Seasonal dynamics of biodegradation activities for dimethylarsinic acid (DMA) in Lake Kahokugata

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Response to Reviewer #1

We thank for the your evaluation for acceptable of our manuscript.

Response to Reviewer #2

I thank for admitting the value of our manuscript as acceptable.

Response to Editor comments:

I thank for admitting the value of our manuscript. Furthermore, I appreciate for your check in manuscript. The sections [Q] indicate your comments and the sections (A) indicate my responses. The changes introduced in the revised manuscript were shown by the page and line numbers with under lines at the sections (A).

[Q1]. page 1, line 1: delete line

(A1) page 1: I deleted the line including "Title".

[Q2]. page 1, line 5: delete line

(A2) page 1: I deleted the line including "Authors".

[Q3]. page 1, lines 6-7: Delete all superscripts "1". Those are redundant since there is only one address for all authors.

(A3) page 1, lines 4-5: I deleted all superscripts "1".

[Q4]. page 1, line 9: delete line

(A4) page 1: I deleted the line including "Affiliation of all authors".

[Q5]. page 1, line 10: delete "1" .

(A5) line 7: I deleted "1".

[Q6]. page 2: delete line

(A6) page 2: I deleted the line including "Revised manuscript without the marked changes".

[Q7]. line 14: correct "degradation is diminished"

(A7) lines 12: I revised " degradation diminished " to " degradation was diminished ".

[Q8]. line 39: insert space and comma "al., 2007]."

(A8) line 37: I inserted space and comma and revised to "al., 2007]."

[Q9]. line 80: delete "at total"

(A9) line 79: I deleted "at total ".

[Q10]. line 82: delete "shallow at"

(A10) line 81: I deleted "shallow at".

[Q11]. line 107: units "hr" should be "h"

(A11) line 106: I revised units "hr" to "h".

[Q12]. line 110: units "hr" should be "h"

(A12) line 109: I revised units "hr" to "h".

[Q13]. line 112: Those cannot be correct units for the light intensity. The light intensity is normally expressed in energy units like W, J or kcal per squared cm. Also "<mu>mol photon" units are ill defined at least since the energy of photons depends on frequency and there is no "universal" photon. Thus, those units do not give information about the light intensity. This must be clarified.

(A13) The units "<mu>mol photon" units do not express the light intensity, as indication, and show the photon densities of lights. In this study, cool white fluorescence lumps were used for the incubation. Therefore, I described the photon densities of cool white fluorescence lumps.
line 111: I revised "a light intensity of 150 <mu>mol photon m^{-2} sec^{-1}" to "a photon flux density of 150 <mu>mol m^{-2} sec^{-1} of cool white fluorescent lamps".

[Q14]. lines 163-165: This sentence sounds confusing and I cannot understand its last part (line 165). It seems to me that you should delete "periods and the analytical process using this employed measurement" and "experiment" should be "experiments".

(A14) lines 162-164: I agree with your comment and removed "periods and the analytical process using this employed measurement". Moreover, I revised "experiment" to "experiments"..

[Q15]. line 214: "days" should be "day" Correct 2 times.

(A15) lines 213: I revised ""days" to "day" at 2 sections.

[Q16]. line 230: delete "of incubation"

(A16) line 229: I deleted "of incubation ".

[Q17]. line 231: correct "degrade in 21 or 35 days"

(A17) lines 230: I revised "degraded at 21days and 28 days" to "degraded in 21 or 35

days".

[Q18]. line 255: "concentrations" should be "concentration"

(A18) lines 254: I revised "concentrations" should be "concentration".

[Q19]. line 302: correct "arsenic in 21 or 35 days (Fig. 3)."

(A19) lines 301: I revised " arsenic at 21 and 28 days (Fig. 3)." to " arsenic in 21 or 35 days (Fig. 4).".

[Q20]. lines 383-384: correct book title "Arsenic Compounds in the Environment, Environmental Chemistry of Arsenic."

(A20) lines 382-384: I revised "Arsenic compounds in the environment, Environmental chemistry of arsenic. Marcel Dekker, New York, " to " Arsenic Compounds in the Environment, In: Frankenberger, W.T. (Eds.). Environmental Chemistry of Arsenic. Marcel Dekker, New York, USA, pp. 51-94.".

[Q21]. Table 1: units "uC" should be "ºC"

(A21) Table 1: I revised units "uC" to "ºC".

Other revised section

(1) line 229: I revised "At 30˚C," to "At 20˚C,".

(2) line 319: I revised "Fig. 4a" to " Fig. 5a ".

(3) line 320: I revised "Fig. 4b" to " Fig. 5b ".

Abstract

The microbial activities in aquatic environments significantly influence arsenic cycles such as the turnover between inorganic arsenic and organoarsenic compounds. In Lake Kahokugata, inorganic arsenic was detected at concentrations ranging from 2.8 to 23 nM in all seasons, while the concentrations of dimethylarsinic acid (DMA) produced by microorganisms such as phytoplankton changed seasonally and showed a peak in winter. The changes in the concentrations of methylarsenic species did not correlate with the changes in phytoplankton abundance (chlorophyll a contents), suggesting that DMA-degradation is related to this inconsistency. One μM of DMA added into the lake water was converted to inorganic arsenic at 20 ºC only under anaerobic and dark conditions, while DMA degradation was diminished under aerobic or light conditions. Moreover, DMA added to the lake water samples collected through four seasons was degraded at the same rates under anaerobic and dark conditions at 20 ºC. However, at 30 ºC, 1 μM of DMA in the summer lake water samples was rapidly degraded in 7 and 21 days. In contrast, DMA degradation was diminished in the winter lake water samples at 4 ºC of incubation. Presumably,

18 DMA-biodegradation activities are mainly controlled by changes in the water
19 temperature in Lake Kahokugata, where the arsenic concentrations change seasonally.
20

Introduction

Arsenic compounds are widely distributed in aquatic environments in a variety of chemical forms, and some of them are known to endanger human health and organism activities at high concentrations (Cullen and Reimer, 1989; Ninh et al., 2008; Peshut et al., 2008). The dynamics of arsenic forms have attracted much attention from those seeking to understand the arsenic cycles in aquatic environments (Oremland and Stolz, 2003). Among the variety of arsenic species, arsenate, arsenite, and methylated arsenic compounds dominate in both fresh water and seawater environments, and the conversion process mainly depends on the bioactivities of microorganisms that readily metabolize the arsenic species (Oremland and Stolz, 2003). The microbial reduction of arsenate in soils enhanced the release of arsenic compounds into ground water, causing the arsenic contamination of drinking water (Stolz et al., 2006). Microorganisms, such as phytoplankton (microalgae) and bacteria, uptake and accumulate ambient arsenate under phosphate-limited conditions through their phosphate-metabolism because arsenate is a chemical analogue of phosphate (Andreae, 1979; Farías et al., 2007). Moreover, the phytoplankton in aquatic environments

38 reduce arsenate into arsenite or methylate it into monomethylarsonic acid
39 ($CH_3AsO(OH)_2$; MMA(V)) and dimethylarsinic acid ($(CH_3)_2AsO(OH)$; DMA(V))
40 (Francesconi and Kuehnelt, 2002). The produced MMA and DMA are subsequently
41 converted to more complex organoarsenic compounds such as tetramethylarsonium ion
42 and arsenosugars by phytoplankton, bacteria, and/or fungi (Francesconi and Kuehnelt.
43 2002).

44 Although phytoplankton produce organoarsenic compounds in aquatic
45 environments, there was not a significant positive correlation between the *in situ*
46 amounts of chlorophyll a (the biomass of phytoplankton) and of organoarsenic
47 compounds in aquatic environments (Hasegawa, 1996). Sohrin et al. (1997)
48 speculated that environmental degradation of organoarsenic compounds by bacteria had
49 led to this inconsistency. The dominant chemical forms in a number of lakes and
50 estuaries have been reported to change seasonally by the degradation and production of
51 organoarsenic compounds (Anderson and Bruland, 1991). Considering the seasonal
52 dynamics and the distribution of arsenic compounds in aquatic environments, the
53 DMA-degradation process is worthy of study. A few reports described that
54 environmental bacteria in marine sediments (Sanders, 1979), seawater (Kaise et al.,

55 1985), and associated consorcia with marine animals, such as crabs (Khokiattiwong et
56 al., 2001) and mussels (Jenkins et al., 2003), could degrade the organoarsenic
57 compounds amended. Bacterial isolates from activated sludge (Quinn and McMullan,
58 1995) and natural environments (Lehr et al., 2003; Maki et al., 2006) also degraded
59 organoarsenic compounds to inorganic arsenic. However, little information is
60 available on the influence of environmental factors on the DMA-biodegradation process
61 in aquatic environments, and the ecological characteristics of DMA biodegradation are
62 unclear. In our previous investigation, the bacterial composition of DMA-degrading
63 bacteria was demonstrated to change seasonally in the lakes of Japan (Maki et al., 2006),
64 but, until the present study, the seasonal dynamics of biodegradation activities for
65 organoarsenic compounds had not been estimated in detail in a single lake.

66 In this study, the seasonal change in the concentrations of arsenic species was
67 investigated in Lake Kahokugata from April 2005 to March 2008 to estimate the
68 interaction of the arsenic dynamics between arsenic compounds and chlorophyll a.
69 Moreover, environmental factors controlling DMA degradation were determined in the
70 lake water samples spiked with DMA, and the DMA-degradation activities in the
71 natural lake water were estimated in all seasons during the investigation period. DMA

72 was selected as a representative organoarsenic compound that is widely distributed in
73 freshwater (Sohrin et al., 1997).
74
75 **Experimental**
76
77 **Sampling at Lake Kahokugata**
78 A lake water sample at a depth of 1 m was collected in polycarbonate bottles
79 from Lake Kahokugata in the Ishikawa Prefecture of Japan 22 times from April 2005 to
80 March 2008. Lake Kahokugata is eutrophic and suffered from wastewater inflow from
81 cities and croplands. The depth of Lake Kahokugata is less than 2 m and the water is
82 frequently mixed throughout the four seasons. The oxygen levels in the lake water
83 sample ranged from 2.0 to 8.3 mg L^{-1} during the investigation period. When the water
84 transparency was measured using a standard 25 cm black and white Secchi disk, the
85 disk depths ranged from 0.1 m to 1 m from water surface during the investigation period,
86 indicating that the sun irradiation hardly reached to the depth of 1 m. For the
87 measurement of arsenic species and chlorophyll a, 50 mL of sample water was filtrated
88 with a GF/C glass fiber filter (ADVANTEC, Tokyo, Japan). The concentrations of

89 arsenic species in the filtrate were determined using a cold trap HG-AA speciation
90 procedure. Chlorophyll a was extracted from the GF/C glass fiber filter with acetone
91 and assessed colorimeterically (Maki et al., 2005). Moreover, surface water samples
92 of Lake Kahokugata in several polycarbonate bottles were used for the determination of
93 the DMA-biodegradation activities of natural lake water. These samples were
94 incubated under different treatments.
95
96 **Experiment design and DMA biodegradation in lake water**
97 The lake water samples collected into polycarbonate bottles from Lake
98 Kahokugata on October 10, 2006, were used for investigating DMA-degradation
99 activities in lake water samples incubated under aerobic and anaerobic conditions and
100 light and dark conditions. Twelve polycarbonate bottles (500 mL) were filled up with
101 lake water and transferred to our laboratory. Within 2 hours of sampling, 500 μL of a
102 1 mM DMA (Nacalai Tesque, Kyoto, Japan) solution was added into 12 bottles at a final
103 concentration of 1 μM. One half of the bottles (6) in each experiment were incubated
104 under anaerobic conditions. To produce the anaerobic conditions, the air phases in the
105 bottles were kept at the lowest possible level, and the water samples were purged with

