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Rising levels of temperature and CO_2 antagonistically affect phytoplankton primary productivity in the South China Sea

Yong Zhang[1], Tifeng Wang[1], He Li[1], Nanou Bao[1], Jason M. Hall-Spencer[1,3,4], Kunshan Gao[1,2*]

[1]State Key Laboratory of Marine Environmental Science and College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China

[2]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China

[3]Marine Biology and Ecology Research Centre, University of Plymouth, United Kingdom

[4]Shimoda Marine Research Centre, University of Tsukuba, Japan

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[*]Corresponding author: State Key Laboratory of Marine Environmental Science and College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China

E-mail address: ksgao@xmu.edu.cn

ABSTRACT

Coastal and offshore waters in the South China Sea are warming and becoming acidified due to rising atmospheric levels of carbon dioxide (CO_2), yet the combined effects of these two stressors are poorly known. Here, we carried out shipboard incubations at ambient (398 μatm) and elevated (934 μatm) pCO_2 at in situ and in situ+1.8 ^oC temperatures and we measured primary productivity at two coastal and two offshore stations. Both warming and increased CO_2 levels individually increased phytoplankton productivity at all stations, but the combination of high temperature and high CO_2 did not, reflecting an antagonistic effect. Warming decreased Chl *a* concentrations in off-shore waters at ambient CO_2, but had no effect in the coastal waters. The high CO_2 treatment increased night time respiration in the coastal waters at ambient temperatures. Our findings show that phytoplankton assemblage responses to rising temperature and CO_2 levels differ between coastal and offshore waters. While it is difficult to predict how ongoing warming and acidification will influence primary productivity in the South China Sea, our data imply that predicted increases in temperature and pCO_2 will not boost surface phytoplankton primary productivity.

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

1. Introduction

Rising atmospheric carbon dioxide (CO_2) concentrations are warming and acidifying the oceans worldwide (Caldeira and Wickett, 2003; IPCC, 2014), including the South China Sea (Ji et al., 2017). On average, surface seawater temperatures are projected to increase by 1.51–3.22 ^{o}C by the end of this century and CO_2 levels to increase from the current level of about 400 μatm up to 1000 μatm (Boyd et al., 2015). Ocean warming and acidification are expected to affect the physiology, distribution and structure of phytoplankton communities (Hare et al., 2007; Feng et al., 2009; Taucher et al., 2012; Sommer et al., 2015; Riebesell et al., 2017).

Rising CO_2 levels can increase the availability of dissolved inorganic carbon (DIC) for phytoplankton carbon fixation, but they are also causing seawater acidification, and this may inhibit algal calcification and photosynthetic carbon fixation (Falkowski and Raven, 2007; Gao and Zheng, 2010; Gao et al., 2012; Brodie et al., 2014). Thus, algal responses to increasing CO_2 levels are dependent on the balance between the positive effects of increasing DIC and the negative effects of decreasing pH (Wu et al., 2008; Bach et al., 2015; Liu et al., 2017). Several studies report that, in comparison to current CO_2 levels, elevated CO_2 (800–1000 μatm) increases productivity of phytoplankton assemblages that are dominated by diatoms (Kim et al., 2006; Tortell et al, 2008; Domingues et al. 2014; Engel et al., 2014; Johnson et al. 2015). Others have found that rising CO_2 levels can decrease the productivity of phytoplankton communities dominated by the coccolithophore *Emiliania huxleyi* (Delille et al., 2005;

Riebesell et al., 2017). Paradoxically, an increase in CO_2 concentrations from 385 to 800 μatm decreased the productivity of surface phytoplankton assemblages dominated by diatoms in the South China Sea under natural fluctuating solar radiation (Gao et al., 2012). These discrepancies highlight the fact that the effects of rising CO_2 on C-fixation are dependent on algal community composition as well as regional environmental conditions (Egge et al., 2009; Gao et al., 2012; Celis-Pla et al. 2015; Holding et al., 2015; Hoppe et al., 2018).

