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1 Rising levels of temperature and CO₂ antagonistically affect phytoplankton primary
2 productivity in the South China Sea

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17 Running head: Temperature and CO₂ on primary productivity

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23 ABSTRACT

24 Coastal and offshore waters in the South China Sea are warming and becoming
25 acidified due to rising atmospheric levels of carbon dioxide (CO₂), yet the combined
26 effects of these two stressors are poorly known. Here, we carried out shipboard
27 incubations at ambient (398 μatm) and elevated (934 μatm) pCO₂ at in situ and in
28 situ+1.8 °C temperatures and we measured primary productivity at two coastal and
29 two offshore stations. Both warming and increased CO₂ levels individually increased
30 phytoplankton productivity at all stations, but the combination of high temperature
31 and high CO₂ did not, reflecting an antagonistic effect. Warming decreased Chl *a*
32 concentrations in off-shore waters at ambient CO₂, but had no effect in the coastal
33 waters. The high CO₂ treatment increased night time respiration in the coastal
34 waters at ambient temperatures. Our findings show that phytoplankton assemblage
35 responses to rising temperature and CO₂ levels differ between coastal and offshore
36 waters. While it is difficult to predict how ongoing warming and acidification will
37 influence primary productivity in the South China Sea, our data imply that predicted
38 increases in temperature and pCO₂ will not boost surface phytoplankton primary
39 productivity.

40

41 *Keywords:* Chl *a*; night time respiration; ocean acidification; ocean warming;
42 primary productivity; South China Sea

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44

45 **1. Introduction**

46

47 Rising atmospheric carbon dioxide (CO₂) concentrations are warming and
48 acidifying the oceans worldwide (Caldeira and Wickett, 2003; IPCC, 2014), including
49 the South China Sea (Ji et al., 2017). On average, surface seawater temperatures are
50 projected to increase by 1.51–3.22 °C by the end of this century and CO₂ levels to
51 increase from the current level of about 400 µatm up to 1000 µatm (Boyd et al., 2015).
52 Ocean warming and acidification are expected to affect the physiology, distribution
53 and structure of phytoplankton communities (Hare et al., 2007; Feng et al., 2009;
54 Taucher et al., 2012; Sommer et al., 2015; Riebesell et al., 2017).

55 Rising CO₂ levels can increase the availability of dissolved inorganic carbon (DIC)
56 for phytoplankton carbon fixation, but they are also causing seawater acidification,
57 and this may inhibit algal calcification and photosynthetic carbon fixation (Falkowski
58 and Raven, 2007; Gao and Zheng, 2010; Gao et al., 2012; Brodie et al., 2014). Thus,
59 algal responses to increasing CO₂ levels are dependent on the balance between the
60 positive effects of increasing DIC and the negative effects of decreasing pH (Wu et al.,
61 2008; Bach et al., 2015; Liu et al., 2017). Several studies report that, in comparison to
62 current CO₂ levels, elevated CO₂ (800–1000 µatm) increases productivity of
63 phytoplankton assemblages that are dominated by diatoms (Kim et al., 2006; Tortell et
64 al, 2008; Domingues et al. 2014; Engel et al., 2014; Johnson et al. 2015). Others have
65 found that rising CO₂ levels can decrease the productivity of phytoplankton
66 communities dominated by the coccolithophore *Emiliana huxleyi* (Delille et al., 2005;

67 Riebesell et al., 2017). Paradoxically, an increase in CO₂ concentrations from 385 to
68 800 µatm decreased the productivity of surface phytoplankton assemblages dominated
69 by diatoms in the South China Sea under natural fluctuating solar radiation (Gao et al.,
70 2012). These discrepancies highlight the fact that the effects of rising CO₂ on
71 C-fixation are dependent on algal community composition as well as regional
72 environmental conditions (Egge et al., 2009; Gao et al., 2012; Celis-Pla et al. 2015;
73 Holding et al., 2015; Hoppe et al., 2018).

74 On a global scale, by using satellite records and in situ monitoring, rising
75 temperatures have been shown to reduce phytoplankton productivity in the open
76 ocean (Boyce et al., 2010; Siegel et al., 2013), because increased thermal stratification
77 of the water column can starve the algae of nutrients (Doney et al., 2006; Kletou and
78 Hall-Spencer, 2012). In general, it seems that photosynthetic C-fixation increases with
79 increasing temperature, reaches a maximum and decreases thereafter (Beardall and
80 Raven, 2004). Optimal temperatures for C-fixation differ between latitudes and
81 seasons, with small phytoplankton species functioning optimally at higher
82 temperatures than larger species (Daufresne et al., 2009; Finkel et al., 2010; Sommer
83 et al., 2015; Wolf et al., 2017). Carbon fixation was reduced when temperatures were
84 experimentally increased in cold adapted phytoplankton assemblages (Wohlers et al.,
85 2009; Wolf et al., 2017). However, increases from 27 °C to 30 °C enhanced
86 photosynthetic C-fixation in incubations of samples of surface phytoplankton
87 assemblages from two stations off China (Gao et al., 2017). Regional differences in
88 physicochemical conditions may drive different responses of phytoplankton to ocean

89 climate change.

90 Temperature affects cellular membrane permeability, cell size of a single
91 phytoplankton cell and the uptake of dissolved inorganic carbon (Beardall and Raven,
92 2004) and so has fundamental control over the effects of changing carbonate
93 chemistry on photosynthetic C-fixation. For example, when CO₂ concentrations were
94 increased from 390 to 690 μatm, C-fixation of a phytoplankton community at 12 °C
95 (in situ temperature) decreased in the North Atlantic spring bloom area, whereas at 16
96 °C rising CO₂ levels enhanced C-fixation (Feng et al., 2009). Increasing CO₂ levels
97 (from 150 to 300 μatm) combined with rising temperature (from -1 °C to 7 °C)
98 synergistically enhanced phytoplankton productivity in the European Arctic Ocean,
99 and the positive effect of rising CO₂ on productivity was lower at 6 °C than at 1 °C
100 (Holding et al., 2015). Furthermore, elevated temperature reversed the positive effect
101 of rising CO₂ on phytoplankton assemblages off Svalbard and did not affect the
102 response of phytoplankton primary productivity in coastal Arctic and subarctic
103 seawater to rising CO₂ (Coello-Camba et al., 2014; Hoppe et al., 2018). These results
104 show that rising temperature and increasing CO₂ can have synergistic or antagonistic
105 effects on the productivity of marine phytoplankton assemblages. Given that the
106 carbon cycle underpins the ecology and fisheries productivity of marine ecosystems,
107 region-specific research is urgently needed to assess whether rising atmospheric CO₂
108 levels will positively or negatively affect photosynthetic production.

