How will the key marine calcifier *Emiliania huxleyi* respond to a warmer and more thermally variable ocean?

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21   **Abstract**

22   Global warming will be combined with predicted increases in thermal variability in

23   the future surface ocean, but how temperature dynamics will affect phytoplankton

24   biology and biogeochemistry is largely unknown. Here, we examine the responses of

25   the globally important marine coccolithophore *Emiliania huxleyi* to thermal variations

26   at two frequencies (one-day and two-day) at low (18.5 ^{o}C) and high (25.5 ^{o}C) mean

27   temperatures. Elevated temperature and thermal variation decreased growth,

28   calcification and physiological rates, both individually and interactively. One-day

29   thermal variation frequencies were less inhibitory than two-day variations under high

30   temperature, indicating that high frequency thermal fluctuations may reduce heat-

31   induced mortality and mitigate some impacts of extreme high temperature events.

32   Cellular elemental composition and calcification was significantly affected by both

33   thermal variation treatments relative to each other, and to the constant temperature

34   controls. The negative effects of thermal variation on *E. huxleyi* growth rate and

35   physiology are especially pronounced at high temperatures. These responses of the

36   key marine calcifier *E. huxleyi* to warmer, more variable temperature regimes have

37   potentially large implications for ocean productivity and marine biogeochemical

38   cycles under a future changing climate.

39

Introduction

Climate-driven changes such as ocean warming alter the productivity and composition of marine phytoplankton communities, thereby influencing global biogeochemical cycles (Boyd et al., 2018; Hutchins & Fu, 2017; Thomas, et al., 2012). Increasing sea surface temperatures have been linked to global declines in phytoplankton concentration (Boyce et al., 2010), changes in spring bloom timing (Friedland et al., 2018), and biogeographic shifts in harmful algal blooms (Fu et al. 2012; Gobler et al., 2017). Warming and acidification may drive shifts away from dinoflagellate or diatom dominance, and towards nanophytoplankton (Hare et al., 2007; Keys et al., 2018). Similarly, Morán et al. (2010) predicted that a gradual shift will occur towards smaller primary producers in a warmer ocean.

Effects of temperature increases on phytoplankton diversity are uncertain. Warming and phytoplankton biodiversity were found to be inversely correlated in a coastal California diatom assemblage, at least on short timescales (Tatters et al., 2018). In contrast, a five-year long mesocosm experiment found that elevated temperature can modulate species coexistence, thus increasing phytoplankton species richness and productivity (Yvon-Durocher et al. 2015). Globally, rising temperatures may result in losses of phytoplankton biodiversity in the tropics, but gains in the polar regions (Thomas et al., 2012). It is thought that ocean warming will lead to a poleward range expansion of warm-water species at the expense of cold-water species (Boyd et al., 2010; Gao et al., 2018; Hallegraeff, 2010; Hutchins & Fu, 2017; Thomas et al., 2012). It is evident that rising ocean temperatures will benefit some groups, while having

62   detrimental consequences for others (Boyd et al., 2010, 2015, 2018; Feng, et al., 2017;

63   Fu et al., 2014). For example, recent decades of satellite observations show a striking

64   poleward shift in the distribution of blooms of the coccolithophore *Emiliania huxleyi*,

65   a species that was previously virtually absent in polar waters (Boyd et al., 2010;

66   Neukermans et al., 2018).

67   Coccolithophores are the most successful calcifying phytoplankton in the ocean,

68   and contribute almost half of global marine calcium carbonate production. They play

69   crucial biogeochemical roles by performing both photosynthesis and calcification, and

70   facilitate carbon export to the deep ocean through the ballasting effects of their

71   calcium carbonate shells (Klaas & Archer, 2002; Krumhardt et al., 2017; Monteiro et

72   al., 2016). *E. huxleyi* (Lohm.) is the most abundant and cosmopolitan coccolithophore,

73   forming prolific blooms in many regions (Holligan, et al., 1983; 1993; Iglesias-

74   Rodríguez et al., 2002; Westbroek et al., 1993).

75   The responses of *E. huxleyi* to global change factors have been intensively

76   investigated. Many *E. huxleyi* strains are sensitive to ocean acidification, which

77   negatively affects their growth rates and calcification (Feng et al., 2018; Hoppe et al.,

78   2011). However, among the many currently changing environmental drivers,

79   temperature may be among the most important in regulating coccolithophore

80   physiology (Boyd et al., 2010). Feng et al. (2008) reported that the growth rate of *E.*

81   *huxleyi* was improved by elevated temperature at low irradiance. Furthermore,

82   temperature was the most important driver controlling both cellular particulate

83   organic and inorganic carbon content of a Southern Hemisphere *E. huxleyi* strain

84 (Feng et al., 2018).

85   Most research about the effects of global warming on *E. huxleyi* and

86 phytoplankton in general has focused on predicted increases in mean temperatures.

87 However, in the natural environment, seawater temperatures fluctuate over timescales

88 ranging from hours, to days, to months (Bozinovic et al., 2011; Jiang et al., 2017).

89 Future climate models predict not only in an increase in mean temperature, but also an

90 increase in temperature variability (frequency and intensity), as well as a higher

91 probability of extreme events (IPCC 2014).

92   The impacts of climatic variability and extremes have been best studied in

93 metazoans, where they may sometimes have a larger effect than increases in climatic

94 averages alone (Vázquez et al., 2017; Vasseur et al., 2014; Zander et al., 2017).

95 Variability can promote greater zooplankton species richness, compared with

96 long-term average conditions (Cáceres 1997; Shurin et al. 2010). In corals,

97 temperature variability could buffer warming stress, elevate thermal tolerance and

98 reduce the risk of bleaching (Oliver & Palumbi, 2011; Safaie et al., 2018).

99   In comparison, we still lack a thorough understanding of how thermal variation

100 affects phytoplankton growth and physiology. Unlike zooplankton, the few available

101 studies suggest increasing thermal variation may decrease phytoplankton biomass and

102 biodiversity, and shift the community towards small phytoplankton (Burgmer &

103 Hillebrand, 2011; Rasconi et al., 2017). Two studies have shown that plastic responses

104 play a key role in acclimation and adaptation to thermal fluctuations in algae (Kremer

105 et al., 2018; Schaum & Collins, 2014). Population growth rates of phytoplankton in

106   fluctuating thermal environments have been quantitatively modeled based on data

107   from thermal response curves obtained under constant temperatures (Bernhardt et al.,

108   2018).

109   In view of this relative lack of information on the effects of non-steady state

110   temperatures on biogeochemically important phytoplankton, we carried out a thermal

111   variability study using the Sargasso Sea *E. huxleyi* isolate CCMP371. Our

112   experiments combined ocean warming with thermal variations, with a focus on the

113   increasing frequency of temperature variations under global climate change. We

114   examined growth rates, photosynthesis, calcification and elemental composition under

115   constant, one-day and two-day temperature variations. This study is intended to

116   provide insights into how different frequencies of thermal variation may influence the

117   physiology and biogeochemistry of this important marine calcifying phytoplankton

118   species under both current and future sea surface temperatures.

119   **Materials and methods**

120   The marine coccolithophore *E. huxleyi* (Lohm.) Hay and Mohler stain CCMP371

121   (isolated from the Sargasso Sea) was maintained in the laboratory as stock batch

122   cultures in Aquil medium (100 μmol L^{-1} NO_3^- , 10 μmol L^{-1} PO_4^{3-}) made with 0.2

123   μM-filtered coastal seawater collected from the California region (Sunda et al., 2005).

124   Cells were grown at 22 $^{\circ}$C under 120 μmol photons m^{-2} s^{-1} cool white fluorescent

125   light with a 12 h/12 h light/dark cycle.

126   **Experimental set-up**

127   An aluminum thermal gradient block with a range of 13 temperatures was used

to perform the thermal response curve and temperature variation experiments. For the thermal curve experiment, the extreme temperatures of the thermal-block were set to 8.5 °C and 28.6 °C, with intermediate temperatures of 10.5 °C, 12 °C, 13.5 °C, 15.5 °C, 17.5 °C, 18.5 °C, 21.3 °C, 22.6 °C, 24.5 °C, 26.6 °C, and 27.6 °C. The *E. huxleyi* cells were transferred from the stock cultures into triplicate 120 ml acid washed polycarbonate bottles in the thermal block under a 12 h light /12h dark cycle at 180 μmol photons m^{-2} s^{-1}. For the light intensity measurement, irradiance was measured individually at each position in the thermal block using a light meter with a small detector bulb to fit into the round holes drilled to fit the experimental bottles (LI-250A light meter, LI-COR). During measurements the detector bulb was positioned identically in each position, and if necessary fluorescent lights were rearranged, added or removed until the light intensity was between 175-185 μmol photons m^{-2} s^{-1} for every experimental replicate.

