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The fatty acid content of plankton is changing in subtropical coastal waters as a result of OA: Results from a mesocosm study

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ABSTRACT

Ocean Acidification (OA) effects on marine plankton are most often considered in terms of inorganic carbon chemistry, but decreasing pH may influence other aspects of cellular metabolism. Here we present the effects of OA on the fatty acid (FA) content and composition of an artificial phytoplankton community (*Phaeodactylum tricornutum*, *Thalassiosira weissflogii*, and *Emiliania huxleyi*) in a fully replicated, ~4 m^3 mesocosm study in subtropical coastal waters (Wuyuan Bay, China, 24.52°N, 117.18°E) at present day (400 μatm) and elevated (1000 μatm) pCO_2 concentrations. Phytoplankton growth occurred in three phases during the 33-day experiment: an initial exponential growth leading to senescence and a subsequent decline phase. Phytoplankton sampled from these mesocosms were fed to mesozooplankton collected by net haul from Wuyuan Bay. Concentrations of saturated fatty acids (SFA) in both phytoplankton and mesozooplankton remained high under acidified and non-acidified conditions. However, polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) increased significantly more under elevated pCO_2 during the late exponential phase (Day 13), indicating increased nutritional value for zooplankton and higher trophic levels. Indeed, uptake rates of the essential FA docosahexaenoic acid (C20:5n3, DHA) increased in mesozooplankton under acidified conditions. However, mesozooplankton grazing rates decreased overall with elevated pCO_2. Our findings show that these selected phytoplankton species have a relatively high tolerance to acidification in terms of FA production, and local mesozooplankton in these subtropical coastal waters can maintain their FA composition under end of century ocean acidification conditions.

1. Introduction

Anthropogenic increases in atmospheric CO_2 have resulted in acidification of the surface ocean, with resulting impacts on marine organisms (Doney et al., 2009). Approximately one third of the anthropogenic CO_2 released to the atmosphere has been absorbed by the oceans (Sabine et al., 2004; Le Quéré et al., 2013), leading to increased pCO_2 in surface seawaters. For example, pCO_2 has increased 18.8 μatm per decade between 1988 and 2008 in the subtropical North Pacific (Station ALOHA), with a corresponding decrease of 0.019 units per decade (Dore et al., 2009; Doney, 2010). The average pH of the surface ocean is predicted to decrease by 0.31 units by the end of the 21st century (IPCC, 2013) which, combined with climate-driven increases in UV exposure, may significantly reduce primary production in subtropical waters (Gao et al., 2012b). These effects may be particularly important for coastal oceans, which host ~15-30% of marine primary production and ~80% of organic matter burial (Gattuso et al., 1998). Moreover, OA in many coastal regions is exacerbated by hypoxia and increased concentrations of dissolved organic matter (DOM) associated with cultural eutrophication (Melzner et al., 2013; Cai et al., 2011). As a consequence, projecting the effects of OA on coastal ecosystems will be more complex than in oceanic environments.

For most marine organisms, lipid fatty acids (FA) are the main form of energy storage for growth and survival, particularly during times of food shortage. Fatty acids are important in membrane structure, energy metabolism, hormone precursors, and, when combined with proteins, as intracellular messengers (Storch and Thumser, 2010). The composition of total FA in marine algae varies in different growth phases (Tonon et al., 2002), in different physiological states, and with environmental conditions, such as temperature (Thompson et al., 1992), pCO_2 (Gordillo et al., 1998), nutrient concentrations (Reitan et al., 1994; Hu and Gao, 2006; Leu et al., 2006, 2010), and light intensity (Thompson et al., 1990). A subset of these fatty acids are the nutritionally important long chain polyunsaturated fatty acids (PUFA), the essential fatty acids (EFA), are synthesized exclusively by phytoplankton (Brett and Müller-Navarra, 1997). EFA are critical

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constituents in the diets of zooplankton and higher trophic levels (Jónasdóttir et al., 2009). Feeding on algae rich in PUFA markedly increases zooplankton growth rates (Müller-Navarra, 1995; Müller-Navarra et al., 2000), highlighting the important roles that phytoplankton PUFA have in the marine food web (Kainz et al., 2004). Diets rich in PUFA are essential for egg production in copepods (Jónasdóttir et al., 2009) as well as in fish growth and reproduction (Watanabe, 1982; Bell et al., 1986; James Henderson and Tocher, 1987).

There is little known about how OA may affect the production and composition of total and nutritional FA in algae species or planktonic communities. Tsuzuki et al. (1990) studied the effects of elevated pCO_2 on FA composition in seven microalgal species and found that acidification altered the FA composition in three green algal species (*Chlorella vulgaris, Chlamydomonas reinhardtii*, and *Dunaliella tertiolecta*), while no differences were observed in four other species (two cyanobacteria species, *Anabaena variabilis* and *Anacystis nidulans*; the red alga, *Porphridium cruentum*; and the green alga *Euglena gracilis*). What's more, they found significant increases in total FA and the relative contents of oleic acid (C18:1n9) in *C. vulgaris* when low pCO_2 acclimated cells were transferred into high pCO_2 conditions. Carvalho and Malcata (2005) also found increased total lipid contents of a chrysophyte (*Pavlova lutheri*) under high pCO_2 conditions but decreased PUFA concentrations. Hu and Gao (2003) found significantly higher concentrations of total FA and EPA (C20:5n3 - eicosapentaenoic acid) in *Nannochloropsis* when cultured at high (2800 μatm) pCO_2 levels.

Testing the effects of elevated pCO_2 on the total and nutritional FA contents of phytoplankton communities are more rare. Most studies use laboratory incubation experiments, which, due to volume constraint, greatly challenge the study of trophic transfer effects on mesozooplankton. Whole planktonic systems can be investigated using mesocosms, but very few of these studies to date have studied OA effects on planktonic FA. Leu et al. (2013) utilized large scale mesocosms to study a post spring bloom phytoplankton community in the Arctic Kongsfjorden. Small positive correlations between increasing pCO_2 and most PUFA were found during the early and mid-stages of the 30-day experiment. These changes were due either to measured shifts in the taxonomic composition of the community or OA-induced physiological effects on the cells. Bermúdez et al. (2016a) showed that increasing pCO_2 at another high latitude site (Ranue Fjord, Norway) also induced shifts in phytoplankton community composition accompanied by a decline in the proportion of PUFA to total FA, with the same trend in the FA content of copepods. The question is how representative are these findings to other regions, particularly at lower latitudes, and whether OA-induced changes in algal physiology alone could cause similar changes in FA production and trophic transfer, even where phytoplankton community composition remains stable.