On a global scale, by using satellite records and in situ monitoring, rising temperatures have been shown to reduce phytoplankton productivity in the open ocean (Boyce et al., 2010; Siegel et al., 2013), because increased thermal stratification of the water column can starve the algae of nutrients (Doney et al., 2006; Kletou and Hall-Spencer, 2012). In general, it seems that photosynthetic C-fixation increases with increasing temperature, reaches a maximum and decreases thereafter (Beardall and Raven, 2004). Optimal temperatures for C-fixation differ between latitudes and seasons, with small phytoplankton species functioning optimally at higher temperatures than larger species (Daufresne et al., 2009; Finkel et al., 2010; Sommer et al., 2015; Wolf et al., 2017). Carbon fixation was reduced when temperatures were experimentally increased in cold adapted phytoplankton assemblages (Wohlers et al., 2009; Wolf et al., 2017). However, increases from 27 ^{o}C to 30 ^{o}C enhanced photosynthetic C-fixation in incubations of samples of surface phytoplankton assemblages from two stations off China (Gao et al., 2017). Regional differences in 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 CO_2 concentrations were
94 increased from 390 to 690 μatm, C-fixation of a phytoplankton community at 12 ^oC
95 (in situ temperature) decreased in the North Atlantic spring bloom area, whereas at 16
96 ^oC rising CO_2 levels enhanced C-fixation (Feng et al., 2009). Increasing CO_2 levels
97 (from 150 to 300 μatm) combined with rising temperature (from –1 ^oC to 7 ^oC)
98 synergistically enhanced phytoplankton productivity in the European Arctic Ocean,
99 and the positive effect of rising CO_2 on productivity was lower at 6 ^oC than at 1 ^oC
100 (Holding et al., 2015). Furthermore, elevated temperature reversed the positive effect
101 of rising CO_2 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 CO_2 (Coello-Camba et al., 2014; Hoppe et al., 2018). These results
104 show that rising temperature and increasing CO_2 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 CO_2
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

C-fixation rates and Chlorophyll *a* (Chl *a*) concentrations. Our aim was to assess how rising levels of pCO_2 and temperature are likely to affect coastal and offshore productivity in the South China Sea.

2. Materials and methods

2.1. Sampling and culture condition

This study was carried out aboard RV 'Shiyan III' in off-shore and coastal waters of the South China Sea from 11th September to 12th October, 2017 (Fig. 1). Surface seawater (0–2 m) was collected with a 8 L acid-cleaned plastic bucket and stored in a 30 L acid-cleaned polycarbonate tank at 9:00 a.m. to 10:00 a.m., at station S1 (12.99° N, 113.50° E) on September 21, station S2 (14.01° N, 113.01° E) on September 22, station S3 (17.75° N, 110.65° E) on October 2, and station S4 (18.30° N, 111.29° E) on October 3, respectively. Surface seawater at each station was filtered through a 200 μm mesh, and then dispensed into twelve 2 L Nalgene bottles. 1 μmol L^{-1} $NaNO_3$ and 0.5 μmol L^{-1} NaH_2PO_4 was added into the seawater in all treatments to stimulate phytoplankton growth (Chen et al., 2004; Tseng et al., 2005; Celis-Plá et al., 2015).

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

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

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

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

10:30 a.m., 100 mL samples for TA measurements were filtered (GF/F filter) by gentle pressure with 200 mbar in the pump (GM-0.5A, JINTENG). 100 μL saturated $HgCl_2$ solution was added into the TA samples which were stored at 4 °C. TA was measured at 25 °C in the laboratory by potentiometric titration (AS-ALK1+, Apollo SciTech) according to Dickson et al. (2003). Carbonate chemistry parameters were calculated from TA, pH_{nbs}, phosphate, silicate, temperature, and salinity using the CO2SYS (Pierrot et al., 2006).

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

2.3. Chlorophyll *a* analysis

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

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

2.4. Primary productivity measurements

Primary productivity was obtained according to the method described by Gao et al. (2017). On the final day of the incubations, at about 5:00 a.m., subsamples were taken from each incubation bottle, dispensed into two 50 mL quartz tubes placed under a plastic plate which allowed 85% PAR and non UVR transmissions, assuring that the light environment was similar to that of incubations. 5 μCi (0.185 MBq) $NaH^{14}CO_3$ (ICN Radiochemical, USA) was added to the subsamples, which were cultured in the corresponding deck incubators for 12 hrs (from 6:00 a.m. to 6:00 p.m.) and 24 hrs (from 6:00 a.m. to 6:00 a.m. next day) under solar radiation. Subsamples were then filtered onto GF/F glass filters, which were darkly stored at –20 ^{o}C until they were analyzed in the laboratory. Each filter was put into a 10 mL scintillation vial, fumed with HCl for 24 hours to remove inorganic carbon, and dried at 60 ^{o}C for 12 hrs. 3 mL scintillation cocktail (Hisafe 3, Perkin Elmer, Shelton, USA) was added to the vial and the activity of the fixed radiocarbon was measured using a liquid scintillation counting (LS 6500, Beckman Coulter, USA). The activity of photosynthetic C-fixation during 12 hrs incubation was defined to be the day-time primary productivity (DPP), and the photosynthetic C-fixation during 24 hours was considered

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

2.5. Data analysis

Effects of temperature, CO_2 and their interactions on Chl *a*, DPP, NPP and night time respiration rates were assessed by a two-way analysis of variance (ANOVA). The normal distribution of all data was assessed by a Shapiro-Wilk's test, and homogeneity of variance was determined by a Levene's test. A Tukey Post hoc test (Tukey HSD) was performed to show difference between temperature or CO_2 treatments. Statistical analysis was tested by using R and significant difference was indicated by $p < 0.05$.

