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Insensitivities of a subtropical productive coastal plankton community and trophic transfer to ocean acidification: Results from a microcosm study

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ABSTRACT

Ocean acidification (OA) has potential to affect marine phytoplankton in ways that are partly understood, but there is less knowledge about how it may alter the coupling to secondary producers. We investigated the effects of OA on phytoplankton primary production, and its trophic transfer to zooplankton in a subtropical eutrophic water (Wuyuan Bay, China) under present day (400 µatm) and projected end-of-century (1000 µatm) pCO_2 levels. Net primary production was unaffected, although OA did lead to small decreases in growth rates. OA had no measurable effect on micro-/mesozooplankton grazing rates. Elevated pCO_2 had no effect on phytoplankton fatty acid (FA) concentrations during exponential phase, but saturated FAs increased relative to the control during declining phase. FA profiles of mesozooplankton were unaffected. Our findings show that short-term exposure of plankton communities in eutrophic subtropical waters to projected end-of-century OA conditions has little effect on primary productivity and trophic linkage to mesozooplankton.

1. Introduction

The oceans have absorbed approximately one third of the released anthropogenic CO_2 (Sabine et al., 2004; Le Quéré et al., 2013), causing surface water pH to decrease at a rate of 0.019 unit per decade in subtropical waters (Dore et al., 2009), and resulting in ocean acidification (OA). Projections suggest pH decreases of > 0.31 unit by the end of the century, with greater rates expected in higher latitude, lower temperature waters (Gattuso et al., 2015). A wide range of planktonic and benthic organisms will be influenced (Doney, 2010), and these effects may be positive or negative. For microalgae, positive, neutral and negative effects of elevated pCO_2 are reported on diatoms, coccolithophores, cyanobacteria and phytoplankton assemblages in terms of growth, photosynthesis, N_2 and CO_2 fixation, respiration, photoinhibition and calcification (Gao et al., 2012a). As a consequence, progressive OA will likely drive shifts in phytoplankton community structure in ways that are difficult to predict (Gao et al., 2012b; Schulz et al., 2013), but may alter the transfer of energy to higher trophic levels.

The effect of OA on phytoplankton assemblages can result from either bottom-up or top-down mechanisms (Bermúdez et al., 2016a; Cripps et al., 2016). The composition and abundance of microzooplankton and mesozooplankton largely appear not to be directly affected by elevated CO_2 levels (Aberle et al., 2013; Niehoff et al., 2013), however, respiration and grazing rate may increase due to required extra energy to cope with the acidic stress (Li and Gao, 2012). On the other hand, OA may influence production of essential fatty acids (FAs) in phytoplankton, altering their food quality for zooplankton and higher trophic levels (Bermúdez et al., 2016a; Cripps et al., 2016) and may enhance accumulation of toxic phenolics in both microalgae and their grazers (Jin et al., 2015). OA-induced changes of biochemical composition in phytoplankton may also alter the growth and reproduction of zooplankton, leading to changes in top-down pressures on the phytoplankton assemblage. For example, some polyunsaturated fatty acids (PUFAs) cannot be synthesized by higher trophic levels *de novo*, and must be acquired from their diets (Brett and Müller-Navarra, 1997). Diets rich in PUFAs markedly increase zooplankton growth (Müller-Navarra et al., 2000; Brett et al., 2006), and are essential for growth and reproduction of copepods (Jónasdóttir et al., 2009) and fishes (Bell et al., 1986; Henderson and Tocher, 1987), which highlight the importance of PUFAs in marine food webs (Kainz et al., 2004).

Although there were a few studies that have examined the influences of OA across trophic levels, little has been documented on how OA affects the composition of natural phytoplankton assemblages, their FA profiles, and the interactive effects on mesozooplankton (Leu et al.,

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Table 1
The carbonate systems of LC controls and HC treatments at the beginning and end of the experimental period. Data are averages ± standard deviation (n = 3) and letters followed the numeral showed significance between treatments and phases.

Treatments		pH_{NBS}	TA μmol L^{-1}	DIC μmol L^{-1}	pCO_2 μatm	CO_3^{2-} μmol L^{-1}
Day 2	LC	8.00 ± 0.01[a]	2078.7 ± 28.8[a]	1944.0 ± 33.1[a]	610.1 ± 32.4[a]	104.6 ± 1.8[a]
	HC	7.82 ± 0.02[b]	2120.9 ± 1.9[a]	2045.6 ± 7.5[ab]	992.1 ± 50.1[b]	73.1 ± 3.0[b]
Day 7	LC	8.11 ± 0.03[c]	2070.1 ± 112.3[a]	1895.9 ± 107.2[ac]	461.8 ± 43.1[c]	129.4 ± 9.4[c]
	HC	7.83 ± 0.01[b]	2140.7 ± 5.4[a]	2062.5 ± 2.2[ab]	972.8 ± 21.9[b]	76.3 ± 1.8[b]

2013; Bermúdez et al., 2016a). The studies to date have been done mainly in high latitude waters (Leu et al., 2013; Bermúdez et al., 2016a, 2016b), with no studies, to the best of our knowledge, investigating these effects in low latitude, subtropical coastal waters. Wang et al. (2017) studied OA effects on an artificially inoculated phytoplankton assemblage in a eutrophic water in the South China Sea (24.52°N, 117.18°E, Wuyuan Bay, Xiamen, China). They found higher PUFAs and monounsaturated fatty acids (MUFAs) in phytoplankton with increased pCO_2 during late exponential phase, while FA contents in mesozooplankton fed algae from these treatments remained constant.

The effects of OA on whole plankton systems in higher latitude coastal systems have been investigated using large mesocosm facilities. Of these studies, OA impacts on planktonic biological or biochemical processes occurred in different ways in these regions. A recent summary of mesocosm studies found that positive or neutral effects of OA were observed for dinophyceae and picoeukaryotes, while negative effects were found for other groups (Gazeau et al., 2017). The stoichiometric ratios (Burkhardt and Riebesell, 1997; Urabe et al., 2003; Riebesell et al., 2007) of phytoplankton and their biochemical composition of nutritional FAs can also be altered by high pCO_2 (Carvalho and Malcata, 2005; Hu and Gao, 2006; Bermúdez et al., 2015). In temperate waters off Korean coast, little or positive effects of OA were found for diatoms and nano-eukaryotes (Kim et al., 2006; Kim et al., 2013). Although microzooplankton was present in these studies, their responses were not reported.

While most OA mesocosm studies to date have been conducted in relatively pristine coastal regions, many coastal waters near large population centers are experiencing faster rates of OA than pristine coastal regions, shelf regions, or open ocean waters due to increased nutrient flux from the terrestrial environments and subsequently enhanced respiration (Cai et al., 2011), yet there is even less known about OA effects on biological processes in these planktonic systems. A recent series of OA mesocosm experiments were carried out with artificial phytoplankton communities in eutrophic Chinese coastal waters (N. Liu et al., 2017; X. Liu et al., 2017). Wang et al. (2017) found decreased mesozooplankton grazing rates when fed with phytoplankton from acidified mesocosms compared to the controls. However, this artificially inoculated assemblage makes it difficult to extrapolate these findings to natural waters without similar testing of natural coastal phytoplankton assemblages adapted to these conditions.