We present the findings of an OA mesocosm study in Wuyuan Bay, Xiamen, a culturally eutrophic, shallow coastal system. The 33-day experiment was designed to study OA-induced changes in the production and trophic transfer of algal FA. Isolates of *Phaeodactylum tricornutum*, *Thalassiosira weissflogii*, and *Emiliania huxleyi* were added to filtered natural waters. In this way, we were able to investigate the effects of OA on cell physiology in naturally eutrophic waters while minimizing the complexity of shifting compositions of natural phytoplankton communities. We measured the effects of high (1000 μatm) and present day (400 μatm) pCO_2 levels on the total and nutritional lipid contents and compositions of the phytoplankton and mesozooplankton grazers. The findings highlight the challenges of studying OA effects in eutrophic systems, and the potential impact OA may have on food quality of the planktonic assemblages.

2. Materials and methods

2.1. Study area and facility

Wuyuan Bay, located on the northeast shore of Xiamen Island, is 5 km long by 0.5–2 km wide, with an average depth of 20 m and salinity ~29 puμ. The bay is influenced by the semi-diurnal tide (~ ± 3 m of sea level changing based on mean sea level) as well as runoff from the Chiu-lung River. The Facility for OA Impacts Study of Xiamen University (FOANIC-XMU), situated in the center of Wuyuan Bay (24.52 °N, 117.18 °E), is fully solar powered with 200 m^2 of workspace, and houses up to nine mesocosms on its southern side.

2.2. Experimental setup

Six mesocosms were used in this study over an experimental period of 33 days (22 Dec 2014 to 24 Jan 2015). Each mesocosm comprised a 3 m deep (2.5 m submerged), 1.5 m wide cylindrical transparent thermoplastic polyurethane (TPU) bag fixed to a steel frame fitted with a conical roof (made of TPU) to minimize contamination from spray and rainfall. All mesocosms were injected simultaneously over 24 h with filtered (0.01 μm) *in-situ* surface seawater (~4000 L in each mesocosm). Three of the six mesocosms then were injected with filtered CO_2 saturated seawater to achieve a pCO_2 level of 1000 μatm. These low carbon (LC) and high carbon (HC) levels of pCO_2 were maintained by bubbling with ambient filtered air (LC; 400 μatm) or with CO_2 enriched air (HC; 1000 μatm) using a CO_2 Enrichlor (CE-100B, Wuhan Ruihua Instrument & Equipment Ltd, China). The two air sources were delivered into the mesocosms (5 L min^{-1}) using air stones at the bottom of the bags.

Three phytoplankton species, two diatoms [*Phaeodactylum tricornutum* (CCMA 106) and *Thalassiosira weissflogii* (CCMP 102)] and one coccolithophore [*Emiliania huxleyi* (PML B92/11)] were inoculated on Day 0 into each mesocosm at concentrations of 10 cells L^{-1}, 10 cells L^{-1}, and 20 cells L^{-1}, respectively.

Chemical conditions, including pH, total alkalinity (TA), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and nutrients were monitored daily during the exponential growth phases and every two days thereafter. The analytical methods used followed recommendations given in Riebesell et al. (2011), and specific details are found in a companion publication on this experiment (Liu et al., 2017).

Phytoplankton were sampled from the mesocosms every two days after Day 10 for FA determination. Mesozooplankton were collected from Wuyuan Bay seawater adjacent to the FOANIC-XMU during the evening of Day 12 by horizontal plankton net trawls (160 μm mesh size). The mesozooplankton were distributed equally into 18 tanks (500 mL, 9 LC controls and 9 HC treatments) (~240 individuals/tank), and maintained at the target pCO_2 levels (400 or 1000 μatm) and fed with the phytoplankton from matched mesocosms (3 tanks for each mesocosm). On the second and third day, 50% of the water was replaced with a fresh aliquot collected from the respective mesocosms. After 3 days of cultivation (Day 12–15), mesozooplankton samples from 12 of the 18 LC/HC tanks were collected on 160 μm mesh nets, rinsed with filtered seawater to remove any remaining phytoplankton, collected onto pre-combusted GF/F filters, and stored at -20 °C for FA analysis. And mesozooplankton from the other 6 tanks (3 for LC and 3 for HC) were collected for dry weight measurements.

2.3. Analyses

2.3.1. Phytoplankton biomass and identification

Samples for measurement of chlorophyll *a* (Chl *a*) and particulate organic carbon (POC) were collected from each mesocosm every two days beginning on Day 4, which was increased to daily collections during the exponential growth phase (Day 6 to Day 15). For Chl *a* measurement, 0.2–0.5 L water samples were filtered (GF/F, Whatman) and extracted with 100% methanol at 4 °C in darkness for 24 h. Extracts were centrifuged (5000 g for 10 min; Universal 320R, Hettich, Germany) and the supernatant scanned from 400 to 700 nm (DU800, Beckman, Fullerton, California, USA). Chl *a* concentrations were calculated following the method of Porra (2002). POC was measured on

0.1–0.5 L water samples filtered onto pre-combusted GF/F filters and dried at 60 °C for 24 h. The dried GF/F filter samples were analyzed using a CHNS/O Analyzer (2400 series II, PerkinElmer) (Liu et al., 2017). Samples for cells counting and identification were fixed with Lugol's iodine (2% final concentration) and examined at 10 × 20 or 10 × 40 magnification. We counted only the innoculated species; some invasive species were observed in very low abundance but were not enumerated.

2.3.2. Mesozooplankton populations and biomass

The in-situ mesozooplankton collection was subsampled and fixed with Lugol's iodine (2% final concentration) for taxonomic identification. Mesozooplankton species identification and enumeration is given in the supplemental materials of Jin et al. (2015). Subsamples of mesozooplankton collected from 6 LC/HC tanks (3 for LC and 3 for HC) were dried at 60 °C for 48 h for dry weight measurement.

For mesozooplankton grazing rate determinations, the collected zooplankton were aliquoted into 12 polycarbonate bottles (1000 mL) containing phytoplankton from each replicate mesocosm and placed into a flowing seawater bath under natural light for 24 h. Grazing rates were calculated from the change in chlorophyll *a* concentration over time according to Frost (1972).

2.3.3. Fatty acid composition

Mesocosm water samples (0.5–1 L) for phytoplankton FA analyses were filtered onto a pre-combusted GF/F filters (Whatman) and stored at -20 °C until analysis. An internal standard (nonadecanoic acid methylester, Sigma, America) was added to each sample before the filters were extracted in chloroform: methanol (2:1, v/v). The FA were methyl esterified with 5% HCl in methanol at 85 °C for 1 h and the resulting fatty acid methyl esters (FAME) extracted with hexane. FAMEs were analyzed by GC/MS (thermos TRACE 1310 GC-ISQ QD MS) on a capillary column (30 m × 0.25 mm × 0.25 μm) at a Hi-purity Helium carried flow rate of 1.2 mL min^{-1} using temperature programming (Injection: splitless at 290 °C; Detection: FID at 80 °C maintained for 1 min, 10 °C min^{-1} to 200 °C, 5 °C min^{-1} to 250 °C, 2 °C min^{-1} to 270 °C and maintained for 3 min). FAMEs were identified and quantified by comparison with known standards.