106 nitrogen (100 mL min^{-1}) for 0.5 h. The remaining half of (6 bottles) were incubated
107 under aerobic conditions. To produce the aerobic conditions, natural air filtrated
108 through a 0.2 μm Nuclepore filter (Whatman, Tokyo, Japan) was continuously supplied
109 at 700 m^3 h^{-1} into the bottle using an air-pump. After the anaerobic and aerobic
110 treatments, 3 bottles under each anaerobic and aerobic condition were incubated under a
111 photon flux density of 150 μmol m^{-2} sec^{-1} of cool white fluorescent lamps with a
112 12:12 light:dark cycle as the light condition. The remaining 3 bottles under each
113 anaerobic and aerobic condition were incubated under dark conditions by covering the
114 bottles with aluminum foil. The experiments consisted of a total of four conditions:
115 anaerobic and light, aerobic and light, anaerobic and dark, and aerobic and dark. The
116 water samples were then incubated in a controlled temperature room (20 ºC).
117 Moreover, for estimating the biosynthesis from arsenate to DMA, arsenate was added to
118 500 mL bottles of lake water samples at a final concentration of 1 μM, and a single
119 bottle of the water samples was incubated at 20 ºC under each of four conditions.
120 On the other hand, the microbial activities in the lake water sample were
121 eliminated using four treatments: the lake water was autoclaved at 120 ºC for 20
122 minutes; an antibiotic mixture was added to each sample of lake water at a final

123 concentration of 10 mg L^{-1}; sodium azide was added to each sample of lake water at a
124 final concentration of 10 mg L^{-1}; and the lake water was filtrated through a 0.02 μm
125 polycarbonate filter. Three bottles (500 mL) of the lake water samples treated by each
126 method and spiked with DMA at a final concentration of 1 μM were incubated at 20 ºC
127 under anaerobic and dark conditions. The oxygen concentrations of the lake water
128 sample under the aerobic condition were always approximately 8.5 mg L^{-1}. In the
129 anaerobic condition, the oxygen levels ranged from 1.2 to 2.3 mg L^{-1} during the
130 experiments.

131 In order to compare the DMA-degradation activities in the lake water in four
132 seasons, spring (March, April, and May), summer (June, July, and August), fall
133 (September, October, and November), and winter (December, January, and February),
134 lake water samples were collected every few months from June 2005 to February 2008
135 in polycarbonate bottles (500 mL). The 500 μL of 1 mM DMA solution was added
136 into bottles at a final concentration of 1 μM, and the bottles were incubated at 20 ºC
137 under anaerobic and dark conditions. Furthermore, to examine the effects of water
138 temperature on the DMA-degradation activities, the lake water samples that were
139 collected in summer (July 1, 2006, July 28, 2006, and August 9, 2007) and winter

140 (December 13, 2006, February 28, 2007, and February 3, 2008) and spiked with DMA
141 added at a final concentration of 1 μM were incubated under anaerobic and dark
142 conditions at temperatures of 30 ºC and 4 ºC, respectively, in controlled-temperature
143 boxes for 56 days. Each experiment was performed in triplicate.
144 During the incubation period (56 days), portions (10 mL) of the water samples
145 were collected, and the concentrations of arsenic species were determined using a
146 cold-trap hydride-generation atomic-absorption (HG-AA) speciation procedure.
147
148 **Measurements of the arsenic compound concentration**
149 The cold-trap HG-AA speciation procedure was employed as the protocol
150 previously reported (Braman and Foreback, 1973; Hasegawa et al., 1994). The water
151 subsamples, which were filtrated through a 0.45 μm cellulose ester filter (ADVANTEC,
152 Tokyo, Japan), were adjusted to 40 mL using pure water and acidified by the addition of
153 5 mL of a 0.2 M EDTA solution and 5 mL of 5 M HCl. Next, 10 mL of a 30% (w v^{-1})
154 $NaBH_4$ solution was gradually added to the sample solution at a speed of 2 mL min^{-1},
155 and the arsenic included in the sample solution was evaporated by reacting with $NaBH_4$.
156 The produced arsines were swept by a flow of nitrogen into a cold-trap column cooled

157 by liquid nitrogen. After the column was gently warmed by electrical heating, the
158 arsines (including inorganic arsenic, MMA, and DMA) released from the column were
159 loaded into a quartz-T tube held at about 900 ˚C in a flame and quantified using an
160 atomic absorption spectrometer Z-8100 (Hitachi, Chiba, Japan). The potential
161 concentrations for detection of arsenic compounds were more than 1.0 nM of measured
162 solution. Moreover, there is a low possibility that other arsenic species, except for
163 inorganic arsenic, MMA, and DMA, are produced in the water samples during the
164 experiments.
165
166 **Results**
167
168 **Seasonal variation in Lake Kahokugata**
169 In Lake Kahokugata, the concentrations of chlorophyll a increased to amounts in
170 excess of 50 μg L^{-1} from spring to summer and decreased to below 15 μg L^{-1} from fall
171 to winter during the investigation period between April 2005 and March 2008,
172 suggesting that the growth of phytoplankton was activated from spring to summer (Fig.
173 1a). The concentrations of inorganic arsenic fluctuated ranging from 2.8 to 23 nM

through all seasons, while DMA was detected at peaks of up to 13 nM only during fall and winter. Moreover, MMA was not detected from water samples during the investigation period. Consequently, the changes in the concentrations of methylarsenic compounds did not correlate with the changes in phytoplankton abundance during the investigation period. Furthermore, the water temperature was below 10 ˚C during winter and early spring (from December to April), while it increased to over 30 ˚C in summer (August) (Fig. 1b).

Incubation condition of DMA biodegradation in the lake water from Lake Kahokugata

When the lake water samples were spiked with DMA at a final concentration of approximately 1 μM and incubated at 20 ˚C under anaerobic and dark conditions, the concentration of DMA at the onset of the experiment decreased from 1020 nM (average) to the detection limit (avg.) during the first 21 days of incubation (Fig. 2d). In accordance with the decrease of DMA, the concentration of inorganic arsenic, which is considered to be the resultant product from DMA degradation, increased from 5.1 to 850 nM during the first 21 days and fluctuated over the concentration of 760 nM until

191 56 days of incubation. In contrast, under the other 3 conditions (anaerobic and light,
192 aerobic and dark, and aerobic and light), the reduction of DMA and the accumulation of
193 inorganic arsenic were not observed through 56 days of incubation (Fig. 2a, b, c).
194 When the microbial activities were eliminated using autoclave sterilization, addition of
195 antibiotics and sodium azide, or filtration, the DMA degradation and the accumulation
196 of inorganic arsenic diminished in the lake water samples with 4 treatments (Table 1).
197 The concentrations of inorganic arsenic and organoarsenic compounds in the lake water
198 without the addition of DMA, on the other hand, were stable below 10 nM during the
199 entire experiment (data not shown). These results indicated that this DMA degradation
200 occurred as a result of a biotic (microbiological) process under anaerobic and dark
201 conditions and that the physical degradation, including photochemical degradation and
202 heat degradation, could be ignored. On the other hand, in the lake water that was
203 spiked with inorganic arsenic, the concentrations of DMA maintained low
204 concentrations ranged below 450 nM from the 14^{th} day to the 56^{th} day (Fig. 3). These
205 results indicated that the rates of DMA synthesis are at relatively low levels, in contrast
206 to those of DMA degradation.
207

208 **Seasonal dynamics of DMA-biodegradation activities in the lake water**

209 In the lake water samples that were collected in four seasons and incubated with
210 the addition of approximately 1 μM DMA at 20 ˚C under anaerobic and dark conditions,
211 the DMA added to most of the lake water samples collected in the four seasons (15
212 samples of 22) decreased to the detection limit and was completely converted to
213 inorganic arsenic between 21st day and 28th day of incubation (Fig. 4). In the other 7
214 samples of lake water collected in spring, summer, and fall (sampling days - 7 June
215 2005, 1 November 2005, 27 April 2006, 1 September 2006, 24 April 2007, 9 August
216 2007, and 26 October 2007), the DMA biodegradation and the accumulation of
217 inorganic arsenic were observed for longer incubation times ranging from 35 to 56 days.
218 Consequently, at 20 ˚C of incubation under anaerobic and dark conditions, DMA added
219 to the lake water samples was degraded at similar rates throughout the four seasons.

220

221 **DMA-degradation activities of lake water samples at different temperatures**

222 The degradation patterns of DMA were significantly different at different
223 incubation temperatures, such as 30 ˚C and 4 ˚C, under anaerobic and dark conditions
224 using lake water collected in the summer (July and August) and winter (February and

March), respectively. In the lake water collected in the summer and incubated at 30˚C, 1 μM of DMA was rapidly degraded and converted to 860 nM of inorganic arsenic for short incubation times ranging from 7 days to 21 days (Fig. 5a). In contrast, DMA degradation was not observed in the winter lake water samples, which was incubated at 4 ˚C (Fig. 5b). At 20˚C, DMA spiked into the same water samples of summer and winter was completely degraded in 21 or 35 days (Fig. 4b, d). These results mean that DMA degradation was activated at a high temperature of 30˚C and reduced at a low temperature of 4 ˚C.

Discussion

Phytoplankton in lake water and coastal seawater incorporate and accumulate inorganic arsenics instead of phosphorus and synthesize organoarsenic compounds for detoxification (Andrete, 1979; Hasegawa et al., 2001; Santosa et al., 1994). In Lake Kahokugata, the concentrations of chlorophyll a in water samples indicated peaks (up to 100 μg L^{-1}) during spring and summer indicating the activity of phytoplankton (Fig. 1). DMA increased to concentrations of up to 13 nM from late fall to winter through the

242 investigation period. These results indicated that the dynamics of methylarsenic
243 species were not related to the dynamics of chlorophyll a in Lake Kahokugata. In
244 lakes and coasted areas, the changes in microalgal abundance (chlorophyll a contents)
245 did not positively correlate with the changes in the concentrations of methylarsenic
246 species (Hasegawa et al., 1996). In contrast, in other aquatic environments, the
247 concentrations of DMA frequently increased in summer positively and correlated with
248 the production of phytoplankton (Sohrin et al., 1997). Some microorganisms, such as
249 fungi and bacteria, have been reported to produce DMA as well as phytoplankton
250 (Francesconi and Kuehnelt, 2002). Except for phytoplankton, these microorganisms
251 might produce DMA during winter in Lake Kahokugata. Sanders (1979) also
252 demonstrated that microbial communities in environmental freshwater system
253 demethylated DMA to inorganic arsenate. In this study, both the biosynthesis and
254 biodegradation of DMA, which vary with time, seemed to determine the concentration
255 of DMA in aquatic environments. The water samples from Lake Kahokugata spiked
256 with DMA were converted to inorganic arsenic only under dark and anaerobic
257 conditions of incubation (Fig. 2d). Furthermore, this DMA degradation was not
258 observed in the lake water in which the bacterial activities were eliminated by four

259 treatments, including autoclave sterilization, filtration, and the addition of sodium azide
260 and antibiotics. These results suggested that this degradation of DMA occurs as a
261 result of a biotic (microbiological) process. Biological demethylation has been
262 reported to be the dominant process for the generation of inorganic arsenic from
263 organoarsenic compounds (Andreae, 1979). In a previous investigation, several
264 species of DMA-degrading bacteria were isolated from Lake Kahokugata (Maki et al.,
265 2005). This study suggested that the DMA-degrading microorganisms generally
266 inhabiting Lake Kahokugata would degrade the methylarsenic compounds produced by
267 microorganisms and influence the arsenic cycling in aquatic ecosystems.

