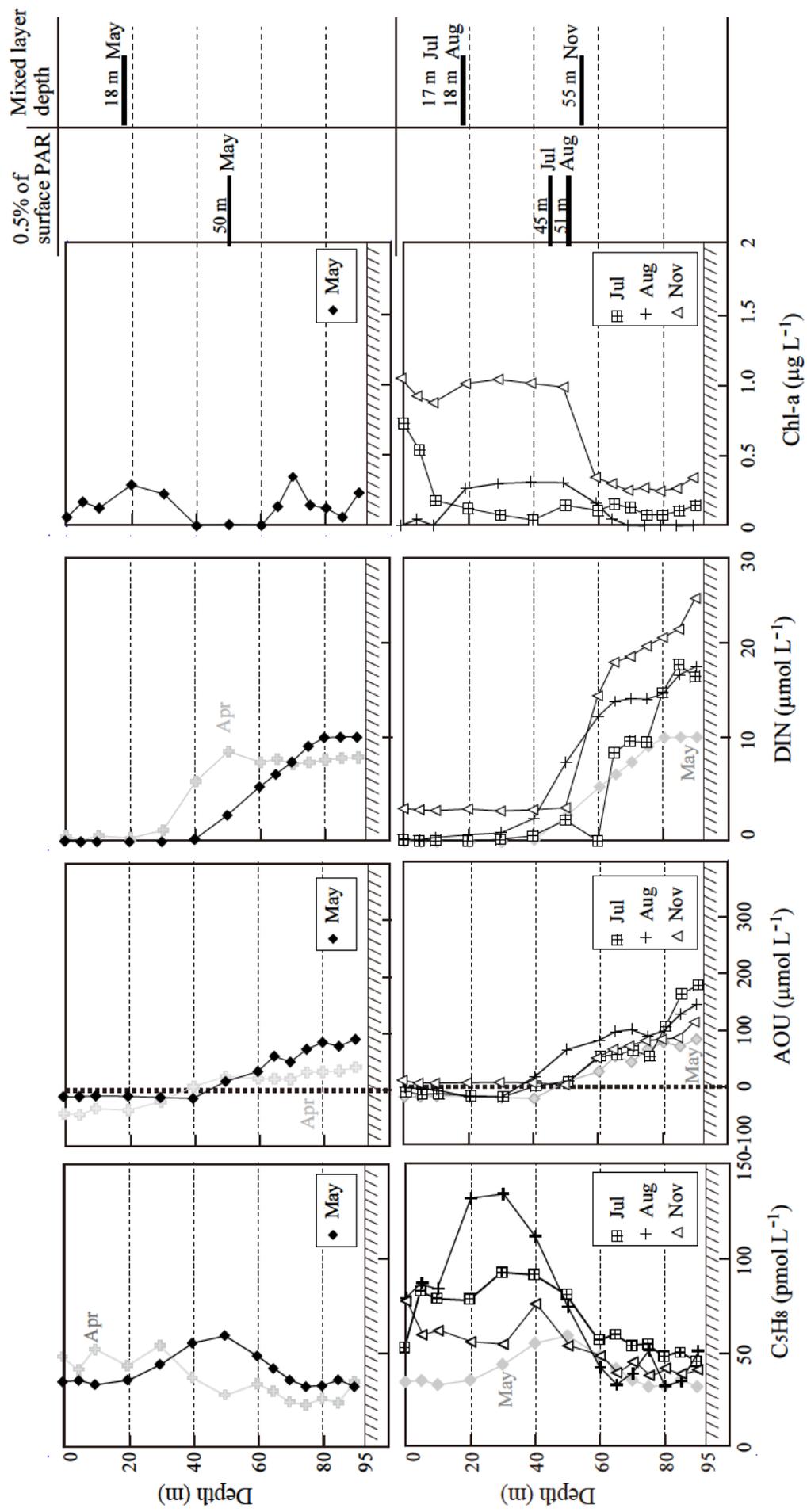


Figure 6b

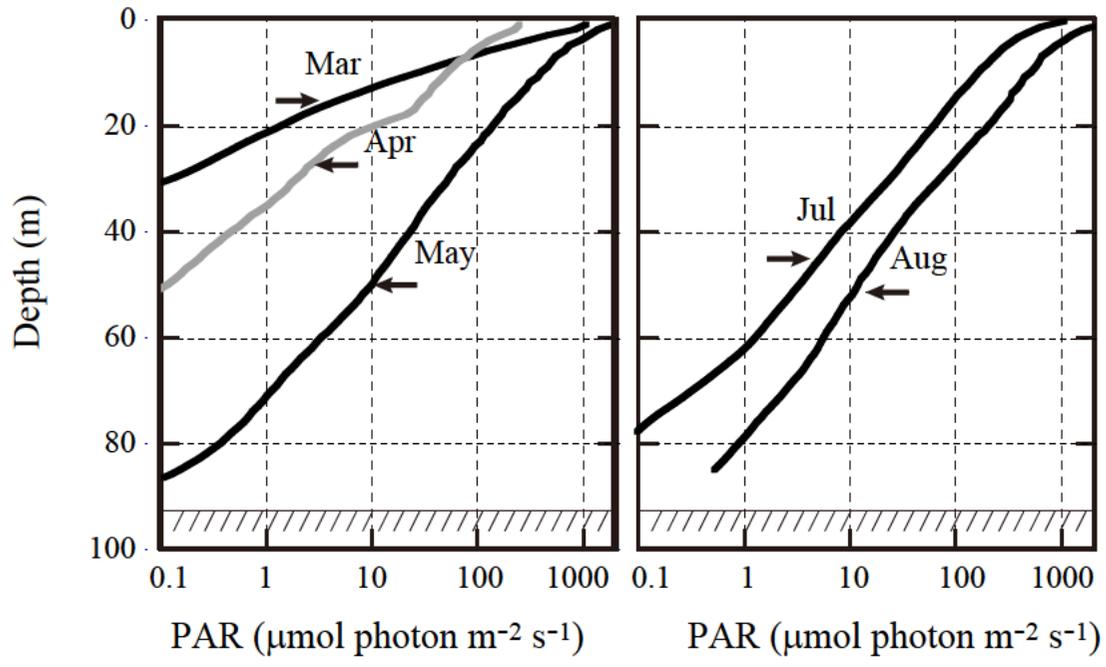