Here we present our results of an *in-situ* short-term 30-L microcosm study in a subtropical coastal eutrophic bay (Wuyuan bay, Xiamen, China) with the aim to investigate the impacts of OA on primary producers and their consumers as well as their trophic transfer. In this system, phytoplankton, microzooplankton and mesozooplankton were considered. Under an acidified level of 1000 μatm pCO_2, the growth and carbon fixation of phytoplankton assemblages, grazing rates of micro-/*meso*zooplankton assemblages and the nutritional FAs of both phytoplankton and mesozooplankton were compared with those parameters under present day level (400 μatm) of pCO_2. The results show the complex effects of short-term ocean acidification and the challenges of studying OA effects in these subtropical eutrophic coastal areas in terms of relative unstable and unmanageable environmental conditions.

2. Materials and methods

2.1. Experimental design

The *in-situ* microcosm experiment was performed in a subtropical coastal water on the Facility for Ocean Acidification Impacts Study of Xiamen University (FOANIC- XMU) (24.52°N, 117.18°E) in winter (25th December 2014 to 1st January 2015). The facility, situated in the center of Wuyuan Bay, was fully solar-powered for all instruments on board with 200 m^2 of workspace and nine 4-m^3 mesocosms on its southern side (http://mel.xmu.edu.cn/en/facility.asp?id = 33). More details of FOANIC-XMU were reported in X. Liu et al. (2017). *In-situ* surface seawater was collected into six 30-L water-jacketed microcosms in the evening through a mesh (160 μm) to remove meso-/macrozooplankton. The cultures were grown under full sunlight and temperature was maintained by circulating surface seawater (0.5 m) through the water-jacket. The microcosms, made of polymethyl methacrylate, allowed 91% photosynthetically active radiation, 63% ultraviolet-A, and 6% ultraviolet-B transmissions (Gao et al., 2012b).

A CO_2 enricher (CE-100D, Wuhan Ruihua Instrument & Equipment Ltd., China) was used to manipulate the carbonate system by bubbling through a diffuser at the bottom of the microcosm using air for the control (LC, 400 μatm) and elevated pCO_2 (HC, 1000 μatm) for the treatment at a continuous flow rate of 1 L min^{-1}. Target CO_2 concentrations, calculated from pH and total alkalinity (TA) by using CO2SYS (Lewis et al., 1998), were reached after 6 h of aerating. The pCO_2 in the eutrophic surface water was 600 μatm at the time of collection, and bubbling with air for the control lowered this to 460 μatm (Table 1). Bubbling began in the early morning after water was collected and was continuous through the 7-day experiment.

The temperature and pH_{NBS} (National Bureau of Standards) (CS1030 3-in-1 pH sensor, PH200, CLEAN Instruments, 2014, China) was measured each day between 08:00–10:00 am. Nitrite, nitrite plus nitrate, phosphate and silicate were determined colorometrically using a four-channel flow injection analyzer (AA3, Bran Luebbe, Germany). Nitrate plus nitrite was measured by reducing nitrate to nitrite with an on-line Cd coil. TA was determined using Gran titration on a 25-mL sample with a Kloehn digital syringe pump at Day 2 and Day 7 (Cai et al., 2004). Photosynthetically active radiation (PAR) intensity was recorded (Eldonet XP, Real Time Computer, Germany) during the experimental period.

2.2. Phytoplankton responses

2.2.1. Phytoplankton biomass, identification, and apparent growth rates

Daily samples (200 mL) for chlorophyll *a* (Chl *a*) analysis were filtered onto a Whatman GF/F glass fiber filters and the filters extracted with 100% methanol at 4 °C in darkness for 24 h (Li et al., 2016). The extracts were centrifuged (5000 *g* for 10 min; Universal 320R, Hettich, Germany) and the supernatant absorption measured (400–700 nm; DU800, Beckman, Fullerton, California, USA). Chl *a* concentrations were calculated following the method of Porra (2002). For phytoplankton identification, sub-samples were fixed with Lugol's iodine (2%) and examined under 200 or 400 magnification (TS100, Nikon, Japan). The apparent growth rates of phytoplankton were calculated as

slope of liner regressions of ln (*Chl a*) during exponential phase (Day 0–4).

2.2.2. Net primary production

Seawater samples taken from each microcosm at Day 3 were dispensed into 50-mL quartz tubes, inoculated with 5 μCi (0.185 MBq) $NaH^{14}CO_3$ (ICN Radiochemicals, USA) and then incubated for 24 h under the same ambient light and temperature as the microcosms. The incubation temperature of each treatment was the same as the corresponding microcosm treatment. After incubation, the cells were filtered onto Whatman GF/F glass fiber filters and immediately frozen and stored at −20 °C for later analysis. The frozen filters were placed individually into 20-mL scintillation vials, exposed to HCl fumes for 12 h, then filters were dried under 55 °C for 6 h. Scintillation cocktail (Perkin Elmer®) was added (3 mL) to each vial and the samples were counted by liquid scintillation (LS 6500, Beckman Coulter, USA). Carbon fixation for 24 h was taken as daily net primary productivity (NPP).

2.3. Zooplankton responses

2.3.1. Microzooplankton grazing rates and phytoplankton instantaneous growth

Microzooplankton grazing rates were measured on Day 3 using the dilution method. Dilution method enables the calculation of instantaneous growth rates of phytoplankton. Unfiltered volumes of the two treatments were diluted (100%, 75%, 50%, and 25%) to 1000 mL in polycarbonate bottles using target pCO_2 (400 or 1000 μatm) bubbled filtered (0.22 μm) surface waters, triplicate for each gradient. Changes in Chl *a* were measured after incubation for 24 h under the same light and temperature as the experimental cultures. The microzooplankton grazing rates and phytoplankton instantaneous growth rates were calculated from the slopes and intercepts of liner regressions of $\ln(Chl\ a_t/Chl\ a_{t0})/(t\text{-}t_0)$ as a function of dilution gradients, respectively (Landry and Hassett, 1982).

2.3.2. Mesozooplankton grazing rates and identification

Mesozooplankton grazing rates on subsamples of the treatment cultures were measured from Day 3 to Day 5. Mesozooplankton, dominated by calanoid copepods, were collected from Wuyuan Bay seawater adjacent to the FOANIC-XMU during the evening of Day 2 by horizontal plankton net trawls (160 μm mesh size) at depth of 0–1 m. The net samples were well mixed and 200 mL of aliquoted samples injected into 121,000-mL polycarbonate bottles (~200 individuals/per bottle, 6 for LC and 6 for HC) containing phytoplankton from each replicate microcosm and were placed into a flowing seawater bath under natural solar light for 12 h. Two replicates and one control (without mesozooplankton) were set for each microcosm. All bottles were gently mixed by multiple inversions several times over the incubation. Grazing rates were calculated from the changes in Chl *a* concentrations over time according to Frost (1972). A subset of the initial samples were fixed (Lugol's iodine) for mesozooplankton identification and examined under 10 or 20 magnification (TS100, Nikon, Japan).