268 Degradation of DMA to inorganic arsenic occurred only under anaerobic and
269 dark conditions and was not observed in the lake water that was incubated under aerobic
270 or light conditions (Fig. 2). Woolson (1977) also reported that, in the soil under
271 aerobic conditions, methylarsenic was not converted to arsenate. Several kinds of
272 organic matter were degraded only under anaerobic environments, including the
273 sediments of lakes, suggesting that the anaerobic microbial population contributes to the
274 degradation (Coates et al., 2001; Bastviken et al., 2004; Fathepure and Vogel, 1991).
275 Anaerobic microbial reactions in the lake water of Lake Kahokugata would be relatively

276 optimal for converting DMA to inorganic arsenic. In Lake Kahokugata, which
277 averages slightly less than 2 m in depth, the water would be vertically mixed in all
278 seasons, and the DMA-degrading bacteria would be transported from the lake sediments,
279 which is under dark and anaerobic conditions.

280 Moreover, under light conditions, phototrophic microorganisms can grow and
281 produce greater amounts of organic matter than under dark conditions and create the
282 dynamics of a microbial population (Takenaka et al., 2007). Organic matter, such as
283 glucose, is known to inhibit the degradation of methylarsenic compounds (Gao et al.,
284 1997). The addition of glucose into the lake water of Lake Kahokugata inhibited the
285 DMA degradation (data not shown). Accordingly, DMA biodegradation under light
286 conditions might be reduced by the products of phototrophic microorganisms.
287 Furthermore, as described, some phototrophic organisms, such as fungi and plankton,
288 are reported to uptake inorganic arsenic and convert it into DMA (Hasegawa et al.,
289 2001; Sntosa et al., 1994). However, in this study, the biosynthesis of DMA in the lake
290 water was at relatively low levels under aerobic and light conditions and was not
291 observed under aerobic and dark and anaerobic and light conditions (Fig. 3). Cheng
292 and Focht (1979) also reported that microorganisms involved in the demethylation

293 process in the soil were more abundant than DMA-synthesizing microorganisms. In
294 Lake Kahokugata, DMA synthesis by phytoplankton grown under aerobic and light
295 conditions should also be at low levels but might offset, to some degree, the DMA
296 decrease by biodegradation.

297 DMA-biodegradation activities are thought to influence the seasonal changes in
298 the concentrations of DMA, which are caused by microorganisms. When lake water
299 collected in all seasons and spiked with 1 μM of DMA was incubated at 20 ºC, the
300 DMA in most of lake water samples in the four seasons was converted to inorganic
301 arsenic in 21 or 35 days of incubation (Fig. 4). The species compositions of
302 DMA-degrading bacteria have been reported to change seasonally in Lake Kahokugata
303 (Maki et al., 2005). Anderson and Brueland (1991) reported that, in a number of lakes
304 and estuaries, the rates of DMA degradation were faster in water in winter when the
305 water layer was mixed. However, the depth of Lake Kahokugata was shallow at less
306 than 2 m and the water was constantly mixed throughout the four seasons. Therefore,
307 the DMA-degradation experiments performed under incubation at 20 ºC indicated that
308 similar rates of potential DMA degradation were obtained in all four seasons regardless
309 of the seasonal changes of bacterial composition. On the other hand, the DMA spiked

310 into some samples of lake water in spring, summer, and fall continued to be degraded
311 for incubation times ranging from 35 and 56 days. In some sampling days of spring,
312 summer, and fall, the low abundance of microorganisms transported from the lake
313 sediments may reduce the DMA-degradation activities. Moreover, phytoplankton
314 activities that synthesize DMA and increase from spring to summer (Fig. 1a) are
315 thought to reduce the rate of DMA decrease and inorganic arsenic accumulation in the
316 natural lake water in the spring, summer, and fall.

317 Furthermore, in the lake water that was collected in the summer and incubated at
318 30 ºC, 1 μM of DMA was rapidly degraded at incubation times ranging from 7 to 21
319 days (Fig. 5a). When the lake winter water samples were incubated at 4 ºC, DMA
320 degradation was negligible (Fig. 5b). The water temperature in aquatic environments
321 was reported to influence the dynamics of bacterial communities and the levels of
322 metabolic activities by microorganisms (Pomeroy and Wiebe, 2000; Simon, 1999). In
323 Lake Kahokugata, the water temperature was below 10 ˚C in fall and winter, while it
324 increased to over 30˚C from spring to summer (Fig. 1b). Although the potential rates
325 of DMA degradation under incubation at 20˚C maintained similar levels in all seasons
326 (Fig. 4), the water temperature could change the DMA-degradation activities in the lake

327 water and overcome the potential activities of DMA degradation in each season. The
328 low temperature in winter would reduce the DMA-biodegradation activities, while the
329 high temperature in summer would activate the DMA biodegradation in Lake
330 Kahokugata. Consequently, organoarsenic compounds might maintain a concentration
331 of up to 20 nM in winter, and the high microbial activities in summer might degrade
332 organoarsenic compounds in the lake water.
333
334 **Conclusions**
335
336 This is the first report directly demonstrating that DMA biodegradation in aquatic
337 environments is enhanced under anaerobic and dark conditions. Although the DMA
338 degradation potentially maintained the same rates throughout the four seasons, the
339 seasonal dynamics of the DMA-biodegradation activities in Lake Kahokugata are
340 thought to depend on changes in the water temperature. In Lake Kahokugata, the
341 residue of DMA was detected only during fall and winter, when the low water
342 temperature would reduce the DMA biodegradation. In summer, DMA in the lake is
343 thought to disappear due to the high activities of DMA-biodegradation at high

344 temperatures. Considering the arsenic cycles in aquatic environments, the
345 biodegradation process of organoarsenic compounds appeared to be as important as the
346 biosynthesis process of organoarsenic compounds. In the future, since the arsenic
347 cycles were composed of a highly complex structure of organoarsenic compounds such
348 as arsenobetaine, which are also produced by microorganisms, the processes of
349 degradation and biosynthesis involving highly complex organoarsenic compounds
350 should be investigated in order to elucidate the arsenic cycles in aquatic environments.
351

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353

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358

359 **References**

360

361 Anderson, L.C.D., Bruland, K.W., 1991. Biogeochemistry of arsenic in natural waters:
362 The importance of methylated species. Environ. Sci. Technol. 25, 420-427.
363 Andreae, M.O., 1979. Arsenic speciation in seawater and interstitial waters: The
364 influence of biological-chemical interactions on the chemistry of a trace element.
365 Limnol. Oceanogr. 24, 440-452.
366 Bastviken, D., Persson, L., Odham, G., Tranvik, L., 2004. Degradation of dissolved
367 organic matter in oxic and anoxic lake water. Limnol. Oceanogr. 49, 109-116.
368 Braman, R.S., Foreback, C.C., 1973. Methylated forms of arsenic in the environment.
369 Science 182, 1247-1249.
370 Cheng C.N., Focht D.D., 1979. Production of arsine and methylarsines in soil and in
371 culture. Appl. Environ. Microb. 38, 494-498.
372 Coates J.D., Chakraborty R., Lack J.G., O'Connor S.M., Cole K., 2001. Anaerobic
373 benzene oxidation coupled to nitrate reduction in pure culture by two novel
374 organism. Nature 411, 1039-1043.
375 Cullen, W.R., Reimer, K.J., 1989. Arsenic speciation in the environment. Chem. Rev. 89,
376 713-764.
377 Farías, S., Smichowski, P., Vélez, D., Montoro, R., Curtosi, A., Vodopívez, C., 2007.

Total and inorganic arsenic in Antarctic macroalgae. Chemosphere 69, 1017-1024.

Fathepure B.Z., Vogel T.M., 1991. Complete degradation of polychlorinated hydrocarbons by a two-stage biofilm reactor. Appl. Environ. Microbiol. 57, 3418-3422.

Francesconi, K.A., Kuehnelt, D., 2002. Arsenic Compounds in the Environment, In: Frankenberger, W.T. (Eds.). Environmental Chemistry of Arsenic. Marcel Dekker, New York, USA, pp. 51-94.

Gao S., Burau, R.G., 1997. Environmental factors affecting rates of arsine evolution from and mineralization of arsenicals in soil. J. Environ. Qual. 26, 753-763.

Hasegawa, H., 1996. Seasonal changes in methylarsenic distribution in Tosa Bay and Uranouchi Inlet. Appl. Organomet. Chem. 10, 733-740.

Hasegawa, H., Sohrin, Y., Matsui, M., Honjo, M., Kawashima, M., 1994. Speciation of arsenic in natural waters by solvent extraction and hydride generation atomic absorption spectrometry. Anal. Chem. 66, 3247-3252.

Hasegawa, H., Sohrin, Y., Seki, K., Sato, M., Norisuye, K., Naito, K., Matsui, M., 2001. Biosynthesis and release of methylarsenic compounds during the growth of freshwater algae. Chemosphere 43, 265-272.

395 Jenkins, R.O., Ritchie, A.W., Edmonds, J.S., Goessler, W., Molenat, N., Kuehnelt, D.,
396 Harrington, C.F., Sutton, P.G., 2003. Bacterial degradation of arsenobetaine via
397 dimethylarsinoylacetate. Arch. Microbiol. 180, 142-150.
398 Kaise, T., Hanaoka, K., Tagawa, S., 1985. The formation of trimethylarsine oxide from
399 arsenobetaine by biodegradation with marine microorganisms. Chemosphere 16,
400 2551-2558.
401 Khokiattiwong, S., Goessler, W., Pedersen, S.N., Cox, R., Francesconi, K.A., 2001.
402 Dimethylarsinoylacetate from microbial demethylation of arsenobetaine in
403 seawater. Appl. Organomet. Chem. 15, 481-489.
404 Lehr, C.R., Polishchuk, E., Radoja, U., Cullen, W.R., 2003. Demethylation of
405 methylarsenic species by *Mycobacterium neoaurum*. Appl. Organomet. Chem. 17,
406 831-834.
407 Maki, T., Hasegawa, H., Ueda, K., 2005. Seasonal dynamics of dimethylarsinic acid
408 (DMAA) decomposing bacteria dominated in Lake Kahokugata. Appl. Organomet.
409 Chem. 19, 231-238.
410 Maki, T., Takeda, N., Hasegawa, H., Ueda, K., 2006. Isolation of monomethylarsonic
411 acid (MMAA)-mineralizing bacteria from arsenic contaminated soils of Island

412 Ohkunoshima. Appl. Organomet. Chem. 20, 538-544.

413 Maki, T., Watarai, H., Kakimoto, T., Takahashi, M., Hasegawa, H., Ueda, K., 2006.

414 Seasonal dynamics of dimethylarsenic acid degrading bacteria dominated in Lake

415 Kibagata. Geomicrobiol. J. 23, 311-318.

416 Ninh, T.D., Nagashima, Y., Shiomi, K., 2008. Unusual arsenic speciation in sea

417 anemones. Chemosphere 70, 1168-1174.

418 Oremland, R.S., Stolz, J.F., 2003. The ecology of arsenic. Science 300, 939-944.

419 Quinn, J.P., McMullan, G., 1995. Carbon-arsenic bond cleavage by a newly isolated

420 gram-negative bacterium, strain ASV2. Microbiol. 141, 721-725.

421 Peshut, P.J., Morrison, R.J., Brooks, B.A., 2008. Arsenic speciation in marine fish and

422 shellfish from American Samoa. Chemosphere 71, 484-492.