Figure 7

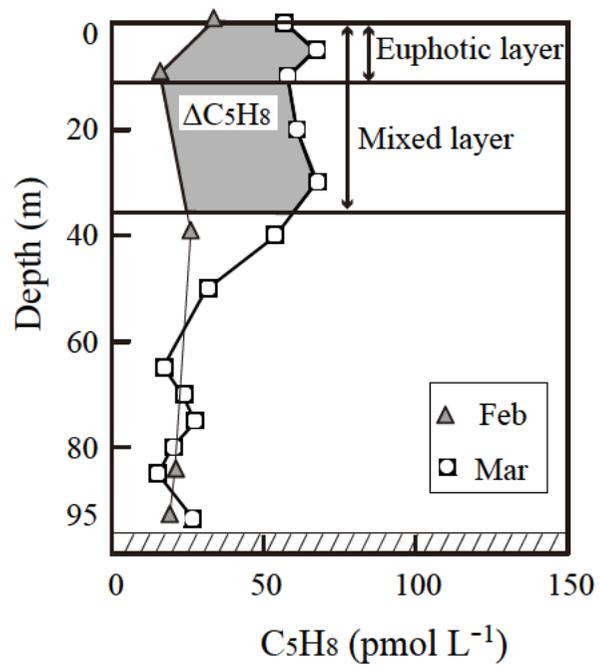


Figure 8