2.3.3. Potential trophic transfer

The potential trophic transfer from phytoplankton to zooplankton was estimated based on the measured grazing rates and initial phytoplankton biomass. For microzooplankton, daily consumption proportion was estimated from the dilution experiments as described by Calbet and Landry (2004). Mesozooplankton daily consumption was recalculated to adjust to the *in-situ* phytoplankton biomass by using the relationship between grazing rates of copepod (*Calanus pacificus*) and diatom (*Thalassiosira fluviatilis*) concentration (Frost, 1972, 1975). Chl *a* cellular quota of *T. fluviatilis* was taken from literatures (Conover, 1975; Laws and Bannister, 1980).

2.4. Fatty acid content and taxonomic markers

For phytoplankton FA determination, 200–500 mL water samples were filtered onto a pre-combusted GF/F glass fiber filters and stored at −20 °C until analysis. An internal standard (nonadecanoic acid methyl ester, Sigma, America) was added to each sample before the filters were extracted in chloroform: methanol (2:1, *v*/v). The FAs were methyl esterified with 5% HCl in methanol at 85 °C for 1 h and the resulting fatty acid methyl esters (FAMEs) extracted with hexane. FAMEs were analyzed by GC/MS (thermos TRACE 1310 GC-ISQ QD MS). More details of FA measurements were reported in Wang et al. (2017).

For mesozooplankton FA determination, the collected mesozooplankton samples were distributed equally into 18 tanks (500 mL, 9 LC controls and 9 HC treatments) (~ 240 individuals/tank) fitted with a solid cover to reduce gas exchange and lower light exposure, and held at ambient air temperatures (~14 °C). Fresh aliquots of microcosm water containing the phytoplankton community, maintained at the target pCO_2 levels, were replaced (50%) each day during the 3-day feeding experiment (3 tanks for each microcosm). After 3 days of cultivation (Day 3–5), mesozooplankton samples from 12 of the 18 LC/HC tanks were rinsed on a mesh (160 μm) with filtered seawater, collected onto pre-combusted GF/F filters, and then stored at −20 °C for FA analysis (6 for HC and 6 for LC). Mesozooplankton from the other 6 tanks (3 for LC and 3 for HC) were collected for dry weight measurements.

The FAs as taxonomic markers, though lack species specificity, were used here to identify diatoms (C16:1n7 + C20:5n3), flagellates (C22:6n3 + C18:4n3), and chlorophytes (C18:2n6 + C18:3n3), while odd numbered FAs (*e.g.*, C15:0) were used as bacterial biomarkers (modified from Dalsgaard et al., 2003).

2.5. Statistical analyses

Liner regression was used to calculate the apparent growth rate and instantaneous growth rates of phytoplankton and microzooplankton grazing rate. One-way ANOVA was used to test the phytoplankton growth, NPP, and zooplankton grazing between treatments. Two-way ANOVA and Principal Component Analysis (PCA) were used to test the FA composition and content in different phase between treatments. The carbonate system was calculated based on TA and pH by using CO2SYS (Lewis et al., 1998). Tukey multiple comparison was used to analyse the physico-chemical parameters at different times between treatments. Data are averages ± standard deviation in triplicate. All the statistical analyses were run by SPSS (PASW Statistics for Windows, Version 18.0, Chicago: SPSS Inc), and figures were graphed by Origin (OriginPro 8 SR0, Northampton: OriginLab Corporation).

3. Results

3.1. Physico-chemical conditions

The daily average PAR intensities ranged from 125.6 to 603.8 μmol m^{-2} s^{-1} during the experimental duration (Fig. 1A). The temperature remained stable (16.5 ± 0.2 °C and 16.5 ± 0.2 °C for LC and HC, respectively) during the whole experiment period (Fig. 1B). The carbonate system was well controlled over the first two days, with pCO_2 of 610 ± 34 μatm and 992 ± 50 μatm for LC and HC treatments, respectively (Table 1), and was well maintained for biological systems through to the end of the experiment (pCO_2 of 461 ± 43 μatm and 972 ± 21 μatm for LC and HC microcosms, respectively). The difference in pH between the LC and HC treatments was ~0.26 pH on Day 2, and this difference ranged from 0.18 ± 0.02 to 0.40 ± 0.02 over the course of the experiment (Fig. 1C). The diurnal pH changes were measured on Day 4, and showed a trend of increasing pH over the daylight hours. The difference between HC and LC treatments increased from morning (0.35 ± 0.01) to evening (0.63 ± 0.02) (Fig. 1D).

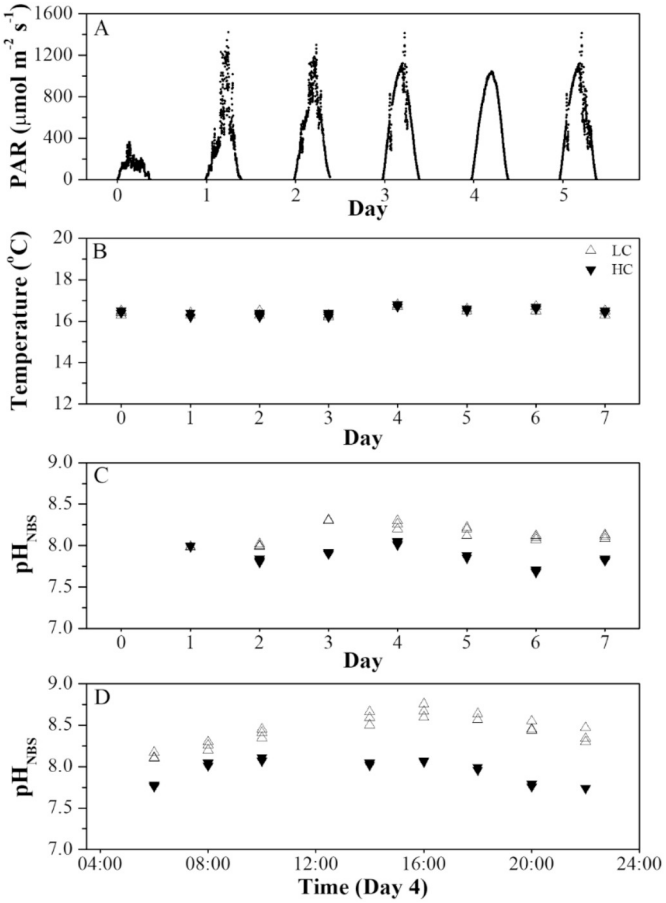

Fig. 1. The physicochemical conditions of microcosms during the experimental period; (A) photosynthetically active radiation (PAR), (B) temperature and (C) pH_{NBS} during the experiment days and (D) diel pH_{NBS} variation measured between 0800 and 0900 on Day 4.