Semi-continuous culturing methods were used for all experiments. Cultures were diluted with Aquil medium every two days to keep them in exponential growth stage while acclimating to the treatment temperatures for two weeks before starting the variation experiment. Dilution volumes were calculated to match growth rates of each individual replicate, as measured using *in vivo* chlorophyll a (Chl *a*) fluorescence. Once steady-state growth rates were recorded for 3–5 consecutive transfers, the cultures were sampled (Zhu et al., 2017). Due to the decrease of cell numbers during cultivation at 28.6 °C (from our preliminary experiment), these cultures were diluted from 22 °C stock cultures. They were then sampled as a batch

150 culture (without dilution) after 4-6 days to estimate the negative growth rates and

151 elemental stoichiometry at this upper limit temperature point.

152 Six treatments were used to determine the responses of *E. huxleyi* growth,

153 photosynthesis and calcification to different frequencies of temperature fluctuation.

154 Temperature fluctuation treatments included: 1) Low temperature, constant (18.5

155 °C). 2) Low temperature, one-day fluctuation cycle (16-21°C, mean = 18.5°C). 3)

156 Low temperature, two-day fluctuation cycle (16-21°C, mean =18.5°C). 4) High

157 temperature, constant (25.5 °C). 5) High temperature, one-day fluctuation cycle

158 (23-28°C, mean = 25.5°C). 6) High temperature, two-day fluctuation cycle (23-28°C,

159 mean = 25.5°C). For the variation treatment cycles, cultures were incubated at the

160 cool phase (16 °C and 23 °C for low and high temperatures, respectively) for either

161 one or two days. They were then transformed to the warm phase (21 °C and 28 °C for

162 low and high temperature, respectively) for the same amount of time. It took about

163 1/2 hour to re-adjust the thermal block to the transformed temperature at the

164 beginning of each new treatment cycle. The experimental *E. huxleyi* cultures were

165 grown in triplicate in 120 ml acid washed polycarbonate bottles using the

166 thermal-block under a 12 h light /12h dark cycle at 180 μmol photons m^{-2} s^{-1}.

167 For the variable temperature experiment, cultures were diluted semi-continuously

168 with Aquil medium every two days the for constant and one-day variation treatments,

169 and every four days for two-day variation treatments. To ensure nutrient-replete

170 conditions in the two-day variation treatments, Aquil nitrate and phosphate stocks

171 were added at the two day midpoint of every four day thermal cycle to make sure

172   that the final nitrate and phosphate concentrations were not depleted and were

173   always maintained >100 μmol L^{-1} and >10 μmol L^{-1}, respectively. Cultures were

174   grown for at least eight dilutions (~16 days for constant and one day variation

175   treatments; ~32 days for two-day variation treatments) to acclimate to the different

176   experimental conditions before final sampling. All variation treatments were

177   sampled twice across the thermal variation cycle, once during the cool phase and

178   once during the warm phase.

179   **Growth rates**

180   *In vivo* fluorescence was measured daily for the one-day variation treatment and

181   every two days for the constant and two-day variation treatments using a Turner

182   10-AU fluorometer (Turner Designs, CA). *In vivo*-derived growth rates were

183   subsequently verified using cell samples counted with a nanoplankton counting

184   chamber on an Olympus BX51 microscope. Specific growth rates (d^{-1}) were

185   calculated using the *in vivo* fluorescence and cell count data as:

186     $\mu=\ln[N(T_2)/N(T_1)]/(T_2-T_1)$, in which $N(T_1)$ and $N(T_2)$ are the *in vivo* fluorescence

187   values (for thermal curve experiments and constant treatments) or cell counts (for

188   variation treatments, because of potential changes in cellular *in vivo* fluorescence

189   during fluctuation) at T_1 and T_2.

190   **Chl *a* analysis**

191   Twenty ml culture samples were filtered onto GF/F glass fiber filters (Whatman,

192   Maidstone, UK) for Chl *a* analysis. In vitro Chl *a* was extracted with 90% aqueous

193   acetone for 24 hours at -20 °C, and then measured using a Turner 10-AU fluorometer

194   (Turner Design, USA). (Fu et al., 2007).

195   **Elemental analysis**

196   Elemental composition sampling included total particulate carbon (TPC),

197   particulate organic carbon (POC), particulate organic nitrogen (PON), particulate

198   inorganic carbon (PIC) and particulate organic phosphorus (POP), allowing

199   calculation of cellular elemental stoichiometry and calcite/organic carbon rations

200   (PIC/POC) (Feng et al.; 2008). Culture samples for TPC, POC and PON, were

201   collected onto pre-combusted GF/F glass fiber filters (Whatman) and dried in a 60

202   ^{o}C oven overnight.   For POC analysis, filters were fumed for 24 hours with

203   saturated HCl (~37%) to remove all inorganic carbon prior to analysis. TPC, PON

204   and POC were then measured by a 440 Elemental Analyzer (Costech Inc, CA)

205   according to the previous studies (Hutchins et al., 1998; Feng et al., 2008). PIC was

206   calculated as the difference between TPC and POC. For POP measurement, culture

207   samples were filtered on onto pre-combusted GF/F filters (Whatman) and analyzed

208   using a molybdate colorimetric method (Solórzano and Sharp, 1980), with minor

209   modifications as in Fu et al. (2007).

210   **Total carbon fixation, photosynthetic and calcification rates & ratios**

211   Total carbon fixation, photosynthetic carbon fixation and calcification rates were

212   measured using a ^{14}C incubation techniques (Platt et al., 1980) with slight

213   modifications as in Feng et al. (2008). Sixty mL culture samples from each treatment

214   were spiked with 0.2 μCi NaH^{14}CO$_3$ and then incubated for 4 h under their

215   respective experimental conditions. After incubation, samples were filtered on two

216   Whatman GF/F filters (30mL each) for total carbon fixation and photosynthetic rate

217   separately. The filters for photosynthetic rate measurement were fumed with

218   saturated HCl (~37%) before adding scintillation fluid. Thirty mL from each

219   treatment (10 mL from each replicate bottle) was filtered immediately, after adding

220   equal amounts of $NaH^{14}CO_3$ for procedural filter blanks. Filters were then placed in

221   7 mL scintillation vials with 4 mL scintillation fluid overnight in the dark. To

222   determine the total radioactivity (TA), 0.2 μCi $NaH^{14}CO_3$ together with 100 μL

223   phenylalanine was placed in scintillation vials with the addition of 4 mL scintillation

224   solution. All samples were counted on a Perkin Elmer Liquid Scintillation Counter to

225   measure the radioactivity. Total carbon fixation and photosynthetic rate were

226   calculated from TA, final radioactivity and total dissolved inorganic carbon (DIC)

227   values. Calcification rate was then calculated as the difference between total carbon

228   fixation and photosynthetic rate for each sample.

229   **Model for population growth of *E. huxleyi***

230   Growth rates measured under constant temperatures in the thermal block were

231   fitted to the Eppley thermal performance curve (Eppley, 1972; Norberg, 2004;

232   Thomas et al., 2012). This function quantifies parameters of growth temperature

233   effects, including the temperature optimum for growth (T_{opt}), and high and low

234   temperature limits (T_{max} and T_{min} respectively) in our strain of *E. huxleyi*. A model

235   based on the Eppley curve but incorporating non-linear averaging and consideration

236   of Jensen's inequality (Bernhardt et al., 2018) was applied to predict the impact that

237   fluctuating temperatures might have on the shape of thermal growth curves at

238 present-day and future mean temperatures, similar to other recent studies (Qu et al.

239 2019, Kling et al. in press).

240 **Statistical analysis**

241 The mean values of most parameters measured under the variation treatments

242 were calculated by averaging the values from the cool and warm phases, including

243 all the elemental content and ratios, photosynthetic and calcification rates and ratios.

244 For the statistical analyses, the Student's t-test and one-way ANOVA were applied

245 to analyze the difference among temperature treatments. p-values were calculated

246 based on two formulas including compare_means() and stat_compare_mean() via the

247 ggpubr package, and the figures were generated via the ggplot package in open

248 source statistical software R version 3.5.0 (R Foundation).

249 **Results**

250 **Responses of *E. huxleyi* to warming**

251 The growth rates of *E. huxleyi* at constant temperature increased significantly

252 with warming from 0.09 ± 0.01 d^{-1} at 8.5 ^{o}C to a maximum value of 0.90 ± 0.02 d^{-1} at

253 21.3 ^{o}C. Growth was optimal up to 24.5 ^{o}C, and then decreased rapidly to -0.46 ± 0.05

254 d^{-1} at 28.6 ^{o}C ($p<0.05$, Fig. 1).

255 The elemental ratios of the cells in the different temperature treatments were

256 compared to the average elemental ratios across the entire temperature range (Fig. 2).

