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1 Change in the elemental composition and cell geometry of the marine
2 diatom *Attheya longicornis* under nitrogen- and iron-depleted
3 conditions

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30 **ABSTRACT**

31 The morphology of the siliceous cell wall (frustule) is fundamental to the identification of
32 diatom species. One of the fundamental questions is the ecophysiological role of the diatom
33 frustule, which often shows morphological plasticity under different growth conditions. In this
34 study, the morphology and elemental composition of the diatom *Attheya longicornis* were
35 investigated under nutrient-replete (control), iron-depleted and nitrogen-depleted conditions.
36 This cylindrical, unicellular species has four siliceous horns per cell. The horns are each
37 formed from a hoop-like structure with a supporting rod, which greatly increases the surface
38 area of the cell. Under the iron-depleted conditions, relative to the controls the surface area to
39 cell volume ratio, silicon cell quota, and siliceous horn length increased 2.3-, 2.3- and 1.4-fold,
40 respectively. Under the nitrogen-depleted conditions, the cell size decreased without an
41 increase in horn length, and the cellular biogenic silica content was the highest between the
42 three growth media. The change in cell geometry and elemental composition modified the
43 sinking behaviour of *A. longicornis*. Estimated sinking rate was fastest in the nitrogen-depleted
44 cells, followed by the controls and iron-depleted cells. The data suggest that the
45 biogeochemical processes of biogenic silica could show vertically opposite direction
46 depending on the growth-limiting factors through a change in the elemental composition and
47 cell morphology of diatoms. Such plastic responses to nitrogen and iron depletion may
48 contribute to the relatively wide distribution of this species from the coastal to open ocean in
49 the subarctic region.

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51 *Key index words:*

52 diatom; frustule ultrastructure; iron availability; morphological plasticity; nitrogen depletion;
53 sinking rate

54

55 Running head: Trade-offs between sinking and silicification

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59 **Introduction**

60 Diatoms contribute to approximately 20% of the Earth's oxygen production
61 (Falkowski et al. 1998) and their organic constituents can potentially sink rapidly below the
62 mixed layer depth depending on their relatively large cell size and heavy siliceous cell wall
63 (frustule). Therefore, diatoms drive a substantial part of the biological carbon pump (Smetacek
64 1999, De La Rocha et al. 2008). After diatom-dominated blooms, sedimentation of their
65 aggregates is a well-known phenomenon (Smetacek 1985). Although frustules are inclined to
66 sink, frustules with eccentric morphologies, especially species with small cells, are thought to
67 resist sinking as the deviation from a sphere increases the surface area to volume ratio
68 (Margalef 1997, Raven and Waite 2004). Previous studies have mainly focused on the
69 relationship between species-specific sinking rates and physiological status (Brzezinski &
70 Nelson 1988, Waite et al. 1992). A change in physiological status under macronutrient- and
71 micronutrient-limited conditions often induces the change in diatom morphology (Sugie &
72 Kuma 2008, Marchetti & Cassar 2009, Wilken et al. 2011, Sugie & Yoshimura 2013). However,
73 testing the effect of morphological variation, frustule morphology and nutritional status in
74 combination is relatively rare.

75 The morphology of siliceous frustules is used to determine species identity in diatoms.
76 Many species have developed specialized frustule shapes and structures, probably reflecting
77 long-term evolutionary processes (Round et al. 1990, Smetacek 2001, Raven & Waite 2004).
78 Certain diatom groups have spines or horns, some of which have surface ornamentation
79 (Round et al. 1990). These structures should further increase the surface area to volume ratio,
80 presumably reducing sinking to aphotic depths in the open ocean, which is fatal for
81 photosynthetic organisms. By contrast, diatoms that sink in shallow coastal environments may
82 be able to return to the photic layer through mixing events. Therefore, sinking behaviour is
83 one of the important factors affecting phytoplankton dynamics in the ocean (Smetacek 1985,
84 Brzezinski & Nelson 1988, Peperzak et al. 2003). Many coastal diatom species, including their
85 resting spores, increase silicification and sink to deeper waters under micronutrient-replete but
86 macronutrient- or light-limited conditions thereby avoiding grazing and photo-oxidative
87 damage at the surface (Hargraves & French 1983, Raven & Waite 2004, Sugie & Kuma 2008).

88 In contrast, under micronutrient (iron)-depleted conditions, a diatom cells can increase their
89 surface area to cell volume ratio by changing their cell morphology or extending their siliceous
90 spines, which may regulate sinking velocity (Timmermans et al. 2001, Sugie & Kuma 2008,
91 Sugie & Yoshimura 2013).

92 The elemental composition of plankton strongly affects the ocean biogeochemical
93 cycling of bioelements (Redfield et al. 1963). A recent study showed that the average nitrogen
94 and phosphate ratios among phytoplankton groups vary and that such variation governs basin-
95 scale variation in seawater nutrient stoichiometry (Weber & Deutsch 2010). Nutrient
96 limitations also affects the cellular nutrient stoichiometry of phytoplankton (Marchetti &
97 Cassar 2009, Sugie & Yoshimura 2013). A deviation in relative nutrient utilization by
98 phytoplankton from the Redfield ratio due to the nutrient limitations could influence the spatial
99 and temporal nutrient availability and therefore productivity (Brzezinski et al. 2002, Sugie et
100 al. 2010b). For example, Sugie et al. (2010a) reported that iron- or nitrate-limited diatoms have
101 high Si:N ratios after the spring bloom peak and that such limitation leads to a decrease in
102 silicic acid availability in the western subarctic Pacific Ocean (Sugie et al. 2010b). Although
103 patterns of limiting nutrients differ spatiotemporally (Tyrrell & Law 1997, Moore et al. 2013),
104 comparisons between the effects of macro- and micro-nutrient limitations on phytoplankton
105 stoichiometry are very limited.