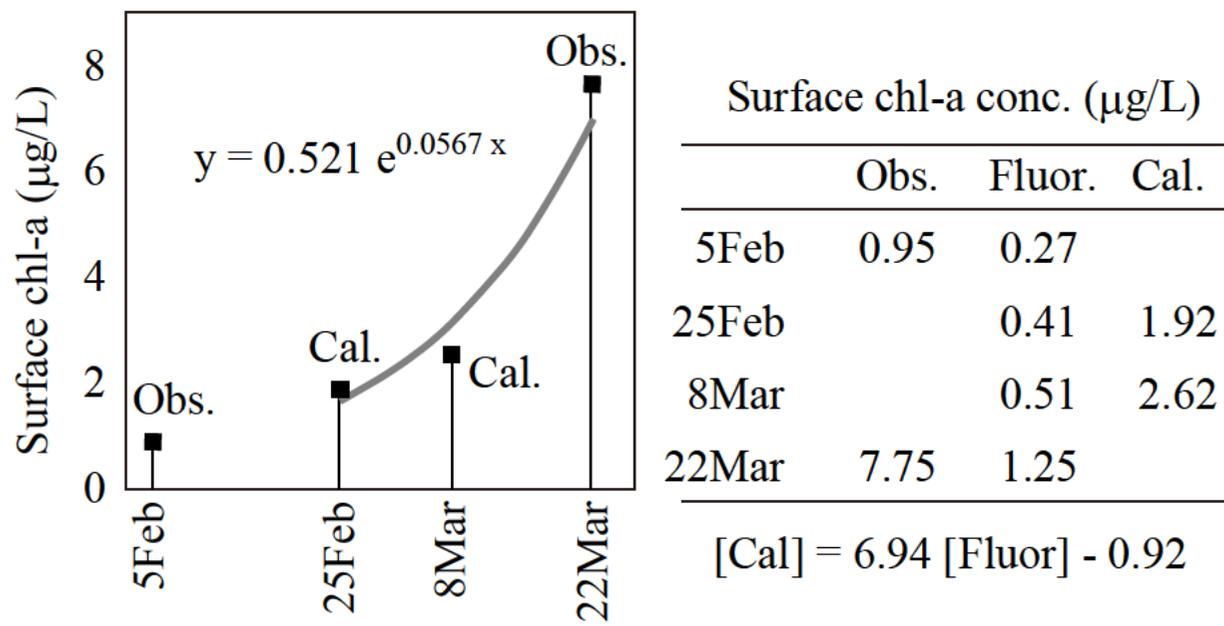


Figure 9

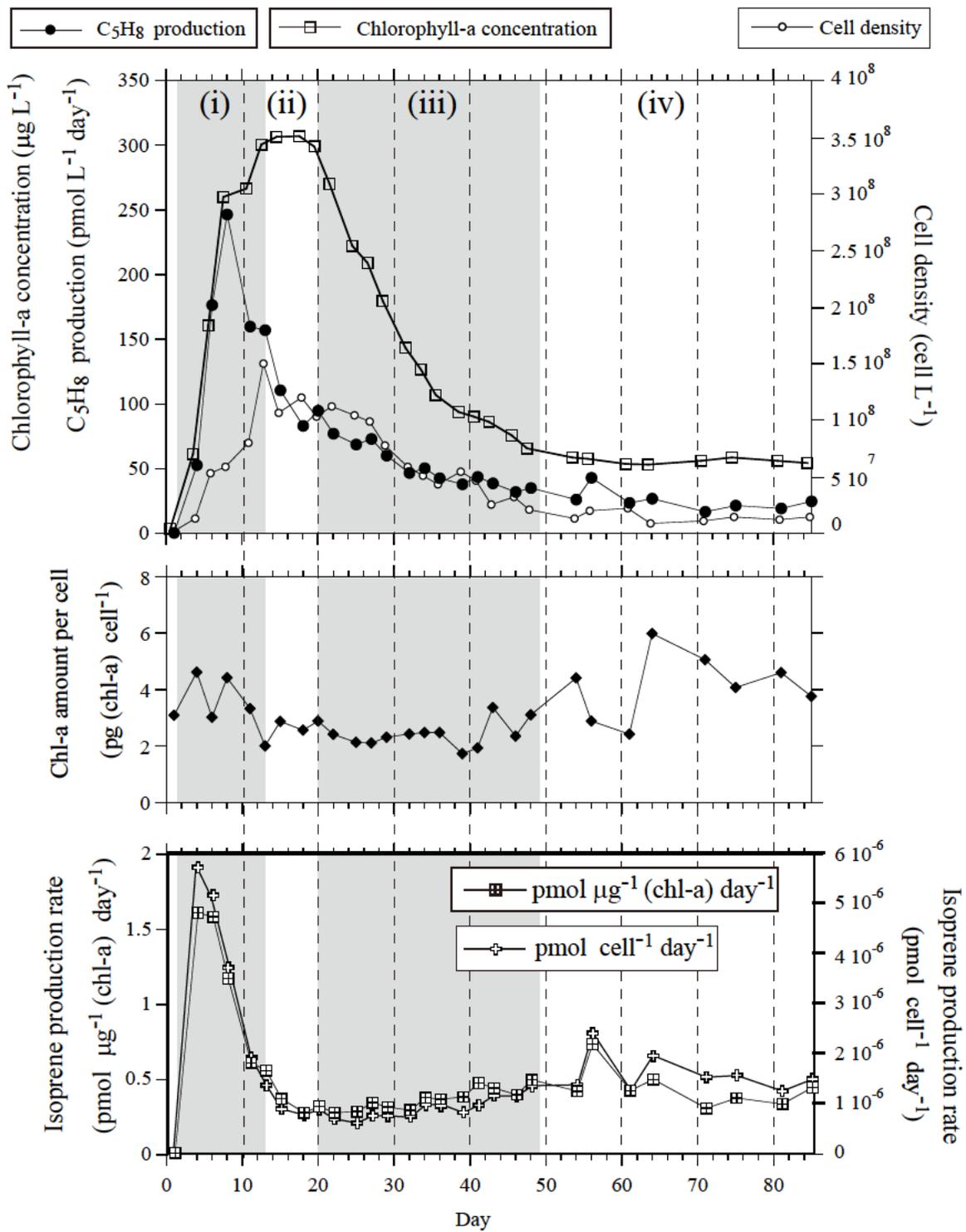