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

2.4. Statistical analyses

Phytoplankton growth was split into three developmental phases (exponential, senescence, and decline) based on calculated growth rates and Chl *a* concentrations. Fatty acid concentrations were normalized to POC and are presented as the means ± SD of 3 LC/HC replicates. The grazing rates of mesozooplankton on phytoplankton were calculated from drawdown of Chl *a*. FA grazing/intake rates of mesozooplankton were calculated from that of FA to Chl *a* ratios (Grazingng/intake rates of Chl *a*). Changes in pH, normalized FA concentration and composition were tested using two-way ANOVA ($\alpha = 0.05$). Grazing rates of mesozooplankton under the different pCO_2 treatments were tested using one-way ANOVA analysis ($\alpha = 0.05$). Principal component analysis (PCA) was used to compare the FA composition in different treatments and experimental days. SPSS was used in all statistical analyses. Origin was used in graphic processing. All data are presented as the mean ± 1 SD.

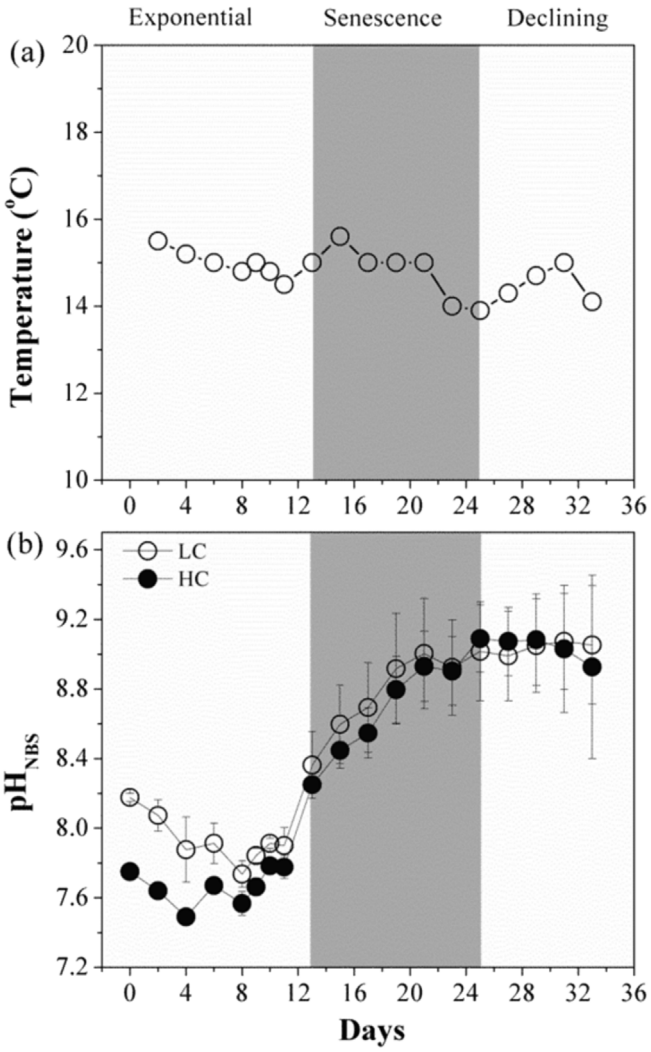

Fig. 1. The (a) Temperature and (b) pH development in the mesocosms during the experimental period. The temperature data are mean values of sampling day, and the pH data are means ± SD (n = 3).

3. Results

3.1. Mesocosm response

3.1.1. Physical conditions

Temperature remained relatively uniform (14.8 ± 0.5 °C) during the 33 day experiment (Fig. 1a). The initial pH in the LC controls (8.17 ± 0.02) and HC treatments (7.75 ± 0.04) decreased in unison over the first 8 days, and then increased substantially between Day 8–20, overwhelming the pH control system (Fig. 1b). The initial pH difference between the LC controls and HC treatments (~0.42 units) was roughly uniform from Day 0–6 but decreased to ~ 0.15 units by Day 12, after which there was no statistically significant difference ($p = 0.38$, Fig. 1b). These changes coincided with substantial increases in Chl *a* concentrations in these eutrophied systems (Fig. 2). Our experience in laboratory tests with these species shows that it is difficult to control the carbonate system when chlorophyll biomass exceeds ~60 μg Chl *a* L^{-1}, consistent with the mesocosm findings here. The DIC concentrations showed significant differences ($p = 0.004$) at the beginning of the experiment between LC controls (2176.8 ± 28.7 μmol kg^{-1}) and HC treatments (2328.2 ± 34.3 μmol kg^{-1}), and decreased to 1637.6 ± 244.9 μmol kg^{-1} (LC) and 1714.4 ± 420.4 μmol kg^{-1} (HC) at the end (Dai, M. unpublished data), and showed no significant

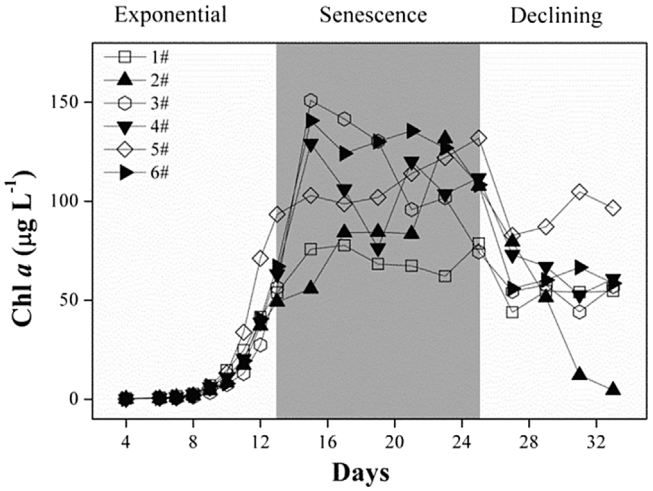

Fig. 2. The Chl *a* concentrations of phytoplankton in LC/HC mesocosms in different phases independently. 1#, 3#, 5# are LC mesocosms and 2#, 4#, 6# are HC mesocosms. Exponential phase, from Day 0 to Day 13; senescence phase, from Day 14 to Day 25 and declining phase, from Day 26 to Day 33.

differences ($p = 0.798$) due to the large consuming of photosynthesis. A full description of the carbonate system during the mesocosm experiment is provided separately (Dai, M. et al., in preparation).

The initial mesocsom waters contained high nutrient concentrations; total dissolved inorganic nitrogen (DIN, nitrate + nitrite + ammonium) of 72.0 ± 5.9 μmol L^{-1} (LC) and 74.7 ± 2.8 μmol L^{-1} (HC), PO_4^{3-} of 2.6 ± 0.2 μmol L^{-1} (LC) and 2.5 ± 0.2 μmol L^{-1}(HC) (Dai, M., unpublished data), and Si of 38.4 ± 1.8 μmol L^{-1} (LC) and 39.4 ± 0.7 μmol L^{-1} (HC) (Li, F., unpublished data). For most mesocosms, DIN and PO_4^{3-} drawdown was essentially complete by about Day 15, while silicate remained in excess in all mesocom replicates throughout the experiment (Day 33, 16.4 ± 11.5 μmol L^{-1} for LC and 24.9 ± 6.7 μmol L^{-1} for HC).