106 In a steady-state marine ecosystem, macro- or micronutrient limitation often regulates
107 primary productivity (Tyrrell & Law 1997, Moor et al. 2013). Nitrate depletion is often found
108 in the coastal region and subtropical gyres, whereas iron depletion is common in the subarctic
109 Pacific and Southern Ocean (Boyd et al. 2007, Moore et al. 2013). The addition of iron to iron-
110 limited waters could dramatically enhance the growth of phytoplankton, mainly diatoms
111 (Tsuda et al. 2005, Boyd et al. 2007). In this respect, some diatoms should survive under
112 unfavourable, iron-limited conditions in the upper mixed layers. However, previous studies
113 reported that iron limitation could increase diatom silicification (Takeda 1998, Timmermans
114 et al. 2004, Sugie et al. 2010a, Wilken et al. 2011, Sugie & Yoshimura 2013), which may
115 accelerate their sinking rate compared to that of healthy growing cells (De La Rocha et al.
116 2008, Sugie & Kuma 2008). Plausible mechanisms are still lacking for the survival of diatoms

117 with heavily silicified frustules at the surface under iron limitation.

118 We conducted a manipulative experiment using the diatom *Attheya longicornis* R. M.
119 Crawford et C. Gardner under nutrient-replete (control), nitrogen-depleted and iron-depleted
120 conditions to test the effects of the nutritional status of the diatom on its morphology and
121 elemental composition. *Attheya* species have four siliceous horns per cell (23–50 μm in length;
122 Orlova et al. 2002, Stonick et al. 2006), which are composed of hoop-like structures and
123 supporting rods, unlike the setae of *Chaetoceros* species, which have a more or less smooth
124 surface (Round et al. 1990, Crawford et al. 1994). The cylindrical cells are 4–12 μm in
125 diameter and 6–12 μm in height (per valve length) (Crawford et al. 1994, Orlova et al. 2002,
126 Stonick et al. 2006). Certain *Attheya* species (mainly *A. longicornis* and *A. septentrionalis*) are
127 often found as solitary phytoplankton in the open ocean (Sugie et al. 2010a, Malviya et al.
128 2016, Sugie and Suzuki in press) or attached by their horns to chain-forming diatoms in the
129 coastal region (Figs 1, 2; Crawford et al. 1994, Orlova et al. 2002, Stonick et al. 2006). The
130 cryophilic species, *A. longicornis*, occurs from subarctic seas to the Arctic Ocean and can
131 survive in darkness for at least several months (Orlova et al. 2002, Sugie et al. 2010a,
132 Tsukazaki et al. 2013). Unravelling the ecophysiology of *A. longicornis* is important to
133 understand their relatively wider distribution in the coastal waters to the open ocean.

134

135 **Materials and methods**

136 *Culture design*

137 The strain of *A. longicornis* used in the experiments was isolated from the western
138 subarctic gyre. The culture was maintained at 10°C under 150 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ fluorescent
139 light (12 h light:12 h dark), using silicic acid-enhanced f/2 medium (Guillard & Ryther 1962).
140 The seawater used for culturing was collected from a coastal region in the northern Japan Sea,
141 near Otaru, Hokkaido Prefecture, Japan. The seawater was filtered using an acid-cleaned 0.22
142 μm membrane filter (Millipore) and autoclaved for 20 min at 121°C and 108 kPa. The filter
143 was soaked in 1N HCl for at least 24h, followed by repeatedly rinsing and soaking for 24 h
144 with Milli-Q water ($>18.0 \text{ M}\Omega \text{ cm}^{-1}$, Millipore). The concentrations of Fe, NO_3+NO_2 , NH_3 ,
145 PO_4 and $\text{Si}(\text{OH})_4$ in the autoclaved filtered seawater were less than 2 nmol L^{-1} , 6 $\mu\text{mol L}^{-1}$,

146 0.1 $\mu\text{mol L}^{-1}$, 0.4 $\mu\text{mol L}^{-1}$, and approximately 240 $\mu\text{mol L}^{-1}$, respectively. The silicic acid-
147 enhanced f/2 medium contained 886 $\mu\text{mol L}^{-1}$ of NO_3 , 38 $\mu\text{mol L}^{-1}$ of PO_4 and approximately
148 350 $\mu\text{mol L}^{-1}$ of Si(OH)_4 as macronutrients, and f/2 metals chelated with EDTA. All the
149 macronutrient stock solutions were passed through a Chelex 100 ion-exchange resin to remove
150 trace metals (Morel et al. 1979). Prior to the experiment, the diatom stock cultures were kept
151 in the exponential growth phase for at least 10 doublings by the semi-continuous transfer of
152 cells into the silicic acid-enhanced f/2 medium. Diatoms showing healthy growth were
153 transferred to a modified f/2 medium, prepared without the addition of f/2 trace metals, EDTA,
154 or vitamins. Ferric iron (100 nmol L^{-1}) and manganese (25 nmol L^{-1}) were added to the
155 medium to remove excess trace metals (Sugie & Kuma 2008, Sugie et al. 2010a, 2011). Before
156 the experiment, the algal strain was grown in exponential growth phase for 7 days (or
157 approximately seven cell divisions) in the modified f/2 medium.