3 Results

3.1. Incubation temperature, nutrient concentrations and carbonate chemistry parameters

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

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

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

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

3.2. Chl *a* concentration

Chl *a* concentrations in situ were 0.080 μg L^{-1} at station S1, 0.091 μg L^{-1} at station S2, 0.130 μg L^{-1} at station S3, and 0.092 μg L^{-1} at station S4 (Fig. 3). At the end of the incubation, temperature and CO_2 concentration did not significantly affect Chl *a* concentrations at stations S1 and S2, individually and interactively (Table S1; Fig. 3A,B). Elevated temperature significantly reduced Chl *a* concentrations at station S3 at both LC and HC levels (Tukey HSD, both $p < 0.05$), and at station S4 at LC level (Tukey HSD, $p = 0.02$) (Table S1; Fig. 3C,D). By the sixth day of the incubation, Chl *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 CO_2 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 CO_2 (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

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

3.5. Night time respiration

Temperature and CO_2 concentration independently and interactively affected night time respiration rate at station S4, but not at the other stations (Table S1). At S1 and S2, at ambient temperature, night time respiration rates increased significantly at elevated CO_2 (Tukey HSD, both $p < 0.05$, Fig. 6A,B); whereas at high temperature, night time respiration rates were not affected by elevated CO_2 levels (Tukey HSD, both $p > 0.05$). At station S3, at HC, night time respiration rate was enhanced by rising temperature (Tukey HSD, $p = 0.03$) (Fig. 6C); at station S4, at LC, night time respiration rate was enhanced by rising temperature (Tukey HSD, $p < 0.01$) (Fig. 6D).

4 Discussion

Warming and increased CO_2 levels both individually boosted primary productivity in samples of phytoplankton communities taken in nearshore and offshore habitats in the western South China Sea, although these were not all statistically significant increases (Figs. 4; 5). The effect of rising CO_2 on primary productivity and respiration was temperature dependent, and the combination of elevated CO_2 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 CO_2 levels at all stations
290 (Figs. 4; 5), a similar result to that obtained in other experiments using shipboard
291 incubations, mesocosm experiments and CO_2 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 dinoflagelates (*Protoperidinium conicum*) dominated at
296 our inshore stations (Zhang et al., 2014). Rising seawater CO_2 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 CO_2 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 pCO_2 could be due to differences
304 in the phytoplankton communities.

305 Temperature increases of about 2^oC significantly increased phytoplankton
306 assemblage productivity in coastal water at ambient levels of CO_2. This can be
307 expected, since warming is known to increase enzyme activity, and enhance cellular
308 metabolic activity and so improve nutrient or CO_2 uptake (Montagnes and Franklin,

2001; Beardall and Raven, 2004). However, warming did not lead to any increase in night time respiration in coastal water, which might indicate less effect of rising temperature on enzyme activity in our study (Fig. 6), suggesting that increased productivity may be due to more efficient nutrient or CO_2 uptake. Another possible reason for greater primary productivity in the warming treatments may be a shift from predominantly large to mainly small sized algal cells during the incubation (Daufresne et al. 2009; Sommer et al. 2015). Unfortunately, we did not determine the community structure at the end of experiments. However, both ambient and elevated temperature treatments in this study are close to the upper thermal limit for growth of most phytoplankton species (Boyd et al. 2013). In this case, rising temperature is expected to shift community composition and cause an increase in the abundance of small-celled phytoplankton. Small species show stronger temperature responses in terms of their photosynthetic C-fixation compared with large species (Sommer et al., 2015), which may lead to higher productivity in warmer coastal water (Figs. 4, 5).

In the present work, we observed higher night respiratory under HC conditions (Fig. 6) in coastal waters at ambient temperature, this could be due to enhanced energy demand against the acidic stress such as maintaining the cell's homoeostasis (Jin et al. 2015). However, such a respiratory enhancement was not observed at elevated temperature. It is possible that such a level of elevated temperature may increase cellular metabolic activity and periplasmic redox activity that counter-acted the acidic stress. On the other hand, small-sized species seem insensitive to increased pCO_2 in terms of carbon fixation (Tortell et al. 2002; Domingues et al., 2014; Wu et