109 In this work, we performed shipboard incubations at two coastal and two off-shore
110 stations in the western South China Sea in autumn 2017 and measured photosynthetic

111 C-fixation rates and Chlorophyll *a* (Chl *a*) concentrations. Our aim was to assess how
112 rising levels of pCO₂ and temperature are likely to affect coastal and offshore
113 productivity in the South China Sea.

114

115 **2. Materials and methods**

116

117 2.1. Sampling and culture condition

118 This study was carried out aboard RV ‘Shiyan III’ in off-shore and coastal waters of
119 the South China Sea from 11th September to 12th October, 2017 (Fig. 1). Surface
120 seawater (0–2 m) was collected with a 8 L acid-cleaned plastic bucket and stored in a
121 30 L acid-cleaned polycarbonate tank at 9:00 a.m. to 10:00 a.m., at station S1 (12.99°
122 N, 113.50° E) on September 21, station S2 (14.01° N, 113.01° E) on September 22,
123 station S3 (17.75° N, 110.65° E) on October 2, and station S4 (18.30° N, 111.29° E)
124 on October 3, respectively. Surface seawater at each station was filtered through a 200
125 µm mesh, and then dispensed into twelve 2 L Nalgene bottles. 1 µmol L⁻¹ NaNO₃ and
126 0.5 µmol L⁻¹ NaH₂PO₄ was added into the seawater in all treatments to stimulate
127 phytoplankton growth (Chen et al., 2004; Tseng et al., 2005; Celis-Plá et al., 2015).

128 Six bottles for ambient temperature treatment were put into one deck incubator
129 (120 cm × 85 cm × 25 cm) bathed with flowing surface seawater. Six bottles for the
130 elevated temperature treatment were put into another deck incubator with an
131 auto-temperature control system (Fig. S1) which fitted with two circulating coolers
132 (AL36G-160, Shenzhen Aolinghengye Ltd., China) during the day, and heated at

133 night (Aqua Zonic, Shanghai AiKe Ltd., China). Temperatures in both incubators
134 were measured hourly (Fig. 2A). Bottles were held in place using wire mesh with a
135 pore size of 11.5 cm (Fig. S1). Three bottles of seawater in each incubator were
136 bubbled with filtered (PVDF 0.22 μm pore size, simplepure, Haining) ambient air
137 ($\sim 400 \mu\text{atm}$) or air of elevated CO_2 ($\sim 1,000 \mu\text{atm}$) during the incubation periods,
138 respectively. The high CO_2 concentration was controlled using a CO_2 enricher
139 (CE100B, Wuhan Ruihua Instrument & Equipment Ltd., China). An Eldonet
140 broadband filter radiometer (ELDONET, Real Time Computer, Germany) was used to
141 measure the incident solar radiation (Fig. 2B), and solar light intensities and weather
142 condition were similar during the incubation periods. The positions of the bottles were
143 changed three times per day to ensure they were exposed equally to sunlight. Our four
144 treatments were: low temperature and low CO_2 (LTLC), low temperature and high
145 CO_2 (LTHC), high temperature and low CO_2 (HTLC), high temperature and high CO_2
146 (HTHC). Each treatment had three replicates and the incubations were run for 6 days.

147

148 2.2. pH_{nbs} , total alkalinity and nutrient concentrations measurements

149 pH_{nbs} (NBS scale) was measured before incubation, 24 hrs after incubation and at
150 the end of the 6 days experiment. At about 10:00 a.m., 20 mL samples for pH_{nbs}
151 measurements were taken from the bottles and measured immediately at 25 $^\circ\text{C}$ with a
152 pH meter (Benchtop pH, Orion 8102BN) calibrated with an equimolar pH buffer (Tris
153 $\cdot\text{HCl}$, Hanna) which is isosmotic with seawater (Dickson, 1993). Total alkalinity (TA)
154 was measured before incubation and at the end of the incubation. At 10:00 a.m. to

155 10:30 a.m., 100 mL samples for TA measurements were filtered (GF/F filter) by
156 gentle pressure with 200 mbar in the pump (GM-0.5A, JINTENG). 100 μ L saturated
157 HgCl₂ solution was added into the TA samples which were stored at 4 °C. TA was
158 measured at 25 °C in the laboratory by potentiometric titration (AS-ALK1+, Apollo
159 SciTech) according to Dickson et al. (2003). Carbonate chemistry parameters were
160 calculated from TA, pH_{nbs}, phosphate, silicate, temperature, and salinity using the
161 CO2SYS (Pierrot et al., 2006).

162 At the beginning of the incubation, dissolved inorganic nitrogen (DIN) and
163 phosphate (DIP) concentrations of seawater in situ were obtained from the dataset of
164 this cruise . At the end of the incubation, at 10:30 a. m. to 11:00 a. m., 50 mL samples
165 for determination of DIN and DIP concentrations were syringe-filtered (0.22 μ m pore
166 size, Haining), stored at -20 °C, measured using a scanning spectrophotometer (Du
167 800, Beckman Coulter) in the laboratory after the nitrate had been reduced to nitrite
168 according to Hansen and Koroleff (1999).

169

170 2.3. Chlorophyll *a* analysis

171 At each station, at about 14:00 p.m., 2 L surface seawater were filtered onto a GF/F
172 glass filter (25 mm, Whatman) for in situ chlorophyll *a* (Chl *a*) measurement. At the
173 end of incubation, at 11:00 a.m to 12:00 a.m., 700 mL samples were filtered onto
174 GF/F glass filters, and all filters were stored at -20 °C until they were analyzed in the
175 laboratory. The filters were placed in 5 mL 100% methanol and stored at 4 °C for 12
176 hours. Then the solutions were centrifuged at 5000 g for 10 min and the absorbances

177 of the supernatant were determined using a scanning spectrophotometer (Du 800,
178 Beckman Coulter). Chl *a* concentrations were determined as follows: $\text{Chl } a = 13.27 \times$
179 $(A_{665} - A_{750}) - 2.68 \times (A_{632} - A_{750})$ ($\mu\text{g mL}^{-1}$) (Ritchie, 2002). A_{632} , A_{665} , and A_{750}
180 represent absorbances of the supernatant at 632 nm, 665 nm and 750 nm.