158 Three types of media were prepared for use in the experiment: (1) control, (2) nitrogen
159 (N)-depleted and (3) iron (Fe)-depleted media (Table 1). Silicic acid-enhanced f/2 medium,
160 which was macro- and micronutrient-replete, was used as the control. The N-depleted medium
161 was prepared by adding 5 $\mu\text{mol L}^{-1}$ of NO_3 (instead of 880 $\mu\text{mol L}^{-1}$) to the silicic acid-
162 enhanced f/2 medium (i.e., 11 $\mu\text{mol L}^{-1}$ of NO_3 in the N-depleted medium). The Fe-depleted
163 medium was prepared by adding 1 $\mu\text{mol L}^{-1}$ of strong iron-binding siderophore
164 desferrioxamine B (DFB) (Sigma Chem. Co. Ltd.) to the modified f/2 medium. In our
165 preliminary experiment, the growth of *A. longicornis* ceased when there was excess DFB
166 relative to ferric iron concentrations, as observed in a previous study using different diatoms
167 (Sugie et al. 2011). Previous studies have demonstrated that the addition of 1 $\mu\text{mol L}^{-1}$ DFB
168 successfully prevents Fe uptake from ambient extracellular Fe by coastal diatoms (Iwade et al.
169 2006, Yoshida et al. 2006). The culture experiments were conducted in triplicate using acid-
170 washed, polycarbonate 100-mL Erlenmeyer flasks. Manipulations were conducted in a Class
171 100 laminar flow cabinet to avoid inadvertent contamination.

172 **Measurements**

173 The algal growth was monitored daily using a hemocytometer and a light microscope.
174 Culturing was stopped during the exponential phase for the control samples (day 8), and the

175 stationary phase for the N-depleted (day 10) and Fe-depleted treatments (day 9). Growth rate
176 (μ , d^{-1}) was determined from the slope of a plot of natural log abundance against time during
177 the exponential growth phase. The Fe and macronutrients were measured by the flow injection
178 method with chemiluminescence detection (Obata et al. 1993) and by continuous flow analysis,
179 respectively (Sugie & Kuma 2008, Sugie & Yoshimura 2013). At the end of the culture period,
180 the cells were harvested for particulate organic carbon (POC), particulate nitrogen (PN),
181 biogenic silica (BSi) and chlorophyll-*a* analysis, as well as for morphometric measurements.
182 For the POC and PN analyses, the samples were passed through a pre-combusted (450°C, 4 h)
183 GF/F filter, and rinsed with 1N HCl-acidified filtered seawater to remove inorganic carbon,
184 and then rinsed with Mill-Q water ($> 18.2 \text{ M}\Omega \text{ cm}^{-1}$, Merck KGaA, Darmstadt, Germany).
185 The filter samples for the POC and PN analyses were dried at 60°C overnight and measured
186 using a CHN analyser. For the BSi analysis, the diatom cells were collected on a 0.8 μm pore
187 size polycarbonate membrane filter and rinsed with Milli-Q water to remove silicic acid from
188 the culture medium. The samples were then digested by heating to 85°C for 2 h in a 0.5%
189 Na_2CO_3 solution (Paasche 1980), and the dissolved $\text{Si}(\text{OH})_4$ was analysed using a QuAAtro
190 continuous flow analyser. For the chlorophyll-*a* measurements, the culture samples were
191 collected on a GF/F filter and soaked in 6 mL of *N,N*-dimethylformamide (DMF) (Suzuki &
192 Ishimaru 1990). The samples were stored at -20°C until analysis. Chlorophyll-*a* was measured
193 using a Turner Design 10-AU fluorometer (Welschmeyer 1994).

194 Cells of *A. longicornis* were observed using a light microscope and photographed for
195 subsequent size analysis. Measurement of the surface area (SA) and cell volume (CV) of this
196 cylindrical species followed the methods described by Hillebrand et al. (1999). Thus, cell
197 diameter (apical axis) and height (peralver axis) were measured. Additionally, the length of
198 horns was measured via image analysis with Image-Pro Plus, or using photographs with
199 calibrated scales and a ruler. We measured one fully in focus horn per cell and assumed all
200 horn in a cell were of the same length (Figs 3–5). The measurements were conducted on at
201 least 15 cells per treatment. Horn ultrastructure of the cells grown in the control medium was
202 observed using a scanning electron microscope (SEM). These cells were cleaned with a
203 commercially available alkali-detergent (Pipe Unish: a drain pipe cleaner, S.C. Johnson Inc.),

204 rinsed with Milli-Q water (Nagumo 1995) and attached to a glass slide. The glass slide was
205 then sputtered with gold prior to examination using the SEM (JSM-6360LA, JEOL Ltd., Tokyo,
206 Japan). Horn (diameter (d_{horn}): 0.51 μm) ultrastructure was measured as the continuum of
207 hoops (or torus) without the supporting rod to simplify the measurements (Fig 3). There were
208 11–14 (average 12.75, $n = 6$) hoops per 1.0 μm and their diameter (d_{hoop}) was 0.06 μm . Hoop
209 volume (V_{hoop}) and surface area of the hoop (SA_{hoop}) were calculated using $V_{\text{hoop}} = 2\pi^2 \times$
210 $(d_{\text{horn}}/2 - d_{\text{hoop}}/2) \times (d_{\text{hoop}}/2)^2$ and $SA_{\text{hoop}} = 4\pi^2 \times (d_{\text{horn}}/2 - d_{\text{hoop}}/2) \times d_{\text{hoop}}/2$, respectively.