423 Pomeroy, L.R., Wiebe, W.J., 2000. Temperature and substrates as interactive limiting

424 factors for marine heterotrophic bacteria. Aquatic Microbial. Ecol. 23, 187-204.

425 Sanders, J.G., 1979. Microbial role in the demethylation and oxidation of methylated

426 arsenicals in seawater. Chemosphere 8, 135-137.

427 Santosa, S.J., Wada, S., Tanaka, S., 1994. Distribution and cycle of arsenic compounds

428 in the ocean. Appl. Organomet. Chem. 8, 273-283.

429 Simon, M., Glöckner, F.O., Amann, R., 1999. Different community structure and
430 temperature optima of heterotrophic picoplankton in various regions of the
431 Southern Ocean. Aquat. Microb. Ecol. 18, 275-284.
432 Sohrin, Y., Matsui, M., Kawashima, M., Honjo, M., Hasegawa, H., 1997. Arsenic
433 biogeochemistry affected by eutrophication in lake Biwa, Japan. Environ. Sci.
434 Technol. 31, 2712-2720.
435 Stolz, J.F., Basu, P., Santini, J.M., Oremland, R.S., 2006. Arsenic and selenium in
436 microbial metabolism. Annu. Rev. Microbiol. 60, 107-130.
437 Takenaka, T., Tashiro, T., Ozaki, A., Takakubo, H., Yamamoto, Y., Maruyama, T.,
438 Nagaosa, K., Kimura, H., Kato, K., 2007. Planktonic bacterial population
439 dynamics with environmental changes in coastal areas of Suruga Bay. Microbes
440 Environ. 22, 257-267.
441 Woolson, E.A., 1977. Generation of alkylarsines from soil. Weed Sci. 25, 412-416.
442
443

444 **Figure legends**

445

446 Fig. 1 Seasonal variation in the concentrations of arsenic species and chlorophyll a
447 and the water temperature in Lake Kahokugata. (a) Open circles, closed circles, and
448 closed triangles indicate the abundance of inorganic arsenic, DMA, and MMA,
449 respectively. (b) Closed squares and closed diamonds show the amount of chlorophyll
450 a and the water temperature, respectively. .

451

452 Fig. 2 Changes in the concentrations of arsenic compounds in lake water samples, to
453 which 1 μM of DMA was added. The lake water samples were incubated at 20 ºC
454 under aerobic and light conditions (a), aerobic and dark conditions (b), anaerobic and
455 light conditions (c), and anaerobic and dark conditions (d). Open circles, closed circles,
456 and closed triangles indicate the abundance of inorganic arsenic, DMA, and MMA,
457 respectively. Each experiment was performed in triplicate.

458

459 Fig. 3 Changes in the concentrations of arsenic compounds in lake water samples to
460 which 1 μM of inorganic arsenic have been added. The lake water samples were
461 incubated at 20 ºC under aerobic and light condition (a), aerobic and dark condition (b),

462 anaerobic and light condition (c), and anaerobic and dark condition (d). Open circles,
463 closed circles, and closed triangles indicate the abundance of inorganic arsenic, DMA,
464 and MMA, respectively.
465
466 Fig. 4 Changes in the concentrations of arsenic compounds in lake water samples that
467 were collected from Lake Kahokugata in the four seasons, spring (March, April, and
468 May) (a), summer (June, July, and August) (b), fall (September, October, and
469 November) (c), and winter (December, January, and February) (d), and spiked with 1
470 μM of DMA. The lake water samples were incubated at 20 ºC under anaerobic and
471 dark conditions. The open and closed symbols indicate the abundance of inorganic
472 arsenic and DMA, respectively. MMA was below the detection limit.
473
474 Fig. 5 Changes in the concentrations of arsenic compounds in lake water samples to
475 which 1 μM of DMA have been added. The lake water samples collected in the
476 summer (July and August) (a) and winter (January and February) (b) were incubated at
477 30 ºC and 4 ºC, respectively, under anaerobic and dark conditions. The open and
478 closed symbols indicate the abundance of inorganic arsenic and DMA, respectively.

MMA was below the detection limit.

Seasonal dynamics of biodegradation activities for dimethylarsinic acid (DMA) in Lake Kahokugata

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Abstract

The microbial activities in aquatic environments significantly influence arsenic cycles such as the turnover between inorganic arsenic and organoarsenic compounds. In Lake Kahokugata, inorganic arsenic was detected at concentrations ranging from 2.8 to 23 nM in all seasons, while the concentrations of dimethylarsinic acid (DMA) produced by microorganisms such as phytoplankton changed seasonally and showed a peak in winter. The changes in the concentrations of methylarsenic species did not correlate with the changes in phytoplankton abundance (chlorophyll a contents), suggesting that DMA-degradation is related to this inconsistency. One μM of DMA added into the lake water was converted to inorganic arsenic at 20 ºC only under anaerobic and dark conditions, while DMA degradation was diminished under aerobic or light conditions. Moreover, DMA added to the lake water samples collected through four seasons was degraded at the same rates under anaerobic and dark conditions at 20 ºC. However, at 30 ºC, 1 μM of DMA in the summer lake water samples was rapidly degraded in 7 and 21 days. In contrast, DMA degradation was diminished in the winter lake water samples at 4 ºC of incubation. Presumably,

18 DMA-biodegradation activities are mainly controlled by changes in the water
19 temperature in Lake Kahokugata, where the arsenic concentrations change seasonally.
20

Introduction

Arsenic compounds are widely distributed in aquatic environments in a variety of chemical forms, and some of them are known to endanger human health and organism activities at high concentrations (Cullen and Reimer, 1989; Ninh et al., 2008; Peshut et al., 2008). The dynamics of arsenic forms have attracted much attention from those seeking to understand the arsenic cycles in aquatic environments (Oremland and Stolz, 2003). Among the variety of arsenic species, arsenate, arsenite, and methylated arsenic compounds dominate in both fresh water and seawater environments, and the conversion process mainly depends on the bioactivities of microorganisms that readily metabolize the arsenic species (Oremland and Stolz, 2003). The microbial reduction of arsenate in soils enhanced the release of arsenic compounds into ground water, causing the arsenic contamination of drinking water (Stolz et al., 2006). Microorganisms, such as phytoplankton (microalgae) and bacteria, uptake and accumulate ambient arsenate under phosphate-limited conditions through their phosphate-metabolism because arsenate is a chemical analogue of phosphate (Andreae, 1979; Farías et al., 2007). Moreover, the phytoplankton in aquatic environments

38 reduce arsenate into arsenite or methylate it into monomethylarsonic acid
39 ($CH_3AsO(OH)_2$; MMA(V)) and dimethylarsinic acid ($(CH_3)_2AsO(OH)$; DMA(V))
40 (Francesconi and Kuehnelt, 2002). The produced MMA and DMA are subsequently
41 converted to more complex organoarsenic compounds such as tetramethylarsonium ion
42 and arsenosugars by phytoplankton, bacteria, and/or fungi (Francesconi and Kuehnelt.
43 2002).

44 Although phytoplankton produce organoarsenic compounds in aquatic
45 environments, there was not a significant positive correlation between the *in situ*
46 amounts of chlorophyll a (the biomass of phytoplankton) and of organoarsenic
47 compounds in aquatic environments (Hasegawa, 1996). Sohrin et al. (1997)
48 speculated that environmental degradation of organoarsenic compounds by bacteria had
49 led to this inconsistency. The dominant chemical forms in a number of lakes and
50 estuaries have been reported to change seasonally by the degradation and production of
51 organoarsenic compounds (Anderson and Bruland, 1991). Considering the seasonal
52 dynamics and the distribution of arsenic compounds in aquatic environments, the
53 DMA-degradation process is worthy of study. A few reports described that
54 environmental bacteria in marine sediments (Sanders, 1979), seawater (Kaise et al.,

55 1985), and associated consorcia with marine animals, such as crabs (Khokiattiwong et
56 al., 2001) and mussels (Jenkins et al., 2003), could degrade the organoarsenic
57 compounds amended. Bacterial isolates from activated sludge (Quinn and McMullan,
58 1995) and natural environments (Lehr et al., 2003; Maki et al., 2006) also degraded
59 organoarsenic compounds to inorganic arsenic. However, little information is
60 available on the influence of environmental factors on the DMA-biodegradation process
61 in aquatic environments, and the ecological characteristics of DMA biodegradation are
62 unclear. In our previous investigation, the bacterial composition of DMA-degrading
63 bacteria was demonstrated to change seasonally in the lakes of Japan (Maki et al., 2006),
64 but, until the present study, the seasonal dynamics of biodegradation activities for
65 organoarsenic compounds had not been estimated in detail in a single lake.

66 In this study, the seasonal change in the concentrations of arsenic species was
67 investigated in Lake Kahokugata from April 2005 to March 2008 to estimate the
68 interaction of the arsenic dynamics between arsenic compounds and chlorophyll a.
69 Moreover, environmental factors controlling DMA degradation were determined in the
70 lake water samples spiked with DMA, and the DMA-degradation activities in the
71 natural lake water were estimated in all seasons during the investigation period. DMA

72 was selected as a representative organoarsenic compound that is widely distributed in
73 freshwater (Sohrin et al., 1997).
74
75 **Experimental**
76
77 **Sampling at Lake Kahokugata**
78 A lake water sample at a depth of 1 m was collected in polycarbonate bottles
79 from Lake Kahokugata in the Ishikawa Prefecture of Japan 22 times from April 2005 to
80 March 2008. Lake Kahokugata is eutrophic and suffered from wastewater inflow from
81 cities and croplands. The depth of Lake Kahokugata is less than 2 m and the water is
82 frequently mixed throughout the four seasons. The oxygen levels in the lake water
83 sample ranged from 2.0 to 8.3 mg L^{-1} during the investigation period. When the water
84 transparency was measured using a standard 25 cm black and white Secchi disk, the
85 disk depths ranged from 0.1 m to 1 m from water surface during the investigation period,
86 indicating that the sun irradiation hardly reached to the depth of 1 m. For the
87 measurement of arsenic species and chlorophyll a, 50 mL of sample water was filtrated
88 with a GF/C glass fiber filter (ADVANTEC, Tokyo, Japan). The concentrations of

89 arsenic species in the filtrate were determined using a cold trap HG-AA speciation
90 procedure. Chlorophyll a was extracted from the GF/C glass fiber filter with acetone
91 and assessed colorimeterically (Maki et al., 2005). Moreover, surface water samples
92 of Lake Kahokugata in several polycarbonate bottles were used for the determination of
93 the DMA-biodegradation activities of natural lake water. These samples were
94 incubated under different treatments.
95
96 **Experiment design and DMA biodegradation in lake water**
97 The lake water samples collected into polycarbonate bottles from Lake
98 Kahokugata on October 10, 2006, were used for investigating DMA-degradation
99 activities in lake water samples incubated under aerobic and anaerobic conditions and
100 light and dark conditions. Twelve polycarbonate bottles (500 mL) were filled up with
101 lake water and transferred to our laboratory. Within 2 hours of sampling, 500 μL of a
102 1 mM DMA (Nacalai Tesque, Kyoto, Japan) solution was added into 12 bottles at a final
103 concentration of 1 μM. One half of the bottles (6) in each experiment were incubated
104 under anaerobic conditions. To produce the anaerobic conditions, the air phases in the
105 bottles were kept at the lowest possible level, and the water samples were purged with

