1   **Combined effects of elevated pCO$_2$ and temperature on biomass and carbon**

2   **fixation of phytoplankton assemblages in the northern South China Sea**

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26   **Abstract**

27   The individual influences of ocean warming and acidification on marine

28   organisms have been investigated intensively, but studies regarding the combined

29   effects of both global change variables on natural marine phytoplankton assemblages

30   are still scarce. Even fewer studies have addressed possible differences in the

31   responses of phytoplankton communities in pelagic and coastal zones to ocean

32   warming and acidification. We conducted shipboard microcosm experiments at both

33   off-shore (SEATS) and near-shore (D001) stations in the northern South China Sea

34   (NSCS) under three treatments, low temperature (30.5 ^{o}C at SEATS and 28.5 ^{o}C at

35   D001) and low pCO$_2$ (390.0 μatm at SEATS and 420.0 μatm at D001) (LTLC), high

36   temperature (33.5 ^{o}C at SEATS and 31.5 ^{o}C at D001) and low pCO$_2$ (390 μatm at

37   SEATS and 420 μatm at D001) (HTLC), and high temperature (33.5 ^{o}C at SEATS

38   and 31.5 ^{o}C at D001) and high pCO$_2$ (1000 μatm at SEATS and 1030 μatm at D001)

39   (HTHC). Biomass of phytoplankton at both stations were enhanced by HT. HTHC did

40   not affect phytoplankton biomass at station D001 but decreased it at station SEATS.

41   At this offshore station HT alone increased daily primary productivity (DPP, μg C (μg

42   chl a)$^{-1}$ d^{-1}) by ~64%, and by ~117% when higher pCO$_2$ was added. In contrast, HT

43   alone did not affect DPP and HTHC reduced it by ~15% at station D001. HT

44   enhanced the dark respiration rate (μg C (μg chl a)$^{-1}$ d^{-1}) by 64% at station SEATS, but

45   had no significant effect at station D001, and did not change the ratio of respiration to

46   photosynthesis at either station. HTHC did not affect dark respiration rate (μg C (μg

47   chl a)$^{-1}$ d^{-1}) at either station compared to LTLC. HTHC reduced the respiration to

48   photosynthesis ratio by ~41% at station SEATS but increased it ~42% at station D001.

49   Overall, our findings indicate that responses of coastal and offshore phytoplankton

50   assemblages in NSCS to ocean warming and acidification are contrasting, with the

51   pelagic phytoplankton communities being more sensitive to these two global change

52   factors.

53

54   **Key words:** ocean acidification, ocean warming, photosynthesis, primary productivity,

55   respiration, South China Sea

56

1 Introduction

57

58   From 1979 to 2012, the mean global sea surface temperature increased at a

59   rate of ~0.12 ℃ per decade, based on in situ data records (IPCC, 2013). Particularly,

60   the warming rate in the South China Sea (~0.26 per decade) from 1982 to 2004 is

61   approximately 2 times faster than the global ocean mean rate (~0.14 per decade) from

62   1960 to 1990 (Casey and Cornillon, 2001; Fang et al., 2006). It is extremely likely

63   that more than half of the observed increase in global average surface temperature

64   from 1951 to 2010 was caused by the anthropogenic increase in greenhouse gas

65   concentrations and other anthropogenic forcings. Ocean warming is projected to rise

66   approximately by 0.6 ℃ (RCP 2.6) to 2.0 ℃ (RCP 8.5), in the upper 100 m by the

67   end of the 21st century (IPCC, 2013).

68   Ocean warming is known to affect primary productivity both directly and

69   indirectly. In situ surface chl a declined exponentially with rise of SST (13–23 ℃) in

70   the northeast Atlantic Ocean from latitudes 29 to 63 °N in spring and summer, which

71   was attributed to enhanced stratification and consequent reduced upward transport of

72   nutrients into the upper mixed layer (Poll et al., 2013). The seawater volume-specific

73   primary produtivity also decreased with temperature rise due to lower phytoplanton

74   biomass (Poll et al., 2013). Mesocosm experiments have also demonstrated that ocean

75   warming (an increase of 6 ℃) can decrease phytoplankton biomass in the Baltic Sea

76   (Lewandowska et al., 2012; Sommer et al., 2015). On the other hand, ocean warming

77   did not affect volume-specific primary produtivity in the Baltic Sea (Lewandowska et

78   al., 2012).

79   Increased atmospheric CO_2 is responsible for global warming and the ocean.

80   As a main sink for CO_2, the ocean has absorbed approximately 30% of the emitted

81   anthropogenic carbon dioxide (IPCC, 2013). Such a dissolution of CO_2 increases

82   seawater CO_2 partial pressure and bicarbonate ion levels and decreases pH and

83   carbonate ion concentrations, leading to ocean acidification (OA). By 2100, the

84   projected decline in global-mean surface pH is projected to be approximately 0.065 to

85   0.31, depending on the RCP scenario (IPCC, 2013). In terms of the South China Sea,

86   an accelerated trend of ocean acidification has been reported and the rate of pH

87   decline almost tripled between 1951 and 2000, compared to that between 1840 and

88   1950 (Liu et al., 2014).