al., 2014b), and they are highly sensitive to high light intensities that cause severe inhibition of C-fixation (Li et al., 2011). Therefore, these effects might contribute to the observed similar response in primary productivity of offshore-water where small-sized species dominated (Zhong et al., 2013), and also contribute to the low primary productivity of coastal water at warming and acidification treatments with high percentage of small sized species (Figs 4, 5). Gao et al. (2012) reported that rising CO_2 decreased phytoplankton productivity in surface seawater under 90% incident solar radiation in the South China Sea, due to enhanced photoinhibition. Different nutrient concentrations can be responsible for the discrepancy between our study and Gao et al., (2012), because seawater was enriched by 1 μmol L^{-1} $NaNO_3$ and 0.5 μmol L^{-1} NaH_2PO_4 in this study whereas initial DIN and DIP concentration were lower than 0.01 μmol L^{-1} and 0.15 μmol L^{-1}, respectively, in the study of Gao et al. (2012). Rising CO_2 is known to increase primary productivity at high nutrient concentrations, but the additional inorganic carbon does not boost productivity in nutrient limited conditions (Yoshimura et al., 2009; Celis-Plá et al., 2015).

The temperature and CO_2 concentrations of surface oceans are rising simultaneously, but the carbonate chemistry of coastal water is complex, due to the local effects of hydrography, metabolic activity, nutrient input and watershed processes (Duarte et al. 2013). The effects of CO_2 on phytoplankton physiology and productivity has important biogeochemical implications. Increased productivity at elevated CO_2 level could accelerate carbon sequestration of phytoplankton which may increase the CO_2 uptake of coastal seawater from the atmosphere. Decreased

chlorophyll concentrations offshore due to warming may limit biological productivity because phytoplankton are the primary energy source for marine food chains. Our study shows that phytoplankton assemblages in different regions respond differently to increases in CO_2 and temperature. However, if our shipboard tests reflect natural responses, then ongoing warming and acidification in the South China Sea is not expected to increase overall regional primary productivity due to a lack of nutrients in offshore waters. Other environmental factors such as changes in solar radiation, wind-speed induced mixing and deposition of dusts may also affect the primary productivity of phytoplankton communities. Therefore, shipboard incubations during different seasons or with waters influenced by episodic events might lead to differential responses to warming and acidification.

5. Conclusion

The present study shows combined effects of ocean warming and acidification on phytoplankton primary productivity, Chl *a* concentration and night respiration of two coastal and two offshore waters in the western South China Sea. Warming and elevated CO_2 levels individually increased primary productivity, especially in the coastal water. However, the combination of elevated temperature and increased CO_2 did not increase primary productivity at all stations. Different responses in primary productivity, Chl *a* concentration and night respiration to warming and acidification between the coastal and offshore waters may be due to differences in the phytoplankton community composition and in their sensitivity to elevated temperature

375 or CO_2 levels.

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595 Figure Legend

596

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

598 autumn 2017.

599

600 Figure 2. Water temperature in the deck incubators for the low and high temperature

601 treatments during the incubations, and solar radiation.

602

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 ± 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 ± standard deviation (error bar) for three replicates

612

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 ± standard deviation (error bar) for three replicates

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

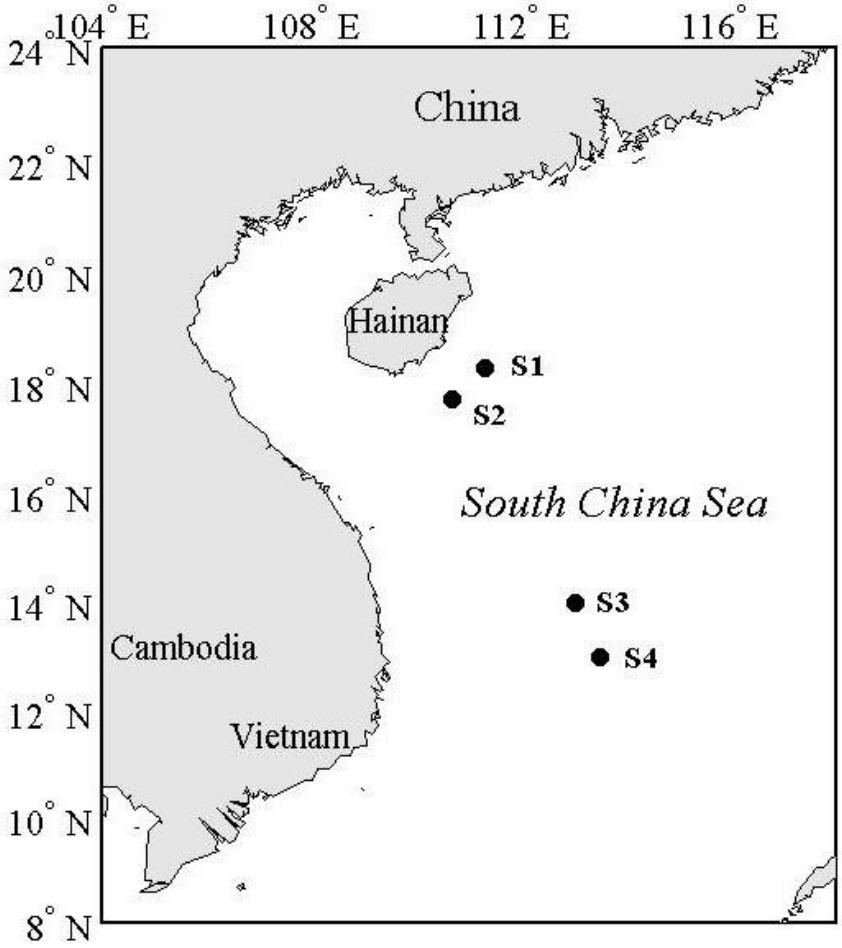
104° E
108° E
112° E
116° E
24° N
22° N
20° N
18° N
16° N
14° N
12° N
10° N
8° N
China
Hainan
S1
S2
South China Sea
S3
S4
Cambodia
Vietnam