211 In the present study, the sinking rate of a cell is estimated by Stokes' law as follows:

$$212 \quad S = g \times R^2 \times (\rho_p - \rho_{\text{sw}}) \times 86,400 / 18\eta, \quad (1)$$

213 where S is the sinking rate (m d^{-1}), g is the gravitational acceleration constant (9.8 m s^{-2}), R
214 is the spherical radius of the particle (m), ρ_p and ρ_{sw} represent density (kg m^{-3}) of the particle
215 and seawater, respectively; 86,400 is the number of seconds in a day and η is the dynamic
216 viscosity of seawater ($\text{kg m}^{-1} \text{ s}^{-1}$). Stokes' law indicates that, where the density of a particle
217 and that of its physical surroundings are the same, the sinking rate increases with the square
218 of the particle radius. Surface area ($4\pi R^2$) to the volume ($4/3\pi R^3$) ratio for a spherical particle
219 increases as its radius (R) decreases, therefore decreasing its sinking rate (S). To estimate
220 sinking rates using Stokes' law, the SA/CV ratios were converted to the corresponding
221 spherical diameter (CSD) of a particle using the following formula: $\text{CSD} = 6 / (\text{SA}/\text{CV})$. The
222 calculated radius (CSD/2) was substituted into Eq. (1). This simple estimation shows the
223 potential sinking behaviour of a single particle, assuming that the cells do not have a stable
224 position while sinking in a water column.

225 *Statistics*

226 Differences in algal cells between the different culture treatments were tested with
227 Tukey's HSD test, using PASW statistical software (version 17.0 SPSS Inc., Chicago, IL,
228 USA). Significant differences are reported at the 95% confidence level. The data are shown as
229 the means ± 1 standard deviation, each calculated using the results from the three replicate
230 cultures.

231

232 **Results**

233 ***Cell growth.***

234 The cells in the control cultures grew exponentially until the end of the experiment
235 (day 8), at a growth rate of $\mu = 0.66 \pm 0.01 \text{ d}^{-1}$ (Fig. 7). In the N-depleted treatment, cell growth
236 decreased from day 7, and the same cell density was maintained until the end of culture (day
237 10). The growth rate of the cells in the Fe-depleted medium decreased from day 3 or 4, but
238 cell density increased gradually at a growth rate of $\mu = 0.22 \pm 0.03 \text{ d}^{-1}$ until the end of the
239 experiment (day 9) (Fig. 7). At the end of culture, the PN concentrations were 40, 11, and 10
240 $\mu\text{mol L}^{-1}$ under the controls, N-depleted, and Fe-depleted conditions, respectively. These
241 results suggest that macronutrients were replete in the controls throughout the course of the
242 experiment, nitrate was exhausted under the N-depleted conditions, and severe iron-limitation
243 reduced nitrate drawdown.

244 ***Cell quota of bioactive elements.***

245 The carbon and nitrogen contents of the algal cells were significantly higher in the
246 Fe-depleted treatments, compared with the N-depleted treatments and the controls (Fig 8a, 8b).
247 Si cell quota differed significantly between treatments, with the highest amounts found in the
248 N-depleted treatments, followed by the Fe-depleted treatments and the controls (Fig 8c). The
249 chlorophyll-*a* cell quota was significantly higher in the controls compared with the N- and Fe-
250 depleted treatments (Fig 8d). There were no significant differences in the carbon to nitrogen
251 ratio among the treatments (6.5 ± 0.8 for the control, 6.8 ± 0.8 for the Fe-depleted, and $8.2 \pm$
252 0.9 for the N-depleted treatment), but there were significant differences in the silicon to carbon
253 (Si:C), and silicon to nitrogen (Si:N) ratios. Si:C and Si:N ratios were highest for cells cultured
254 in the N-depleted media (Si:C: 0.80 ± 0.10 , Si:N: 6.5 ± 0.4), followed by cells from the Fe-
255 depleted treatments (Si:C: 0.46 ± 0.02 , Si:N: 3.1 ± 0.5) and the controls (Si:C: 0.28 ± 0.03 ,
256 Si:N: 1.8 ± 0.4).

257 ***Cell geometry.***

258 Cells from the controls had significantly greater surface areas (SA) and cell volumes
259 (CV), than those from the N- and Fe-depleted treatments (Fig. 9a, 9b). The smaller cell volume
260 and surface area of cells grown in the N- and Fe-depleted media were mainly due to a reduction
261 in cell height (controls: 15.8–31.5 μm , N-depleted: 10.9–21.3 μm , Fe-depleted: 5.0–14.3 μm).

262 In contrast, horn length was significantly greater in the algal cells cultured under Fe-depleted
263 conditions (52.2–67.8 μm), compared with those in the N-depleted treatment (35.6–56.5 μm)
264 and the controls (33.8–47.9 μm) (Figs 4–6 and 9c). The SA/CV ratios were significantly higher
265 in cells from the Fe-depleted treatment, followed by the N-depleted treatment and the controls
266 (Fig. 4d).