181

182 2.4. Primary productivity measurements

183 Primary productivity was obtained according to the method described by Gao et al.
184 (2017). On the final day of the incubations, at about 5:00 a.m., subsamples were taken
185 from each incubation bottle, dispensed into two 50 mL quartz tubes placed under a
186 plastic plate which allowed 85% PAR and non UVR transmissions, assuring that the
187 light environment was similar to that of incubations. 5 μCi (0.185 MBq) $\text{NaH}^{14}\text{CO}_3$
188 (ICN Radiochemical, USA) was added to the subsamples, which were cultured in the
189 corresponding deck incubators for 12 hrs (from 6:00 a.m. to 6:00 p.m.) and 24 hrs
190 (from 6:00 a.m. to 6:00 a.m. next day) under solar radiation. Subsamples were then
191 filtered onto GF/F glass filters, which were darkly stored at -20°C until they were
192 analyzed in the laboratory. Each filter was put into a 10 mL scintillation vial, fumed
193 with HCl for 24 hours to remove inorganic carbon, and dried at 60°C for 12 hrs. 3 mL
194 scintillation cocktail (Hisafe 3, Perkin Elmer, Shelton, USA) was added to the vial
195 and the activity of the fixed radiocarbon was measured using a liquid scintillation
196 counting (LS 6500, Beckman Coulter, USA). The activity of photosynthetic
197 C-fixation during 12 hrs incubation was defined to be the day-time primary
198 productivity (DPP), and the photosynthetic C-fixation during 24 hours was considered

199 to be the net primary productivity (NPP) (Delille et al., 2005). The difference between
200 DPP and NPP was taken as night time respiratory C loss.

201

202 2.5. Data analysis

203 Effects of temperature, CO₂ and their interactions on Chl *a*, DPP, NPP and night
204 time respiration rates were assessed by a two-way analysis of variance (ANOVA). The
205 normal distribution of all data was assessed by a Shapiro-Wilk's test, and
206 homogeneity of variance was determined by a Levene's test. A Tukey Post hoc test
207 (Tukey HSD) was performed to show difference between temperature or CO₂
208 treatments. Statistical analysis was tested by using R and significant difference was
209 indicated by $p < 0.05$.

210

211 **3 Results**

212

213 3.1. Incubation temperature, nutrient concentrations and carbonate chemistry 214 parameters

215 Incubation temperatures varied from 29.1 °C to 31.2 °C in our low temperature
216 treatment (to match the surface seawater temperature at the time of sampling); and
217 varied from 30.6 °C to 34.0 °C in our high temperature treatments (Fig. 2A). Average
218 temperatures were 29.7 ± 0.29 °C for the low temperature treatments and $31.5 \pm$
219 0.41 °C for the high temperature treatments, respectively.

220 Dissolved inorganic nitrogen (DIN) and phosphate (DIP) concentrations in situ

221 surface water of the South China Sea were 0.03–0.12 $\mu\text{mol L}^{-1}$ and 0.14–0.21 μmol
222 L^{-1} , respectively (Table 1). By adding NaNO_3 and NaH_2PO_4 to the seawater, DIN and
223 DIP concentrations at the beginning of the incubation were 1.03–1.12 $\mu\text{mol L}^{-1}$ and
224 0.64–0.71 $\mu\text{mol L}^{-1}$, respectively. DIN concentrations at all treatments decreased
225 below the detection limit ($< 0.04 \mu\text{mol L}^{-1}$) and DIP concentrations were about 0.05
226 $\mu\text{mol L}^{-1}$ at the end of the experiments. This means that DIN and DIP concentrations
227 appeared to be replete at the beginning of incubations, and low DIN concentration
228 could have limited the phytoplankton abundance at the end of incubations.

229 CO_2 concentrations were 354–439 μatm at low CO_2 levels and were 804–1059
230 μatm at high CO_2 levels (Table 2). Correspondingly, pH_{nbs} values were 8.17–8.25 at
231 low CO_2 levels, and 7.85–7.95 at high CO_2 levels. Total alkalinities ranged
232 2319–2381 $\mu\text{mol L}^{-1}$ in all treatments.

233

234 3.2. Chl *a* concentration

235 Chl *a* concentrations in situ were 0.080 $\mu\text{g L}^{-1}$ at station S1, 0.091 $\mu\text{g L}^{-1}$ at station
236 S2, 0.130 $\mu\text{g L}^{-1}$ at station S3, and 0.092 $\mu\text{g L}^{-1}$ at station S4 (Fig. 3). At the end of
237 the incubation, temperature and CO_2 concentration did not significantly affect Chl *a*
238 concentrations at stations S1 and S2, individually and interactively (Table S1; Fig.
239 3A,B). Elevated temperature significantly reduced Chl *a* concentrations at station S3
240 at both LC and HC levels (Tukey HSD, both $p < 0.05$), and at station S4 at LC level
241 (Tukey HSD, $p = 0.02$) (Table S1; Fig. 3C,D). By the sixth day of the incubation, Chl
242 *a* concentrations at station S3 were 47%–55% lower at HT than at LT (Tukey HSD, p

243 < 0.05) (Fig. 3C). At LC level, Chl *a* concentration at station S4 reduced by 52% with
244 rising temperatures, while at HC Chl *a* concentration was not significantly affected by
245 rising temperatures (Tukey HSD, $p = 0.7$) (Fig. 3D).

246

247 3.3. Day-time primary productivity

248 On the final day of the incubations, temperature and CO₂ concentration
249 interactively affected day-time primary productivity at stations S1 and S2, but not at
250 stations S3 and S4 (Table S1). Compared to low temperature and low CO₂ (LTLC)
251 treatments, daytime productivity at station S1 was 41% higher at LTHC (Tukey HSD,
252 $p = 0.02$) and 44% higher at HTLC (Tukey HSD, $p = 0.01$) (Fig. 4A). At station S2,
253 daytime primary productivity was 12% higher at LTHC (Tukey HSD, $p = 0.08$) and
254 39% higher at HTLC (Tukey HSD, $p = 0.04$) than at LTLC. Daytime productivity at
255 stations S1 and S2 was similar between LTLC and HTHC treatments (Tukey HSD, $p >$
256 0.1). At stations S3 and S4, daytime productivity was not significantly different
257 between all treatments (Tukey HSD, all $p > 0.05$) (Fig. 4C,D).