89   CO_2 may be a potentially limiting factor for marine primary productivity

90   because of the low CO_2 level in seawater and the low affinity of the enzyme Rubisco

91   for dissolved CO_2 (Falkowski and Raven, 2013). In addition, CO_2 in seawater diffuses

92   approximately 10,000 times slower than in air, leading to its supply rate being much

93   lower than the demand of photosynthetic carbon fixation (Raven, 1993; Riebesell et

94   al., 1993). Although phytoplankton have evolved carbon-concentrating mechanisms

95   (CCMs) to cope with these problems (Giordano et al., 2005; Raven et al., 2012;

96   Reinfelder, 2011), increased CO_2 concentration may still be beneficial since energy

97   saved due to down-regulation of CCMs under elevated CO_2 can be utilized in other

98   metabolic processes (Gao et al., 2012a). Early laboratory and shipboard experiments

99   suggested that increased CO_2 indeed could enhance phytoplankton growth rates and

100   thus marine primary productivity (Riebesell et al., 1993; Hein and Sand-Jensen, 1997;

101   Schippers et al., 2004). Since then, neutral effects of increased CO_2 on growth of

102   phytoplankton assemblages have also been reported (Tortell and Morel, 2002; Tortell

103   et al., 2000). Furthermore, ocean acidification can even reduce primary productivity

104   of surface phytoplankton assemblages when exposed to incident solar radiation (Gao

105   et al., 2012b). Therefore, the effects of ocean acidification on marine primary

106   productivity remain controversial and its interactions with other environmental factors,

107   such as warming, solar UV radiation, hypoxia, etc. are incompletely understood (Gao

108   et al., 2012a; Häder and Gao, 2015; Mostofa, 2016).

109   Ocean warming and acidification, both caused by increasing atmospheric CO_2,

110   are proceeding simultaneously. The interactive or combined effects of warming and

111   OA could be completely different from that of either one stressor (Hare et al. 2007,

112   Feng et al., 2009; Gao et al., 2012a, Tatters et al. 2013). Several oceanographic cruises

113   and ship board experiments in the European sector of the Arctic Ocean, showed that

114 gross primary production increased with pCO$_2$ (145–2099 µatm) and the greatest

115 increase was observed in lower temperature regions, indicating CO$_2$-enhanced

116 primary production in the European Arctic Ocean is temperature-dependent (Holding

117 et al., 2015).

118   The South China Sea (SCS) is located between the equator and 23.8 °N, from

119 99.1 to 121.1 °E, and is one of the largest marginal seas in the world, with a total area

120 of about 3.5×10^6 km^2. Therefore, understanding the effects of ocean warming and

121 acidification on primary production in SCS would help us to define the role of

122 marginal seas in the global carbon cycle. However, only a very few studies on the

123 effects of ocean acidification or warming on primary productivity in the SCS have

124 been reported. Wu and Gao (2010) reported that CO$_2$ enrichment (700 µatm) did not

125 affect the assimilation number of phytoplankton at a near-shore site in SCS, compared

126 to the ambient CO$_2$ level (380 µatm). Gao et al. (2012b) demonstrated that increased

127 pCO$_2$ (800 or 1000 µatm) reduced primary productivity in off-shore stations of the

128 SCS. Therefore, we hypothesized that the effects of ocean acidification or/and

129 warming on primary productivity in SCS would be site-dependent. None of the

130 previous studies have examined co-effects of warming and increased CO$_2$ on primary

131 production in the SCS. In this study, to test this hypothesis we conducted shipboard

132 microcosm experiments at both near-shore and off-shore stations to determine the

133 combined effects of ocean warming and acidification on biomass, photosynthetic

134 carbon fixation, and dark respiration of phytoplankton assemblages in the SCS.

135 **2 Methods**

136 2.1 **Experimental setup**

137   The experiments were conducted at one off-shore station SEATS (17.9963^o N,

138 115.9621^o E) and one near-shore station D001 (18.9740^o N, 110.7166^o E) in the NSCS

139 (Fig. 1). Surface seawater (0–2 m) was collected before sunrise with a 10 L

140 acid-cleaned plastic bucket, filtered (180 µm) to remove large grazers and dispensed

141 into nine microcosms. Microcosms consisted of cylindrical polymethyl methacrylate

142 tanks (32 L, 0.34 m water depth) with water-jacketed space for circulating cooled

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143 water, allowing 91% photosynthesis active radiation (PAR, 400–700 nm), 63%

144 ultraviolet-A (UVA, 315–400 nm) and 6% ultraviolet-B (UVB, 280–315 nm)

145 transmission under incident solar radiation. Two levels of temperature (in situ, in situ

146 + 3 ^{o}C) and pCO$_2$ (ambient, ambient + 610 μatm) were used. There were three

147 triplicated treatments: low temperature and low pCO$_2$, LTLC; high temperature and

148 low pCO$_2$, HTLC; high temperature and high pCO$_2$, HTHC. The treatment of low

149 temperature and high pCO$_2$ was missing due to the lack of microcosms. Microcosm

150 temperature was controlled with circulating coolers (CTP-3000, EYELA, Japan) and

151 stable CO$_2$ equilibrium with the sea water (variation of pCO$_2$ < 5%) was achieved

152 within 24 hours using a CO$_2$ enricher (CE-100, Wuhan Ruihua Instrument &

153 Equipment Ltd, China). The incubations were conducted for seven days for station

154 SEATS (Aug 3rd–9th 2012) and six days for station D001 (Aug 14th–19th 2012).

155 **2.2 Solar radiance**

156 The incident solar radiation was continuously monitored using an Eldonet

157 broadband filter radiometer (Eldonet XP, Real Time Computer, Germany) that was

158 fixed at the top of the ship. It measured every second and recorded the means over

159 each minute.

160 **2.3 Carbonate chemistry parameters**

161 The seawater pH in the microcosm was recorded with a pH meter (FE20, Mettler

162 Toledo, Greifensee, Switzerland) every hour during the first day of incubation and

163 daily afterwards. The pCO$_2$ in seawater was maintained with the CO$_2$ enricher and

164 measured by an automated flowing pCO$_2$ measuring system (Model 8050, GO, USA).