257 The thermal trends of TPC/PON ratios were generally similar with those of growth

258 rates, in that ratios increased from 8.5 to 17.5 ^{o}C, and then decreased from 24.5 to

259 27.6 ^{o}C. The TPC/PON ratios at 8.5, 10.5 and 27.6 ^{o}C were significantly lower than

260   the average level of all the temperature points ($p < 0.05$, Fig 2A). The POC/PON

261   ratios of most temperature points were very close to the mean value of 6.3, except at

262   27.6 °C (7.1) and 28.6 °C (7.4), which were significantly higher than the average

263   ($p < 0.05$, Fig 2B). The highest PIC/POC ratio was 0.49 ± 0.07 at 22.6 °C, and the

264   lowest PIC/POC ratio was 0.05 ± 0.04 at 27.6 °C, a value that was almost 90% less

265   than the highest value. The PIC/POC ratios at the lowest temperature tested (10.5 °C)

266   and at the high end of the temperature range (26.6 and 27.6 °C) were significantly

267   lower than the average level (Fig. 2C). Chl *a*/POC ratios were significant lower at

268   8.5, 10.5 and 27.6 °C than the mean, and at 17.5, 21.3, 22.6 and 24.5 °C were

269   significantly higher than the average ($p < 0.05$, Fig. 2C). The trends of PIC/POC and

270   Chl *a*/POC ratio were similar, in that they gradually increased from low temperature

271   and to the highest value at 22.6 °C, and then dropped rapidly as temperature

272   increased further. (Fig. 2C, D).

273   **Responses of *E. huxleyi* to temperature variations**

274   **Growth rate**

275   In low temperature experiments, both one-day and two-day temperature

276   variations had a negative effect on growth rate. The mean growth rates of the

277   one-day (0.71 ± 0.01 d^{-1}) and two-day (0.72 ± 0.01 d^{-1}) variation treatments were not

278   significantly different from each other ($p > 0.05$), but both were lower than that of the

279   constant 18.5 °C treatment (0.76 ± 0.01, $p < 0.05$) (Fig. 3A). Growth rates were low

280   during the cool phase (16 °C) of the experiment (~0.5-0.6 d^{-1}), but those of the

281   two-day variation cycle were not significantly different from the constant control at

282   this temperature (p>0.05). However, the growth rates during the cool phase of the

283   one-day variation cycle were lower than those of the constant 16 ^{o}C treatment

284   (p<0.05). During the warm phase of the thermal cycle (21°C), there were no

285   significant differences in the elevated growth rates (~0.85-0.9 d^{-1}) of the constant

286   control and those of either variable treatment (p>0.05, Fig. 3A).

287   In the high temperature experiments, as in the low temperature experiments, both

288   temperature variation frequencies had a negative effect on mean growth rates. The

289   growth rates in the two-day variation treatment were (0.20±0.02 d^{-1}), a decrease of

290   ~74% compared with the constant 25.5 ^{o}C (p<0.05), and ~62% of the one-day

291   variation treatment value (p<0.05, Fig. 3B). During the cool phase (23 ^{o}C), the

292   growth rate of the one-day variation treatment was slightly lower (p<0.05) than the

293   constant 23 ^{o}C, but there were no significant changes between two-day variations

294   and the constant 23 ^{o}C treatment (p > 0.05, Fig. 3B). During the warm phase (28 ^{o}C),

295   the constant 28 ^{o}C and two-day variation treatment both had negative growth rates of

296   -0.45±0.05 d^{-1} and -0.45±0.04 d^{-1}, respectively. However, the one-day variation

297   treatment had a low but positive warm phase growth rate at 0.25±0.02 d^{-1} (Fig. 3B).

298   There was a time lag of ~1/2 hour to switch to the transformed temperature for each

299   new growth phase, which should thus have had only minimal effects on overall

300   growth   rates across the one-day and two-day thermal variations.

301   **Cellular PIC and POC contents and ratios**

302   In low temperature experiments, the cellular PIC content of the constant 18.5 ^{o}C

303   treatment was 3.5±0.3 pg/cell, and there were no significant differences with

304   temperature variation treatments (p> 0.05, Table 1). However, the cellular POC

305   content of the constant 18.5 $^{\circ}$C treatment was 8.0±0.6 pg/cell, which was lower than

306   in the two-day variation treatment, but significantly higher than in the one-day

307   variation treatment (p<0.05).

308   Like POC, the PIC/POC ratio was significantly affected by temperature

309   variations (Fig. 4A). The lowest PIC/POC ratio was found in the one-day variation

310   treatment (0.38 ±0.07), which was significantly lower than the two-day variation

311   treatment value (p < 0.05), but close to that in the constant 18.5 $^{\circ}$C (p > 0.05). A

312   similar trend was found in both the cool (16 $^{\circ}$C) and warm phases (21 $^{\circ}$C) of the two

313   variation treatments, in that the PIC/POC ratio of the one-day variation treatment

314   was lower than of the two-day variation treatment (p < 0.05, Fig. 4A). Both variation

315   treatments had lower PIC/POC ratios during the warm phase than during the cool

316   phase, although these differences were not significant (p>0.05).

317   High temperature experiments showed particulate carbon trends that were

318   contrary to those of the low temperature treatments. The PIC content and PIC/POC

319   ratios were significantly decreased by temperature variation. The cellular PIC

320   content of the constant treatment (25.5 $^{\circ}$C) was 5.5±0.3 pg/cell, which was ~ 200%

321   higher than that of the one-day variation and ~ 160% higher than in the two-day

322   variation treatments (p<0.05, Table 1). The same trend was found for PIC/POC

323   ratios in one-day variation and two-day variation treatments, which decreased ~ 67%

324   and 33% compared with the constant 25.5 $^{\circ}$C treatment, respectively (p<0.05, Fig.

325   4B). However, the POC content of one-day and two-day variation treatments was

326   higher than in the constant 25.5 °C treatment (p < 0.05, Table 1). During the cool

327   phase (23 °C), the PIC content and PIC/POC ratio of the one-day variation treatment

328   was significantly lower than in the two-day variation treatment, but contrary to PIC

329   content, the POC content of the one-day variation treatment was significantly higher

330   than that in the two-day variation treatment. During the warm phase (28 °C), there

331   were no significant differences of PIC content, POC content, or PIC/POC ratio

332   between the one-day and two-day variation treatments (Fig. 4B, Table 1).

333   **Photosynthetic and calcification rates and ratios**

334   In low temperature treatments, there were no differences between total carbon

335   fixation rates (photosynthesis plus calcification) for the two variable treatments

336   relative to the constant control (Fig. 5A). However, during the cool phase total

337   carbon fixation rates were higher in the one-day variation than in the two-day

338   variation (p<0.05, Fig 5A), while this rate was the same in both variation treatments

339   during the warm phase (p > 0.05, Fig. 5A). In high temperature experiments, the

340   total carbon fixation rates of the one-day and two-day variation treatments were

341   significantly decreased by about ~20% and ~18% respectively, compared with the

342   constant 25.5 °C treatment (p<0.05, Fig. 5 B).

343   The photosynthetic and calcification rates of the constant 18.5 °C treatment were

344   0.04 ± 0.00 pmol C cell^{-1} hr^{-1} and 0.02 ± 0.00 pmol C cell^{-1} hr^{-1}, respectively, which

345   were not significantly different from both of the temperature variation treatments (p >

346   0.05, Fig. 5 C,E). Photosynthetic rates changed within the thermal cycle for both

347   one-day and two-day variation treatments, with a decrease of 22% and 28% from the

348   warm phase to the cool phase, respectively (Fig. 5C). However, there were no

349   significant changes in calcification rates under either variation frequency treatment

350   between the cool and warm phases of the thermal cycles ($p > 0.05$).

351   In the mean 25.5 $^{\circ}$C experiment, photosynthetic rates were not significantly

352   different between the one-day variation and constant treatments ($p > 0.05$), while the

353   photosynthetic rate of the two-day variation was slightly higher than that of the

354   constant 25.5 $^{\circ}$C treatment ($p<0.05$, Fig. 5D). In contrast, calcification rates of

355   one-day and two-day variation treatments at a mean temperature of 25.5 $^{\circ}$ were

356   significantly decreased by about ~46% and ~51%, respectively, relative to the

357   constant control ($p<0.05$, Fig. 5F). There were no significant differences in total

358   carbon fixation, photosynthetic and calcification rates between the one-day variation

359   and two-day variation treatments during both the cool (23 $^{\circ}$C) and warm (28 $^{\circ}$C)

360   phases ($p>0.05$, Fig. 5 B,D,F).

361   In the low temperature treatments, there were no significant differences in

362   Cal/Photo ratios between the constant and the two variable treatments ($p > 0.05$, Fig

363   6A). In contrast, in the high temperature experiments, the Cal/Photo ratio of the

364   one-day variation and two-day variation treatments were decreased by ~40% and

365   49%, respectively, compared with the constant 25.5 $^{\circ}$C treatment ($p<0.05$, Fig. 6B).