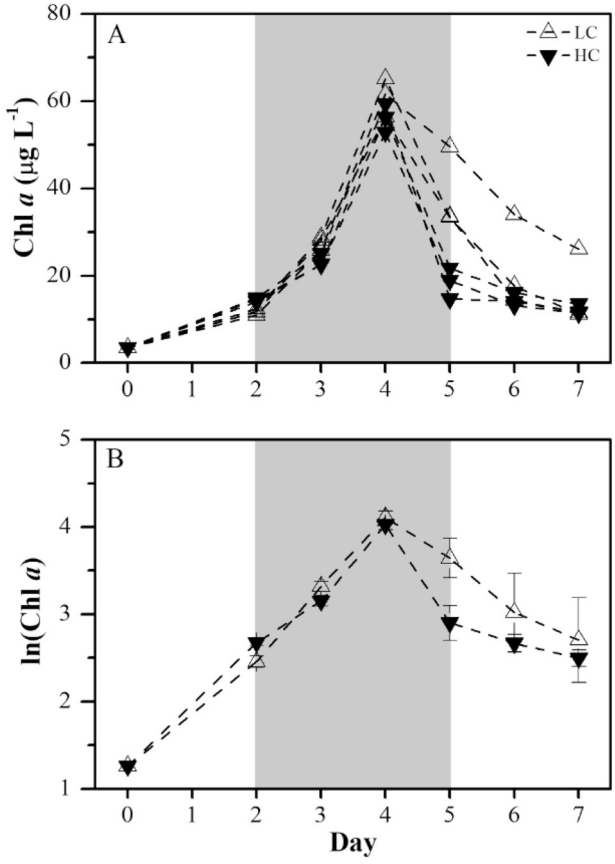

Fig. 2. (A) Chl *a* concentrations in each microcosm and (B) Chl *a* concentrations in *ln* scale. Data are averages ± standard deviation, $n = 3$. The gray area shows the duration of mesozooplankton feeding experiment.

The initial microcosm water (Day 0) contained high concentrations of inorganic nutrients; ~54 μmol L^{-1} for N (nitrate and nitrite), ~2 μmol L^{-1} for P (phosphate), and ~26 μmol L^{-1} for Si (silicate) (Table 2). Over the course of the experiment there was almost complete drawdown of N and Si in both treatments, while P remained at very low concentrations (0.18 ± 0.07 μmol L^{-1} and 0.07 ± 0.02 μmol L^{-1} for LC and HC, respectively) (Table 2).

3.2. Phytoplankton responses

The phytoplankton growth responses were divided into two phases; an exponential growth phase followed by a decline phase (Fig. 2A). High pCO_2 significantly affected the growth of phytoplankton (two-way ANOVA, $CO_2 \times$ Time, $F = 13.74$, $p < 0.01$, $n = 3$), and lower apparent growth rates were observed under HC treatments (HC: 0.82 ± 0.02 d^{-1}, LC: 0.77 ± 0.02 d^{-1}, one-way ANOVA, $F = 10.90$, $p = 0.03$, n = 3). However, there were no significant differences in the maximum Chl *a* biomass between the control and high pCO_2 treatment (one-way ANOVA, $F = 2.43$, $p = 0.19$, $n = 3$).We also estimated the instantaneous growth rates by using dilution method, and our data showed significant higher instantaneous growth rates when compared with apparent growth rates (LC: 1.85 ± 0.43 d^{-1}: HC: 2.09 ± 0.31 d^{-1}) with no significant differences between LC and HC treatments (one-way ANOVA, $F = 0.64$, $p = 0.47$, n = 3). Similarly, net primary production did not differ significantly between treatments (LC: 51.6 ± 13.8 μg C μg-Chl a^{-1} d^{-1}; HC: 42.1 ± 6.2 μg C μg-Chl a^{-1} d^{-1}) (Fig. 3C).

The FA biomarkers were used to indicate the relative phytoplankton community structures by comparison of their percentage of the total FA content. We found that diatoms, chlorophytes, and flagellates occurred at the beginning in both treatments in terms of the FA biomarkers, followed by an increase in diatoms (from 9.5% to 24.7% on average) and a decrease almost to null in flagellates and chlorophytes (Fig. 4A, B, C). Bacteria biomarkers in contrast remained relatively stable over the course of the experiment (Fig. 4D). However, there were no significant differences in taxonomic biomarkers between LC and HC treatments. These findings were consistent with the microscopic analyses, which showed that diatoms (dominant species: *Skeletonema costatum* and *Chaetoceros* spp.) completely dominated at the end of the experiment.

Table 2
The nutrients concentrations of LC controls and HC treatments at the beginning and end of the experimental period. Data showed averages ± standard deviation (n = 3) and letters followed the numeral showed significance between treatments and phases.

Treatments		NO_3^- (μmol L^{-1})	NO_2^- (μmol L^{-1})	P(μmol L^{-1})	Si(μmol L^{-1})
Day 0		49.23 ± 0.28^a	5.02 ± 0.00^a	2.08 ± 0.01^a	26.52 ± 0.07^a
Day 7	LC	0.12 ± 0.06^b	0.07 ± 0.02^b	0.18 ± 0.07^b	0.22 ± 0.30^b
	HC	0.05 ± 0.01^b	0.06 ± 0.01^b	0.07 ± 0.02^c	0.17 ± 0.12^b