165 Other carbonate system parameters were derived via CO2SYS (Pierrot et al., 2006),

166 using the equilibrium constants of K$_1$ and K$_2$ for carbonic acid dissociation (Roy et al.,

167 1993).

168 **2.4 Chlorophyll *a* analysis**

169 For the measurement of chlorophyll *a* (chl *a*), 500 mL of seawater were filtered

170 onto a Whatman GF/F glass fiber filter (25 mm).Then the filter was placed in 5 ml 93%

171 acetone at -20 ℃ for 24 h. Chl *a* concentration was determined with a fluorometer

172 (Trilogy, Turner Designs, USA), following the protocol of Welschmeyer (1994).

2.5 Primary productivity and dark respiration

173

174 Seawater samples taken from each microcosm were dispensed into 50 mL quartz

175 tubes inoculated with 5 μCi (0.185 MBq) NaH^{14}CO$_3$ (ICN Radiochemicals, USA) and

176 incubated for 12 h and 24 h under natural light and day-night conditions. The

177 incubation temperature of every treatment was the same as the corresponding

178 microcosm treatment. After the incubation, the cells were filtered onto a Whatman

179 GF∕F glass fiber filter (25 mm), which was immediately frozen and stored at -20°C

180 for later analysis. In the laboratory, each frozen filter was placed into a 20 mL

181 scintillation vial, exposed to HCl fumes overnight, and dried (55 ^{o}C, 6 h) to expel

182 non-fixed ^{14}C (Gao et al., 2007). Then 3 mL scintillation cocktail (Perkin Elmer®)

183 was added to each vial and incorporated radioactivity was counted by a liquid

184 scintillation counting (LS 6500, Beckman Coulter, USA). The daytime primary

185 productivity (DPP) was defined as the amount of carbon fixation during 12 h

186 incubation. The dark respiration was defined as the difference in amount of carbon

187 fixation between 12 h and 24 h. Carbon fixation over 24 h was taken as daily net

188 primary productivity (NPP). Ratio of respiration to photosynthesis (R/P) was

189 expressed as that of respiratory carbon loss to daytime carbon fixation.

2.6 Statistical analyses

190

191 Results were expressed as means of replicates ± standard deviation. Data were

192 analyzed using the software SPSS v.21. The data from each treatment conformed to a

193 normal distribution (Shapiro-Wilk, $P > 0.05$) and the variances could be considered

194 equal (Levene's test, $P > 0.05$). One-way ANOVAs were conducted to assess the

195 significant differences in carbonate chemistry parameters, chl a, DPP, NPP, dark

196 respiration, ratio of dark respiration to photosynthesis between three treatments.

197 Tukey HSD was conducted for post hoc investigation. Independent samples t-tests

198 were conducted to compare in situ chl a, DPP, NPP, dark respiration, and ratio of dark

199 respiration to photosynthesis between both stations. The threshold value for

200 determining statistical significance was $P < 0.05$.

3 Results

201

202 The incident solar radiation during the experiment was recorded (Table 1). The

203   daytime (12 h) mean solar radiation ranged from 927 to 1592 μmol photons m^{-2} s^{-1} at

204   station SEATS while the lowest solar radiance was only 111 μmol photons m^{-2} s^{-1},

205   with the highest of 1583 μmol photons m^{-2} s^{-1}, at station D001. The average of

206   daytime mean solar radiation over the microcosm incubation at station SEATS was

207   15.52% higher than that at station D001.

208   The changes of the seawater carbonate system under different conditions are

209   shown in Table 2. At station SEATS, an increase of 3 ^{o}C in temperature (HTLC) did

210   not alter carbonate parameters except leading to enhanced CO_3^{2-} (Tukey HSD, P =

211   0.009). HTHC resulted in a significant decrease in CO_3^{2-} (Tukey HSD, $P < 0.001$) and

212   TA (Tukey HSD, P = 0.016) but an increase in CO_2 (Tukey HSD, $P < 0.001$)

213   compared with LTLC. The effect of temperature on carbonate parameters at station

214   D001 were similar to station SEATS, while HTHC increased HCO_3^- (Tukey HSD, P =

215   0.046) and did not affect TA (Tukey HSD, P = 0.203).

216   The in situ chl a level at station D001 (0.37 ±0.05 μg L^{-1}) was higher than that at

217   station SEATS (0.15 ±0.02 μg L^{-1}) (Independent samples t-test, t = −7.483, df = 4, P

218   = 0.002; Fig. 2a). After seven days incubation in the microcosms at station SEATS,

219   Tukey comparison $(P = 0.05)$ showed that higher temperature (0.46 ± 0.04 μg L^{-1})

220   increased chl a compared with LTLC (0.23 ±0.03 μg L^{-1}) while HTHC (0.07 ±0.01

221   μg L^{-1}) reduced it (Fig. 2b). The higher temperature (0.72 ± 0.07 μg L^{-1}) also

222   increased chl a compared with LTLC (0.41 ± 0.05 μg L^{-1}) at station D001 (Tukey

223   HSD, P = 0.001; Fig. 1b), but with no effect of HTLC (0.41 ±0.04 μg L^{-1}) (Tukey

224   HSD, P = 0.988).

225   The in situ DPP at station D001 (49.4 ±4.5 μg C L^{-1} d^{-1} or 133.4 ±12.1 μg C (μg

226   chl a)$^{-1}$ d^{-1}) was dramatically higher than that at station SEATS (5.1 ±0.5 μg C L^{-1} d^{-1}

227   or 34.1 ± 3.1 μg C (μg chl a)$^{-1}$ d^{-1}), whether normalized to volume of seawater

228   (Independent samples t-test, t = −17.056, df = 4, $P < 0.001$; Fig. 3a) or chl a