258

259 3.4. Net primary productivity

260 On the final day of the incubations, at station S1, net primary productivity was
261 lower at LTLC than at LTHC or HTLC conditions (Tukey HSD, $p = 0.3$ between
262 LTLC and LTHC treatments; $p = 0.04$ between LTLC and HTLC treatments) (Fig.
263 5A). Net primary productivity was not significantly different between LTLC and
264 HTHC treatments at station S1. Similarly, at station S2, net primary productivity at

265 LTLC was significantly lower than at HTLC (Tukey HSD, $p = 0.03$), whereas it was
266 not significantly different between LTLC, LTHC and HTHC (Tukey HSD, all $p > 0.05$)
267 (Fig. 5B). At stations S3 and S4, net primary production did not differ between all
268 treatments (Tukey HSD, all $p > 0.05$) (Fig. 5C,D).

269

270 3.5. Night time respiration

271 Temperature and CO₂ concentration independently and interactively affected night
272 time respiration rate at station S4, but not at the other stations (Table S1). At S1 and
273 S2, at ambient temperature, night time respiration rates increased significantly at
274 elevated CO₂ (Tukey HSD, both $p < 0.05$, Fig. 6A,B); whereas at high temperature,
275 night time respiration rates were not affected by elevated CO₂ levels (Tukey HSD,
276 both $p > 0.05$). At station S3, at HC, night time respiration rate was enhanced by
277 rising temperature (Tukey HSD, $p = 0.03$) (Fig. 6C); at station S4, at LC, night time
278 respiration rate was enhanced by rising temperature (Tukey HSD, $p < 0.01$) (Fig. 6D).

279

280 **4 Discussion**

281

282 Warming and increased CO₂ levels both individually boosted primary productivity
283 in samples of phytoplankton communities taken in nearshore and offshore habitats in
284 the western South China Sea, although these were not all statistically significant
285 increases (Figs. 4; 5). The effect of rising CO₂ on primary productivity and respiration
286 was temperature dependent, and the combination of elevated CO₂ and temperature

287 resulted in antagonistic effects on production and respiration of the phytoplankton
288 assemblages (Figs. 4; 5; 6).

289 There were enhanced carbon fixation rates at elevated CO₂ levels at all stations
290 (Figs. 4; 5), a similar result to that obtained in other experiments using shipboard
291 incubations, mesocosm experiments and CO₂ seeps (Tortell et al., 2008; Engel et al.,
292 2014; Holding et al., 2015; Johnson et al., 2015). The dominant phytoplankton groups
293 at our offshore stations were *Synechococcus*, *Prochlorococcus* and picoeukaryotes
294 (Zhong et al., 2013; Wu et al., 2014a) whereas diatoms (*Pseudonitzschia pungens* and
295 *Chaetoceros pseudocurvisetus*) and dinoflagellates (*Protoperdinium conicum*) dominated at
296 our inshore stations (Zhang et al., 2014). Rising seawater CO₂ levels are expected to
297 increase carbon fixation rates of larger species more than small phytoplankton species
298 because it is more difficult for large species to take up sufficient inorganic carbon as
299 they have a smaller cell surface:volume quotient (Wu et al., 2014b). Furthermore,
300 elevated CO₂ levels tend to increase the percentage of diatoms in phytoplanktonic and
301 sessile algal communities (Tortell et al., 2002; Domingues et al., 2014). In our
302 experiments, the different responses of offshore and inshore surface phytoplankton
303 assemblages to increased levels of temperature and pCO₂ could be due to differences
304 in the phytoplankton communities.

305 Temperature increases of about 2°C significantly increased phytoplankton
306 assemblage productivity in coastal water at ambient levels of CO₂. This can be
307 expected, since warming is known to increase enzyme activity, and enhance cellular
308 metabolic activity and so improve nutrient or CO₂ uptake (Montagnes and Franklin,

309 2001; Beardall and Raven, 2004). However, warming did not lead to any increase in
310 night time respiration in coastal water, which might indicate less effect of rising
311 temperature on enzyme activity in our study (Fig. 6), suggesting that increased
312 productivity may be due to more efficient nutrient or CO₂ uptake. Another possible
313 reason for greater primary productivity in the warming treatments may be a shift from
314 predominantly large to mainly small sized algal cells during the incubation
315 (Daufresne et al. 2009; Sommer et al. 2015). Unfortunately, we did not determine the
316 community structure at the end of experiments. However, both ambient and elevated
317 temperature treatments in this study are close to the upper thermal limit for growth of
318 most phytoplankton species (Boyd et al. 2013). In this case, rising temperature is
319 expected to shift community composition and cause an increase in the abundance of
320 small-celled phytoplankton. Small species show stronger temperature responses in
321 terms of their photosynthetic C-fixation compared with large species (Sommer et al.,
322 2015), which may lead to higher productivity in warmer coastal water (Figs. 4, 5).

323 In the present work, we observed higher night respiratory under HC conditions
324 (Fig. 6) in coastal waters at ambient temperature, this could be due to enhanced
325 energy demand against the acidic stress such as maintaining the cell's homeostasis
326 (Jin et al. 2015). However, such a respiratory enhancement was not observed at
327 elevated temperature. It is possible that such a level of elevated temperature may
328 increase cellular metabolic activity and periplasmic redox activity that counter-acted
329 the acidic stress. On the other hand, small-sized species seem insensitive to increased
330 pCO₂ in terms of carbon fixation (Tortell et al. 2002; Domingues et al., 2014; Wu et

331 al., 2014b), and they are highly sensitive to high light intensities that cause severe
332 inhibition of C-fixation (Li et al., 2011). Therefore, these effects might contribute to
333 the observed similar response in primary productivity of offshore-water where
334 small-sized species dominated (Zhong et al., 2013), and also contribute to the low
335 primary productivity of coastal water at warming and acidification treatments with
336 high percentage of small sized species (Figs 4, 5). Gao et al. (2012) reported that
337 rising CO₂ decreased phytoplankton productivity in surface seawater under 90%
338 incident solar radiation in the South China Sea, due to enhanced photoinhibition.
339 Different nutrient concentrations can be responsible for the discrepancy between our
340 study and Gao et al., (2012), because seawater was enriched by 1 μmol L⁻¹ NaNO₃
341 and 0.5 μmol L⁻¹ NaH₂PO₄ in this study whereas initial DIN and DIP concentration
342 were lower than 0.01 μmol L⁻¹ and 0.15 μmol L⁻¹, respectively, in the study of Gao et
343 al. (2012). Rising CO₂ is known to increase primary productivity at high nutrient
344 concentrations, but the additional inorganic carbon does not boost productivity in
345 nutrient limited conditions (Yoshimura et al., 2009; Celis-Plá et al., 2015).