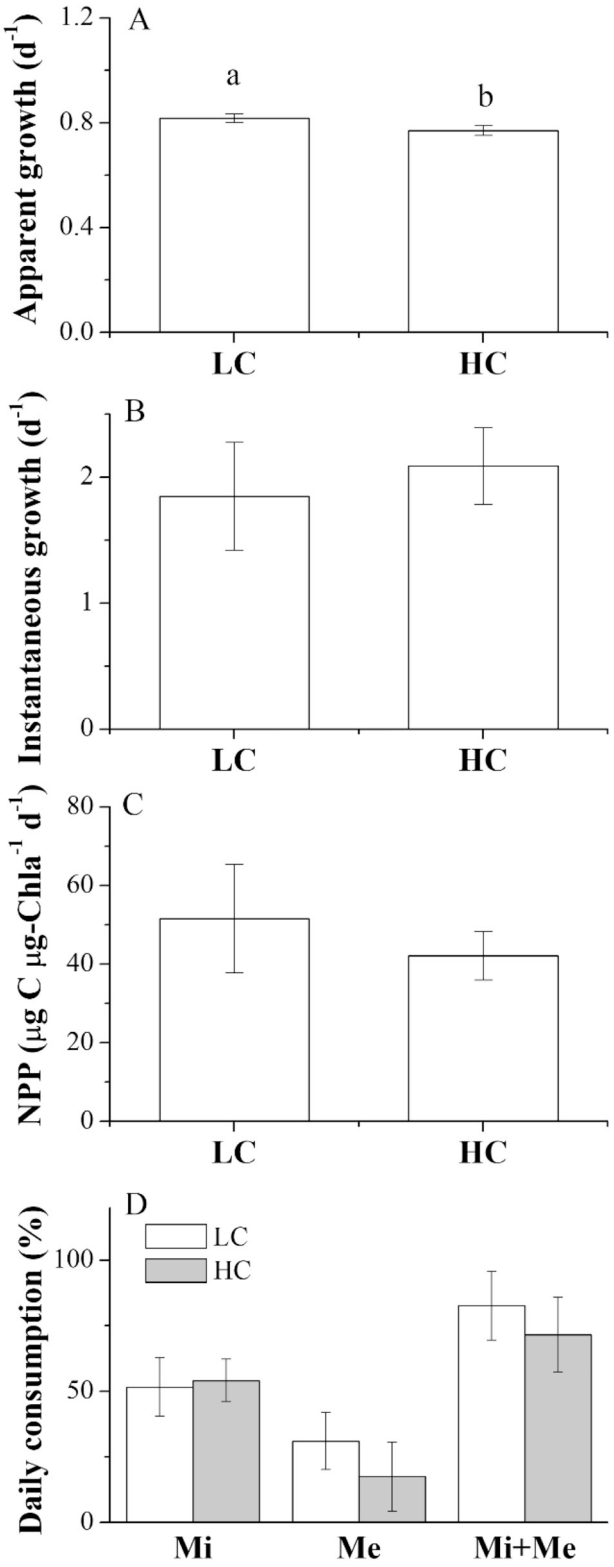

Fig. 3. The (A) apparent growth rates between Day 0 and Day 4, (B) instantaneous growth rates on Day 3, (C) net primary production (NPP) on Day 3. Different letters above the bars show significant difference between treatments. And (D) percentages of microzooplankton (Mi) and mesozooplankton (Me) consumption to daily primary production in LC controls and HC treatments. Data are averages ± standard deviation, n = 3.

3.3. Zooplankton grazing and identification

Our results showed that zooplankton consumed almost 80% of daily primary production (Fig. 3D). Microzooplankton (dominated by *Mesodinium rubrum* and other ciliates) grazing rates for LC and HC were 0.99 ± 0.41 d^{-1} (~52% of daily PP) and 1.15 ± 0.32 d^{-1} (~54% of daily PP), respectively, with no significant differences between LC and HC treatment (one-way ANOVA, F = 0.29, $p = 0.62$, $n = 3$) (Table 3). Mesozooplankton (dominated by calanoid copepods) (Jin et al., 2015) grazing rates for LC and HC treatments were 2.16 ± 0.92 μg Chl *a* $ind^{-1} h^{-1}$ (~31% of PP) and 1.72 ± 1.34 μg Chl *a* $ind^{-1} h^{-1}$ (~17% of PP), respectively, and no significant differences were detected between treatments (one-way ANOVA, F = 0.44, $p = 0.52$, n = 3) (Table 3).

3.4. Nutritional fatty acids

Total fatty acids (TFAs), saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) showed similar patterns, which showed little change during the exponential growth phase followed by a slight increase during decline phase (Fig. 5A, B, D). The trends in polyunsaturated fatty acids (PUFAs) and decosahexaenoic acid (DHA) concentrations were much the opposite, with either low (PUFAs) or generally decreasing (DHA) concentrations during the 7-day experiment (Fig. 5C, F). Increases in SFA contents coincided with increased diatom markers, while DHA decreased with decreased flagellate markers. Significant higher SFA contents were found in HC treatments (two-way ANOVA, CO_2 × Time, F = 6.78, $p = 0.016$, n = 3) during the decline phase. The principal component analysis (PCA) showed that the FA profiles of phytoplankton changed significantly with the culture time (Fig. 6).

The mesozooplankton FAs after feeding on phytoplankton from matched microcosms for 3 days were characterized by high SFA composition (75.97 ± 5.95% for LC and 74.65 ± 4.25% for HC) and low PUFA composition (12.96 ± 5.99% for LC and 14.37 ± 3.48% for HC) (Table 4). No significant differences between LC and HC treatments were observed in neither the percent composition nor concentration of zooplankton FAs (one-way ANOVA, $p > 0.05$, n = 3). The PCA showed that the FA profiles of mesozooplankton differed with that of phytoplankton (Fig. 6), which mainly due to their high scores in PC1.

4. Discussion

4.1. Phytoplankton responses

Ocean acidification presents multiple challenges and advantages to marine phytoplankton, leading to increased or decreased competitive advantages among taxa that can shape different plankton assemblages. Our study is the first to investigate the impact of OA on a natural plankton community in culturally eutrophic waters, and their potential consequences on zooplankton feeding rates. The short-term study focused on determining whether elevated pCO_2 generated acute responses in either the phytoplankton or zooplankton, and therefore it may not reflect the potential impacts that could develop after acclimation of the plankton community to longer term exposure to elevated pCO_2.

The phytoplankton assemblages in Wuyuan Bay are dominated by diatoms and surface water here have persistently high dissolved nutrients (Table 2) and are exposed to high solar radiation (Fig. 1A). As a consequence, there were rapid growth rates of phytoplankton in all microcosms (Fig. 3A, B) during the first 4 days of the experiment, after which decreases in nutrient availability would have limited growth rates (Table 2). The maximum Chl *a* concentrations (Fig. 2), instantaneous growth rates (Fig. 3B), net primary production (Fig. 3C) and taxonomic biomarkers (Fig. 4) did not differ between HC and LC treatments. These findings differed from experiments at two sites in the northern region of the South China Sea, where at lower starting nutrient