267 *Estimated sinking rate.*

268 To estimate the cell sinking rates applying Stokes' law, constant values were used for
269 all the parameters ($\rho_{\text{sw}} = 1025 \text{ kg m}^{-3}$ and $\eta = 0.00151 \text{ kg m}^{-1} \text{ s}^{-1}$; Peperzak et al. 2003) except
270 cell radius and particulate density. Assuming that excess cell density relative to that of
271 seawater was derived solely from the Si content (Sommer 1988), the excess density (i.e., $\rho_p -$
272 ρ_{sw} in eq. 1) of vegetative cells in the control would be 75 kg m^{-3} (i.e., $\rho_p = 1100 \text{ kg m}^{-3}$;
273 Peperzak et al. 2003, Si content of $0.28 \text{ pmol cell}^{-1}$). That of N-depleted cells would be 206
274 kg m^{-3} ($0.77 \text{ pmol Si cell}^{-1}$) and 174 kg m^{-3} ($0.65 \text{ pmol Si cell}^{-1}$) for Fe-depleted cells. The
275 estimated sinking rate was fastest under the N-depleted conditions (Fig. 9e), as this treatment
276 showed the highest Si content. The estimated sinking rate of the cells with prolonged horns
277 and a more than 2-fold increase in Si quota under Fe-depleted conditions was slower than that
278 under the controls (Fig. 9e).

279

280 **Discussion**

281 This study shows that *A. longicornis* could reduce its estimated sinking rate with a
282 simultaneous increase in horn length, SA/CV ratio and Si cell quota under Fe-depleted
283 conditions. These results support previous assumptions that the spines of *Chaetoceros* species
284 may improve the regulation of sinking rate relative to that attainable by spine-free cells
285 (Timmermans et al. 2001, Raven & Waite 2004). Sugie & Kuma (2008) and Sugie &
286 Yoshimura (2013) also showed an increase in the SA/CV ratio of the diatoms *Thalassiosira*
287 *nordenskioldii* and *Pseudo-nitzschia pseudodelicatissima* under Fe-depleted/limited
288 conditions. However, the actual sinking rate of Fe-depleted *T. nordenskioldii* resting cells and
289 resting spores was about twice as fast as that of vegetative cells (Sugie & Kuma 2008). The
290 difference in the SA/CV ratio between the vegetative and Fe-depleted cylindrical *T.*

291 *nordenskioeldii* cells was small (approximately 25%), compared with the difference in *A.*
292 *longicornis*, with its finely structured horns (128%). Turbulent mixing is a key factor
293 regulating the sinking behaviour of particles in the ocean (e.g. Margalef 1997, Huisman et al.
294 2002). Increasing the surface area to volume ratio increases the physical resistance between
295 the cell surface and the water. For diatoms to actively regulate their sinking rate, substantial
296 respiratory energy would be required (Waite et al. 1992). Iron-limitation depresses
297 photosynthesis and therefore limits the energy available for respiration (Muggli et al. 1996).
298 However, silicon uptake may be independent of iron-requiring processes, and silicification
299 requires less energy than the formation of cellulose cell walls (Martin-Jézéquel et al. 2000).
300 Elongation of siliceous horns under Fe-depleted and respiratory energy-limited conditions
301 may be an ideal adaptive strategy to regulate the sinking rates in the open ocean.

302 In previous studies, *A. longicornis* was often found in the iron-limited open subarctic
303 Pacific Ocean during spring and summer (Sugie et al. 2010a). As Fe-limited regions are
304 generally located in the open ocean (Moor et al. 2013), where the seafloor is permanently
305 aphotic, a slow sinking rate under Fe-limited conditions should increase the rate of survival.
306 In some Fe-limited regions such as the western subarctic gyre, chain-forming diatoms are
307 usually scarce (Tsuda et al. 2005, Sugie & Suzuki in press). Micronutrient-limitation induces
308 resting stages in coastal chain-forming species, which have fast sinking rates (Sugie & Kuma
309 2008, Sugie et al. 2010a). Under iron-limited conditions, the long horns of *A. longicornis* could
310 act to increase the SA/CV ratio, rather than entanglement with the *Chaetoceros* chain. Small
311 cell size can be beneficial for the uptake of limiting nutrient due to a small diffusive boundary
312 layer (Pahlow et al. 1997, Raven 1998). In addition, small-celled species can assimilate
313 nutrients more efficiently than large-celled species (Raven 1998, Raven & Waite 2004). The
314 low chlorophyll-*a* content of *A. longicornis* cells under Fe-depleted conditions could also
315 reduce cellular photo-oxidative damages in sunlit surface waters during summer, as suggested
316 previously (Sugie et al. 2011).