Figure 10

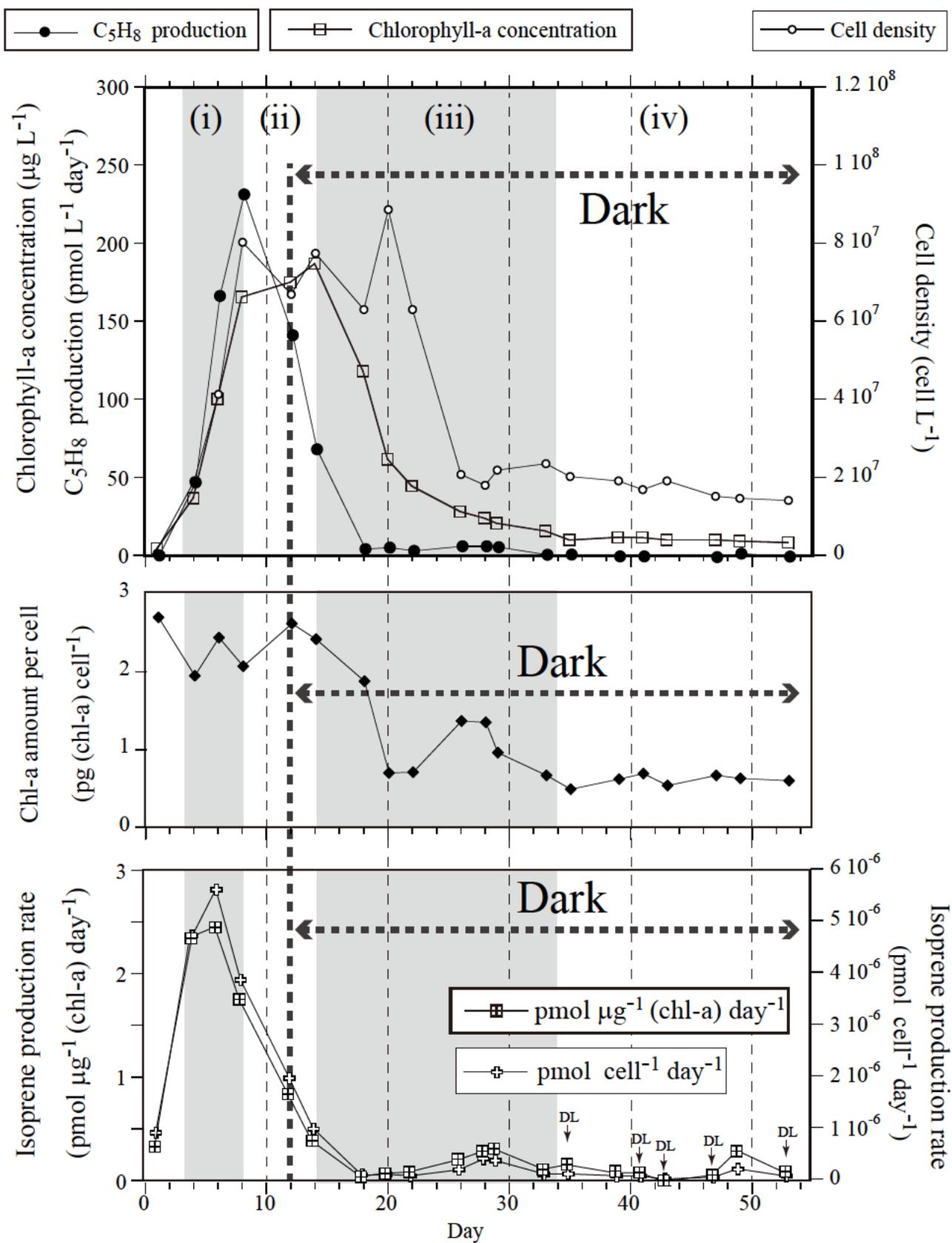


Figure 11