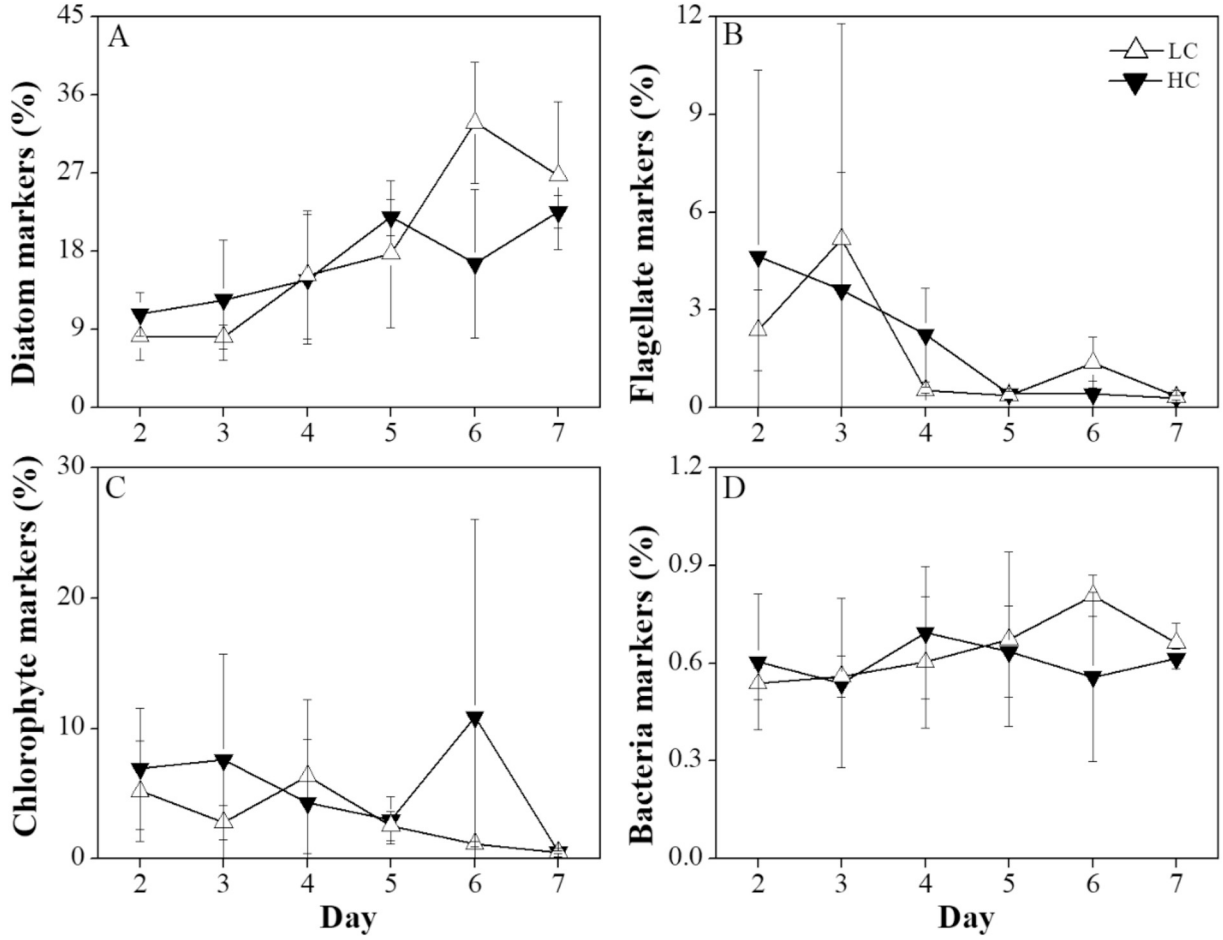

Fig. 4. The fatty acid biomarkers percent contents of (a) diatom, (b) flagellate, (c) chlorophyte, and (d) bacteria in the LC/HC microcosms. Data are averages ± standard deviation, n = 3.

Table 3
Microzooplankton and mesozooplankton grazing experiment results of LC controls and HC treatments. The dominant mesozooplankton taxa were *Acartia pacifica* (60%), *Paracalanus* sp. (20%), *Schmackeria* sp. (15%), and Cirriped and Ascidian larvae (5%) Data are averages ± standard deviation (n = 3).

		LC	HC
Microzooplankton	Grazing (d^{-1})	0.99 ± 0.14	1.15 ± 0.32
Mesozooplankton	Initial Chl *a* ($\mu g\,L^{-1}$)	16.22 ± 6.54	13.95 ± 1.40
	FINAL Chl *a* ($\mu g\,L^{-1}$)	17.17 ± 3.01	13.68 ± 4.37
	Control Chl *a* ($\mu g\,L^{-1}$)	24.18 ± 4.50	19.21 ± 0.49
	Grazing (μg Chl *a* $ind^{-1}\,h^{-1}$)	2.16 ± 0.92	1.72 ± 1.34

concentrations there was a significant decrease of Chl *a* concentration under elevated pCO_2 (Gao et al., 2017). It is possible that higher levels of nutrients and self-shading in these cultures due to the high Chl *a* biomass (Fig. 2A), influenced this outcome, as light dependency on OA effects has been proposed (Gao et al., 2012b). Furthermore, it is also possible that the composition of the species assemblage contributed to the absence of pCO_2 effects. Here, the diatoms *Skeletonema costatum* and *Chaetoceros* spp. were the most dominant species in the microcosms, and previous studies have shown that elevated pCO_2 (1000 μatm) increased the growth of *S. costatum* under low light conditions while reducing its growth rate under high light (Burkhardt and Riebesell, 1997; Gao et al., 2012b). Conversely, Ihnken et al. (2011) showed low light treated *Chaetocera muelleri* had lower growth rates in elevated pCO_2 or reduced pH conditions, but there was no CO_2 or pH effect under high light exposure. Similarly, elevated pCO_2 had no effect on the growth of *Chaetocera debilis* at a light intensity of 90 $\mu mol\,m^{-2}\,s^{-1}$ (Hoppe et al., 2015). Alternatively, the diel changes in pH (Fig. 1D), despite continuous bubbling with the mixed gases, may have helped to mitigate the effect of a sustained lower pH. Fluctuations in multiple drivers may alter OA impacts (Duarte et al., 2013), as shown in the coastal diatom *Thalassiosira weissflogii* (Li et al., 2016). These results highlight that high light exposure and high nutrients availability may counteract the positive or negative effects of OA to *S. costatum* and *Chaetoceros*. spp., as well as phytoplankton assemblages in the present study.

4.2. Zooplankton responses

Microzooplankton are the main consumer of phytoplankton production in the oceans, consuming ~70% and 60% of primary production per day in oceanic and coastal zones, respectively (Calbet and Landry, 2004; Calbet, 2008). They also serve as intermediaries between primary producers and copepods (Gifford, 1991; Calbet and Saiz, 2005), being key components of the microbial loop (Azam et al., 1983; Sherr and Sherr, 2002), and thus their responses to OA are of ecological significance. In winter time, the microzooplankton community in Xiamen coastal waters is dominated by *Mesodinium rubrum* and other ciliates. In our study, there was no apparent effect of increased pCO_2 on microzooplankton grazing, with microzooplankton consuming ~52% and ~54% of daily PP for LC and HC treatments, respectively. This grazing intensity is similar to, or slightly lower than that expected in coastal waters. We did not identify and quantify the microzooplankton community in this study and so are unable to normalize this consumption to the abundance of micrograzers. Rose et al. (2009)