317 Nitrogen is a primary limiting nutrient in the coastal region, where phytoplankton can
318 exhaust nitrate under high iron availability (Tyrrell & Law 1997, Sugie et al. 2010b). Under
319 N-depleted conditions, heavily silicified *A. longicornis* may have a faster sinking rate and

320 therefore tended to sink to deeper waters. Coastal diatoms often form resting stages under
321 macronutrient-depleted conditions, which is an ecological strategy to sink faster in the neritic
322 regions, to avoid grazing by zooplankton and photo-oxidative stresses in the nutrient-depleted,
323 sunlit surface waters (Hargraves & French 1983, Smetacek 1985, Sugie & Kuma 2008, Sugie
324 et al. 2010a). Diatoms surviving on the neritic seafloor can return to the surface layer via
325 mixing events and increase populations under the favourable growth conditions. Although we
326 did not observe the resting spores of *A. longicornis* under the N-depleted conditions, previous
327 reports suggest that the resting cells of this species could survive at least several months on
328 the seafloor (McQuoid 2005, Tsukazaki et al. 2013).

329 The insignificant change in the cellular C:N ratio under the N- and Fe-depleted
330 conditions indicates that the intracellular carbon and nitrogen metabolism are tightly coupled.
331 According for the change in cell size, compared to the controls the intracellular carbon and
332 nitrogen concentrations increased about 2- and 5-fold under the N- and Fe-depleted conditions,
333 respectively. Although the possible mechanisms for high intracellular N concentrations remain
334 uncertain, *A. longicornis* may store nutrients under unfavourable growth conditions. Unlike
335 the increase in *A. longicornis*, Fe-limitation generally decreases the cellular nitrogen content
336 of diatoms such as *Pseudo-nitzschia* spp. (e.g. Marchetti & Cassar 2009, Sugie & Yoshimura
337 2013). In previous studies under N-limited conditions, the C:N ratio increased because of a
338 large increase in C content, whereas the N content remained constant, or was only slightly
339 higher than in vegetative cells (French & Hargraves 1980, Kuwata et al. 1993). This suggests
340 that the relatively small change in C content under N- and Fe-depleted conditions in *A.*
341 *longicornis* is unique and may contribute to the stable cellular C:N ratio under different growth
342 conditions. In contrast, Si:C and Si:N ratios increased under both the N- and Fe-depleted
343 conditions. The higher Si cell quota with a smaller SA under N- and Fe-depleted conditions
344 indicates that *A. longicornis* is more heavily silicified under the unfavourable growth
345 conditions than control conditions. Longer horns under Fe-depletion may contribute to the
346 higher Si content compared with the controls. An increase in the Si:N and Si:C ratios in
347 diatoms grown under an N- or Fe-limited/depleted conditions is well-known (French &
348 Hargraves 1980, Kuwata et al. 1993, Marchetti & Cassar 2009, Sugie et al. 2010a, Sugie &

349 Yoshimura 2013). Diatoms increase the Si:C and Si:N ratios in two ways; Type-1 increases
350 silicification, e.g. *Actinocyclus* sp., *Chaetoceros pseudocurvisetus* and *Thalassiosira*
351 *nordenskioeldii*, (Kuwata et al. 1993, Timmermans et al. 2004, Sugie et al. 2010a) and Type-
352 2 decreases cellular C and N content, e.g. *Chaetoceros dicheta*, and *Pseudo-nitzschia* spp.,
353 (Takeda 1998, Marchetti & Cassar 2009, Sugie & Yoshimura 2013). *Attheya longicornis* is a
354 Type-1 species under both N- and Fe-depleted conditions. These results suggest that *A.*
355 *longicornis* took up dissolved silicic acid from ambient seawater after both N and Fe were
356 depleted. Similar over-consumption of Si compared to N uptake under N- and Fe-depletion
357 has been reported previously from the natural phytoplankton community in the Oyashio region
358 during the spring diatom bloom (Sugie et al. 2010a). However, the different estimated sinking
359 behaviour of *A. longicornis* under the N- and Fe-depleted conditions could result in
360 biogeochemical cycling of Si in opposite directions; the former might sequester Si in deeper
361 waters, whereas the latter might retain Si in the surface.

362 ***Insights into the autecology of A. longicornis***

363 Diatom biogeography in the open ocean is strongly affected by current systems
364 originating from coastal regions where species are abundant, with species-specific ecological
365 traits modifying the subsequent distribution patterns (Sugie & Suzuki in press). *Attheya*
366 *longicornis* is often found in the subarctic seas in the coastal region, tangled by its hon with
367 chain-forming diatoms by its horns (Orlova et al. 2002, Stonik et al. 2006), which significantly
368 increases particle size. Because grazing pressure decreases with increasing cell size (Thingstad
369 et al. 2005), entanglement with, or attachment to a large cell or chain by small species could
370 decrease the grazing pressure by zooplankton. Many chain-forming diatoms are coastal
371 species, such as *Chaetoceros* subgenus *Hyalochaete* (Smetacek 1985, Round et al. 1990, Sugie
372 et al. 2010a) and chain formation may allow diatoms to live as plankton only under high
373 nutrient and turbulent conditions based on nutrient uptake models (Pahlow et al. 1997). After
374 either macro- or micronutrient depletion, such large chain-forming diatoms would decrease
375 their abundance partly due to the formation of fast-sinking resting spores (Smetacek 1985,
376 Sugie & Kuma 2008). *Attheya longicornis* increased its Si quota under Fe- and N-depleted
377 conditions, but the probable fate of biogenic Si may differ depending on the sinking behaviour.