3.1.2. The phytoplankton response

The changes in Chl *a* concentrations showed that the growth responses comprised three stages in all mesocosms (Fig. 2): the initial exponential growth phase (Day 0 to Day 13), a slowing of growth into the senescence (Day 14 to Day 25) that coincided with the nearly complete consumption of inorganic nutrients (Day ~ 15), followed by declining chlorophyll biomass (Day 26 to Day 33). However, Chl *a* concentrations remained elevated (~60 μg L^{-1}) even at the end of the experiment (Day 33). There were no significant differences ($p = 0.97$) in maximum chlorophyll biomass accumulation between the LC and HC treatments (Fig. 2). More details of the phytoplankton response are given in Liu et al. (2017).

Microscopic analyses confirmed that all three added phytoplankton species showed growth at the beginning and during the exponential phase, but *P. tricornutum* came to dominate the biomass during the exponential and senescence phase, with *T. weissflogii* remaining present but at much lower cell concentrations. The numerical dominance corresponded to the strong signal of diatom FA markers (C16:1n7 + C20:5n3) during the experiment (Fig. 3a). In contrast, *E. huxleyi* decreased to very low concentrations during the late exponential phase in both the LC controls and HC treatments. Although not readily observed in microscopic examination, low levels of FA biomarkers for chlorophytes (C18:2n6 + C18:3n3) and flagellates (C22:6n3 + C18:4n3) were measured after Day 12 (Fig. 3b and c). These likely were local invasive species, probably introduced by sea spray. Increased pCO_2 levels had no discernable effect on phytoplankton growth rates, composition (Liu et al., 2017), or lipid biomarkers between the HC and LC treatments (Fig. 3a, b, c; $p = 0.52$, 0.84, 0.38, respectively) during the exponential phase, but it affected phytoplankton species composition, cumulative primary productivity, and POC in late senescence (Liu et al., 2017). Bacterial FA markers (C15:0 + C17:0) increased over time reaching a maximum during senescence in all mesocosms, with no significant difference between the LC controls and HC treatments (Fig. 3d; $p = 0.14$), which was consistent with the flow cytometry measured bacteria abundance (Huang, Y. et al., submitted).

3.2. Phytoplankton fatty acids

Low cell abundance during the early stage of the experiment logistically prevented collecting enough biomass for FA analyses before Day 10 of the experiment, so our FA results begin in the mid-exponential phase and progress through the senescent, and declining phases of the bloom. The community-level fatty acid (FA) contents of the phytoplankton varied over the course of the experiment ($p < 0.01$), and between the LC controls and HC treatments at Day 13 ($p = 0.05$) (Fig. 4, Fig. 6). Although the concentrations of total FA, SFA, and MUFA were variable, there was close correspondence among their respective patterns over time in all mesocosms (Fig. 4a, b, c). The total (TFA) and saturated fatty acid (SFA) contents decreased from ~200 μg mg^{-1} C (TFA) and ~180 μg mg^{-1} C (SFA), to ~ 75 μg mg^{-1} C (TFA) and ~40 μg mg^{-1} C (SFA) during the latter portion of the exponential phase (Day 10–13), and then remained low throughout the remaining portion of the experiment (Fig. 4a and b). Initial MUFA concentrations (~35 μg mg^{-1} C) decreased by 3–4 fold through the late exponential phase but then increased to ~ 20 μg mg^{-1} C during senescence and remained stable during the latter declining phase (Fig. 4c). In contrast, the PUFA contents were low initially (~6 μg mg^{-1} C) but roughly doubled in late exponential to ~ 12 μg mg^{-1} C and then remained unchanged, though variable, through the later phases of the experiment (Fig. 4d).

The EFA contents of the phytoplankton increased over the course of the experiment (Fig. 4e and f). Concentrations of DHA increased sharply in the HC treatments at the end of the exponential phase (Fig. 4e), consistent with the increase seen in PUFA at this growth stage (Fig. 4d). EPA concentrations also showed a very similar pattern to PUFA over the experiment, increasing most in the HC treatments during the exponential phase (Fig. 4f).

There were no significant differences ($p = 0.22$) in the relative composition of the PUFA, as shown by the percent PUFA contents between the LC controls and HC treatments, where both increased during the late exponential phase from 3% to almost 20%, and displayed a slight, though variable decrease during the senescence and declining phases (Fig. 5a). The essential fatty acids DHA and EPA were the most abundant overall and increased through the exponential phase to their maximum during senescence, where they comprised ~3% and ~15% of the total FA, respectively (Fig. 5b and c). The mid exponential phase was characterized by relatively high levels of the important saturated FA C16:0 and C18:0, which ranged from 30 to 46% and 25 - 38% of the total FA, respectively (Fig. 5d and e). However, their relative abundance decreased sharply over the late exponential phase and early senescence phase to account for ~30% (C16:0) and ~10% (C18:0) of total FA by the end of the experiment (Fig. 5d and e). In contrast, the percent contents of C14:0 showed an inverse pattern, increasing from ~2% in the mid exponential phase to ~7% during senescence and the declining phase (Fig. 5f).

Among the MUFA measured, the percent contents of C18:1n9 decreased rapidly during the late exponential phase followed by gradual increase across the senescence and declining phases (Fig. 5g). Conversely, the percent C16:1 contents increased from ~5% at mid-exponential to ~25% of the total FA at the end of the experiment (Fig. 5h). There were no consistent differences ($p = 0.72$) in unsaturated FA between the LC and HC treatments.

The one phase where consistent differences were observed between the LC controls and HC treatments was late exponential phase. While