106 nitrogen (100 mL min^{-1}) for 0.5 h. The remaining half of (6 bottles) were incubated
107 under aerobic conditions. To produce the aerobic conditions, natural air filtrated
108 through a 0.2 μm Nuclepore filter (Whatman, Tokyo, Japan) was continuously supplied
109 at 700 m^3 h^{-1} into the bottle using an air-pump. After the anaerobic and aerobic
110 treatments, 3 bottles under each anaerobic and aerobic condition were incubated under a
111 photon flux density of 150 μmol m^{-2} sec^{-1} of cool white fluorescent lamps with a
112 12:12 light:dark cycle as the light condition. The remaining 3 bottles under each
113 anaerobic and aerobic condition were incubated under dark conditions by covering the
114 bottles with aluminum foil. The experiments consisted of a total of four conditions:
115 anaerobic and light, aerobic and light, anaerobic and dark, and aerobic and dark. The
116 water samples were then incubated in a controlled temperature room (20 ºC).
117 Moreover, for estimating the biosynthesis from arsenate to DMA, arsenate was added to
118 500 mL bottles of lake water samples at a final concentration of 1 μM, and a single
119 bottle of the water samples was incubated at 20 ºC under each of four conditions.
120 On the other hand, the microbial activities in the lake water sample were
121 eliminated using four treatments: the lake water was autoclaved at 120 ºC for 20
122 minutes; an antibiotic mixture was added to each sample of lake water at a final

123 concentration of 10 mg L^{-1}; sodium azide was added to each sample of lake water at a
124 final concentration of 10 mg L^{-1}; and the lake water was filtrated through a 0.02 μm
125 polycarbonate filter. Three bottles (500 mL) of the lake water samples treated by each
126 method and spiked with DMA at a final concentration of 1 μM were incubated at 20 ºC
127 under anaerobic and dark conditions. The oxygen concentrations of the lake water
128 sample under the aerobic condition were always approximately 8.5 mg L^{-1}. In the
129 anaerobic condition, the oxygen levels ranged from 1.2 to 2.3 mg L^{-1} during the
130 experiments.

131 In order to compare the DMA-degradation activities in the lake water in four
132 seasons, spring (March, April, and May), summer (June, July, and August), fall
133 (September, October, and November), and winter (December, January, and February),
134 lake water samples were collected every few months from June 2005 to February 2008
135 in polycarbonate bottles (500 mL). The 500 μL of 1 mM DMA solution was added
136 into bottles at a final concentration of 1 μM, and the bottles were incubated at 20 ºC
137 under anaerobic and dark conditions. Furthermore, to examine the effects of water
138 temperature on the DMA-degradation activities, the lake water samples that were
139 collected in summer (July 1, 2006, July 28, 2006, and August 9, 2007) and winter

140 (December 13, 2006, February 28, 2007, and February 3, 2008) and spiked with DMA
141 added at a final concentration of 1 μM were incubated under anaerobic and dark
142 conditions at temperatures of 30 ºC and 4 ºC, respectively, in controlled-temperature
143 boxes for 56 days. Each experiment was performed in triplicate.
144 During the incubation period (56 days), portions (10 mL) of the water samples
145 were collected, and the concentrations of arsenic species were determined using a
146 cold-trap hydride-generation atomic-absorption (HG-AA) speciation procedure.
147
148 **Measurements of the arsenic compound concentration**
149 The cold-trap HG-AA speciation procedure was employed as the protocol
150 previously reported (Braman and Foreback, 1973; Hasegawa et al., 1994). The water
151 subsamples, which were filtrated through a 0.45 μm cellulose ester filter (ADVANTEC,
152 Tokyo, Japan), were adjusted to 40 mL using pure water and acidified by the addition of
153 5 mL of a 0.2 M EDTA solution and 5 mL of 5 M HCl. Next, 10 mL of a 30% (w v^{-1})
154 $NaBH_4$ solution was gradually added to the sample solution at a speed of 2 mL min^{-1},
155 and the arsenic included in the sample solution was evaporated by reacting with $NaBH_4$.
156 The produced arsines were swept by a flow of nitrogen into a cold-trap column cooled

157 by liquid nitrogen. After the column was gently warmed by electrical heating, the
158 arsines (including inorganic arsenic, MMA, and DMA) released from the column were
159 loaded into a quartz-T tube held at about 900 ˚C in a flame and quantified using an
160 atomic absorption spectrometer Z-8100 (Hitachi, Chiba, Japan). The potential
161 concentrations for detection of arsenic compounds were more than 1.0 nM of measured
162 solution. Moreover, there is a low possibility that other arsenic species, except for
163 inorganic arsenic, MMA, and DMA, are produced in the water samples during the
164 experiments.
165
166 **Results**
167
168 **Seasonal variation in Lake Kahokugata**
169 In Lake Kahokugata, the concentrations of chlorophyll a increased to amounts in
170 excess of 50 μg L^{-1} from spring to summer and decreased to below 15 μg L^{-1} from fall
171 to winter during the investigation period between April 2005 and March 2008,
172 suggesting that the growth of phytoplankton was activated from spring to summer (Fig.
173 1a). The concentrations of inorganic arsenic fluctuated ranging from 2.8 to 23 nM

through all seasons, while DMA was detected at peaks of up to 13 nM only during fall and winter. Moreover, MMA was not detected from water samples during the investigation period. Consequently, the changes in the concentrations of methylarsenic compounds did not correlate with the changes in phytoplankton abundance during the investigation period. Furthermore, the water temperature was below 10 ˚C during winter and early spring (from December to April), while it increased to over 30 ˚C in summer (August) (Fig. 1b).

Incubation condition of DMA biodegradation in the lake water from Lake Kahokugata

When the lake water samples were spiked with DMA at a final concentration of approximately 1 μM and incubated at 20 ˚C under anaerobic and dark conditions, the concentration of DMA at the onset of the experiment decreased from 1020 nM (average) to the detection limit (avg.) during the first 21 days of incubation (Fig. 2d). In accordance with the decrease of DMA, the concentration of inorganic arsenic, which is considered to be the resultant product from DMA degradation, increased from 5.1 to 850 nM during the first 21 days and fluctuated over the concentration of 760 nM until

191 56 days of incubation. In contrast, under the other 3 conditions (anaerobic and light,
192 aerobic and dark, and aerobic and light), the reduction of DMA and the accumulation of
193 inorganic arsenic were not observed through 56 days of incubation (Fig. 2a, b, c).
194 When the microbial activities were eliminated using autoclave sterilization, addition of
195 antibiotics and sodium azide, or filtration, the DMA degradation and the accumulation
196 of inorganic arsenic diminished in the lake water samples with 4 treatments (Table 1).
197 The concentrations of inorganic arsenic and organoarsenic compounds in the lake water
198 without the addition of DMA, on the other hand, were stable below 10 nM during the
199 entire experiment (data not shown). These results indicated that this DMA degradation
200 occurred as a result of a biotic (microbiological) process under anaerobic and dark
201 conditions and that the physical degradation, including photochemical degradation and
202 heat degradation, could be ignored. On the other hand, in the lake water that was
203 spiked with inorganic arsenic, the concentrations of DMA maintained low
204 concentrations ranged below 450 nM from the 14^{th} day to the 56^{th} day (Fig. 3). These
205 results indicated that the rates of DMA synthesis are at relatively low levels, in contrast
206 to those of DMA degradation.
207

208 **Seasonal dynamics of DMA-biodegradation activities in the lake water**

209 In the lake water samples that were collected in four seasons and incubated with
210 the addition of approximately 1 μM DMA at 20 ˚C under anaerobic and dark conditions,
211 the DMA added to most of the lake water samples collected in the four seasons (15
212 samples of 22) decreased to the detection limit and was completely converted to
213 inorganic arsenic between 21st day and 28th day of incubation (Fig. 4). In the other 7
214 samples of lake water collected in spring, summer, and fall (sampling days - 7 June
215 2005, 1 November 2005, 27 April 2006, 1 September 2006, 24 April 2007, 9 August
216 2007, and 26 October 2007), the DMA biodegradation and the accumulation of
217 inorganic arsenic were observed for longer incubation times ranging from 35 to 56 days.
218 Consequently, at 20 ˚C of incubation under anaerobic and dark conditions, DMA added
219 to the lake water samples was degraded at similar rates throughout the four seasons.

220

221 **DMA-degradation activities of lake water samples at different temperatures**

222 The degradation patterns of DMA were significantly different at different
223 incubation temperatures, such as 30 ˚C and 4 ˚C, under anaerobic and dark conditions
224 using lake water collected in the summer (July and August) and winter (February and

March), respectively. In the lake water collected in the summer and incubated at 30˚C, 1 μM of DMA was rapidly degraded and converted to 860 nM of inorganic arsenic for short incubation times ranging from 7 days to 21 days (Fig. 5a). In contrast, DMA degradation was not observed in the winter lake water samples, which was incubated at 4 ˚C (Fig. 5b). At 20˚C, DMA spiked into the same water samples of summer and winter was completely degraded in 21 or 35 days (Fig. 4b, d). These results mean that DMA degradation was activated at a high temperature of 30˚C and reduced at a low temperature of 4 ˚C.

Discussion

Phytoplankton in lake water and coastal seawater incorporate and accumulate inorganic arsenics instead of phosphorus and synthesize organoarsenic compounds for detoxification (Andrete, 1979; Hasegawa et al., 2001; Santosa et al., 1994). In Lake Kahokugata, the concentrations of chlorophyll a in water samples indicated peaks (up to 100 μg L^{-1}) during spring and summer indicating the activity of phytoplankton (Fig. 1). DMA increased to concentrations of up to 13 nM from late fall to winter through the

242 investigation period. These results indicated that the dynamics of methylarsenic
243 species were not related to the dynamics of chlorophyll a in Lake Kahokugata. In
244 lakes and coasted areas, the changes in microalgal abundance (chlorophyll a contents)
245 did not positively correlate with the changes in the concentrations of methylarsenic
246 species (Hasegawa et al., 1996). In contrast, in other aquatic environments, the
247 concentrations of DMA frequently increased in summer positively and correlated with
248 the production of phytoplankton (Sohrin et al., 1997). Some microorganisms, such as
249 fungi and bacteria, have been reported to produce DMA as well as phytoplankton
250 (Francesconi and Kuehnelt, 2002). Except for phytoplankton, these microorganisms
251 might produce DMA during winter in Lake Kahokugata. Sanders (1979) also
252 demonstrated that microbial communities in environmental freshwater system
253 demethylated DMA to inorganic arsenate. In this study, both the biosynthesis and
254 biodegradation of DMA, which vary with time, seemed to determine the concentration
255 of DMA in aquatic environments. The water samples from Lake Kahokugata spiked
256 with DMA were converted to inorganic arsenic only under dark and anaerobic
257 conditions of incubation (Fig. 2d). Furthermore, this DMA degradation was not
258 observed in the lake water in which the bacterial activities were eliminated by four

259 treatments, including autoclave sterilization, filtration, and the addition of sodium azide
260 and antibiotics. These results suggested that this degradation of DMA occurs as a
261 result of a biotic (microbiological) process. Biological demethylation has been
262 reported to be the dominant process for the generation of inorganic arsenic from
263 organoarsenic compounds (Andreae, 1979). In a previous investigation, several
264 species of DMA-degrading bacteria were isolated from Lake Kahokugata (Maki et al.,
265 2005). This study suggested that the DMA-degrading microorganisms generally
266 inhabiting Lake Kahokugata would degrade the methylarsenic compounds produced by
267 microorganisms and influence the arsenic cycling in aquatic ecosystems.