366   For both low and high temperature experiments, there were no significant differences

367   between the one-day and two-day variation treatments in either the cool or warm

368   phases of the thermal cycle ($p > 0.05$, Fig. 6B). However, in both temperature

369   treatments the lower photosynthetic rates during the cool phase (Fig. 5C,D) resulted

370   in an increase in the Cal/Photo ratio during the cool phase for both the one-day and

371   two-day variation treatments (p<0.05 Fig. 6A,B).

372

373   **Elemental content and stoichiometry**

374   In the low temperature experiments, the one-day variation and two-day thermal

375   variations had different effects on cellular elemental contents and ratios, relative to

376   the constant 18.5 °C treatment. One-day variation increased most of the cellular

377   elemental and biochemical contents (TPC, PON, and Chl a) but with no significant

378   difference (p>0.05), except for POP content (p<0.05), compared with the constant

379   18.5 °C treatment (Table 1). In contrast, the two-day variation treatment decreased

380   all the measured cellular elemental and biochemical contents (TPC, PON, POP and

381   Chl a, p<0.05) in relation to the constant 18.5 °C treatment (Table 1). However, the

382   TPC/PON and Chl a/POC ratios of the two-day variation treatment were higher than

383   those of the one-day variation and constant 18.5 °C treatments (p<0.05, Fig. 7A,E),

384   while the PON/POP ratio was lower than in the one-day variation and constant 18.5

385   °C treatments (p<0.05, Fig. 7C). There were no significant differences in TPC/PON,

386   PON/POP and Chl a/POC ratios between the constant 18.5 °C and the one-day

387   variation treatments (p > 0.05, Fig. 7A).

388   In high temperature experiments, the highest cellular TPC, PON and POP

389   contents were all obtained under the one-day variation treatment, which was

390   significantly higher than under constant 25.5 °C conditions (p<0.05, Table 1).

391   However, there were no significant differences in cellular Chl a content between the

constant 25.5 $^{\circ}$C and both variation treatments (p > 0.05, Table 1). The TPC/PON

ratio of the constant 25.5 $^{\circ}$C treatment was ~22% and ~35% higher than that of the

two-day variation and one-day variation treatments, respectively (p<0.05, Fig. 7B),

while the PON/POP ratio was highest in the one-day variation, followed by the

two-day variation and finally by the constant control (Fig. 7D). The Chl *a*/POC ratio

of the one-day variation treatment was significantly lower than that of the constant

25.5 $^{\circ}$C and two-day variation treatments (p<0.05), but there were no significant

differences between the constant 25.5 $^{\circ}$C and two-day variation treatments (p > 0.05,

Fig. 7F).

During the cool phase of the high temperature experiments (23 $^{\circ}$C), the cellular

TPC, PON, POP and Chl *a* content of two-day variation were all significantly lower

than in the one-day variation treatment (p<0.05). Similar decreasing trends during

the cool phase were observed for the TPC/PON ratios (Fig. 7B), but not the Chl

a/POC ratio, which was ~32% higher than in the one-day variation treatment (p<0.05,

Fig. 7F). During the warm phase (28 $^{\circ}$C), there were no significant differences of

cellular TPC, PON and POP contents between one-day and two-day variation

treatments (p > 0.05, Table 1) as well as the TPC/PON ratio (Fig 7B). However, the

Chl *a* content of the one-day variation treatment was ~20% lower than that of the

two-day variation treatment (p<0.05). The Chl *a*/POC ratio was not significantly

different between the one-day and two-day variation treatments at the warm phase

(p > 0.05, Table 1, Fig. 7F).

Experimental constant temperature performance curves and measured and

modeled fluctuating temperature performance curves

The experimentally-determined constant condition temperature performance curves and the predicted fluctuating temperature condition temperature performance curves based on the Bernhardt et al. (2018) non-linear averaging model are shown in Fig. 8 for *E. huxleyi*. Compared with the measured temperature performance curve under constant thermal conditions, the modeled curve of the fluctuating temperature condition showed a leftward shift towards lower temperatures at optimum temperatures and above. The maximum and optimal temperature of the modeled fluctuating temperature performance curve were all lower than those of the measured constant condition curve. In particular, the optimal temperature for growth decreased from 22°C in constant conditions to 21 °C under fluctuating temperature conditions. At the same time, the maximum growth rate (μ_{max}) of the fluctuating temperature condition was 0.8 d^{-1}, which was lower than the constant condition value of 0. 9 d^{-1}. The measured growth rates of experimental one-day (0.71 d^{-1}) and two-day (0.72 d^{-1}) variation treatments at the relatively low mean temperature of 18.5 °C closely matched the model-predicted fluctuating temperature growth rate at this temperature (0.74^{-1}, Fig. 8). However, measured and predicted growth rates did not match as well at the higher mean temperature. At 25.5 °C, the measured growth rate of the one-day variation was 0.52 d^{-1}, 30% higher than the predicted fluctuating temperature growth rate of 0.40 d^{-1}. In contrast, the measured growth rate of the experimental two-day variation treatment was 0.20 d^{-1}, a decrease of 50% compared to the model-predicted fluctuating temperature growth rate of 0.40 d^{-1} at this temperature (Fig. 8).

Discussion

Effects of warming on *Emiliania huxleyi* growth rates and elemental ratios

Thermal response curves and optimum growth temperatures describe the importance of temperature as a control on the distribution of *E. huxleyi* strains in the ocean (Buitenhuis et al., 2008; Paasche, 2001). The optimal temperature range of 21.3-24.5 $^{\circ}$C found in our study is similar to that of some other *E. huxleyi* strains (De Bodt et al., 2010; Feng et al., 2017; Rosas-Navarro et al., 2016; Zhang et al., 2014). Most studies have focused on the lower part of the temperature curve where growth rates increase with rising temperatures (Feng et al., 2017; Matson et al., 2016), with relatively few examining stressfully warm temperatures where growth is inhibited (Zhang et al., 2014). In our study, the descending portion of the upper temperature performance curve ranged from 24.5 $^{\circ}$C to 28.6 $^{\circ}$C, at which point growth rates became negative. This *E. huxleyi* strain was isolated from the Sargasso Sea where the sea surface temperature can reach 29 $^{\circ}$C in the summer, and will be higher in the future with global warming (https://seatemperature.info/sargasso-sea-water-temperature.html). This suggests that this strain may be currently living near its upper thermal limit for part of the year, as are many other tropical and subtropical phytoplankton (Thomas et al. 2012), and that it may therefore be vulnerable to further warming.

Calcification is the key biogeochemical functional trait of this species, and the PIC/POC ratio of *E. huxleyi* can be influenced by factors that include CO_2 concentration, nutrient status, irradiance and temperature (Feng et al., 2008, 2017;

Raven & Crawfurd, 2012). The cellular PIC/POC of *E. huxleyi* has been reported to decrease as irradiance and CO_2 concentration rises, but increase under nitrate and phosphate limitation (Feng et al., 2017; Paasche, 1999; Riegman et al., 2000). The effect of temperature on *E. huxleyi* cellular PIC/POC ratio is however more complex. De Bodt et al. (2010) and Gerecht et al. (2014) observed that higher cellular PIC/POC ratios were obtained at lower temperatures for both *E. huxleyi* and *Coccolithus pelagicus*. Sett et al. (2014), however, found an opposite trend, whereby the PIC/POC ratio increased with temperature in another strain of *E. huxleyi*. Feng et al. (2017) reported that the cellular PIC/POC of *E. huxleyi* was increased as the temperature rose from 4 °C to 11 °C, but decreased with warming from 11 °C to 15 °C and remained steady afterwards.

In our study, the cellular PIC/POC ratio of *E. huxleyi* was positively correlated to growth rate (R^2=0.73), and increased with warming from 8.5 °C to a maximum at 22.6 °C, and then decreased with further warming to 27.6 °C. In a meta-analysis of studies using different coccolithophore subgroups, Krumhardt et al. (2017) found that the highest PIC/POC ratios were observed between 15 °C and 20 °C, in the same thermal range where the highest growth rates of *E. huxleyi* are found, as seen here and in Sett et al. (2014). In contrast, Rosas-Navarro et al. (2016) reported that the cellular PIC/POC ratio showed a minimum at optimal growth temperature (between 20 and 25 °C) for three strains of *E. huxleyi*. However, the *E. huxleyi* strain used here was isolated from a warmer area (the Sargasso Sea) compared with isolates from coastal Japan and New Zealand in previous studies (Rosas-Navarro et al. 2016; Feng et al.