378 To sustain the local population of *A. longicornis* under unfavourable growth conditions, a
379 change in sinking behaviour appears to be beneficial, both in the coastal and open ocean of
380 the subarctic. Such physiological plasticity may contribute to their wide distribution in these
381 regions (Orlova et al. 2002, Stonik et al. 2006, Sugie et al. 2010a, Malviya et al. 2016, Sugie
382 and Suzuki in press). These suggested ecophysiological strategies of diatoms with spiny
383 siliceous structures need additional study, but the available data suggest that the *A. longicornis*
384 increases its survival rate by altering its ecological strategies depending on the nutrient-
385 limiting conditions.

386

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394 FACILITY, Hokkaido University Sousei Hall.

395

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563 *Figure captions*

564 Figs 1–6. Images of *Attheya longicornis*. Cells attached to Fig 1. *Chaetoceros diadema* and
565 Fig 2. *Chaetoceros socialis* (samples were collected from the Oyashio region in April 2007).
566 Scanning electron micrograph of Fig 3. *A. longicornis* (scale bar is 5 μm), with a detailed
567 image of a siliceous horn in the insert (scale bar is 1 μm). Cells grown under Fig 4. nutrient-
568 replete conditions (control), Fig 5. N-depleted conditions and Fig 6. Fe-depleted conditions.

569

570 Fig. 7. Temporal change in cell density of *Attheya longicornis* grown under nutrient-replete
571 (control), N-depleted and Fe-depleted conditions.

572

573 Fig. 8. Change in the cell quota of (a) carbon, (b) nitrogen, (c) biogenic silica and (d)
574 chlorophyll-*a* for *Attheya longicornis* in controls and N- and Fe- depleted conditions. Alphabet
575 above the bars indicate when the data are significantly different (Tukey's HSD test).

576

577 Fig. 9. Values for (a) cell volume, (b) surface area, (c) spine length (d) surface area to cell
578 volume ratio and (e) estimated sinking rate of *Attheya longicornis* cells grown in nutrient-
579 replete (control) and N- and Fe-depleted conditions. Alphabet above the bars indicate when
580 the data are significantly different (Tukey's HSD test).

1 Table 1. Tree treatments examining the effect of nitrogen- and Fe-depletion on the elemental
 2 composition and morphology of *Attheya longicornis*.

3 Treatment	Nutrients	Iron
4 Control	Replete	Replete (+100 nmol L ⁻¹)
5 N-depleted	11 μmol L ⁻¹ NO ₃ (P and Si replete)	Replete (+100 nmol L ⁻¹)
7 Fe-depleted	Replete	1 μmol L ⁻¹ DFB (No Fe additon)