Figure 1

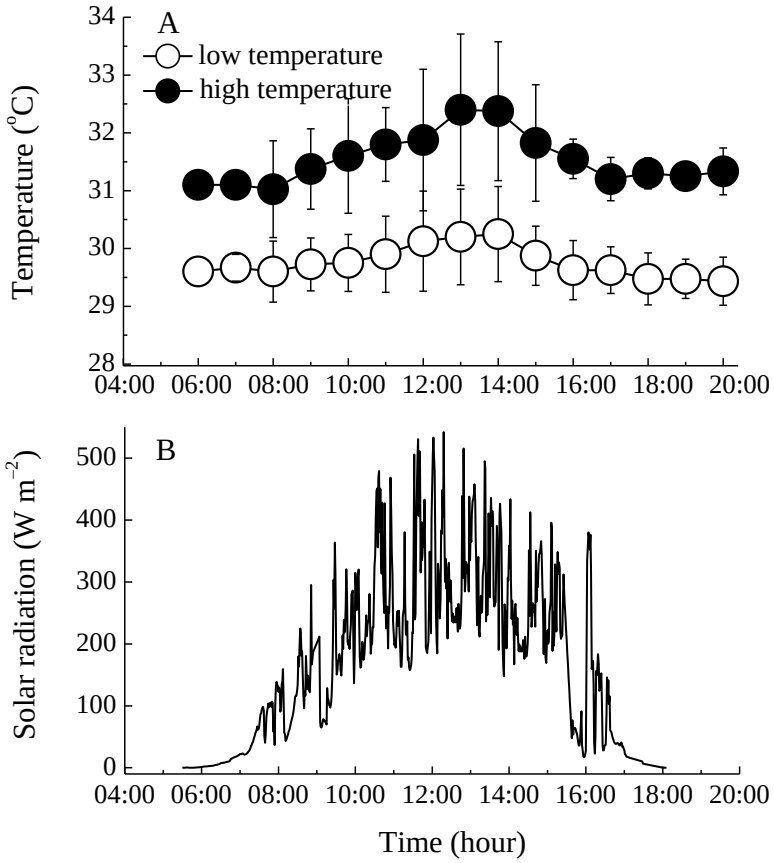
A
low temperature
high temperature
Temperature (°C)
34
33
32
31
30
29
28
04:00 06:00 08:00 10:00 12:00 14:00 16:00 18:00 20:00
B
Solar radiation (W m⁻²)
500
400
300
200
100
0
04:00 06:00 08:00 10:00 12:00 14:00 16:00 18:00 20:00
Time (hour)