480 2017). The growth temperature for our stock cultures was 22-24°C, higher than that of

481 the other two *E. huxleyi* strains. Feng et al. (2017) also found that the optimal

482 temperature for calcification was close to the stock culture maintenance temperature

483 in their study. Our results also support suggestions that stressful high temperatures

484 may lead to decreases in cellular PIC/POC ratios and calcification (De Bodt et al.,

485 2010; Feng et al., 2017; Gerecht et al., 2014; Krumhardt et al., 2017). The cellular

486 PIC/POC ratio of *E. huxleyi* was much more plastic than the other ratios we measured,

487 including TPC/PON and POC/PON. Indeed, PIC/POC ratios may change

488 dramatically (>2-fold) with temperature for some coccolithophore subgroups

489 (Krumhardt et al., 2017). The plasticity in PIC/POC ratios of *E. huxleyi* during

490 temperature changes in our study may have implications for shifts in the ballasting of

491 coccolith-containing particles during sinking, thus affecting the ocean carbon cycle.

492 The cellular Chl *a*/POC ratio of *E. huxleyi* showed a similar pattern with the

493 PIC/POC ratio, as it was also positively correlated to growth rate. Zhu et al. (2017)

494 reported the cellular Chl *a*/POC ratio of a Southern California diatom was also

495 correlated to growth rate across a very similar temperature range. In contrast, Feng et

496 al. (2017) found that the cellular Chl *a*/POC ratio of *E. huxleyi* dramatically decreased

497 with warming. However, in our experiments, the cellular Chl *a*/POC ratio was lower

498 at 27.6 °C than at 28.6 °C, likely due to the negative growth rates and consequent lack

499 of acclimation of the cultures maintained at the highest temperature. Traits such as

500 PIC/POC ratios, Chl *a*/POC ratios and TPC/PON ratios also showed some evidence

501 for possible carryover from the stock cultures (22-24 °C) in this 28.6 °C treatment, as

502   we were forced to sample before the cells died completely, after only 2-3 cycles of

503   dilution.

Effect of thermal variation on *Emiliania huxleyi* growth and physiology

Constant vs variable temperature

506   Thermal variability in the surface ocean is becoming an increasingly relevant

507   topic as global warming proceeds. In our study, we found that the growth rates of a

508   subtropical *E. huxleyi* strain were quite sensitive to temperature variation under both

509   low (18.5 °C, "winter") and high (25.5 °C, "summer") mean temperatures. In both

510   low and high temperature experiments, growth rates always decreased under

511   temperature variation, compared with the constant mean temperature. This result

512   agrees with previous studies showing that temperature variation slowed the growth

513   rates of the fresh water green alga *Chlorella pyrenoidosa* and the marine diatom

514   *Cyclotella meneghiniana*, as observed in laboratory work but also during long-term

515   field observations (Zhang et al., 2016).

516   This growth rate inhibition under temperature variation was more pronounced at

517   high temperature than at low temperature, indicating that variability at the warm

518   range boundary will have a stronger negative effect on population growth rate than

519   variability near the lower thermal limits (Bernhardt et al., 2018). This trend suggests

520   that acclimation to high temperature (whether constant or variable) may require

521   greater investment in cellular repair machinery, such as heat shock proteins, thus

522   potentially diverting nutrient and energy supplies and thereby reducing growth rates

523   (O'Donnell et al., 2018). However, following Jensen's inequality model to predict

524   the thermal performance curve, there should be an inflection point where the transfer

525   between positive and negative effects of temperature variability will occur compared

526   with the constant thermal curve. Conversely, for phytoplankton living in regions of

527   suboptimal temperatures, thermal variation can enhance growth (Bernhardt et al.,

528   2018). Thus, for some polar phytoplankton or for temperate species extending their

529   ranges poleward, such as *E. huxleyi* (Neukermans et al., 2018), not only warming but

530   also thermal variability may need to be taken into consideration in order to

531   understand changes in high latitude microbial communities and biogeochemistry

532   cycles.

533   Temperature variation affected the physiology of *E. huxleyi* differently

534   compared with constant temperature. Physiological traits that were affected by

535   thermal fluctuations also differed at low temperature ("winter") and high temperature

536   ("summer"), suggesting different response mechanisms. Under low temperature

537   variations (16-21 $^{\circ}$C), photosynthesis and calcification were correlated with

538   temperature, leading to rates similar to those observed with constant temperature.

539   However, elemental contents and ratios under thermal variations differed from

540   constant temperature. For instance, the cellular POC, PON, POP and Chl *a* contents

541   increased during one-day variations but decreased during two-day variations,

542   compared with constant temperature.

543   These cellular quota changes were reflected in elemental ratio differences

544   (PIC/POC, Chl a/POC and TPC/POC) between the thermal variation treatments and

545   constant temperature. However, the changes between thermal variation and constant

treatments were not significant under low temperature ("winter"), indicating that the thermal variation wouldn't significantly influence biogeochemical cycles under these conditions. Unlike constant temperature treatments where selection may favor a higher growth rate, the trade-off for the thermal variation treatments may involve sacrificing increased growth rate in order to adjust cellular stoichiometry to adapt to the fluctuating environment.

In contrast, photosynthetic and calcification rates under high temperature thermal variations (23-28 °C) were significantly different from those seen under constant temperature (25 °C), especially the calcification rate. Thermal variation treatments transiently but repeatedly experienced the extreme high temperature point (28 °C), leading to extremely low calcification rates and PIC contents, and thus relatively low PIC/POC and Cal/Photo ratios. Previous *E. huxleyi* studies agree that high temperature decreases PIC content, PIC/POC ratios and Cal/Photo ratios (Feng et al., 2017; 2018; Gerecht et al., 2014). The two different patterns of responses to thermal variation we observed under low and high temperatures imply a seasonal pattern in the ways that thermal variations will affect the elemental stoichiometry of *E. huxleyi* .

Under other stresses such as nutrient limitation, trade-offs between growth rates and resource affinities may be necessary to adapt to thermal changes. For instance, nitrate affinity declines in cultures of the large centric diatom *Coscinodiscus* acclimated to warmer temperatures (Qu et al. 2018), while warming decreases cellular requirements for iron in the nitrogen-fixing cyanobacterium *Trichodesmium*

568   (Jiang et al. 2018). In nitrogen-limited cultures of the marine diatom *Thalassiosira*

569   *pseudonana*, long-term thermal adaptation acted most strongly on systems other than

570   those involved in nitrate uptake and utilization (O'Donnell et al., 2018). Thus, it is

571   possible that our thermal response results with *E. huxleyi* might have differed under

572   nutrient-limited growth conditions.

573   ***One-day vs two-day thermal variation***

574   As temperature fluctuations in the surface ocean increase along with climate

575   change, phytoplankton will be influenced by the frequencies and intensities of these

576   thermal excursions. We found that the responses of *E. huxleyi* to one-day versus

577   two-day temperature variations were different at both low and high temperature. For

578   instance, under low temperature the transition from the warm phase to the cool phase

579   during the thermal variation could be treated as a low temperature stress leading to a

580   lag phase in growth. The growth rate of the one-day variation treatment at the cool

581   phase was lower than that of the two-day variation, suggesting that physiological

582   acclimation is not rapid enough to accommodate to the shorter variation treatment,

583   while the two day variation allows enough time for growth to recover. However, at

584   the warm phase (21 °C) there was no difference in growth rates between the one-day

585   and two-day variations compared with the constant 21-degree treatment. These

586   results imply that there was a shorter lag phase after transfer at the optimal

587   temperature point (21 °C at the warm phase) than during low temperature stress (16

588   °C at the cool phase).

589   There was no significant difference in photosynthetic rates between the one-day

590   and two-day variation during the warm phase (21 $^{\circ}$C), but both were higher than

591   during the cool phase, indicating the photosynthetic rate was correlated to the

592   thermal variation cycle. However, for the calcification rate there was no significant

593   difference between one-day and two-day variations during either the cool or warm

594   phases. These results suggested that photosynthesis was more responsive to

595   temperature variations than calcification, and so ultimately determined the growth

596   rate in both cool and warm phases. Feng et al. (2017) reported a similar relationship

597   between growth and photosynthetic rates of a Southern Hemisphere   cultured at

598   different temperatures.

599   Temperature variation frequencies also strongly influenced elemental

600   composition. In low temperature experiments, the cellular contents of PON, POP and

601   POC in the one-day variation treatment were all higher than under two-day

602   variations. A notable exception to this trend was the cellular PIC content, which was

603   not significantly different between one-day and two-day variation treatments. The

604   PIC content was positively correlated to calcification and relatively stable, indicating

605   that coccolith production and storage of *E. huxleyi* was relatively independent of the

606   frequency of thermal variation.