8

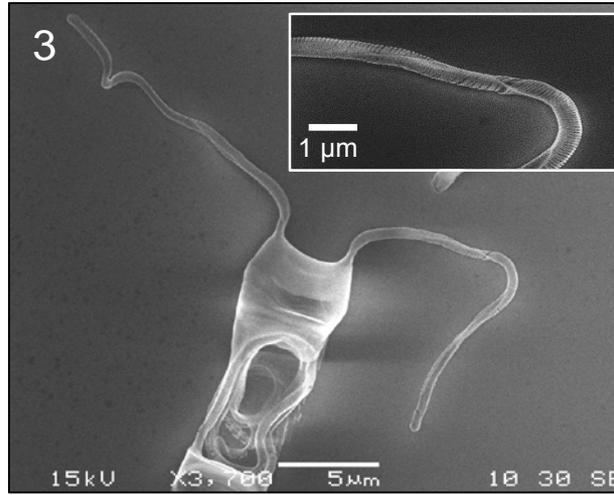


Fig. 7

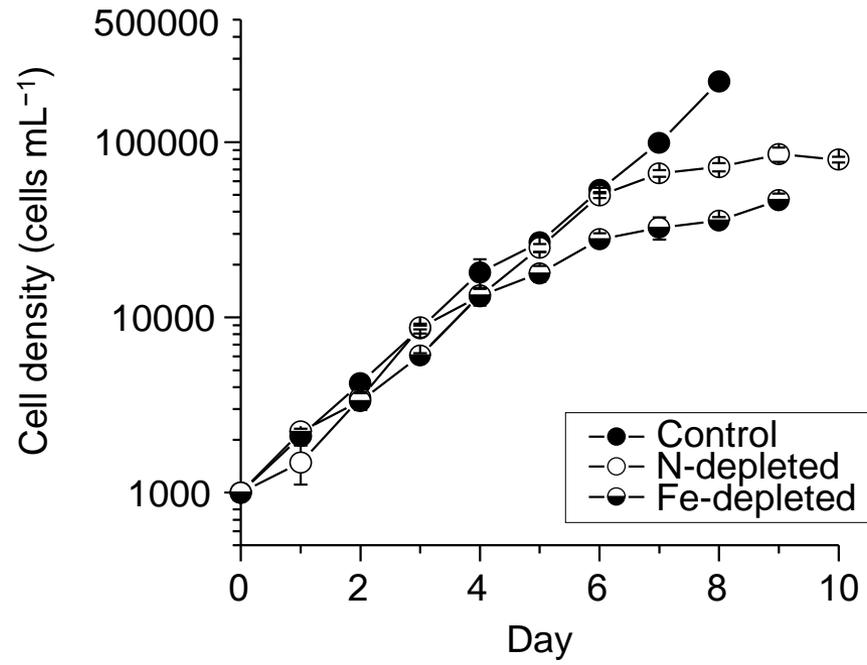


Fig. 8

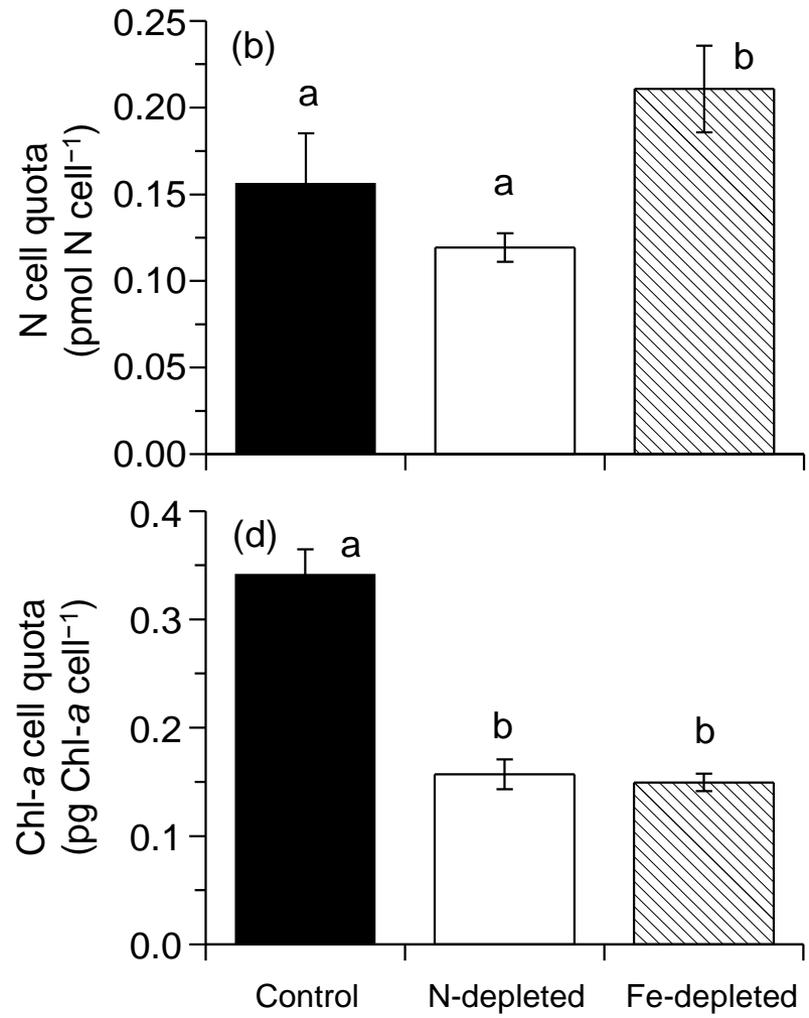
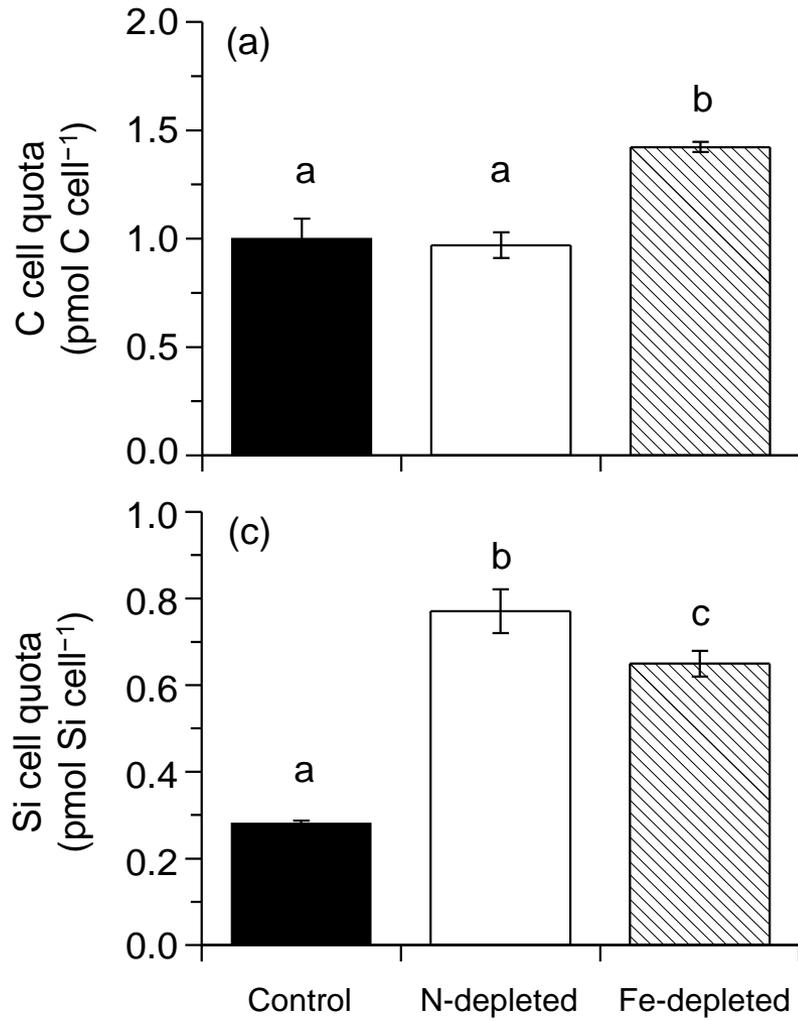


Fig. 9