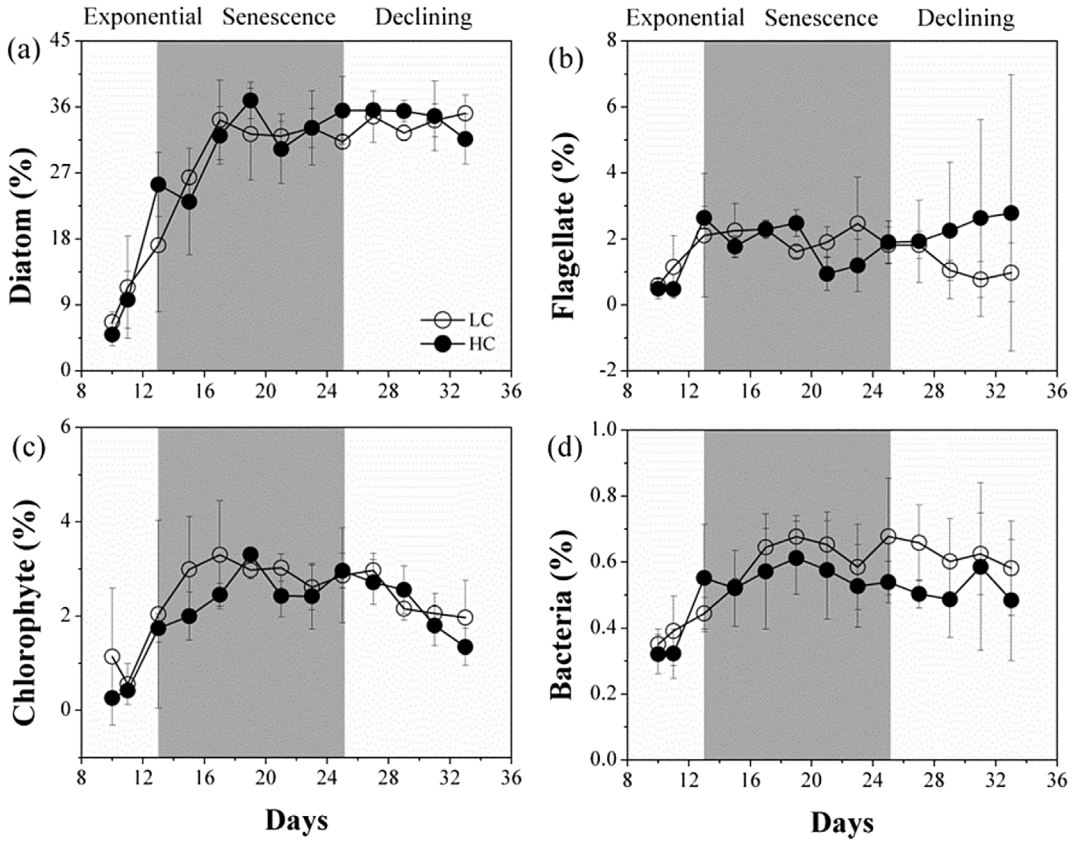

Fig. 3. The fatty acid biomarkers percent contents of (a) diatom, (b) flagellate, (c) chlorophyte and (d) bacteria in the mesocosms during different growth phase. Data are means ± SD (n = 3).

concentrations of total FA, SFA and MUFA decreased during the exponential phase, they remained significant higher in the HC treatments ($p = 0.05$, $p = 0.12$, and $p = 0.04$, respectively) (Fig. 4a, b, c; Fig. 6a, c, g). Similarly, PUFA, DHA, and EPA concentrations increased to a greater extent in the HC treatments at the end of the exponential phase ($p = 0.05$, $p = 0.09$, and $p = 0.02$) (Fig. 4d, e, f; Fig. 6e, i, k; Table 2).

3.3. Zooplankton fatty acids

The mesozooplankton feeding experiment was done at the end of the exponential phase of phytoplankton growth (Day 12). These zooplankton, collected from surface waters adjacent to the mesocosm facility, comprised ~95% copepods (Jin et al., 2015). However, despite the significantly higher fatty contents of phytoplankton at this growth stage, there were no significant differences ($p > 0.05$) in mesozooplankton total FA, SFA, PUFA, MUFA or DHA and EPA concentrations between the LC controls and HC treatments in these short-term feeding experiments (Fig. 6). There also were no significant differences in the wider suite of specific FA between the LC controls and HC treatments (Table 1). The grazing rates of mesozooplankton decreased ($p = 0.049$) by ~ 28% in HC treatments (Fig. 7a). Even so, the calculated intake rates of FA remained constant (total FA, SFA, PUFA, and EPA; $p > 0.05$) or increased (DHA, $p = 0.02$) (Fig. 7).

4. Discussion

Atmospheric and eutrophication-driven OA is a global phenomenon that increasingly will impact many coastal regions. This mesocosm study is the first to investigate OA effects on FA contents and profiles of plankton communities in eutrophic subtropical coastal waters. We used three model species (two diatoms and a coccolithophore) as a preliminary step to provide context for future planned studies using whole phytoplankton communities. This study also served as an opportunity to test the use of mesocosms for OA studies in subtropical eutrophic coastal waters. Although these mesocosms are not intended to represent the natural system, they are useful tools for studying how ocean acidification of natural waters can influence the FA profiles of phytoplankton, and, in turn, the potential effects on zooplankton feeding rates and lipid compositions.

The biological responses in these eutrophic mesocosms overwhelmed the capacity to regulate the carbonate system at two stages. First, the pH steadily decreased in the LC controls and HC treatments over the first 8 days, when phytoplankton biomass was comparatively low. This change may have been partially driven by bacterial remineralization of the presumably high DOM concentrations in these eutrophic waters (Huang, Y. et al., submitted). After Day 8, phytoplankton biomass increased substantially in all mesocosms, along with pH, and the pH difference between the LC controls and HC treatments diminished. This pH increase is attributable to the progressive drawdown of pCO_2 by phytoplankton production, and it reflects how conditions would change in future culturally eutrophic coastal waters.

Shifting community composition can alter FA composition substantially (Galloway and Winder, 2015), however, the diatom *P. tricornutum* became numerically dominant here in both the LC controls and HC treatments throughout most of the experimental period (Liu et al., 2017), consistent with the high relative levels of diatom markers (9%–36%) (Fig. 3a). *T. weissflogii,* remained a significant component of the phytoplankton assemblage, and *E. huxleyi* was observed frequently but at low levels in both the HC and LC treatments. Nevertheless, the measured changes in FA concentrations predominantly reflect the response of *P. tricornutum* to OA. Fatty acid profiles of *P. tricornutum* characteristically have high percentages of EPA and C16 (mostly C16:0 and C16:1) FA and a low DHA content, consistent with previous studies (Zhukova and Aizdaicher, 1995; Tonon et al., 2002; Patil et al., 2007).