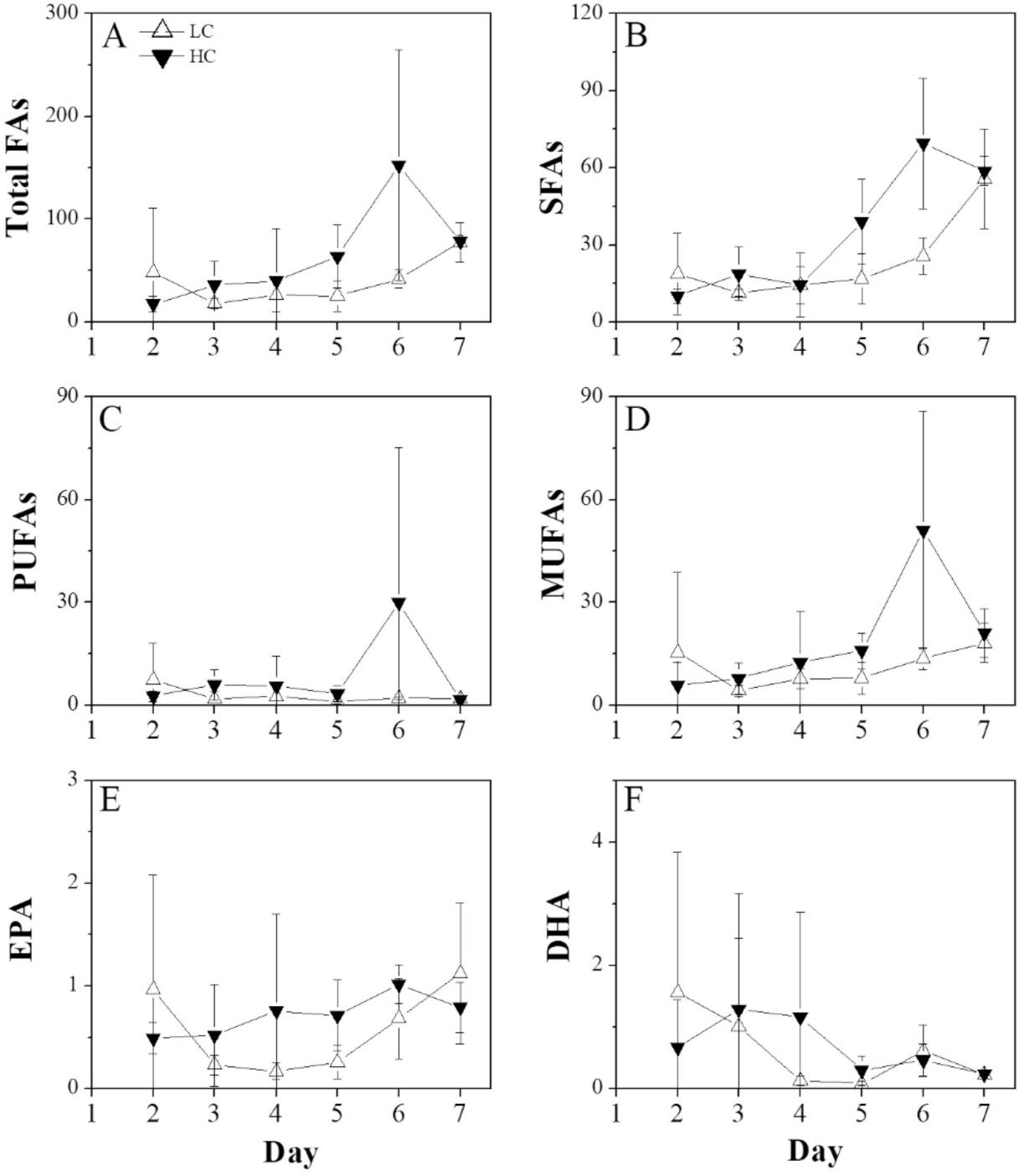

Fig. 5. Phytoplankton nutritional fatty acid contents: (A) total fatty acids (FAs), (B) saturated fatty acids (SFAs), (C) polyunsaturated fatty acids (PUFAs), (D) monounsaturated fatty acids, (E) eicosapentaenoic acid (EPA) and (F) docosahexaenoic acid (DHA) content (μg μg Chl a^{-1}) in LC controls and HC treatments during the experiment days. Data are averages ± standard deviation, n = 3.

demonstrated that indirect effects on microzooplankton community structure from changes in phytoplankton community composition as a result of changing temperature or pCO_2 were likely more important than direct effects on microzooplankton physiology, although they found microzooplankton community unaltered by elevated pCO_2 under ambient temperature. A recent mesocosm study also observed no significant relationship between microzooplankton community structure and pCO_2 in an arctic fjord (Aberle et al., 2013).

Mesozooplankton consumed ~31 ± 10% and ~17 ± 13% of daily primary production (PP) in LC and HC treatments, respectively. Overall, these consumption proportions are somewhat higher than the ~10% consumption of daily PP reported for highly productive waters (Calbet, 2001). In this study, copepods dominated the mesozooplankton (~95%), with *A. pacifica* accounting for 60% of the total mesozooplankton (Jin et al., 2015). This finding is inconsistent with that of Li and Gao (2012), which showed that the calanoid copepod (*Centropages tenuiremis*) enhanced their respiration rates by 22.7% at 1000 μatm pCO_2 level, and decreased the grazing rates by 31.6% in 24 h. However, these grazing and respiration rates increased over 36 h to 90 h of exposure. Copepods have been shown to acclimate to high levels of pCO_2 (1000 μatm) over longer periods of exposure than tested here (Whiteley, 2011; Wittmann and Portner, 2013). Among

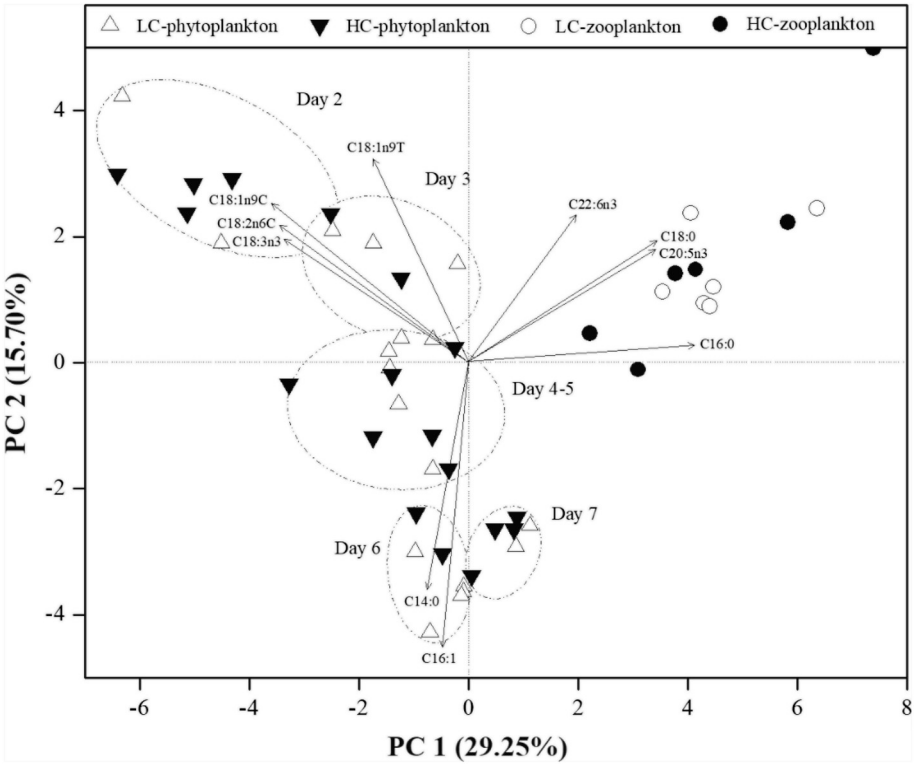

Fig. 6. The principal component analysis (PCA) of fatty acids based on mean compositions of LC and HC microcosms. The PC1 and PC2 explain 29.25% and 15.70% of variation, respectively. The oval circle shows samples from different sampling times. The solid arrows show the importance of FAs in explaining the variances in length.

mesozooplankton community tested, cirriped larvae and ascidian larvae accounted for ~5% of the total mesozooplankton. Larva stage often are the most vulnerable life stage affected by elevated pCO_2 (Kurihara, 2008; Kurihara and Ishimatsu, 2008; Whiteley, 2011). For example, the mollusk larvae *Concholepas concholepas* decreased their grazing rates and shifted their feeding selectivity to bigger cells as a consequence of OA (Vargas et al., 2013). Nonetheless, the small relative contribution of larvae here would have little effect on the response of mesozooplankton grazing to increased pCO_2.