229   (Independent samples t-test, t = −13.786, df = 4, $P < 0.001$; Fig. 3b). After seven days

230   incubation in microcosms, the DPP normalized to volume of seawater under HTLC

231   (33.2 ±4.8 μg C L^{-1} d^{-1}) at station SEATS was significantly higher than that under

232   LTLC (9.9 ±1.2 μg C L^{-1} d^{-1}) and HTHC (6.6 ±0.6 μg C L^{-1} d^{-1}) (Tukey HSD, $P <$

233 0.001) while the difference between LTLC and HTHC was insignificant (Tukey HSD,

234 P = 0.380; Fig. 3c). The pattern at station D001 was similar to SEATS (Fig. 2c). When

235 DPP was normalized to chl a, the higher temperature increased primary productivity

236 from 43.2 $\pm$ 5.1 to 70.7 $\pm$ 10.1 µg C (µg chl a)$^{-1}$ d^{-1} (Tukey HSD, P = 0.014) and

237 further to 93.9 $\pm$ 8.1 µg C (µg chl a)$^{-1}$ d^{-1} (Tukey HSD, P < 0.001) when higher CO_2

238 was combined at station SEATS (Fig. 2d). In contrast, temperature did not affect DPP

239 (Tukey HSD, P = 0.0924) and HTHC reduced it from 150.3 $\pm$ 4.9 to 128.0 $\pm$ 11.5 µg

240 C (µg chl a)$^{-1}$ d^{-1} (Tukey HSD, P = 0.039) at station D001 (Fig. 3d).

241   The in situ NPP at stations SEATS and D001 were 3.5 $\pm$ 0.1 µg C L^{-1} d^{-1} (23.2 $\pm$

242 1.0 µg C (µg chl a)$^{-1}$ d^{-1}) and 37.4 $\pm$ 3.1 µg C L^{-1} d^{-1} (91.2 $\pm$ 7.5 µg C (µg chl a)$^{-1}$ d^{-1})

243 respectively, which indicates that station D001 has higher NPP, irrespective of

244 normalizing to volume of seawater (Independent samples t-test, t = −18.998, df = 4, P

245 < 0.001; Fig. 4a) or chl a (Independent samples t-test, t = −15.511, df = 4, P < 0.001;

246 Fig. 4b). After a seven-day incubation in the microcosms, the higher temperature

247 increased NPP to 23.9 $\pm$ 5.3 µg C L^{-1} d^{-1} (Tukey HSD, P = 0.001) while HTHC (5.5 $\pm$

248 0.4 µg C L^{-1} d^{-1}) did not change it (Tukey HSD, P = 0.793) compared with LTLC (7.2

249 $\pm$ 0.8 µg C L^{-1} d^{-1}). The effects of temperature and CO_2 on NPP at station D001 were

250 similar to that at station SEATS. When NPP was normalized to chl a, the higher

251 temperature increased NPP from 31.1 $\pm$ 3.5 to 50.9 $\pm$ 11.3 (Tukey HSD, P = 0.044)

252 and further to 78.3 $\pm$ 5.9 µg C (µg chl a)$^{-1}$ d^{-1} with the addition of higher CO_2 (Tukey

253 HSD, P < 0.001) at station SEATS. On the other hand, neither HT (Tukey HSD, P =

254 0.707) nor HTHC (Tukey HSD, P = 0.057) affected NPP at station D001.

255   The in situ dark respiration rate at station SEATS was remarkably lower than that

256 at station D001 regardless of normalizing to volume of seawater (Independent

257 samples t-test, t = −11.568, df = 4, P < 0.001; Fig. 5a) or chl a (Independent samples

258 t-test, t = −8.019, df = 4, P = 0.001; Fig. 5b). The higher temperature increased dark

259 respiration rate from 2.8 $\pm$ 1.2 to 9.3 $\pm$ 0.6 µg C L^{-1} d^{-1} (Tukey HSD, P < 0.001) at

260 station SEATS while HTHC reduced it to 1.1 $\pm$ 0.2 µg C L^{-1} d^{-1} (Tukey HSD, P =

261 0.009; Fig. 5c). The higher temperature also promoted dark respiration rate at station

262 D001, from 16.9 $\pm$ 2.0 to 31.5 $\pm$ 5.1 µg C L^{-1} d^{-1} (Tukey HSD, P = 0.007) but HTHC

263   did not alter it (Tukey HSD, P = 0.516; Fig. 5c). When it was normalized to chl a,

264   higher temperature still increased dark respiration rate from 12.0 ± 1.8 to 19.7 ± 1.4

265   µg C (µg chl a)$^{-1}$ d^{-1} (Tukey HSD, P = 0.006) while the effect of temperature on

266   respiration rate at station D001 was insignificant (Tukey HSD, P = 0.891; Fig. 5d).

267   Compared to LTLC, HTHC did not affect respiration rate at either station (Tukey

268   HSD, P = 0.131 at station SEATS, P = 0.348 at station D001; Fig. 5d).

269   The in situ ratio of respiration to photosynthesis was 31.9 ± 3.5% at stations

270   SEATS, significantly higher than that (24.2 ± 1.3%) at station D001 (Independent

271   samples t-test, t = 3.537, df = 4, P = 0.0024; Fig. 6a). After seven days incubation in

272   microcosms, Tukey HSD comparison (P = 0.05) showed that higher temperature did

273   not affect the ratio of respiration to photosynthesis but HTHC reduced it from 27.8 ±

274   1.6% to 16.5 ± 1.3% at station SEATS (Fig. 6b). On the contrary, HTHC (38.7 ± 3.1%)

275   increased the ratio compared to LTLC (27.3 ± 2.4%), with insignificant effect of

276   temperature alone (29.5 ± 3.3%) at station D001 (Fig. 6b).