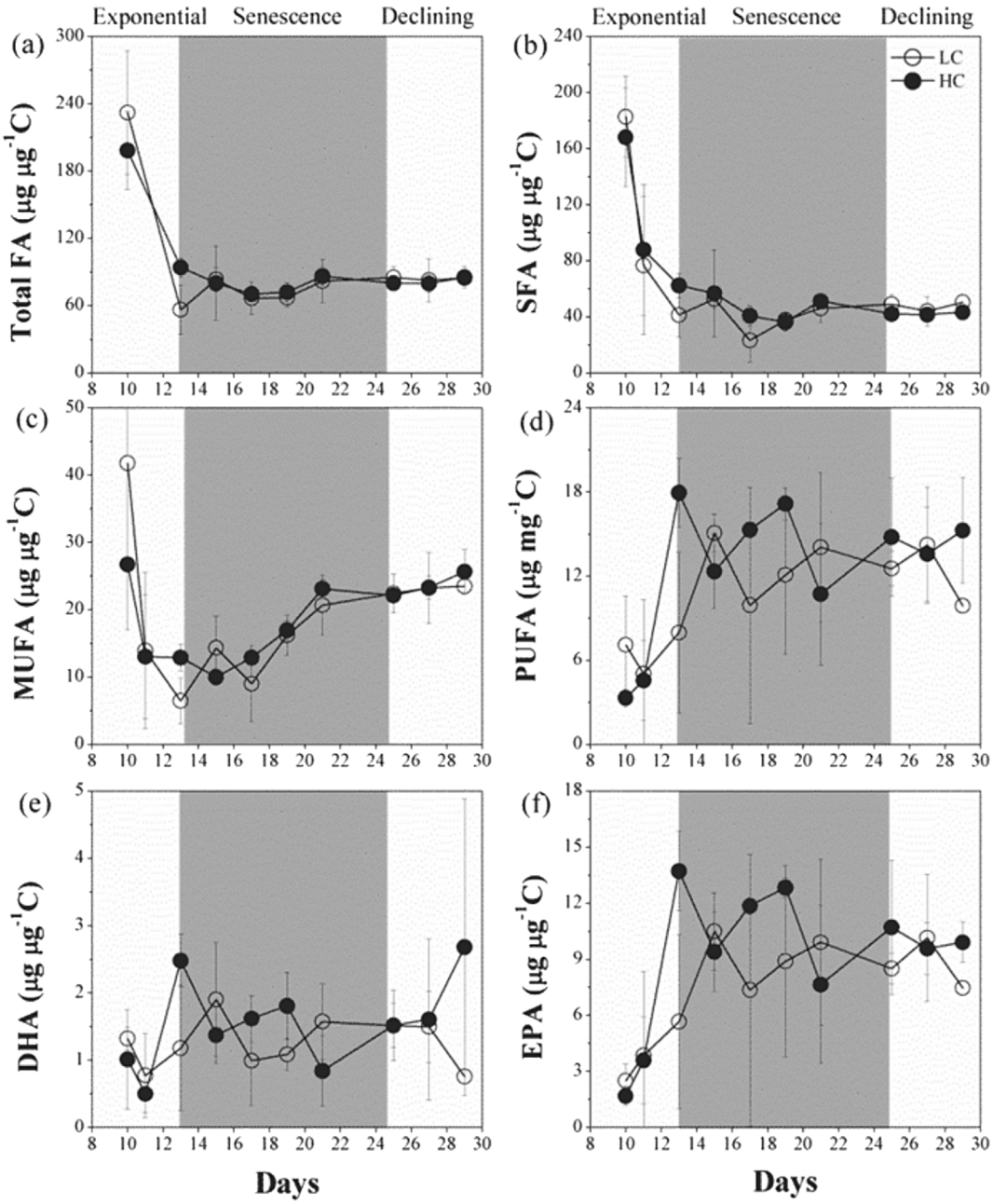

Fig. 4. The development of the contents of (a) total FA, (b) SFA, (c) MUFA, (d) PUFA, (e) DHA and (f) EPA during the experimental days. Data are means ± SD (n = 3).

DHA concentrations increased in one HC mesocosm during the decline phase, reflected by the sharp increase in analysis variance (Fig. 4e; Fig. 5b). This change was coincident with increased flagellate biomarker in this replicate HC mesocosm, suggesting an un-identified flagellate "contaminant" was introduced by sea spray or other means.

The shift in FA contents and profiles over time is consistent with cellular responses to growth and environmental stress. Principal component analysis showed a substantial change in phytoplankton FA compositions between exponential, senescence, and the declining stages of the bloom (Fig. 8). These findings are consistent with the results of other studies showing that different developmental phases significantly affect FA content (Yongmanltchal and Ward, 1992; Tonon et al., 2002; Lin et al., 2007). Changes occurred rapidly through the exponential phase, slowed during the early senescence to become stable in late senescence and the early decline phase before shifting again as phytoplankton became visually unhealthy after Day 29 (Fig. 8). Even so, there were no notable differences between the LC controls and HC treatments in our experiment. This apparent uniformity is noteworthy considering that the HC treatments had greater inorganic carbon inputs, so there would have been differences in cell physiology and cellular carbon processes, but these alone were not sufficient to alter cellular FA compositions.

The maximum concentrations of cellular total FA, SFA and MUFA occurred on Day 10 (the first day of analysis) and decreased sharply shortly after cells shifted into exponential growth (Fig. 4). SFA initially accounted for the majority of the high FA content (> 80%), but decreased to ~ 55% as the cells entered the senescence phase (Fig. 5). The percentages of C18:0, C18:1n9 and C16:0 also were at their highest on Day 10 (~36%, ~10% and ~45%, respectively) before declining with increased cell growth (Fig. 5). In contrast, PUFA, and the essential DHA and EPA all increased through exponential to their maximum concentrations in senescence, and represented an increasing percentage of the total FA. These findings are consistent with those from laboratory batch culture experiments where PUFA and DHA increase during