607   Unlike the photosynthetic rate, the cellular elemental content of one-day and

608   two-day variations were significantly different, but were not changed during

609   temperature variation when transitioning from the warm phase to the cool phase or

610   vice versa.   The temperature dependent photosynthetic enzyme activity likely

611   determined the similar photosynthetic rate of one-day and two-day variation

treatments at both cool and warm phase in our short-term experiment, but the divergent responses of cellular stoichiometry in one-day and two-day thermal variations indicated different mechanisms of rapid acclimation to different thermal fluctuation frequencies. Our results imply that the responses of *E. huxleyi* to one-day and two-day thermal variations have different patterns, but both reach stable states during extended periods of temperature fluctuation. Due to decreasing POC content, the PIC/POC ratio increased in the two-day variation compared with the one-day variation, suggesting that more rapid thermal fluctuations might lead to a decrease in calcite ballasting of sinking organic carbon.

Under the high temperature scenario, thermal variation forces the microalgae to intermittently deal with a lethal high temperature during the warm phase (28 $^{\circ}$C), with potentially irreversible damage to the cells. In the "summer" experiments, the mean growth rate of the two-day variation was much lower than that of the one-day variation. This mainly resulted from the negative growth rate of two-day variation cultures during the warm phase (28 $^{\circ}$C), whereas the growth rate of the one-day variation was >0.20 d^{-1}. This result demonstrates that high frequency temperature variations (one-day) can partly mitigate growth inhibition by high temperatures in *E. huxleyi,* and so allow tolerance to extreme thermal events relative to longer exposures. This observation agrees with previous studies of other marine organisms such as corals (Oliver & Palumbi, 2011; Safaie et al., 2018). In the case of our experiments, the lag phase and metabolic inertia would help to maintain the microalgae during short exposures (one-day) to high temperature when transitioning

634　　from the cool phase (23 $^{\circ}$C) to the warm phase (28 $^{\circ}$C).

635　　　Likewise, the particulate organic element contents (PON, POP and POC) of *E.*

636　*huxleyi* were more stable in one-day than in two-day temperature variation treatments.

637　The relatively steady status of cellular particulate organic matter content in the high

638　frequency temperature variation treatment may conserve energy, compared to the

639　energy-intensive redistribution of major cellular components under lower frequency

640　temperature variations. This differential energetic cost may help to explain the

641　differences in growth rates between the two treatments.　Adaptation to high

642　temperature may also require higher investment in repair machinery, such as heat

643　shock proteins, leading to an increased demand for nitrogen and other nutrients, thus

644　increasing cellular POC, PON and POP contents (O'Donnell et al., 2018).

645　**Prediction and modelling of *E. huxleyi* responses to thermal variation**

646　　　Mathematical curves based on population growth rates from laboratory studies

647　have been used to predict future population abundance, persistence or fitness in a

648　changing world (Bernhardt et al., 2018; Deutsch et al., 2008; Jiang et al. 2017). We

649　applied a modified version of the Eppley thermal performance curve model with the

650　addition of non-linear averaging (Bernhardt et al., 2018) to predict the influence of

651　thermal variation on the growth rate of *E. huxleyi* (Fig. 8). *E. huxleyi* growth rates

652　were predicted to be much lower at warmer temperatures under variable conditions

653　compared to constant conditions, but there were no significant differences at cooler

654　temperatures. Thus, the effect of thermal variation on population growth at the upper

655　thermal limit was predicted to be stronger than that in the lower portion of the thermal

range (Bernhardt et al., 2018; Sunday et al., 2012). This phenomenon has been widely observed in ectothermic animal taxa (Dell et al., 2011), but this model for the effect of thermal variation on population growth rate may lack the ability to predict species responses at the extreme edges of their ranges (Bernhardt et al., 2018).

Our results showed that the measured effects of a variable thermal regime on *E. huxleyi* growth rate fitted well with model-predicted values at a relatively low temperature (mean=18.5 ℃), but differed considerably at high temperature (mean=25.5 ℃). This was especially evident under the two-day variation conditions at a mean of 25.5 ℃, where the growth rate was sharply lower than predicted from the constant temperature performance curve-based model. This result suggests that transient heat waves may erode thermal tolerances of *E. huxleyi* populations already growing near their upper thermal limits, and that the frequency and duration of such extreme events is critically important in determining the magnitude of this stress. Qu et al. (2019) reported that the tropical cyanobacterium *Trichodesmium erythraeum* only showed a slight decrease of growth rate with thermal variation treatments at high temperature (average 30℃), compared with constant 30℃ treatments. In contrast, the sensitivity of *E. huxleyi* to increasing thermal variability may reduce its fitness and its ability to compete with other taxa such as diatoms and cyanobacteria in the future ocean.

Although thermal variation at high temperature negatively impacted the growth rate of *E. huxleyi* in our experiment, our relatively short-term study didn't address the potential for *E. huxleyi* to evolve under selection by frequent extreme heat events.

678   Evolutionary change in the thermal optimum and the maximum growth temperature in

679   response to ocean warming may reduce heat-induced mortality, and mitigate some

680   ecological impacts of global warming (O'Donnell et al., 2018, Thomas et al., 2012).

681   For example, Schlüter et al. (2014) found that after one year of experimental

682   adaptation to warming (26.3°C), the marine coccolithophore *E. huxleyi* evolved a

683   higher growth rate when assayed at the upper thermal tolerance limit. Similar results

684   were reported for the marine diatom *Thalassiosira pseudonana* in recent studies

685   (O'Donnell et al., 2018; Schaum et al., 2018). Schaum et al. (2018) also found that the

686   evolution of thermal tolerance in marine diatoms can be particularly rapid in

687   fluctuating environments. Furthermore, populations originating from more variable

688   environments are generally more plastic (Schaum & Collins, 2014; Schaum et al.,

689   2013). Long-term evolutionary experiments with *E. huxleyi* will be necessary to

690   determine how the thermal performance curve of this important marine calcifier may

691   diverge under selection by different frequencies and durations of extreme thermal

692   variation events.

693   Understanding the combination of ocean warming and magnified thermal

694   variability may be a prerequisite to accurately predicting the effects of climate change

695   on the growth and physiology of the key marine calcifier *E. huxleyi.* This information

696   will help to inform biogeochemical models of the marine and global carbon cycles,

697   and ecological models of phytoplankton distributions and primary productivity. How

698   changing thermal variation frequencies and heat wave events will affect marine

699   phytoplankton remains a relatively under-explored topic, but one that is likely to

700   become increasingly important in the future changing ocean.

701

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Figure legends:

Fig. 1 Thermal performance curve showing cell-specific growth rates (d^{-1}) of *Emiliania huxleyi* CCMP371 across a temperature range from 8.5 to 28.6 °C. Symbols represent means and error bars are the standard deviations of three replicates at each temperature, but in many cases the errors bars are smaller than the symbols.

Fig. 2 Changes in *Emiliania huxleyi* TPC/PON ratios (**A**), POC/PON ratios (**B**), PIC/POC ratios (**C**) and Cha/POC ratios (**D**) across a temperature range from 8.5 to 28.6 °C. Dashed lines represent the average ratios for the entire temperature range. Bars represent means and error bars are the standard deviations of three replicates at each temperature. Symbols * represent the significant difference ($p<0.05$) between average ratios and the ratio at each temperature.

Fig. 3 *Emiliania huxleyi* growth rate responses to constant temperatures, and during the warm and cool phases of the two thermal variation frequencies (one-day and two-day), under low (**A**) and high (**B**) mean temperatures. The thick black line in the boxplots represent median values for each experimental treatment; whiskers on boxplots indicate $1.5 \times$ interquartile range. Listed p-values with their respective brackets are the statistical significance between two treatments.

Fig. 4 Responses of *Emiliania huxleyi* PIC/POC ratios to constant temperatures, and during the warm and cool phases of two thermal variation frequencies (one-day and two-day), under low (**A**) and high (**B**) mean temperatures. LT: Low temperature; HT: High temperature. The thick black line in the boxplots represent median values for each experimental treatment; whiskers on boxplots indicate $1.5 \times$ interquartile range.

Listed p-values with their respective brackets denote the statistical significance

between two treatments.

Fig. 5 Responses of *Emiliania huxleyi* photosynthetic carbon fixation and

calcification at constant temperatures and during the warm and cool phases of two

thermal variation frequencies (one-day and two-day), including: total carbon fixation

(photosynthesis + calcification) at low (**A**) and high (**B**) temperatures; photosynthetic

carbon fixation at low (**C**) and high (**D**) temperatures; and calcification rates at low (**E**)

and high (**F**) temperatures.   LT: Low temperature; HT: High temperature. The thick

black line in the boxplots represent median values for each experimental treatment;

whiskers on boxplots indicate 1.5 × interquartile range. Listed p-values with their

respective brackets denote the statistical significance between two treatments.

Fig. 6 Responses of *Emiliania huxleyi* calcification to photosynthesis ratios (cal/photo)

to constant temperatures, and during the warm and cool phases of two thermal

variation frequencies (1 day and 2 day), under low (**A**) and high (**B**) mean

temperatures. LT: Low temperature; HT: High temperature. The thick black line in the

boxplots represent median values for each experimental treatment; whiskers on

boxplots indicate 1.5 × interquartile range. Listed p-values with their respective

brackets denote the statistical significance between two treatments.