268 Degradation of DMA to inorganic arsenic occurred only under anaerobic and
269 dark conditions and was not observed in the lake water that was incubated under aerobic
270 or light conditions (Fig. 2). Woolson (1977) also reported that, in the soil under
271 aerobic conditions, methylarsenic was not converted to arsenate. Several kinds of
272 organic matter were degraded only under anaerobic environments, including the
273 sediments of lakes, suggesting that the anaerobic microbial population contributes to the
274 degradation (Coates et al., 2001; Bastviken et al., 2004; Fathepure and Vogel, 1991).
275 Anaerobic microbial reactions in the lake water of Lake Kahokugata would be relatively

276 optimal for converting DMA to inorganic arsenic. In Lake Kahokugata, which
277 averages slightly less than 2 m in depth, the water would be vertically mixed in all
278 seasons, and the DMA-degrading bacteria would be transported from the lake sediments,
279 which is under dark and anaerobic conditions.

280 Moreover, under light conditions, phototrophic microorganisms can grow and
281 produce greater amounts of organic matter than under dark conditions and create the
282 dynamics of a microbial population (Takenaka et al., 2007). Organic matter, such as
283 glucose, is known to inhibit the degradation of methylarsenic compounds (Gao et al.,
284 1997). The addition of glucose into the lake water of Lake Kahokugata inhibited the
285 DMA degradation (data not shown). Accordingly, DMA biodegradation under light
286 conditions might be reduced by the products of phototrophic microorganisms.
287 Furthermore, as described, some phototrophic organisms, such as fungi and plankton,
288 are reported to uptake inorganic arsenic and convert it into DMA (Hasegawa et al.,
289 2001; Sntosa et al., 1994). However, in this study, the biosynthesis of DMA in the lake
290 water was at relatively low levels under aerobic and light conditions and was not
291 observed under aerobic and dark and anaerobic and light conditions (Fig. 3). Cheng
292 and Focht (1979) also reported that microorganisms involved in the demethylation

293 process in the soil were more abundant than DMA-synthesizing microorganisms. In
294 Lake Kahokugata, DMA synthesis by phytoplankton grown under aerobic and light
295 conditions should also be at low levels but might offset, to some degree, the DMA
296 decrease by biodegradation.

297 DMA-biodegradation activities are thought to influence the seasonal changes in
298 the concentrations of DMA, which are caused by microorganisms. When lake water
299 collected in all seasons and spiked with 1 μM of DMA was incubated at 20 ºC, the
300 DMA in most of lake water samples in the four seasons was converted to inorganic
301 arsenic in 21 or 35 days of incubation (Fig. 4). The species compositions of
302 DMA-degrading bacteria have been reported to change seasonally in Lake Kahokugata
303 (Maki et al., 2005). Anderson and Brueland (1991) reported that, in a number of lakes
304 and estuaries, the rates of DMA degradation were faster in water in winter when the
305 water layer was mixed. However, the depth of Lake Kahokugata was shallow at less
306 than 2 m and the water was constantly mixed throughout the four seasons. Therefore,
307 the DMA-degradation experiments performed under incubation at 20 ºC indicated that
308 similar rates of potential DMA degradation were obtained in all four seasons regardless
309 of the seasonal changes of bacterial composition. On the other hand, the DMA spiked

310 into some samples of lake water in spring, summer, and fall continued to be degraded
311 for incubation times ranging from 35 and 56 days. In some sampling days of spring,
312 summer, and fall, the low abundance of microorganisms transported from the lake
313 sediments may reduce the DMA-degradation activities. Moreover, phytoplankton
314 activities that synthesize DMA and increase from spring to summer (Fig. 1a) are
315 thought to reduce the rate of DMA decrease and inorganic arsenic accumulation in the
316 natural lake water in the spring, summer, and fall.

317 Furthermore, in the lake water that was collected in the summer and incubated at
318 30 ºC, 1 μM of DMA was rapidly degraded at incubation times ranging from 7 to 21
319 days (Fig. 5a). When the lake winter water samples were incubated at 4 ºC, DMA
320 degradation was negligible (Fig. 5b). The water temperature in aquatic environments
321 was reported to influence the dynamics of bacterial communities and the levels of
322 metabolic activities by microorganisms (Pomeroy and Wiebe, 2000; Simon, 1999). In
323 Lake Kahokugata, the water temperature was below 10 ˚C in fall and winter, while it
324 increased to over 30˚C from spring to summer (Fig. 1b). Although the potential rates
325 of DMA degradation under incubation at 20˚C maintained similar levels in all seasons
326 (Fig. 4), the water temperature could change the DMA-degradation activities in the lake

327 water and overcome the potential activities of DMA degradation in each season. The
328 low temperature in winter would reduce the DMA-biodegradation activities, while the
329 high temperature in summer would activate the DMA biodegradation in Lake
330 Kahokugata. Consequently, organoarsenic compounds might maintain a concentration
331 of up to 20 nM in winter, and the high microbial activities in summer might degrade
332 organoarsenic compounds in the lake water.
333
334 **Conclusions**
335
336 This is the first report directly demonstrating that DMA biodegradation in aquatic
337 environments is enhanced under anaerobic and dark conditions. Although the DMA
338 degradation potentially maintained the same rates throughout the four seasons, the
339 seasonal dynamics of the DMA-biodegradation activities in Lake Kahokugata are
340 thought to depend on changes in the water temperature. In Lake Kahokugata, the
341 residue of DMA was detected only during fall and winter, when the low water
342 temperature would reduce the DMA biodegradation. In summer, DMA in the lake is
343 thought to disappear due to the high activities of DMA-biodegradation at high

344 temperatures. Considering the arsenic cycles in aquatic environments, the
345 biodegradation process of organoarsenic compounds appeared to be as important as the
346 biosynthesis process of organoarsenic compounds. In the future, since the arsenic
347 cycles were composed of a highly complex structure of organoarsenic compounds such
348 as arsenobetaine, which are also produced by microorganisms, the processes of
349 degradation and biosynthesis involving highly complex organoarsenic compounds
350 should be investigated in order to elucidate the arsenic cycles in aquatic environments.
351

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353

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358

359 **References**

360

361 Anderson, L.C.D., Bruland, K.W., 1991. Biogeochemistry of arsenic in natural waters:
362 The importance of methylated species. Environ. Sci. Technol. 25, 420-427.
363 Andreae, M.O., 1979. Arsenic speciation in seawater and interstitial waters: The
364 influence of biological-chemical interactions on the chemistry of a trace element.
365 Limnol. Oceanogr. 24, 440-452.
366 Bastviken, D., Persson, L., Odham, G., Tranvik, L., 2004. Degradation of dissolved
367 organic matter in oxic and anoxic lake water. Limnol. Oceanogr. 49, 109-116.
368 Braman, R.S., Foreback, C.C., 1973. Methylated forms of arsenic in the environment.
369 Science 182, 1247-1249.
370 Cheng C.N., Focht D.D., 1979. Production of arsine and methylarsines in soil and in
371 culture. Appl. Environ. Microb. 38, 494-498.
372 Coates J.D., Chakraborty R., Lack J.G., O'Connor S.M., Cole K., 2001. Anaerobic
373 benzene oxidation coupled to nitrate reduction in pure culture by two novel
374 organism. Nature 411, 1039-1043.
375 Cullen, W.R., Reimer, K.J., 1989. Arsenic speciation in the environment. Chem. Rev. 89,
376 713-764.
377 Farías, S., Smichowski, P., Vélez, D., Montoro, R., Curtosi, A., Vodopívez, C., 2007.

Total and inorganic arsenic in Antarctic macroalgae. Chemosphere 69, 1017-1024.

Fathepure B.Z., Vogel T.M., 1991. Complete degradation of polychlorinated hydrocarbons by a two-stage biofilm reactor. Appl. Environ. Microbiol. 57, 3418-3422.

Francesconi, K.A., Kuehnelt, D., 2002. Arsenic Compounds in the Environment, In: Frankenberger, W.T. (Eds.). Environmental Chemistry of Arsenic. Marcel Dekker, New York, USA, pp. 51-94.

Gao S., Burau, R.G., 1997. Environmental factors affecting rates of arsine evolution from and mineralization of arsenicals in soil. J. Environ. Qual. 26, 753-763.

Hasegawa, H., 1996. Seasonal changes in methylarsenic distribution in Tosa Bay and Uranouchi Inlet. Appl. Organomet. Chem. 10, 733-740.

Hasegawa, H., Sohrin, Y., Matsui, M., Honjo, M., Kawashima, M., 1994. Speciation of arsenic in natural waters by solvent extraction and hydride generation atomic absorption spectrometry. Anal. Chem. 66, 3247-3252.

Hasegawa, H., Sohrin, Y., Seki, K., Sato, M., Norisuye, K., Naito, K., Matsui, M., 2001. Biosynthesis and release of methylarsenic compounds during the growth of freshwater algae. Chemosphere 43, 265-272.

395 Jenkins, R.O., Ritchie, A.W., Edmonds, J.S., Goessler, W., Molenat, N., Kuehnelt, D.,
396 Harrington, C.F., Sutton, P.G., 2003. Bacterial degradation of arsenobetaine via
397 dimethylarsinoylacetate. Arch. Microbiol. 180, 142-150.
398 Kaise, T., Hanaoka, K., Tagawa, S., 1985. The formation of trimethylarsine oxide from
399 arsenobetaine by biodegradation with marine microorganisms. Chemosphere 16,
400 2551-2558.
401 Khokiattiwong, S., Goessler, W., Pedersen, S.N., Cox, R., Francesconi, K.A., 2001.
402 Dimethylarsinoylacetate from microbial demethylation of arsenobetaine in
403 seawater. Appl. Organomet. Chem. 15, 481-489.
404 Lehr, C.R., Polishchuk, E., Radoja, U., Cullen, W.R., 2003. Demethylation of
405 methylarsenic species by *Mycobacterium neoaurum*. Appl. Organomet. Chem. 17,
406 831-834.
407 Maki, T., Hasegawa, H., Ueda, K., 2005. Seasonal dynamics of dimethylarsinic acid
408 (DMAA) decomposing bacteria dominated in Lake Kahokugata. Appl. Organomet.
409 Chem. 19, 231-238.
410 Maki, T., Takeda, N., Hasegawa, H., Ueda, K., 2006. Isolation of monomethylarsonic
411 acid (MMAA)-mineralizing bacteria from arsenic contaminated soils of Island

412 Ohkunoshima. Appl. Organomet. Chem. 20, 538-544.

413 Maki, T., Watarai, H., Kakimoto, T., Takahashi, M., Hasegawa, H., Ueda, K., 2006.

414 Seasonal dynamics of dimethylarsenic acid degrading bacteria dominated in Lake

415 Kibagata. Geomicrobiol. J. 23, 311-318.

416 Ninh, T.D., Nagashima, Y., Shiomi, K., 2008. Unusual arsenic speciation in sea

417 anemones. Chemosphere 70, 1168-1174.

418 Oremland, R.S., Stolz, J.F., 2003. The ecology of arsenic. Science 300, 939-944.

419 Quinn, J.P., McMullan, G., 1995. Carbon-arsenic bond cleavage by a newly isolated

420 gram-negative bacterium, strain ASV2. Microbiol. 141, 721-725.

421 Peshut, P.J., Morrison, R.J., Brooks, B.A., 2008. Arsenic speciation in marine fish and

422 shellfish from American Samoa. Chemosphere 71, 484-492.

423 Pomeroy, L.R., Wiebe, W.J., 2000. Temperature and substrates as interactive limiting

424 factors for marine heterotrophic bacteria. Aquatic Microbial. Ecol. 23, 187-204.