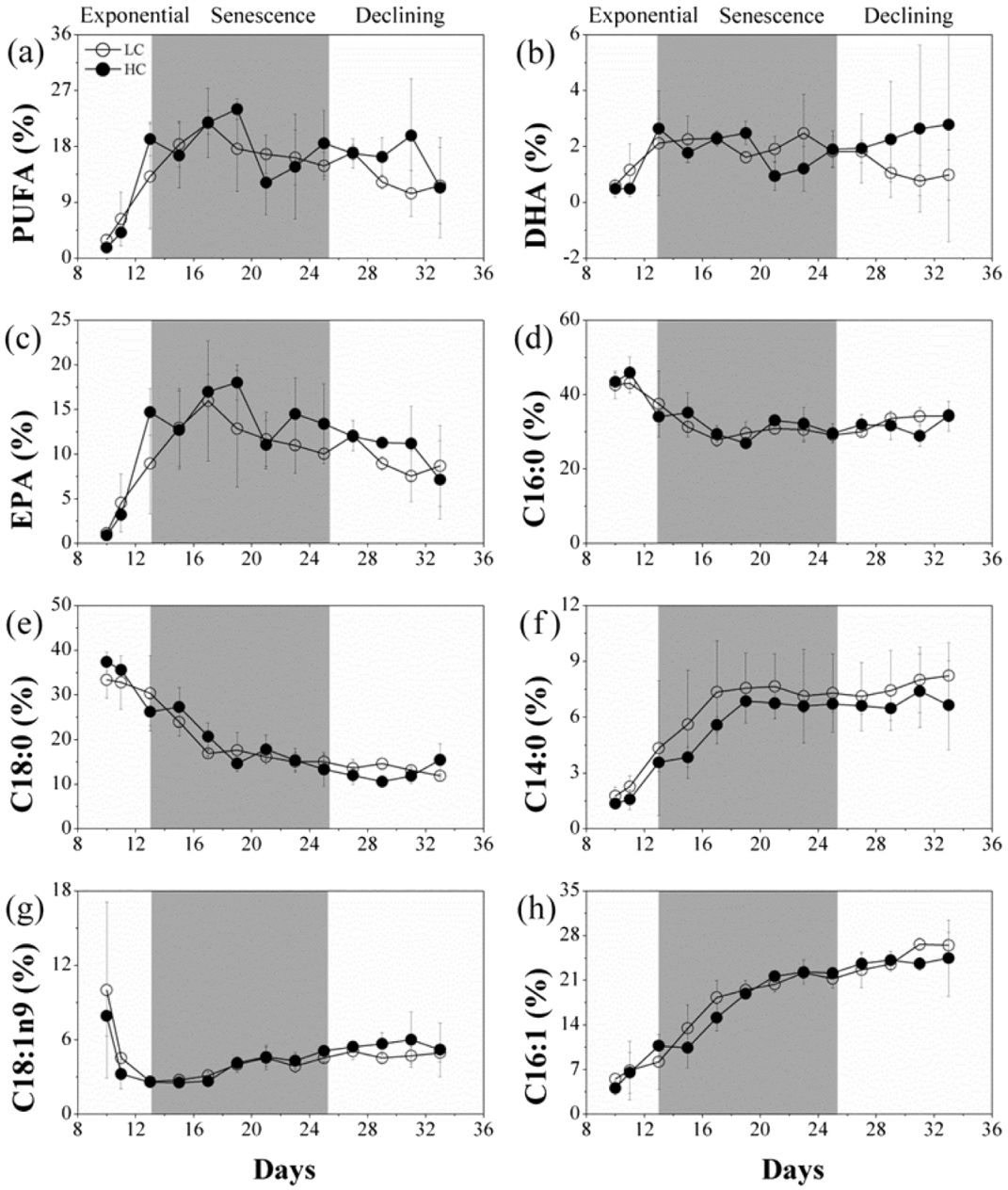

Fig. 5. Proportions of some essential fatty acids, (a) PUFA, (b) DHA, (c) EPA and (d) C16:0, (e) C18:0, (f) C14:0, (g) C18:1n9 in the LC/HC mesocosm phytoplankton. Among these, C16:1 and EPA were diatom biomarkers, while DHA is the main biomarker of flagellates. Data are means ± SD (n = 3).

exponential phase then maintain constantly or decrease slightly during senescence (Yongmanltchal and Ward, 1992; Fidalgo et al., 1998; Lin et al., 2007). However, higher nitrate availability can lead to higher total FA (*P. tricornutum*, *Chaetoceros* sp., *Isochysis galbana*, and *Pavlova lutheri*) (Reitan et al., 1994), while low nitrate concentrations can lead to lower or constant DHA and EPA contents (*I. galbana*, *Chaetoceros calcitrans*, and *Thalassiosira pseudonana*) (Harrison et al., 1990).

Phytoplankton FA composition did not differ significantly between the LC controls and HC treatments at Day 10, when there was difference in pH, or during the senescence and declining phases, when there was no difference in pH. However, coincident and marked OA-induced differences in FA composition were observed during the late exponential phase between Day 10–13 (Fig. 6). But the responses of different phytoplankton species and communities vary under different pCO_2 conditions. Elevated pCO_2 generated increasing total FA and oleic acid (C18:1n9) in the green algal species (*Chlorella vulgaris*) (Tsuzuki et al., 1990), increasing FA and decreasing PUFA in the chrysophyte *Pavlova lutheri* (Carvalho and Malcata, 2005), increasing FA and increasing PUFA and EPA in a *Nannochloropsis* sp. (Hu and Gao, 2003), and decreasing FA and PUFA contents in the diatom *Thalassiosira pseudonana* (Rossoll et al., 2012). The non-uniform effect of OA on FA composition among phytoplankton species highlights why it is necessary to study both the direct effects of OA on cell physiology as well as indirect effects arising from changes in phytoplankton community composition.

The decline in total FA, SFA, and MUFA contents during exponential growth phase were faster in the LC controls than the HC treatments (Fig. 4a, b, c). The contrasting increases in PUFA, DHA and EPA concentrations during this period were significantly greater in HC treatments relative to the LC controls (Fig. 4d, e, f). These findings clearly show that OA had a positive impact on FA synthesis overall, contributing to greater energy storage and FA reserves for membrane integrity. Acidification enhances a key process in FA synthesis, the TCA cycle that provides energy and acetyl-CoA to fuel cellular responses to