Figure 2

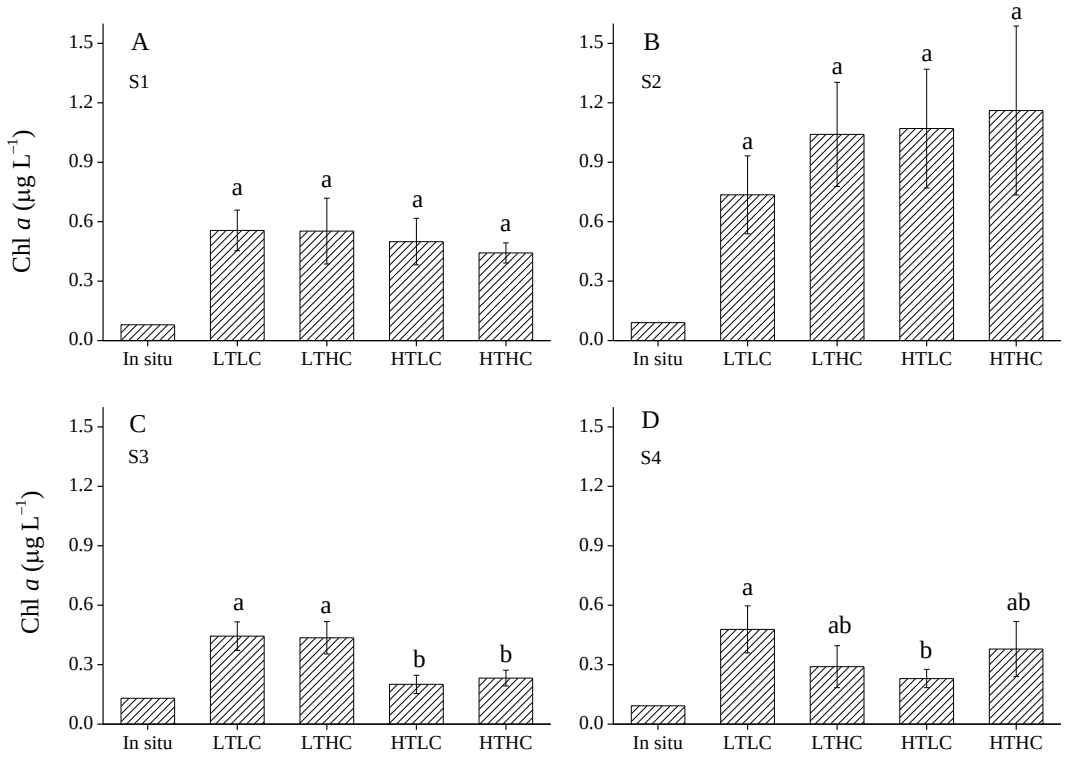
A
S1
B
S2
C
S3
D
S4
Chl a (μg L−1)
1.5
1.2
0.9
0.6
0.3
0.0
a
ab
b
In situ
LTLC
LTHC
HTLC
HTHC

Figure 3

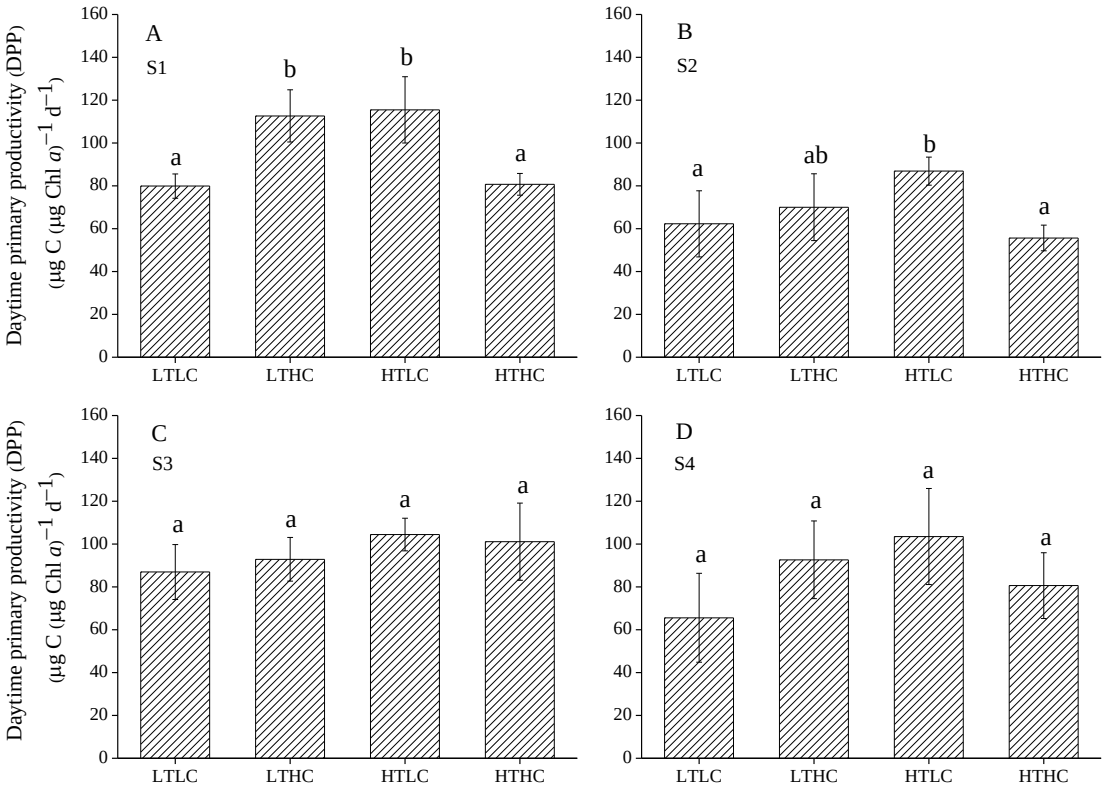
A
S1
B
S2
C
S3
D
S4
Daytime primary productivity (DPP)
(μg C (μg Chl a)$^{-1}$ d^{-1})
0
20
40
60
80
100
120
140
160
a
b
b
a
a
ab
b
a
a
a
a
a
a
a
a
a
LTLC
LTHC
HTLC
HTHC