425 Sanders, J.G., 1979. Microbial role in the demethylation and oxidation of methylated

426 arsenicals in seawater. Chemosphere 8, 135-137.

427 Santosa, S.J., Wada, S., Tanaka, S., 1994. Distribution and cycle of arsenic compounds

428 in the ocean. Appl. Organomet. Chem. 8, 273-283.

429 Simon, M., Glöckner, F.O., Amann, R., 1999. Different community structure and
430 temperature optima of heterotrophic picoplankton in various regions of the
431 Southern Ocean. Aquat. Microb. Ecol. 18, 275-284.
432 Sohrin, Y., Matsui, M., Kawashima, M., Honjo, M., Hasegawa, H., 1997. Arsenic
433 biogeochemistry affected by eutrophication in lake Biwa, Japan. Environ. Sci.
434 Technol. 31, 2712-2720.
435 Stolz, J.F., Basu, P., Santini, J.M., Oremland, R.S., 2006. Arsenic and selenium in
436 microbial metabolism. Annu. Rev. Microbiol. 60, 107-130.
437 Takenaka, T., Tashiro, T., Ozaki, A., Takakubo, H., Yamamoto, Y., Maruyama, T.,
438 Nagaosa, K., Kimura, H., Kato, K., 2007. Planktonic bacterial population
439 dynamics with environmental changes in coastal areas of Suruga Bay. Microbes
440 Environ. 22, 257-267.
441 Woolson, E.A., 1977. Generation of alkylarsines from soil. Weed Sci. 25, 412-416.
442
443

444 **Figure legends**

445

446 Fig. 1 Seasonal variation in the concentrations of arsenic species and chlorophyll a
447 and the water temperature in Lake Kahokugata. (a) Open circles, closed circles, and
448 closed triangles indicate the abundance of inorganic arsenic, DMA, and MMA,
449 respectively. (b) Closed squares and closed diamonds show the amount of chlorophyll
450 a and the water temperature, respectively. .

451

452 Fig. 2 Changes in the concentrations of arsenic compounds in lake water samples, to
453 which 1 μM of DMA was added. The lake water samples were incubated at 20 ºC
454 under aerobic and light conditions (a), aerobic and dark conditions (b), anaerobic and
455 light conditions (c), and anaerobic and dark conditions (d). Open circles, closed circles,
456 and closed triangles indicate the abundance of inorganic arsenic, DMA, and MMA,
457 respectively. Each experiment was performed in triplicate.

458

459 Fig. 3 Changes in the concentrations of arsenic compounds in lake water samples to
460 which 1 μM of inorganic arsenic have been added. The lake water samples were
461 incubated at 20 ºC under aerobic and light condition (a), aerobic and dark condition (b),

462 anaerobic and light condition (c), and anaerobic and dark condition (d). Open circles,
463 closed circles, and closed triangles indicate the abundance of inorganic arsenic, DMA,
464 and MMA, respectively.
465
466 Fig. 4 Changes in the concentrations of arsenic compounds in lake water samples that
467 were collected from Lake Kahokugata in the four seasons, spring (March, April, and
468 May) (a), summer (June, July, and August) (b), fall (September, October, and
469 November) (c), and winter (December, January, and February) (d), and spiked with 1
470 μM of DMA. The lake water samples were incubated at 20 ºC under anaerobic and
471 dark conditions. The open and closed symbols indicate the abundance of inorganic
472 arsenic and DMA, respectively. MMA was below the detection limit.
473
474 Fig. 5 Changes in the concentrations of arsenic compounds in lake water samples to
475 which 1 μM of DMA have been added. The lake water samples collected in the
476 summer (July and August) (a) and winter (January and February) (b) were incubated at
477 30 ºC and 4 ºC, respectively, under anaerobic and dark conditions. The open and
478 closed symbols indicate the abundance of inorganic arsenic and DMA, respectively.

MMA was below the detection limit.

Table

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Table 1. Concentrtaions of arsenic compounds, such as inorganic arsenic, MMA and DMA, in the lake water of Lake Kahokugata, which were treated for removing microbial activities and spiked with DMA at final concentrations of 938±63 nM. The lake water samples were incubated under the anaerobic and dark conditon for 56 days. Each experiment was performed in triplicate.

Treatments	Concentrations of arsenic species (nM)		
	Inorganic arsenic	MMA	DMA
Autoclave*1	<10	<10	971±71
Antibiotics addition*2	<10	<10	837±43
NaN_3 addition*3	<10	<10	779±50
Filtration*4	<10	<10	899±95

*1 Lake water was autoclaved at 120 ºC for 20 minutes.

*2 Antibiotics mixture was added to lake water at a each final concentration of 10 mg L^{-1}.

*3 NaN_3 was added to lake water at a final concentration of 10 mg L^{-1}.

*4 Lake water was filtrated with 0.02 µm polycarbonatefilter.

Figure

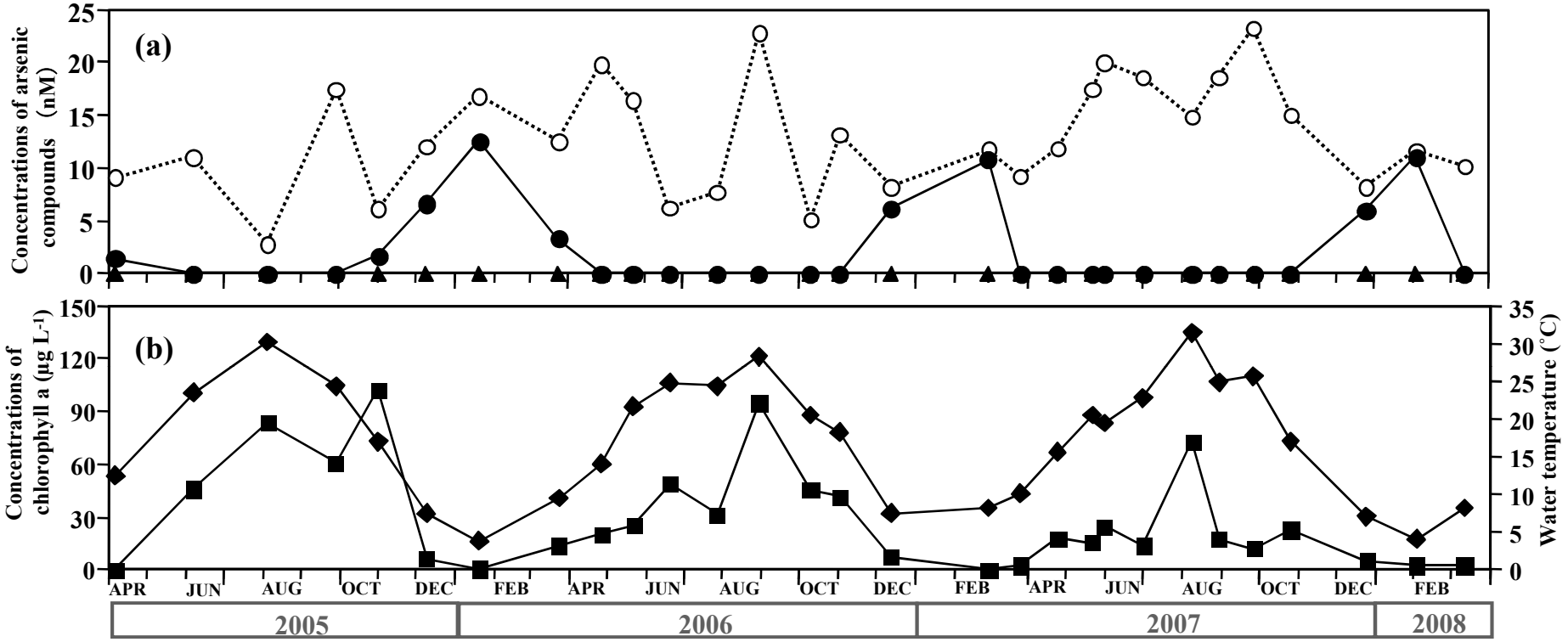
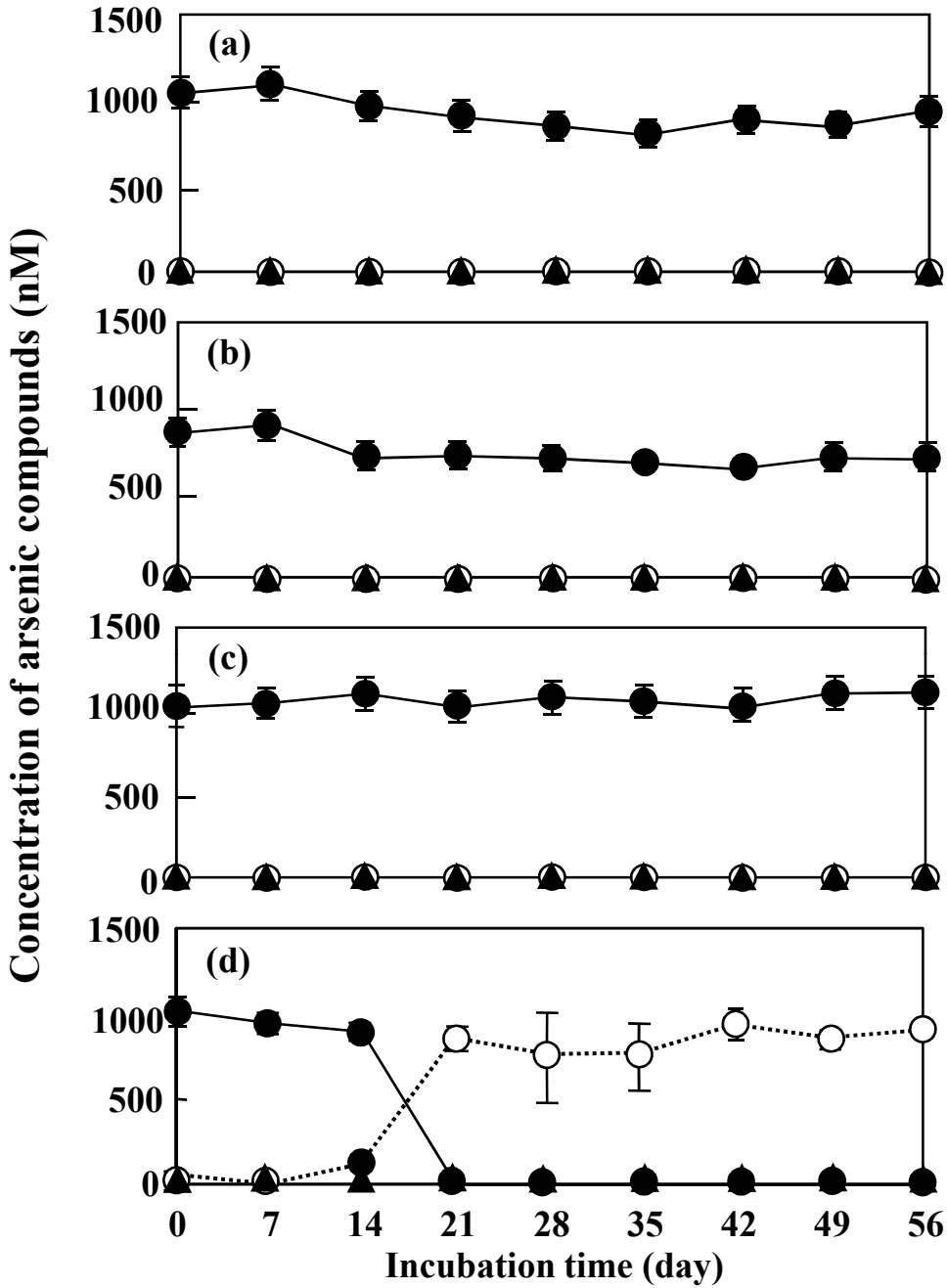


Fig. 1 T.Maki et al.