277   **4 Discussion**

278   **4.1 Effects of increased temperature and CO_2 on biomass**

279   The in situ chl a concentration at station D001 was higher than that at SEATS,

280   indicating more phytoplankton biomass at station D001. The chl a concentration

281   decreases with distance from the coast in the NSCS, mainly due to the change of

282   nutrients (Li et al., 2011). The higher temperature increased chl a concentration at

283   both stations, which might be attributed to increased active uptake of nutrients at the

284   elevated   temperatures   through   enhanced   enzymatic   activities.   Algal   and

285   cyanobacterial growth commonly increases with temperature within a suitable range

286   (Goldman and Carpenter, 1974; Montagnes and Franklin, 2001; Savage et al., 2004;

287   Boyd et al. 2013) and optimum temperatures for growth of marine phytoplanton are

288   usually several degrees higher than the environmental temperatures (Thomas et al.,

289   2012).

290   On the other hand, the elevated CO_2 offset the positive effect of the higher

291   temperature on chl a at station D001, and even reduced chl a at station SEATS. High

292   CO_2 can sometimes enhance algal photosynthesis and growth, since CO_2 in seawater

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293   is suboptimal for full operation of Rubisco enzymes (Wu et al., 2008 and references

294   therein). On the other hand, positive effects of elevated CO_2 can be affected by other

295   environmental factors. Gao et al. (2012b) demonstrated that rising CO_2 could enhance

296   growth of diatoms at low light intensity, but decrease it at high light intensity. It was

297   found that rising CO_2 concentration lowered the threshold for diatom growth above

298   which photosynthetic active radiation becomes excessive or stressful, owing to

299   reduced energy requirements for inorganic carbon acquisition at elevated CO_2 (Gao et

300   al., 2012b). In the present study, the mean daily solar radiation levels during

301   incubation were 1312 and 1136 μmol photons m^{-2} s^{-1} (Table 1), which were far above

302   the threshold light intensity reported for diatoms (Gao et al., 2012b). Consequently,

303   the higher CO_2 combined with the high solar radiation in summer of NSCS may have

304   imposed negative effects on phytoplankton biomass at station SEATS and D001. In

305   addition, the inhibitory effect of higher CO_2 on biomass was more significant at

306   station SEATS than D001. This can be attributed to the higher sensitivity of

307   picoplankton to high solar radiation (Li et al., 2011; Wu et al., 2015), which could be

308   delivered to the interaction of high solar radiation and high CO_2. As shown in Li et

309   al.'s (2011) study, the proportion of picoplankton in phytoplankton assemblages

310   increased with distance off the coasts. Therefore, the dominant species at station

311   SEATS are pico- and nano-phytoplankton, but micro-phytoplankton at station D001

312   (Table 3).

313   **4.2 Effects of increased levels of temperature and CO_2 on primary productivity**

314   The seawater volume-specific DPP at station D001 was higher than station

315   SEATS. This should result from both higher chl *a* concentration and chl *a*-specific

316   DPP at D001. It has been shown that smaller cells exist at SEATS than at D001 (Table

317   3). Smaller cells have been considered to have larger DPP, according to Laws's model

318   (1975). The discrepancy between our finding and Laws's model may be due to the

319   availability of nutrients. Laws's model was based on growth rates obtained from the

320   same nutrient level. Nevertheless, the nutrient level at station D001 is higher than at

321   SEATS (Table 3), leading to higher DPP. The higher temperature increased seawater

322   volume-specific DPP at both stations. This could be attributed to more biomass

323   produced at the warmer conditions, as indicated by chl *a*. High temperature enhanced

324   the chl *a*-specific DPP at station SEATS. However, no positive effects of temperature

325   were found on chl *a*-specific DPP at station D001. The differential effects of

326   temperature on chl *a*-specific DPP between the stations may be due to the

327   phytoplankton community composition, since cyanobacteria and/or

328   pico-phytoplankton have the strongest temperature response in terms of

329   photosynthetic carbon fixation compared to micro- and nano-phytoplankton

330   (Andersson et al., 1994). This finding contributes to the explanation of the dominance

331   of pico-phytoplankton in a warmer ocean (Chen et al., 2014; Montagnes and Franklin,

332   2001; Hare et al. 2007; Morán et al., 2010). HTHC reduced chl *a*-specific DPP at

333   station D001, but increased it at station SEATS. High CO_2 also reduced chl *a*-specific

334   DPP in a previous study, which could result from the interaction of high CO_2 and high

335   solar radiation during the summer in the NSCS (Gao et al., 2012b). The reason that

336   HTHC stimulated chl *a*-specific DPP at station SEATS may be due to a dramatic

337   decline in chl *a* concentration under the HTHC treatment.

338   **4.3 Effects of increased temperature and CO_2 on respiration**

339   The dark respiration rate of phytoplankton at station D001 was higher than that at

340   SEATS, regardless of normalizing to seawater or chl *a*. The respiration rate of algae or

341   cyanobacteria usually increases with cell size (López-Sandoval et al., 2014). Station

342   SEATS has more pico-phytoplankton, which would lead to a lower chl *a*-specific dark

343   respiration rate and then lower seawater volume-specific dark respiration, particularly

344   when combined with lower chl *a* level. The higher temperature increased seawater

345   volume-specific dark respiration at both stations, which could be related to increased

346   chl *a* concentration and/or enhanced respiratory carbon loss at the higher temperature.