Figure 4

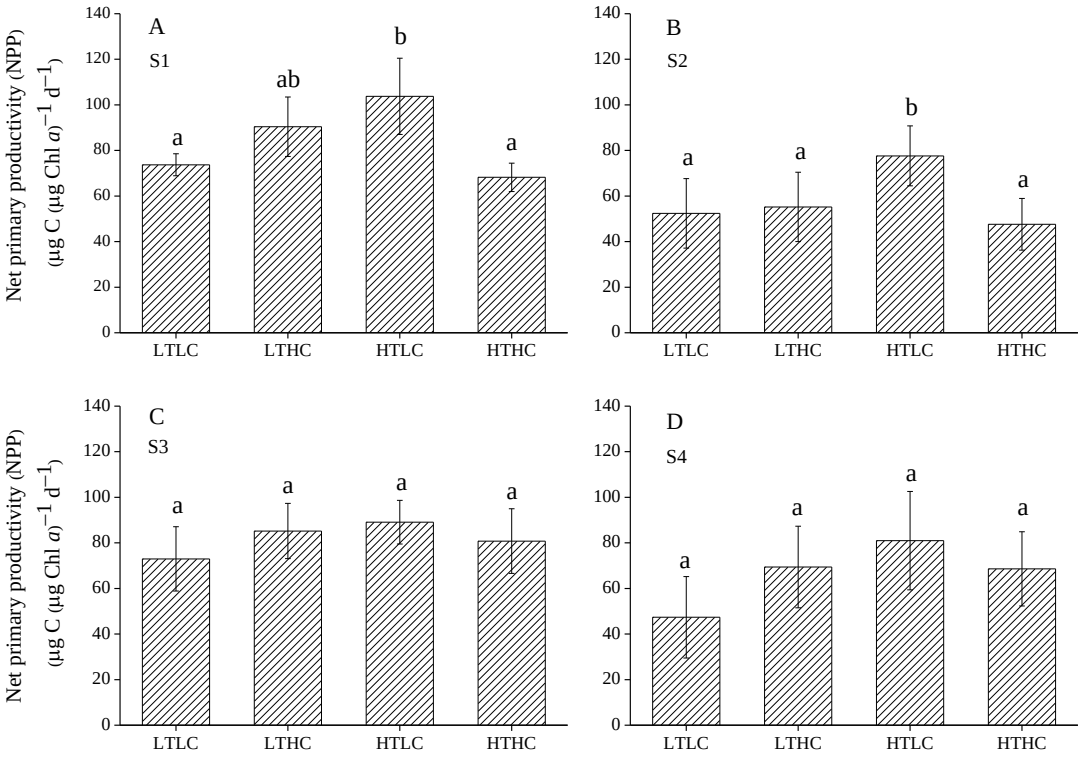
A
S1
B
S2
C
S3
D
S4
Net primary productivity (NPP)
(μg C (μg Chl a)$^{-1}$ d^{-1})
0
20
40
60
80
100
120
140
a
ab
b
LTLC
LTHC
HTLC
HTHC

Figure 5

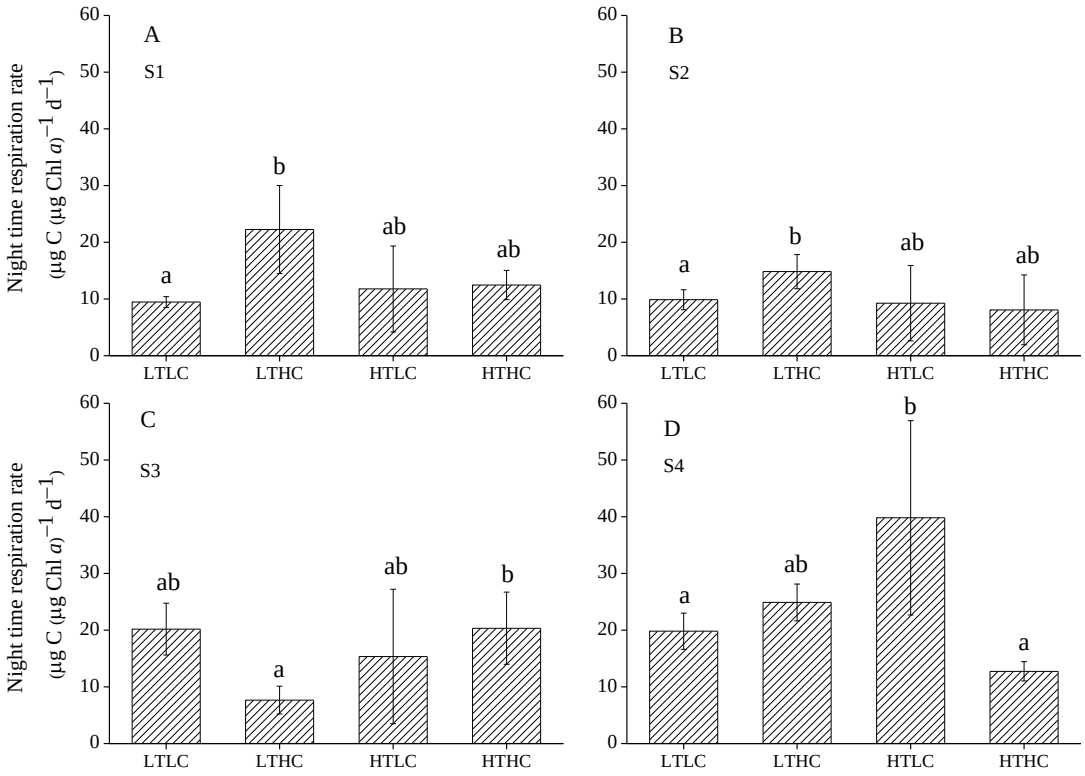
A
S1
B
S2
C
S3
D
S4
Night time respiration rate
(μg C (μg Chl a)$^{-1}$ d^{-1})
LTLC
LTHC
HTLC
HTHC
a
b
ab