346 The temperature and CO₂ concentrations of surface oceans are rising
347 simultaneously, but the carbonate chemistry of coastal water is complex, due to the
348 local effects of hydrography, metabolic activity, nutrient input and watershed
349 processes (Duarte et al. 2013). The effects of CO₂ on phytoplankton physiology and
350 productivity has important biogeochemical implications. Increased productivity at
351 elevated CO₂ level could accelerate carbon sequestration of phytoplankton which may
352 increase the CO₂ uptake of coastal seawater from the atmosphere. Decreased

353 chlorophyll concentrations offshore due to warming may limit biological productivity
354 because phytoplankton are the primary energy source for marine food chains. Our
355 study shows that phytoplankton assemblages in different regions respond differently
356 to increases in CO₂ and temperature. However, if our shipboard tests reflect natural
357 responses, then ongoing warming and acidification in the South China Sea is not
358 expected to increase overall regional primary productivity due to a lack of nutrients in
359 offshore waters. Other environmental factors such as changes in solar radiation,
360 wind-speed induced mixing and deposition of dusts may also affect the primary
361 productivity of phytoplankton communities. Therefore, shipboard incubations during
362 different seasons or with waters influenced by episodic events might lead to
363 differential responses to warming and acidification.

364

365 **5. Conclusion**

366 The present study shows combined effects of ocean warming and acidification
367 on phytoplankton primary productivity, Chl *a* concentration and night respiration of
368 two coastal and two offshore waters in the western South China Sea. Warming and
369 elevated CO₂ levels individually increased primary productivity, especially in the
370 coastal water. However, the combination of elevated temperature and increased CO₂
371 did not increase primary productivity at all stations. Different responses in primary
372 productivity, Chl *a* concentration and night respiration to warming and acidification
373 between the coastal and offshore waters may be due to differences in the
374 phytoplankton community composition and in their sensitivity to elevated temperature

375 or CO₂ levels.

376

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378

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381

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595 Figure Legend

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597 Figure 1. Sampling stations in the western South China Sea in the cruise during
598 autumn 2017.

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600 Figure 2. Water temperature in the deck incubators for the low and high temperature
601 treatments during the incubations, and solar radiation.

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603 Figure 3. Chl *a* concentration of surface phytoplankton assemblages in situ and in the
604 bottle after 6 days of incubation at different experiment conditions. Different letters
605 indicated statistically difference based on Tukey post hoc test. The values represent
606 the mean \pm standard deviation (error bar) for three replicates.

607

608 Figure 4. Daytime primary productivity (DPP) of surface phytoplankton assemblages
609 in the bottle after 6 days of incubation at different experiment conditions. Different
610 letters indicated statistically difference based on Tukey post hoc test. The values
611 represent the mean \pm standard deviation (error bar) for three replicates

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613 Figure 5. Net primary productivity (NPP) of surface phytoplankton assemblages in the
614 bottle after 6 days of incubation at different experiment conditions. Different letters
615 indicated statistically difference based on Tukey post hoc test. The values represent
616 the mean \pm standard deviation (error bar) for three replicates

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618 Figure 6. Night time respiration rate of surface phytoplankton assemblages in the
619 bottle after 6 days of incubation at different experiment conditions. Different letters
620 indicated statistically difference based on Tukey post hoc test. The values represent
621 the mean \pm standard deviation (error bar) for three replicates