The non-detectable effect of OA on zooplankton consumption of daily PP (Fig. 3D) in this study may suggest high tolerance of zooplankton community to future acidified ocean. However, this finding does not take into account whether grazing rates would change in response to any longer-term alterations in the phytoplankton community composition or nutritional status.

4.3. Nutritional fatty acids

Fatty acids (FAs), the main components of lipids, play a key role in energy storage for growth and reproduction of zooplankton and higher trophic levels. Our question here was whether increased pCO_2 altered the fatty acid profiles of the phytoplankton and whether, over this short-term feeding experiment, these changes may be expressed in the fatty acid content of the mesozooplankton.

Phytoplankton FA content and composition changed significantly between the exponential and declining phases of the bloom (Fig. 5, Fig. 6), which was in good agreement with previous studies (Yongmanltchal and Ward, 1992; Tonon et al., 2002; Lin et al., 2007; Galloway and Winder, 2015). While diatoms became to dominate the phytoplankton assemblage in this study (Fig. 3B, C), diatom abundance decreased in an Arctic mesocosm study under increasing pCO_2 (Leu et al., 2013; Schulz et al., 2013). During the declining growth phase of our experiment, SFA concentrations were significantly higher in the HC treatments (Fig. 5B), indicating a physiological response (Mayzaud

Table 4
The compositions and concentrations of mesozooplankton FAs after fed with phytoplankton of LC controls and HC treatments. Data are averages ± standard deviation (n = 3).

FA	LC		HC	
	Composition (%)	Content (μg mg-DW^{-1})	Composition (%)	Content (μg mg-DW^{-1})
C14:0	5.18 ± 1.11	8.27 ± 1.77	4.90 ± 0.98	8.83 ± 2.42
C16:0	39.81 ± 2.97	63.83 ± 8.59	40.28 ± 2.77	71.43 ± 5.99
C16:1	6.66 ± 1.31	10.68 ± 2.52	6.62 ± 1.54	11.98 ± 3.73
C18:0	28.20 ± 3.71	44.75 ± 3.31	26.65 ± 2.47	47.22 ± 4.47
C18:1n9	2.45 ± 0.44	4.01 ± 1.27	2.77 ± 0.53	4.89 ± 0.82
C20:4	0.18 ± 0.03	0.29 ± 0.08	0.22 ± 0.05	0.40 ± 0.09
EPA	5.87 ± 2.27	9.90 ± 5.42	6.43 ± 1.55	11.67 ± 3.77
DHA	6.53 ± 3.58	11.24 ± 8.03	7.08 ± 2.02	12.91 ± 4.77
PUFAs	12.96 ± 5.99	22.07 ± 13.86	14.37 ± 3.48	26.09 ± 8.56
MUFAs	11.02 ± 1.02	17.75 ± 3.06	10.86 ± 0.96	19.40 ± 3.13
SFAs	75.97 ± 5.95	121.32 ± 11.52	74.65 ± 4.25	132.45 ± 10.75
Total FAs	–	161.23 ± 26.24	–	178.14 ± 20.15

et al., 1990; Hayakawa et al., 1996). Even so, the essential PUFAs [DHA and eicosapentaenoic acid (EPA)] remained unchanged, or even decreased during the experimental period, and were not affected by OA. The studies of OA impacts on phytoplankton community FA profiles are limited, but previous work has shown that elevated pCO_2 can affect monocultured algal FA content and profile composition (Carvalho and Malcata, 2005; Hu and Gao, 2006; Bermúdez et al., 2015). The impacts of OA on natural planktonic FAs also have been reported by studies in high latitude waters (Leu et al., 2013; Bermúdez et al., 2016a, 2016b). Our previous study with an artificial phytoplankton community in eutrophic waters showed that increased pCO_2 resulted in higher PUFAs and monounsaturated fatty acids (MUFAs) during the late exponential phase (Wang et al., 2017), although these differences were not observed in the mesozooplankton FAs. It seems that the FA profiles are more species-specific and they were more sensitive to nutrient availability than pCO_2 levels (Mayzaud et al., 1990; Leu et al., 2006, 2010).

Comparatively 3-day incubation were used because copepods in warm water have much faster turnover rates than that in cold waters, typically storing less FAs, and using ingested food immediately for growth and reproduction. So, the 3-day incubation should be sufficient for zooplankton FA composition change to reflect any changes in phytoplankton FAs, as reflected in our previous study (Wang et al., 2017). It is not surprising that after 3 days of this phytoplankton diet, there were no apparent effect of elevated pCO_2 on mesozooplankton fatty acid content or composition (Table 4). Similar results were also observed in mesozooplankton from Arctic fjord (Leu et al., 2013), Baltic sea (Bermúdez et al., 2016b) and this area (Wuyuan Bay) (Wang et al., 2017). Indeed, the FA content of diet affect the FA content in zooplankton (Brett and Müller-Navarra, 1997; Müller-Navarra et al., 2000; Brett et al., 2006). However, shifts in phytoplankton community could also cause smaller–scale changes in the FA composition of zooplankton (Hiltunen et al., 2015). Nonetheless, under our short-term growth conditions, there was no indication that increased pCO_2 had either direct or indirect effects on their feeding rates or their content of nutritional fatty acids.

5. Conclusions

Our finding show that net primary production was unaffected by the moderate OA in the eutrophicate water of the South China Sea, though there was a slight decrease in apparent growth rates of phytoplankton. No effect of OA on the trophic transfer was detected as the micro-/mesozooplankton grazing rates did not change. The nutritional FAs of phytoplankton were unchanged until the late decline phase, when there was an increase in saturated SFAs in the elevated pCO_2 treatment. The FA profiles of mesozooplankton were quite different with that of the phytoplankton, but there were no significant differences between two pCO_2 conditions. Our results suggest that plankton communities in this subtropical coastal water are insensitive to ocean acidification in terms of primary production and trophic transfer from phytoplankton to zooplankton. Further research under more severe OA conditions, and over longer-term exposures, are recommended to better delineate the potential for OA to impact marine ecosystems, along with more biome and multiple environmental factors considered.

Author contributions

This study was designed by K. Gao and T. Wang; T. Wang and P. Jin conducted the experiment and the measurements. T. Wang drafted the paper. K. Gao and M. L. Wells were involved in data analysis and paper writing. All the authors were involved in discussion about the data.

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