Figure 6

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

		DIN (μmol L^{-1})	DIP (μmol L^{-1})
S1	Before culture	1 (0.08)	0.5 (0.17)
	After culture	ND	0.05±0.01
S2	Before culture	1 (0.03)	0.5 (0.21)
	After culture	ND	0.04±0.02
S3	Before culture	1 (0.03)	0.5 (0.14)
	After culture	ND	0.05±0.01
S4	Before culture	1 (0.12)	0.5 (0.16)
	After culture	ND	0.05±0.01

Table 2. Carbonate chemistry parameters of the seawater in the final day of the incubations at different temperature and pCO_2 conditions. TA and pH samples were collected and measured. Different letters (a and b) indicated statistically difference based on Tukey post hoc test. pH_{nbs} means the pH measurements in seawater on the 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

Table S1. Results of two-way ANOVAs of the effects of temperature and pCO_2 on Chl *a*, day-time primary productivity (DPP), net primary productivity (NPP) and night time respiration rate. Temp indicates temperature and significant difference was setup to $p < 0.05$.

Station	Parameter	Treatment	d*f*	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

		Temp × CO_2	1	1.03	0.34
S4	Chl *a*	Temp	1	7.53	<0.05
		CO_2	1	0.005	0.95
		Temp × CO_2	1	7.53	<0.05
	DPP	Temp	1	0.39	0.55
		CO_2	1	0.0001	0.99
		Temp × CO_2	1	5.45	<0.05
	NPP	Temp	1	1.64	0.23
		CO_2	1	0.46	0.56
		Temp × CO_2	1	2.50	0.16
	Respiration	Temp	1	17.01	<0.05
		CO_2	1	17.97	<0.05
		Temp × CO_2	1	28.04	<0.05

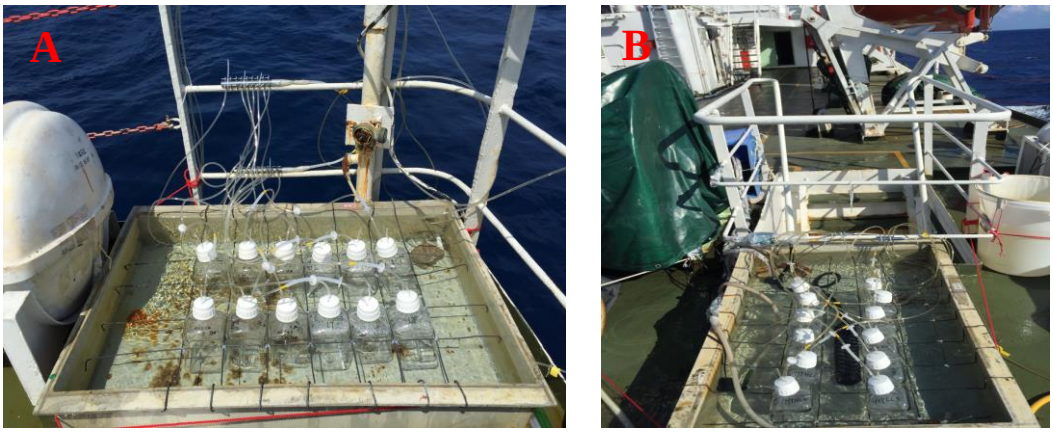

Figure S1. Phytoplankton assemblages were cultured at low temperature (in situ temperature, A) and high temperature (in situ + 1.8 ^{o}C, B) treatments.