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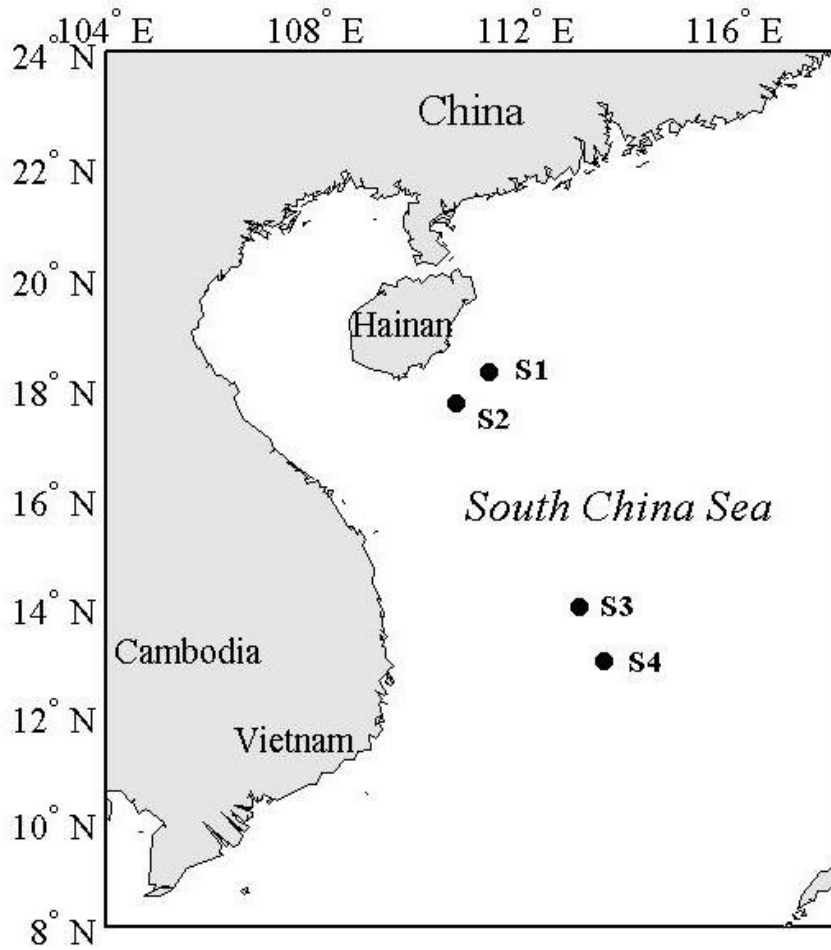
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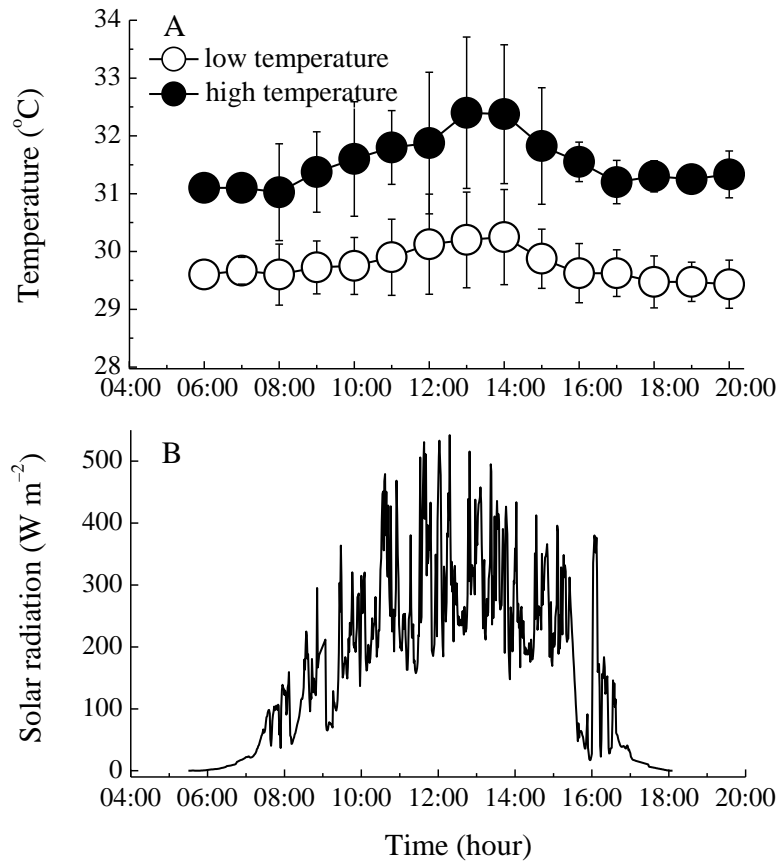
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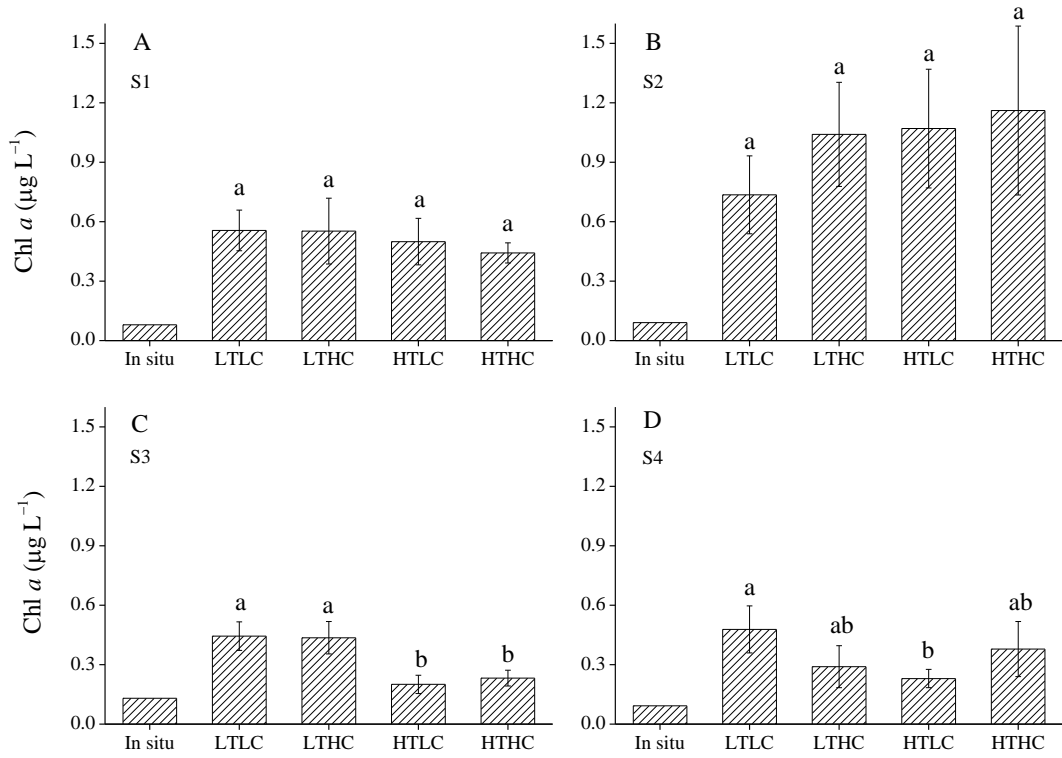