347   The higher temperature also increased chl *a*-specific dark respiration rate at station

348   SEATS. This is consistent with Butrón et al.'s (2009) study, in which respiration rates

349   of phytoplankton along Nervioń–Ibaizabal estuary showed a positive correlation with

350   temperature. Robarts and Zohary (1987) also found that respiration rate of

351   bloom-forming cyanobacteria was temperature-dependent, with optima over 25 ℃.

352   HTHC reduced seawater volume-specific dark respiration at both stations, which

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353 should be the consequence of the decreased chl *a* in this treatment. The higher

354 temperature increased chl *a*-specific dark respiration rate at station SEATS, but there

355 was no significant difference between HTHC and LTLC, indicating the higher CO_2

356 inhibited the chl *a*-specific dark respiration rate. Similarly, reduced respiration was

357 found in mesocosm studies (Spilling et al., 2016). In theory, higher CO_2 would inhibit

358 respiratory release of CO_2. Nevertheless, enhanced respiration rate at higher CO_2

359 conditions have been found in laboratory-grown diatoms (Wu et al., 2010),

360 coccolithophores (Jin et al., 2015), mixed phytoplankton assemblages (Jin et al.,

361 2015), and macroalgae as well (Zou et al., 2011). Such increased respiration has been

362 attributed to extra energy demand to cope with increased seawater acidity caused by

363 higher CO_2 (Gao and Campbell, 2014; Raven et al., 2014). Therefore, the effect of

364 increased CO_2 on phytoplankton respiration could be due to the combined effects of

365 CO_2 diffusive resistance and seawater acidity stress. Meanwhile, neither higher

366 temperature alone nor HTHC significantly affected chl *a*-specific dark respiration rate

367 at station D001. One possible reason could be that larger cells are less sensitive to

368 CO_2 diffusive resistance and acidic stress due to thicker diffusion boundary layers

369 around the cells. This hypothetical explanation is worthy of future testing.

370 **4.4 Effects of increased temperature and CO_2 on R/P**

371   Laws's model (1975) has suggested that large phytoplankton cells are likely to

372 consume a smaller fraction of their biomass to compete with small phytoplankton

373 cells in terms of the growth rate, considering small cells have higher gross production

374 rates. Our finding that phytoplankton in station SEATS had a higher R/P than station

375 D001 confirms Laws's model. It was theorized that autotrophic respiration is more

376 sensitive to temperature than photosynthesis and the ratio of R/P was predicted to

377 increase with temperature (Ryan, 1991; Woodwell, 1990; Woodwell et al., 1983).

378 However, the assumption that plant respiration is highly temperature dependent was

379 primarily based on short-term (a few minutes or hours) responses of plants to changes

380 of temperature (Gifford, 1994). In long-term experiments (days or months), the

381 increase in respiration with temperature tended to disappear due to acclimation

382 (Gifford, 1995; Jones, 1977; Reich et al., 2016; Slot and Kitajima, 2015; Ziska and

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383 Bunce, 1998). Photosynthetic acclimation to warming is variable (Chalifour and

384 Juneau, 2011; Hancke and Glud, 2004; Schlüter et al., 2014; Staehr and Birkeland,

385 2006). However, a general acclimation response to long-term increased temperature is

386 a rise in optimal temperature of photosynthesis (Gunderson et al., 2010; Kattge and

387 Knorr, 2007; Staehr and Birkeland, 2006). Such shifts in the temperature response of

388 photosynthesis and respiration via physiological acclimation can dampen the increase

389 in R/P at high temperatures, or climate warming would not increase R/P (Reich et al.,

390 2016). In other words, phytoplankton would down-regulate the high sensitivity of

391 respiration to temperature, and maintain a relatively consistent net primary production

392 and hence food web structure in a warming ocean. Although the ratio of R/P did not

393 vary with increased temperature at both stations in our work either, both

394 photosynthesis and respiration were enhanced by the higher temperature. The constant

395 ratio was due to the similar amplitude of increase in photosynthesis and respiration.

396 The incubation period of 7–8 days might not be enough for phytoplankton to

397 acclimate to the increased temperature completely, and therefore the stimulated effects

398 of high temperature on photosynthesis and respiration were still notable. Opposite

399 effects of HTHC on R/P were detected at station SEATS and D001, negative at

400 SEATS and positive at D001. This can be attributed to differential responses of

401 photosynthesis at both stations to HTHC, considering the responses of respiration

402 were similar.

5 Conclusions

404 This study demonstrates that ocean warming expected to occur by the end of the

405 century would simulate the DPP and also dark respiration of phytoplankton

406 assemblages in NSCS, but this positive effect can be damped or offset when ocean

407 acidification is combined. The responses of phytoplankton assemblages locating

408 different areas to ocean warming and acidification could be contrasting due to various

409 phytoplankton compositions and physical and chemical environments. It seems that

410 phytoplankton assemblages in pelagic areas are more sensitive to ocean warming and

411 acidification. More exhaustive investigations are needed to obtain an accurate view of

412 primary production under future ocean environment.

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627
628

629   Table 1. The daytime (12 h) mean solar radiation (PAR, μmol photons m^{-2} s^{-1}) during

630   incubation at stations SEATS and D001.

SEATS		D001	
Date	Solar radiation	Date	Solar radiation
03/08/2012[a]	1454	14/08/2012[a]	1512
04/08/2012	1304	15/08/2012	1480
05/08/2012	1146	16/08/2012	400
06/08/2012	1113	17/08/2012	111
07/08/2012	927	18/08/2012	1520
08/08/2012	1592	19/08/2012	1583
09/08/2012	1582	20/08/2012[b]	1346
10/08/2012[b]	1381	Mean[c]	1136
Mean[c]	1312		

631   [a]The dates for measurements of photosynthetic carbon fixation in situ. [b]The dates for

632   measurements of photosynthetic carbon fixation experiencing temperature and pCO$_2$

633   treatments. [c]Mean represents the average of daytime mean solar radiation over seven

634   or six days microcosm incubation.