Fig. 7 Responses of *Emiliania huxleyi* elemental ratios in two thermal variation

frequency treatments (1 day and 2 day) compared to constant temperatures, for:

TPC/PON (**A**, cool phase and **B**, warm phase), PON/POP (**C**, cool phase and **D**, warm

phase) and Chl *a*/POC ratios (**E**, cool phase and **F**, warm phase). LT: Low temperature;

1016 HT: High temperature. The thick black line in the boxplots represent median values

1017 for each experimental treatment; whiskers on boxplots indicate $1.5 \times$ interquartile

1018 range. Listed p-values with their respective brackets denote the statistical significance

1019 between two treatments.

1020 **Fig. 8** Thermal performance curves based on specific growth rates (d^{-1}) of *Emiliania*

1021 *huxleyi*, including our experimentally determined constant condition temperature

1022 performance curve (black symbols and solid line) and a predicted fluctuating

1023 condition temperature performance curve (dashed line) according to the model of

1024 Bernhardt et al. (2018). Measured growth rates from the two low and high

1025 temperature experiments are shown for constant thermal conditions (red symbols),

1026 one-day (green symbols) and two-day (blue symbols) variation treatments.

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1028 **Table 1** The effect of temperature variation under low and high temperature on total carbon (pg/cell), cellular POC (pg/cell), cellular PIC (pg/cell), cellular PON

1029 (pg/cell), cellular POP (pg/cell) and cellular Chl *a* (pg/cell) of *Emiliania huxleyi.*

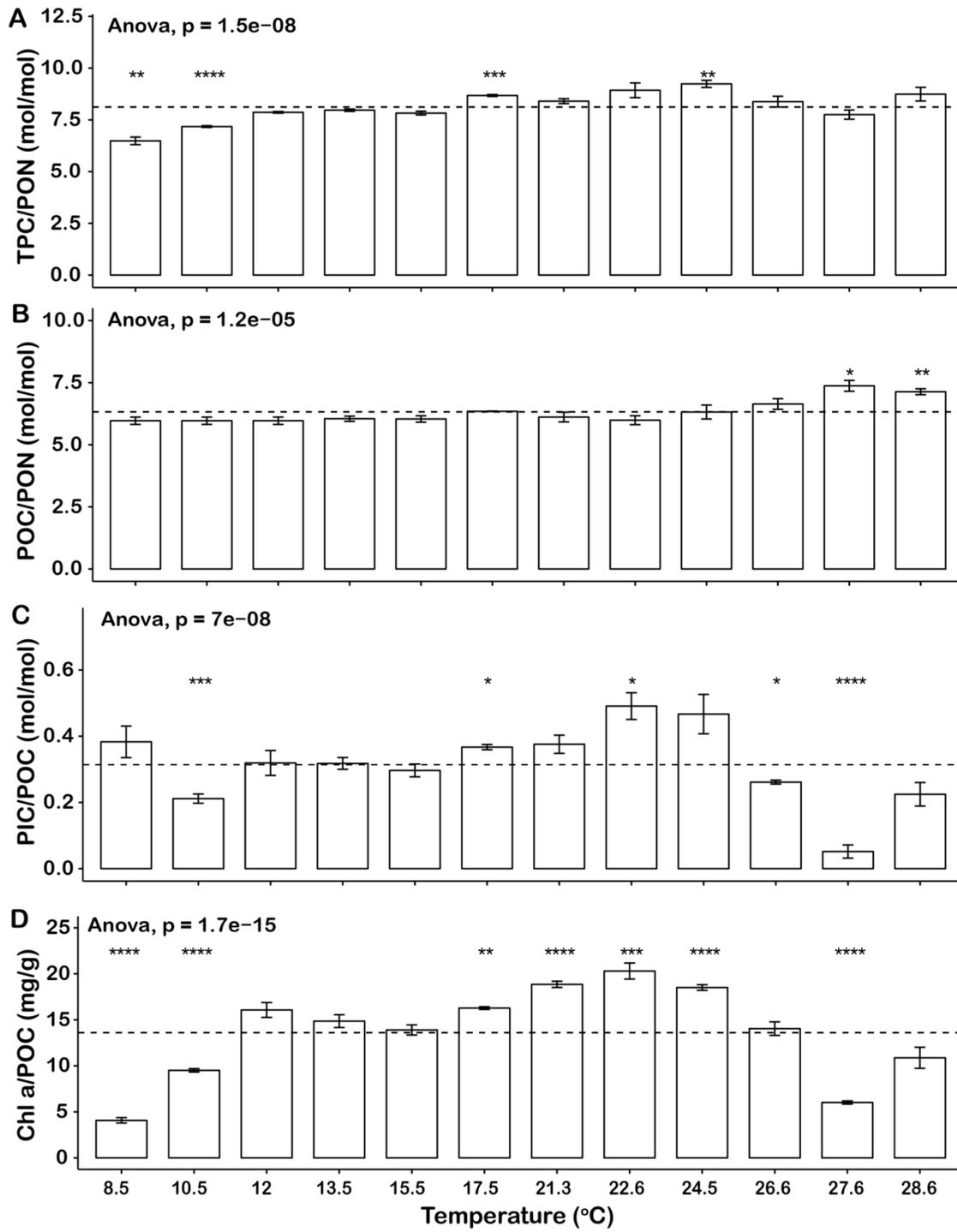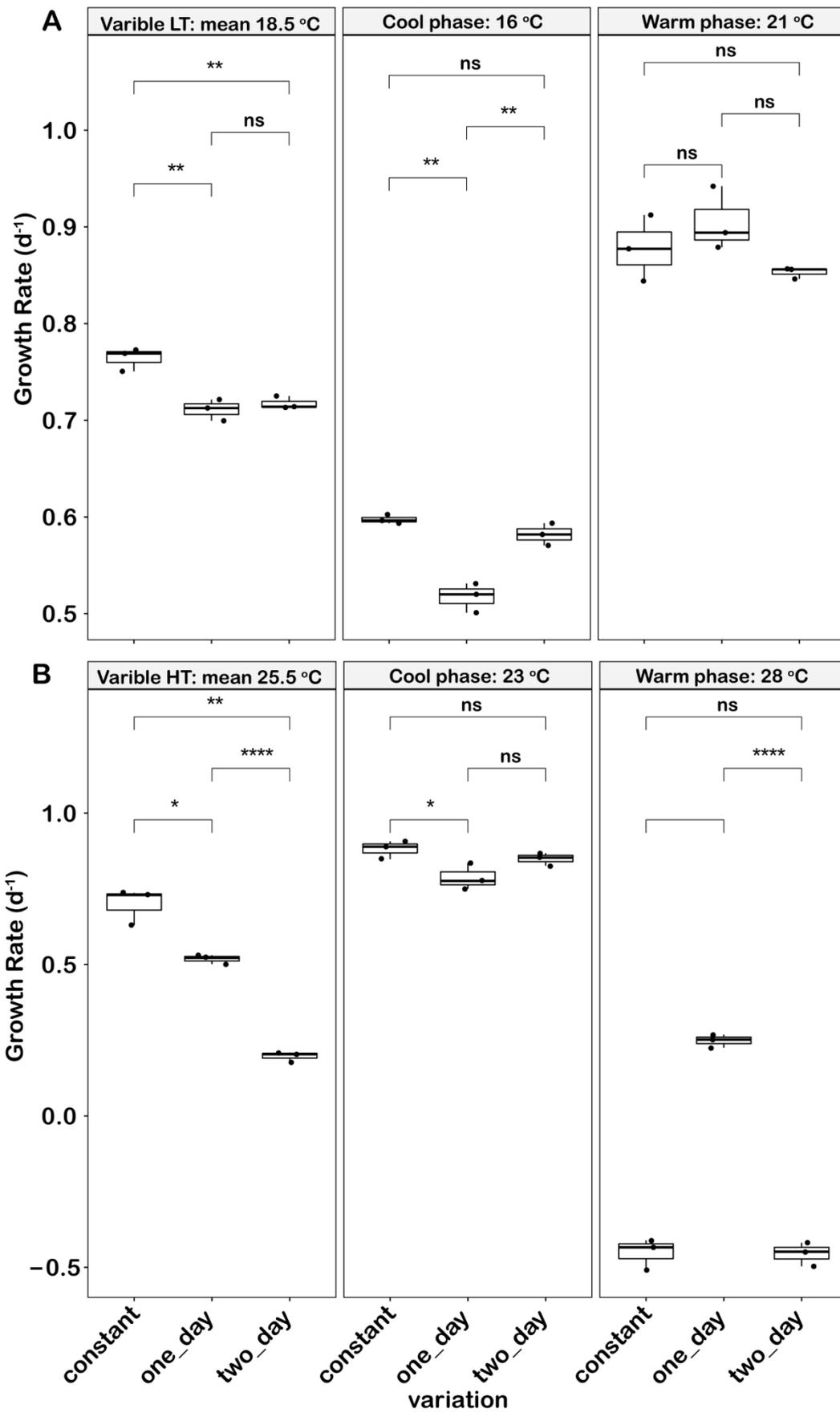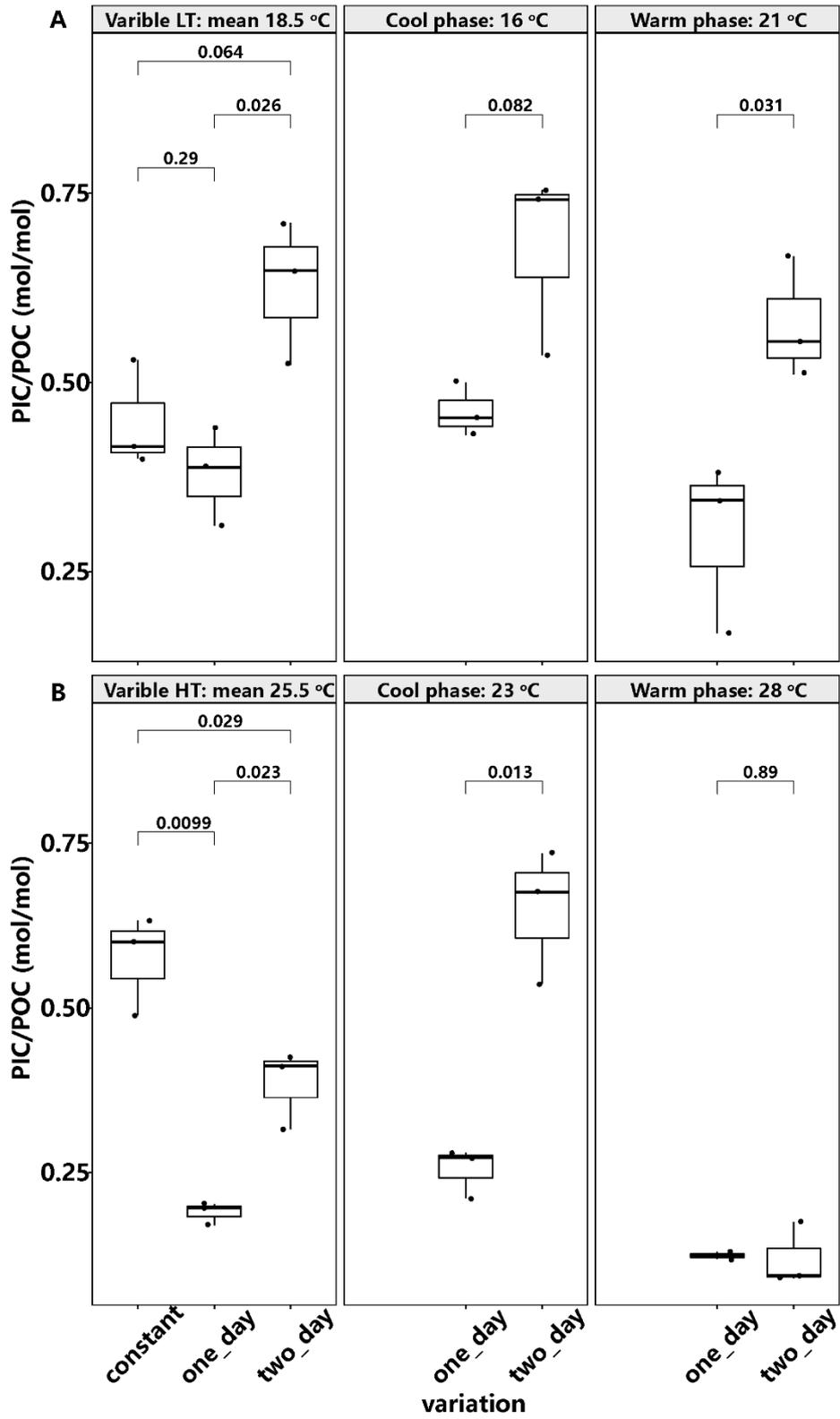| Treatment		Total Carbon	Cellular PON	Cellular POP	Cellular POC	Cellular PIC	Cellar Chl *a*
Low temperature	18.5 °C	11.5±0.4	1.8±0.2	0.17±0.00	8.0±0.6	3.5±0.3	0.14±0.00
	One-day cool point (16)	13.0±0.5	2.2±0.3	0.18±0.00	8.9±0.3	4.1±0.3	0.15±0.01
	One-day warm point (21)	12.0±0.7	2.1±0.3	0.19±0.00	9.3±0.9	2.7±0.9	0.19±0.00
	Two-day cool point (16)	10.1±0.7	1.3±0.2	0.16±0.01	6.0±0.9	4.0±0.3	0.12±0.01
	Two-day warm point (21)	10.4±0.5	1.5±0.2	0.17±0.01	6.6±0.5	3.8±0.3	0.15±0.01
High temperature	25.5 °C	15.0±0.7	2.0±0.1	0.21±0.01	9.5±0.3	5.5±0.7	0.18±0.02
	One-day cool point (23)	16.1±1.4	3.0±0.2	0.21±0.00	12.9±1.5	3.2±0.2	0.15±0.01
	One-day warm point (28)	19.1±0.8	4.4±0.3	0.24±0.01	17.0±0.6	2.1±0.2	0.20±0.02
	Two-day cool point (23)	12.4±1.0	1.9±0.2	0.19±0.01	7.5±1.0	4.8±0.3	0.13±0.01
	Two-day warm point (28)	19.4±2.0	3.9±0.8	0.25±0.03	18.3±3.7	2.1±0.9	0.25±0.02