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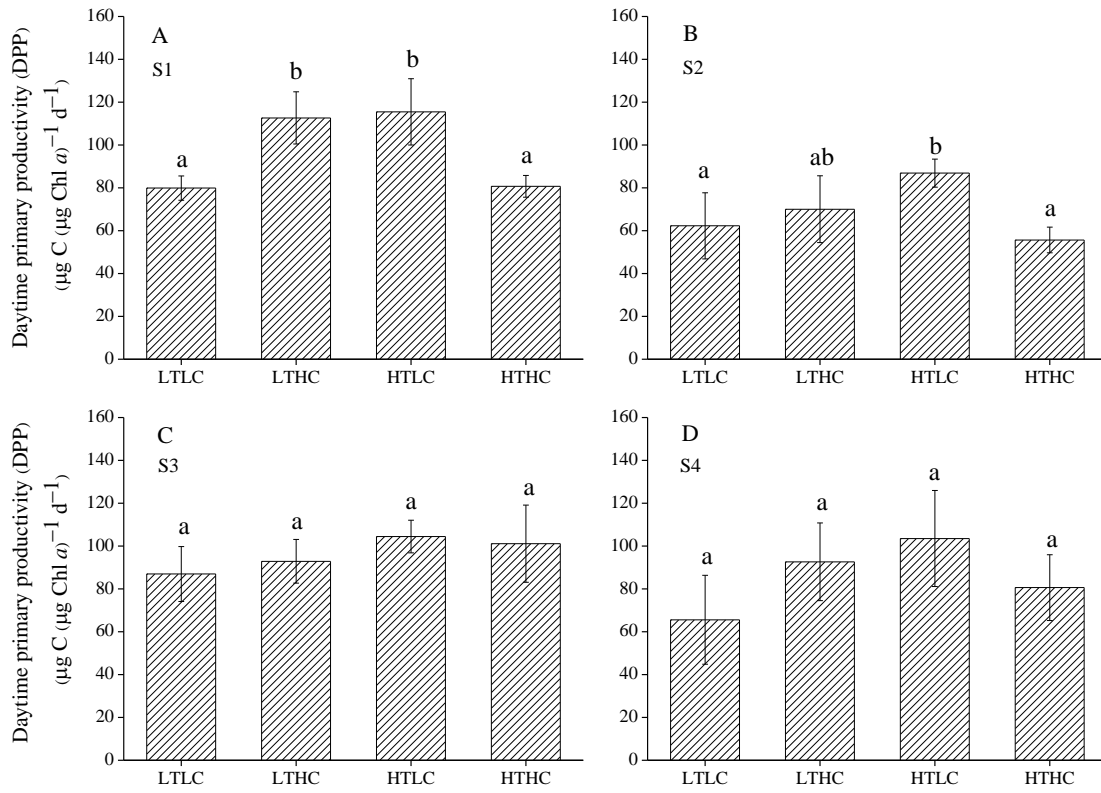
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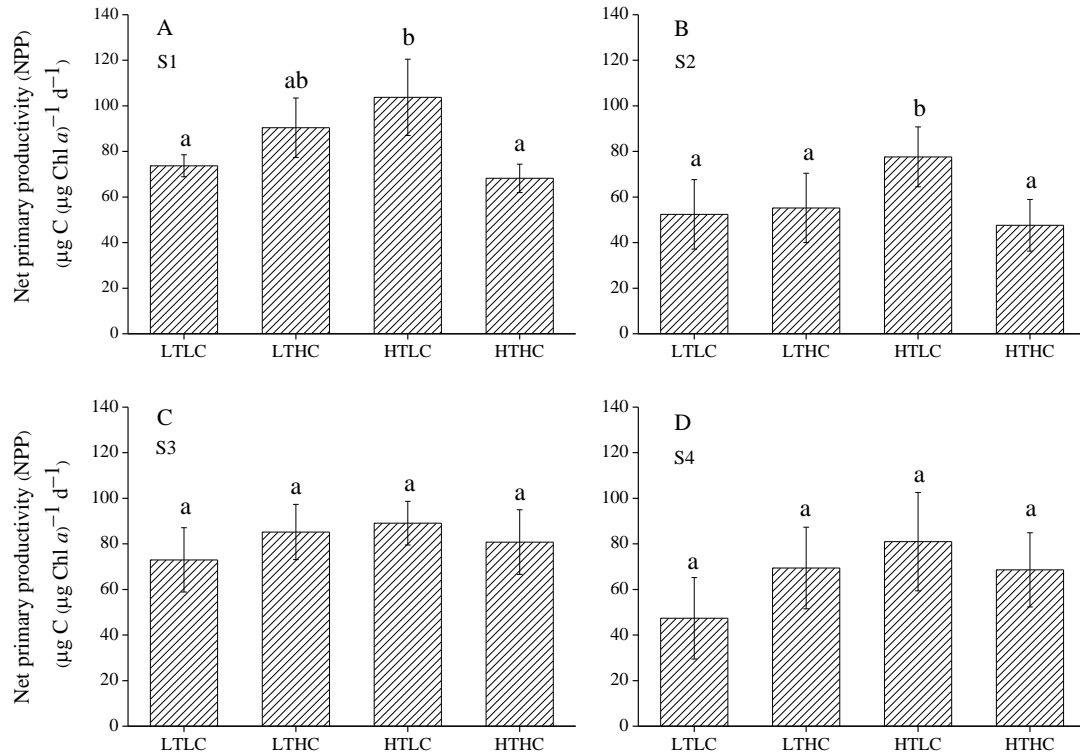
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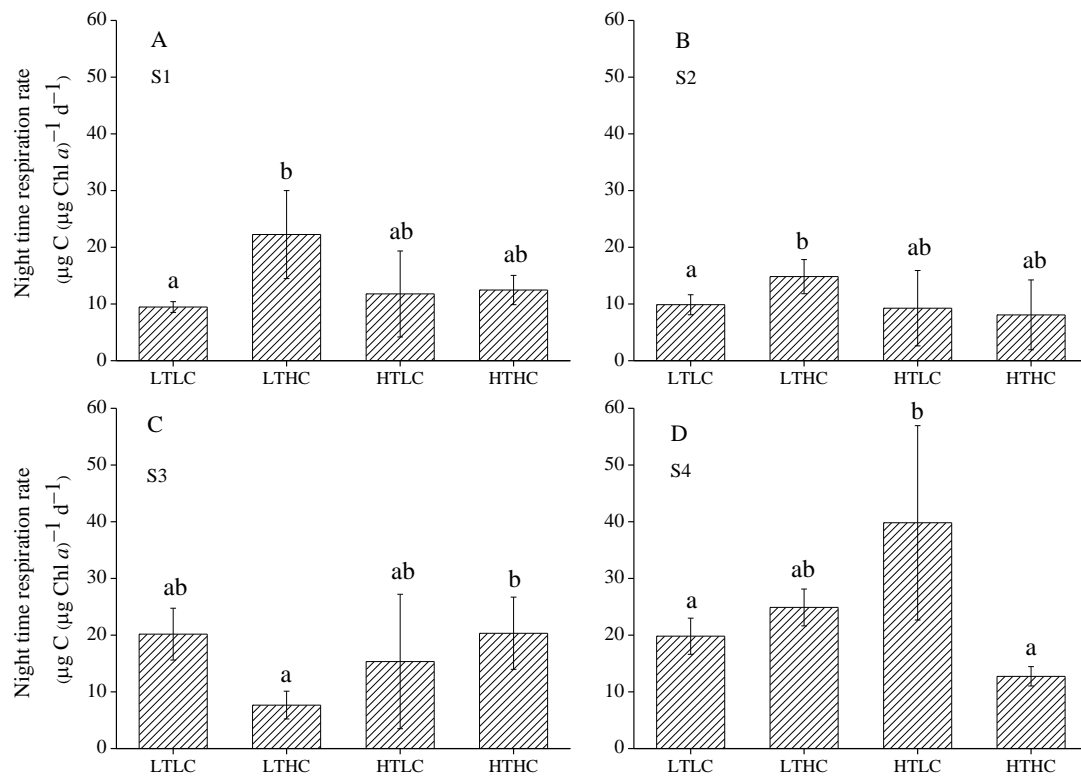
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708 Figure 6