635

636   Table 2. Parameters of the seawater carbonate system at different incubation

637   conditions. Measurements and estimation of the parameters were described in

638   Methods. Data are the means ± SD (n = 3). LTLC, low temperature and low pCO$_2$;

639   HTLC, high temperature and low pCO$_2$; HTHC, high temperature and high pCO$_2$.

640   DIC = dissolved inorganic carbon, TA = total alkalinity. Different superscript letters

641   indicate significant differences between treatments within one station.

642

	SEATS			D001		
	LTLC	HTLC	HTHC	LTLC	HTLC	HTHC
Temperature (°C)	30.5±1.0	33.5±1.0	33.5±1.0	28.5±1.0	31.5±1.0	31.5±1.0
pH$_T$	8.07±0.01	8.05±0.01	7.68±0.01	8.02±0.01	8.01±0.01	7.68±0.01
pCO$_2$ (µatm)	390.0±19.5	390.0±19.5	1000.0±70.0	420.0±25.2	420.0±25.2	1030.0±60.0
DIC (µmol kg^{-1})	2056.4±49.2[a]	1986.6±47.1[a]	1999.9±91.4[a]	1969±67.7[a]	1896.5±64.8[a]	2039.1±69.5[a]
HCO$_3^-$ (µmol kg^{-1})	1758.4±47.5[ab]	1681.2±45.4[a]	1838.4±86.5[b]	1719.2±63.7[a]	1640.9±60.7[a]	1882.3±66.4[b]
CO$_3^{2-}$ (µmol kg^{-1})	288.2±1.2[b]	296.2±1.2[c]	138.1±3.3[a]	238.8±3.4[b]	245.3±3.5[b]	131.6±1.7[a]
CO$_2$ (µmol kg^{-1})	9.8±0.5[a]	9.1±0.5[a]	23.5±1.7[b]	11.0±0.7[a]	10.3±0.7[a]	25.2±1.5[b]
TA (µmol kg^{-1})	2443.5±47.9[a]	2387.7±45.8[a]	2170.7±92.0[b]	2294.2±68.6[a]	2260.0±33.9[a]	2199.4±68.5[a]

643

644   Table 3. Physical, chemical, and biological parameters at stations SEATS and D001.

645   SST: seawater surface temperature; N: $NO_3^- + NO_2$ (μmol L^{-1}); P: PO_4^{3-} (μmol L^{-1}).

646   Data of nutrients and phytoplankton composition are derived from literatures.

647

Station	SST	Salinity	pH_T	N	P	Dominant phytoplankton
SEATS	28.7	32.9	8.07	<0.1[a]	<0.01[b]	Pico- and nano-phytoplankton[c]
D001	26.8	33.5	8.03	>1[d]	>0.1[d]	Micro-phytoplankton[e]

648   [a]Du et al. (2013); [b]Wu et al. (2003); [c]Li et al. (2011); [d]Li et al. (2014); [e]Zhang et al. (2014).

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658   **Figure captions**

659   **Figure 1.** Experimental stations in the northern South China Sea.

660   **Figure 2.** Chl *a* concentration in situ (a) and after temperature and pCO_2 treatments in

661   microcosms (b). The microcosm incubations lasted seven days at station SEATS and

662   six days at station D001.The error bars indicate the standard deviations (n = 3). The

663   different letters above the error bars represent significant ($P < 0.05$) differences

664   between stations in panel (a) and between treatments in panel (b).

665   **Figure 3.** Daytime primary productivity (DPP) in situ (a, b) and after temperature and

666   pCO_2 treatments in microcosms (c, d). The microcosm incubations lasted seven days

667   at station SEATS and six days at station D001. The error bars indicate the standard

668   deviations (n = 3). The different letters above the error bars represent significant ($P <$

669   0.05) differences between stations in panels (a, b) and between treatments in panels (c,
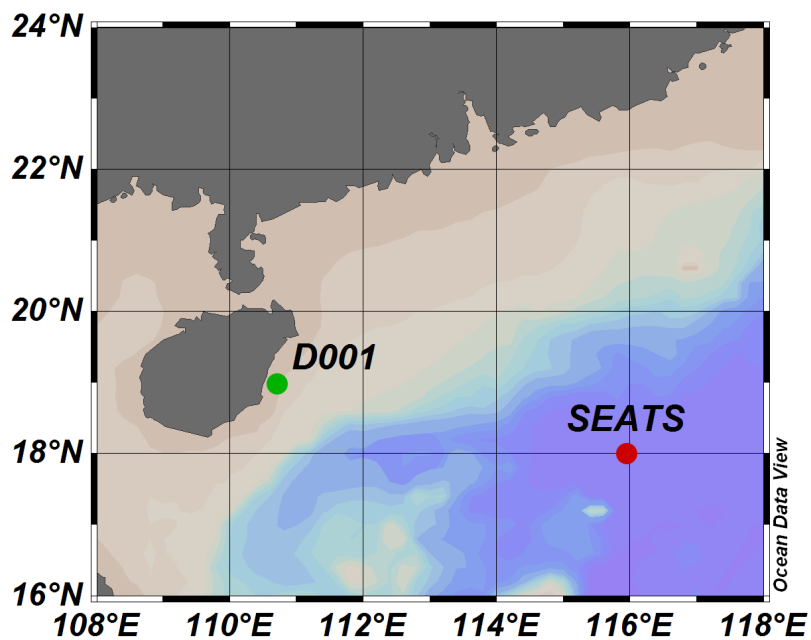
670   d).