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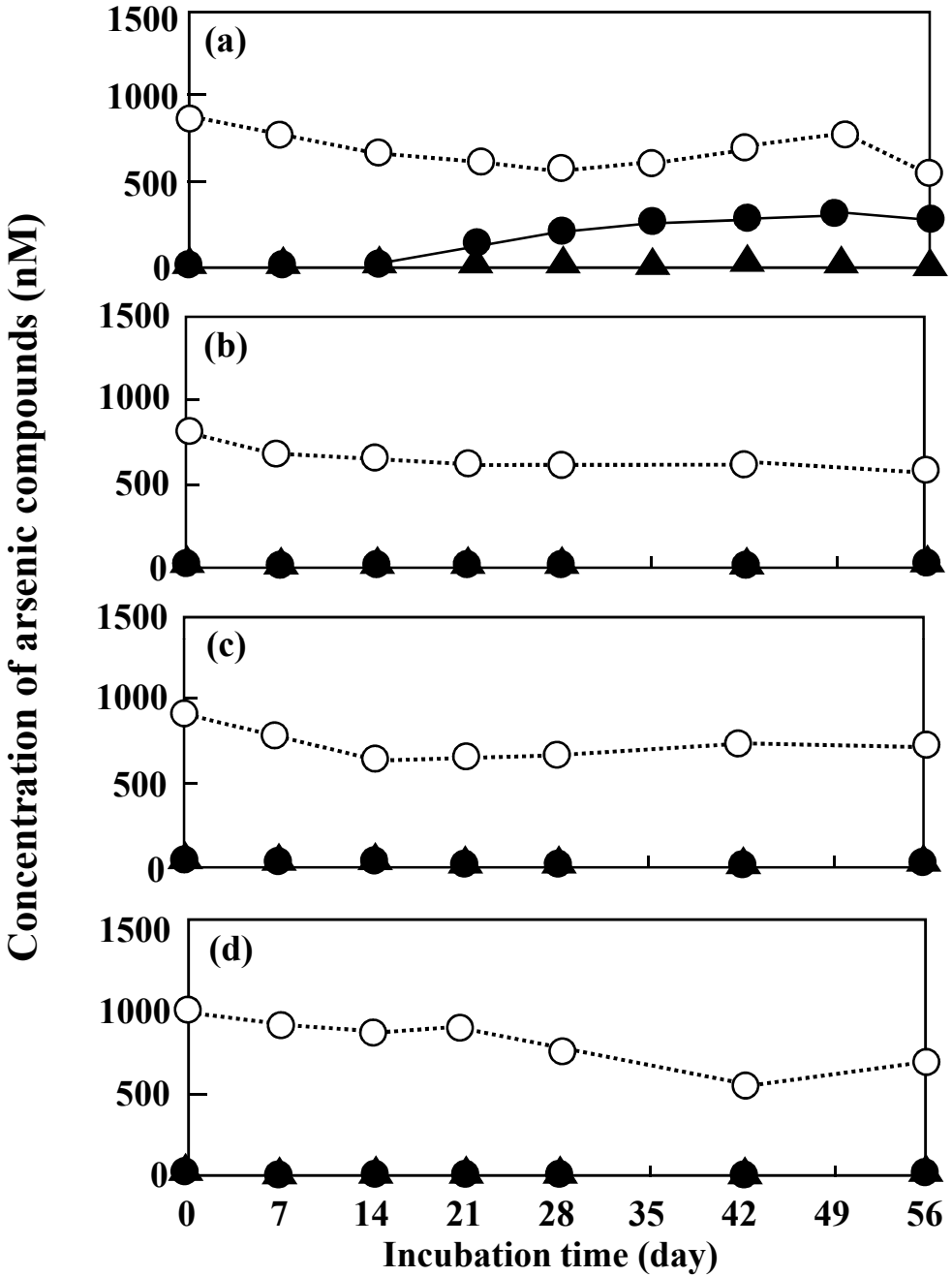

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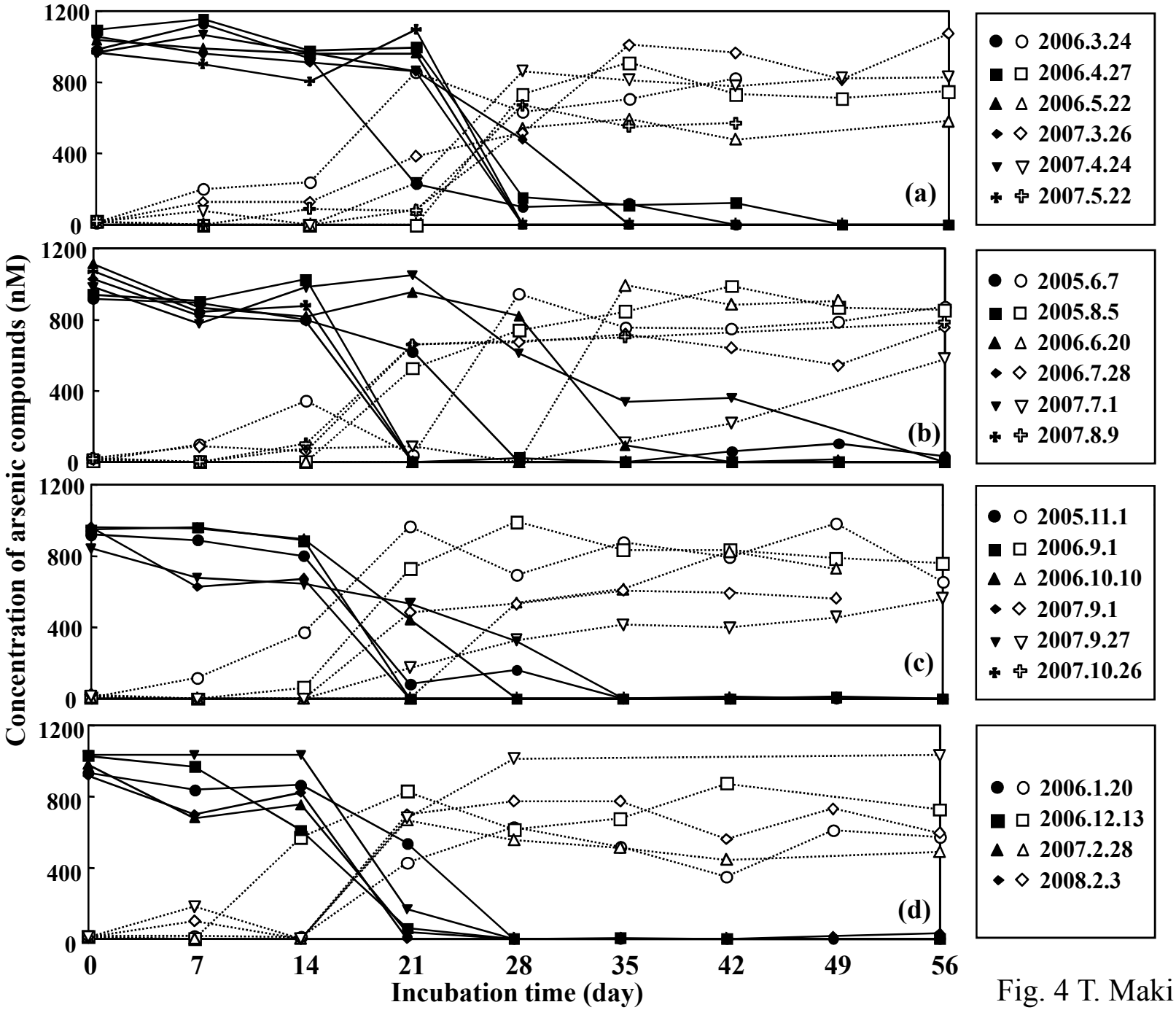

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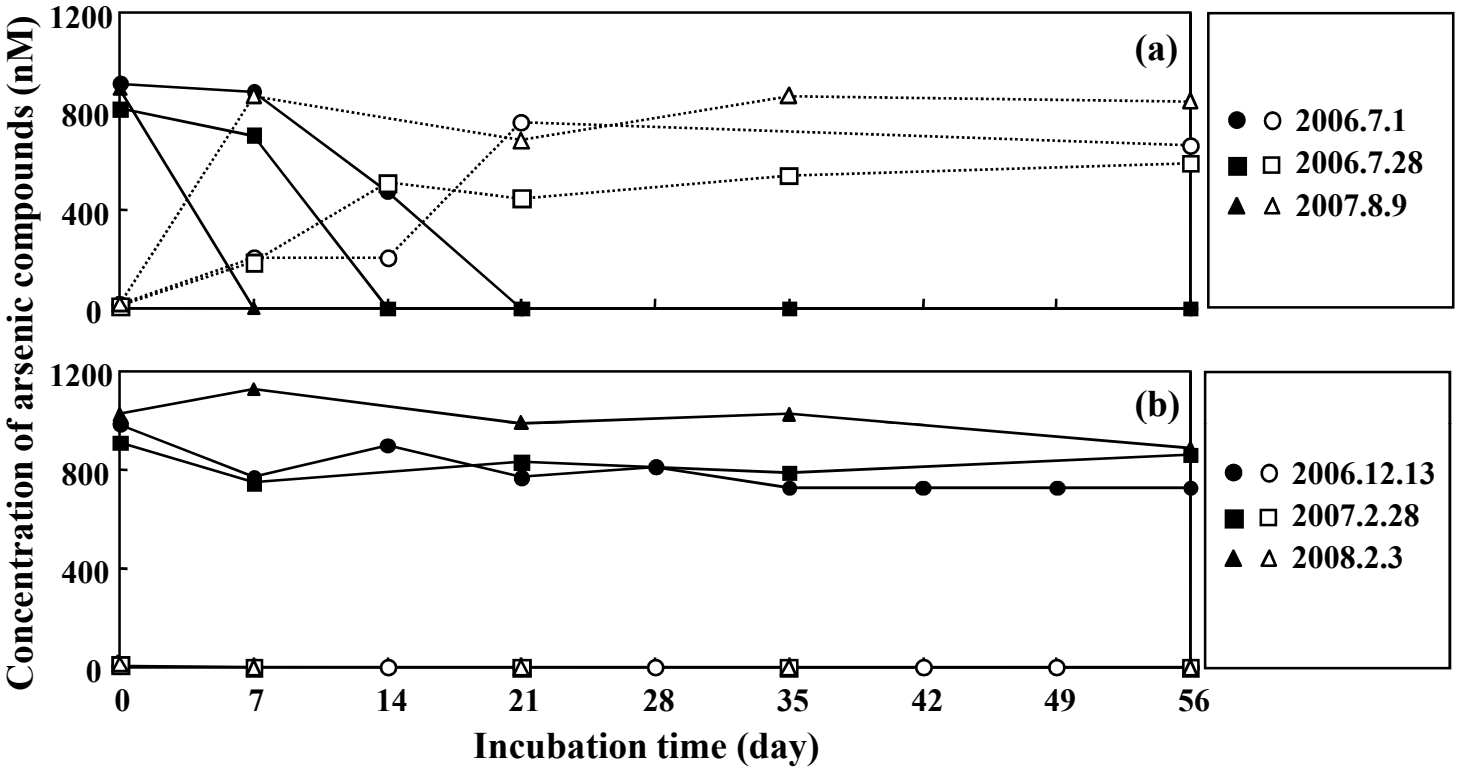

Fig. 5 T. Maki et al.