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714 **Table 1.** Dissolved inorganic nitrogen (DIN) and phosphate (DIP) concentrations at
715 the beginning and end of the incubation. 1 $\mu\text{mol L}^{-1}$ NaNO_3 and 0.5 $\mu\text{mol L}^{-1}$
716 NaH_2PO_4 was added into the seawater in the beginning of the incubation. Data in the
717 bracket were DIN and DIP concentrations *in situ*. ND indicates that concentration was
718 below the detection limit ($< 0.04 \mu\text{mol L}^{-1}$).

		DIN ($\mu\text{mol L}^{-1}$)	DIP ($\mu\text{mol L}^{-1}$)
S1	Before culture	1 (0.08)	0.5 (0.17)
	After culture	ND	0.05 \pm 0.01
S2	Before culture	1 (0.03)	0.5 (0.21)
	After culture	ND	0.04 \pm 0.02
S3	Before culture	1 (0.03)	0.5 (0.14)
	After culture	ND	0.05 \pm 0.01
S4	Before culture	1 (0.12)	0.5 (0.16)
	After culture	ND	0.05 \pm 0.01

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731 **Table 2.** Carbonate chemistry parameters of the seawater in the final day of the
732 incubations at different temperature and pCO₂ conditions. TA and pH samples were
733 collected and measured. Different letters (a and b) indicated statistically difference
734 based on Tukey post hoc test. pH_{nbs} means the pH measurements in seawater on the
735 NBS scale.

	pCO ₂ (μatm)	pH _{nbs}	TA (μmol L ⁻¹)	DIC (μmol L ⁻¹)	HCO ₃ ⁻ (μmol L ⁻¹)	CO ₃ ²⁻ (μmol L ⁻¹)	CO ₂ (μmol L ⁻¹)	Ω calcite
LTLC	419±13 ^a	8.19±0.01 ^a	2342±15 ^a	2050±12 ^a	1818±11 ^a	220±5 ^a	12±0.4 ^a	5.5±0.1 ^a
LTHC	977±64 ^b	7.88±0.03 ^b	2349±18 ^a	2210±16 ^b	2060±17 ^b	121±7 ^b	28±1.8 ^b	3.0±0.2 ^b
HTLC	376±14 ^a	8.23±0.01 ^a	2343±16 ^a	2028±8 ^a	1782±7 ^a	235±8 ^a	11±0.4 ^a	5.8±0.2 ^a
HTHC	891±61 ^b	7.91±0.03 ^b	2348±22 ^a	2194±18 ^b	2038±18 ^b	130±8 ^b	26±1.8 ^b	3.2±0.2 ^b

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749 **Table S1.** Results of two-way ANOVAs of the effects of temperature and $p\text{CO}_2$ on Chl
750 a , day-time primary productivity (DPP), net primary productivity (NPP) and night
751 time respiration rate. Temp indicates temperature and significant difference was setup
752 to $p < 0.05$.

Station	Parameter	Treatment	df	F-value	p
S1	Chl a	Temp	1	2.80	0.13
		CO ₂	1	0.30	0.61
		Temp × CO ₂	1	0.14	0.71
	DPP	Temp	1	2.38	0.15
		CO ₂	1	0.68	0.43
		Temp × CO ₂	1	31.53	<0.01
	NPP	Temp	1	1.65	0.21
		CO ₂	1	0.14	0.75
		Temp × CO ₂	1	14.77	<0.01
	Respiration	Temp	1	1.36	0.26
		CO ₂	1	4.43	0.07
		Temp × CO ₂	1	3.56	0.09
S2	Chl a	Temp	1	2.43	0.15
		CO ₂	1	2.20	0.18
		Temp × CO ₂	1	0.38	0.53
	DPP	Temp	1	0.006	0.94
		CO ₂	1	20.74	<0.01
		Temp × CO ₂	1	7.62	<0.05
	NPP	Temp	1	0.37	0.57
		CO ₂	1	4.03	0.08
		Temp × CO ₂	1	3.98	0.08
	Respiration	Temp	1	0.92	0.37
		CO ₂	1	4.65	0.06
		Temp × CO ₂	1	1.16	0.31
S3	Chl a	Temp	1	38.58	<0.01
		CO ₂	1	0.67	0.41
		Temp × CO ₂	1	0.32	0.61
	DPP	Temp	1	2.43	0.17
		CO ₂	1	0.02	0.93
		Temp × CO ₂	1	0.34	0.59
	NPP	Temp	1	0.88	0.39
		CO ₂	1	0.050	0.82
		Temp × CO ₂	1	1.77	0.21
	Respiration	Temp	1	1.52	0.20
		CO ₂	1	0.14	0.71

S4	Chl <i>a</i>	Temp × CO ₂	1	1.03	0.34	
		Temp	1	7.53	<0.05	
		CO ₂	1	0.005	0.95	
	DPP	Temp × CO ₂	1	7.53	<0.05	
		Temp	1	0.39	0.55	
		CO ₂	1	0.0001	0.99	
	NPP	Temp × CO ₂	1	5.45	<0.05	
		Temp	1	1.64	0.23	
		CO ₂	1	0.46	0.56	
	Respiration	Temp × CO ₂	1	2.50	0.16	
		Temp	1	17.01	<0.05	
		CO ₂	1	17.97	<0.05	
			Temp × CO ₂	1	28.04	<0.05

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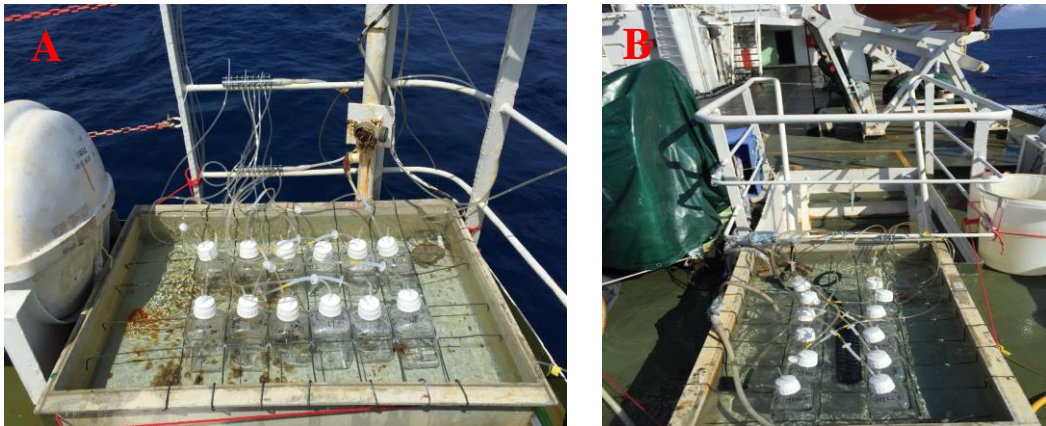
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774 Figure S1. Phytoplankton assemblages were cultured at low temperature (in situ

775 temperature, A) and high temperature (in situ + 1.8 °C, B) treatments.

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