671   **Figure 4.** Net primary productivity (NPP) in situ (a, b) and after temperature and

672   pCO_2 treatments in microcosms (c, d). The microcosm incubations lasted seven days

673   at station SEATS and six days at station D001. The error bars indicate the standard

674   deviations (n = 3). The different letters above the error bars represent significant ($P <$

675   0.05) differences between stations in panels (a, b) and between treatments in panels (c,

676   d).

677   **Figure 5.** Dark respiration in situ (a, b) and after temperature and pCO_2 treatments in

678   microsoms (c, d). The microcosm incubations lasted seven days at station SEATS and

679   six days at station D001. The error bars indicate the standard deviations (n = 3). The

680   different letters above the error bars represent significant ($P < 0.05$) differences

681   between stations in panels (a, b) and between treatments in panels (c, d).

682   **Figure 6.** The ratio of respiration to photosynthesis in situ (a, b) and after temperature

683   and pCO_2 treatments (c, d) in microcosms. The microcosm incubations lasted seven

684   days at station SEATS and six days at station D001. The error bars indicate the

685   standard deviations (n = 3). The different letters above the error bars represent

686   significant ($P < 0.05$) differences between stations in panels (a, b) and between
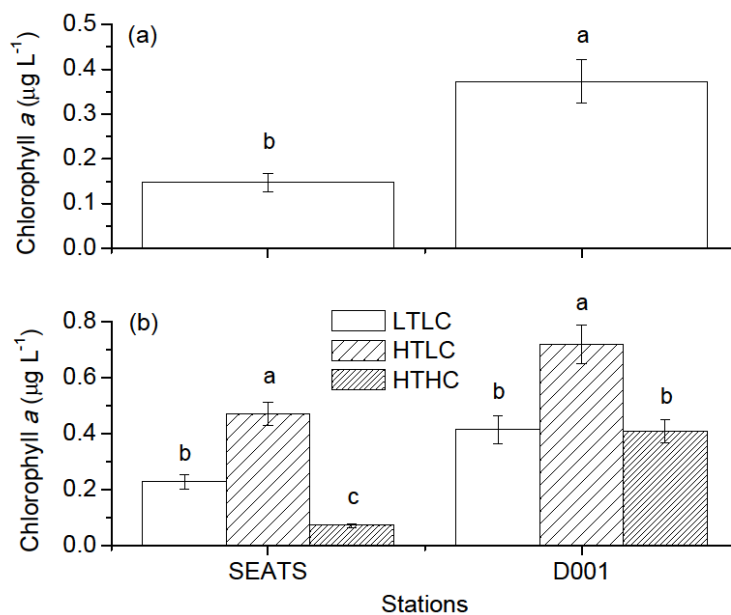
687   treatments in panels (c, d).

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Figure 1

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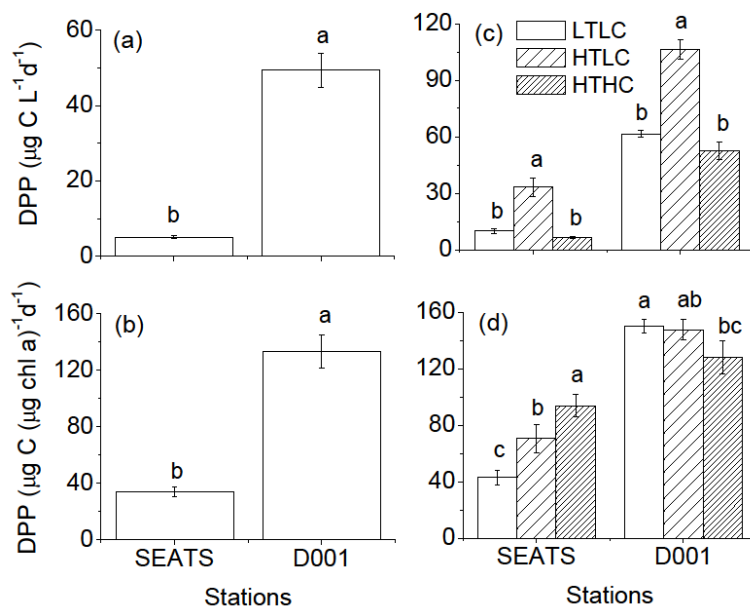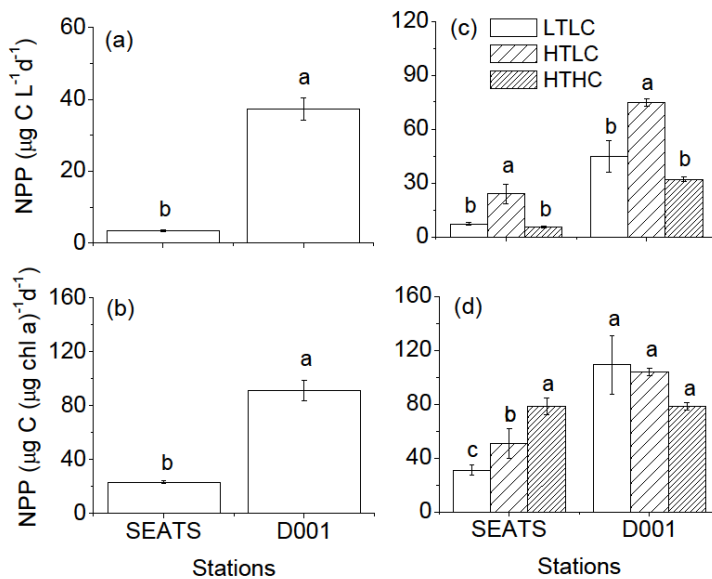695   Figure 2
696

Figure 3

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Figure 4

Figure 5

Figure 6