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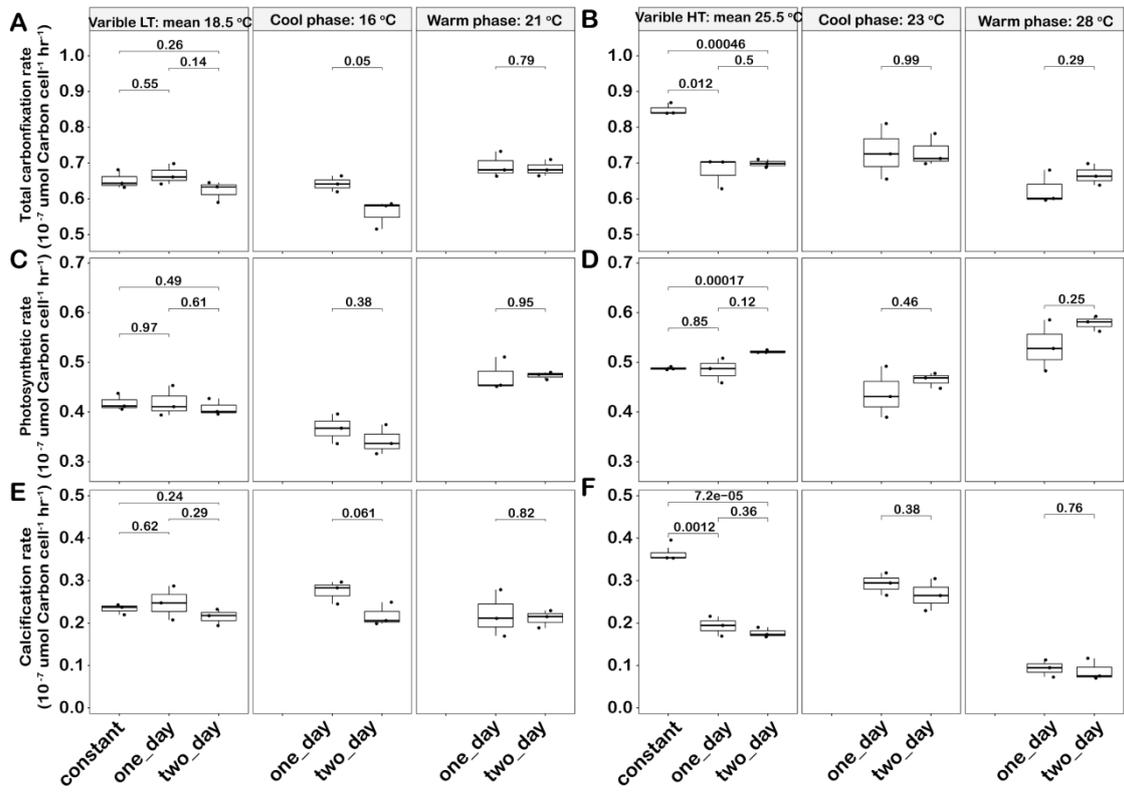
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1032   **Fig. 1**

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