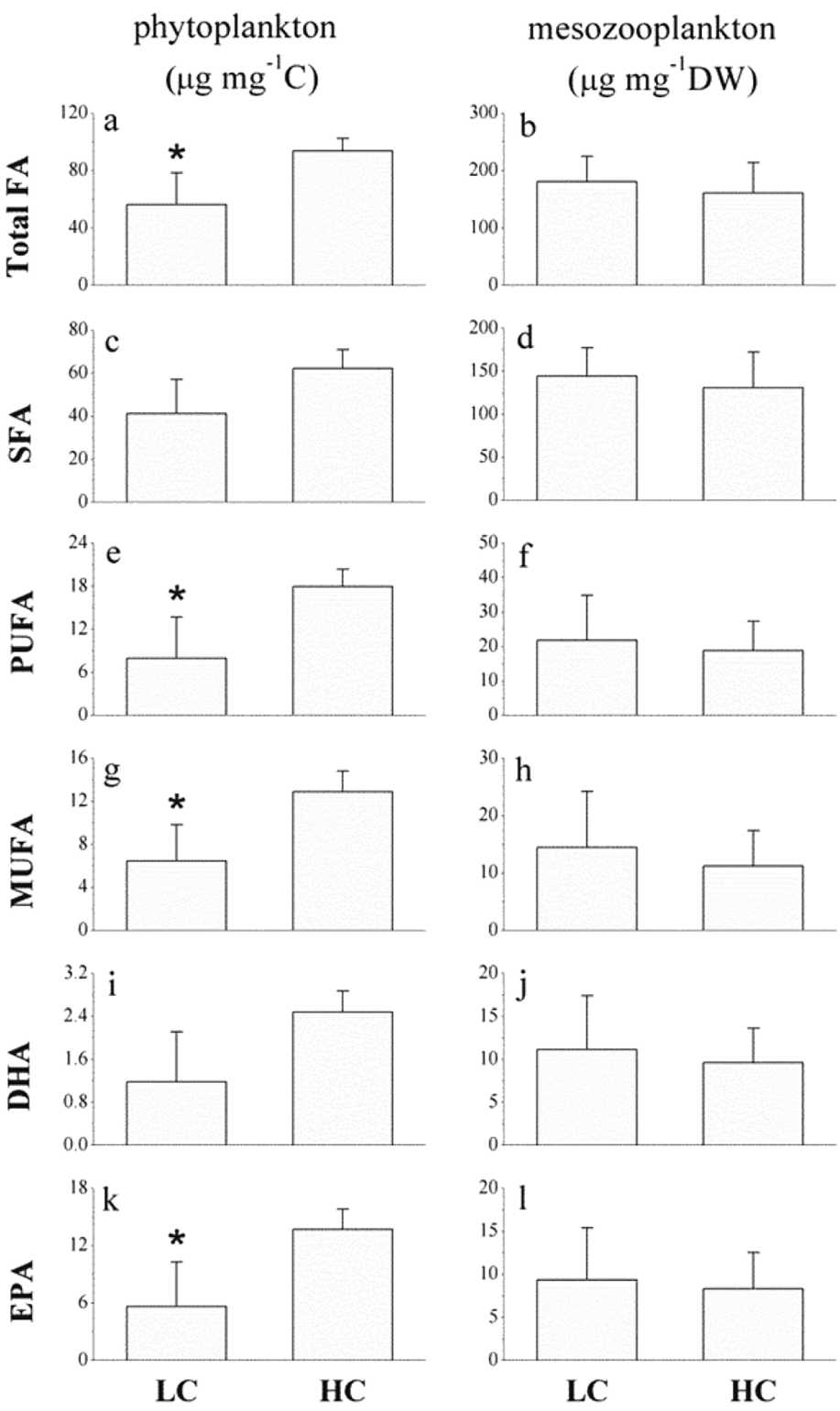

Fig. 6. The contents of total FA, SFA, PUFA, MUFA, DHA and EPA of the phytoplankton (a, c, e, g, i and k) on Day 13 and mesozooplankton (b, d, f, h, j and l) after fed with phytoplankton in matched mesocosms for three days. The symbols "*" in the panel show significant differences ($p < 0.05$) between LC control and HC treatments, and the data are means ± SD (n = 3 – 6).

acidic stress (Jin et al., 2015). Dark respiration also increases under high pCO_2 conditions (Gao et al., 2012a; Wu et al., 2010), which, in turn, would increase acetyl-CoA production and thus support greater FA synthesis. The increased contents of PUFA and MUFA in the HC treatments during late exponential phase (Fig. 6e, g) might be related to enhanced FA desaturating processes that have been observed under high CO_2 concentrations (Tsuzuki et al., 1990).

There is a marked lack of agreement among mesocosm studies on the effects that elevated pCO_2 have on the FA content of natural phytoplankton communities. Leu et al. (2013) found that increasing pCO_2 led to a small but significant increase in the proportion of PUFA as total FA content in post spring bloom waters of the Arctic Norwegian coast, while Bermúdez et al. (2016a) found the opposite trend in a more southern Norwegian fjord. These changes in both cases were attributed primarily to shifts in the phytoplankton community composition under the elevated pCO_2 conditions. Conversely, Bermúdez et al. (2016b) showed that increasing pCO_2 in brackish coastal waters of the Baltic Sea, which experience naturally high fluctuations in pCO_2, had no

Table 1
The fatty acids composition and content of mesozooplankton fed phytoplankton from LC and HC mesocosms from Day 12–15. Data are means ± SD (n = 3).

Fatty acids	LC		HC	
	Composition	Content	Composition	Content
	(%)	(μg FA mg^{-1} DW)	(%)	(μg FA mg^{-1} DW)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
C8:0	0.26 ± 0.05	0.48 ± 0.16	0.28 ± 0.05	0.44 ± 0.15
C10:0	0.92 ± 0.15	1.66 ± 0.47	0.98 ± 0.16	1.57 ± 0.51
C12:0	0.77 ± 0.16	1.38 ± 0.43	0.84 ± 0.11	1.37 ± 0.50
C14:0	2.19 ± 0.315	3.97 ± 1.28	2.26 ± 0.13	3.64 ± 1.23
C15:0	0.36 ± 0.11	0.67 ± 0.34	0.30 ± 0.16	0.48 ± 0.34
C15:1	0.15 ± 0.02	0.26 ± 0.06	0.14 ± 0.01	0.23 ± 0.09
C16:0	41.52 ± 3.36	74.35 ± 16.52	42.60 ± 1.79	68.24 ± 21.08
C16:1	4.89 ± 3.50	9.50 ± 8.64	4.13 ± 1.57	6.76 ± 4.37
C17:0	0.33 ± 0.02	0.61 ± 0.18	0.32 ± 0.03	0.52 ± 0.19
C18:0	33.67 ± 5.49	60.03 ± 15.23	33.43 ± 2.51	53.81 ± 17.59
C18:1n9C	1.03 ± 0.11	1.84 ± 0.44	1.09 ± 0.16	1.78 ± 0.78
C18:1n9T	1.26 ± 0.22	2.34 ± 0.90	1.19 ± 0.23	1.98 ± 0.98
C18:2n6C	0.47 ± 0.10	0.86 ± 0.36	0.42 ± 0.07	0.69 ± 0.29
C20:0	0.36 ± 0.08	0.64 ± 0.21	0.30 ± 0.03	0.49 ± 0.17
C20:5n3	5.04 ± 2.34	9.36 ± 6.04	5.12 ± 1.55	8.32 ± 4.22
C22:6n3	5.97 ± 2.32	11.12 ± 6.29	5.89 ± 1.17	9.59 ± 4.02
C24:0	0.12 ± 0.02	0.21 ± 0.07	0.10 ± 0.03	0.17 ± 0.08
C24:1	0.21 ± 0.07	0.39 ± 0.18	0.22 ± 0.03	0.35 ± 0.11
Total FA	100	180.60 ± 44.50	100	161.07 ± 53.08
PUFA	11.72 ± 4.9	21.81 ± 13.11	11.58 ± 2.71	18.84 ± 8.51
MUFA	7.60 ± 3.66	14.44 ± 9.78	6.82 ± 1.81	11.21 ± 6.15
SFA	80.68 ± 8.43	144.34 ± 32.97	81.59 ± 4.19	131.02 ± 41.49

Table 2
The fatty acids composition and content of phytoplankton of LC/HC mesocosms on Day 13. Data are means ± SD (n = 3). The symbols "*" in table show significant differences between LC controls and HC treatments ($p < 0.05$).

Fatty acids	LC		HC	
	Composition	Content	Composition	Content
	(%)	(μg FA mg^{-1}C)	(%)	(μg FA mg^{-1}C)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
C8:0	0.10 ± 0.02	0.06 ± 0.03	0.09 ± 0.03	0.08 ± 0.03
C10:0	0.33 ± 0.08	0.20 ± 0.13	0.30 ± 0.08	0.28 ± 0.10
C12:0	0.69 ± 0.12	0.40 ± 0.22	0.68 ± 0.10	0.65 ± 0.16
C13:0	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C14:0	4.33 ± 3.62	2.40 ± 1.78	3.58 ± 0.49	3.38 ± 0.75
C15:0	0.28 ± 0.07	0.16 ± 0.08	0.38 ± 0.15	0.36 ± 0.13
C15:1	0.11 ± 0.02	0.06 ± 0.02	0.10 ± 0.01	0.10 ± 0.02
C16:0	37.50 ± 8.89	20.71 ± 8.37	34.07 ± 2.06	32.03 ± 4.10*
C16:1	8.20 ± 4.33	4.64 ± 2.56	10.72 ± 1.80	10.03 ± 1.66
C17:0	0.16 ± 0.03	0.09 ± 0.04	0.17 ± 0.0	0.16 ± 0.00
C18:0	30.35 ± 8.44	16.77 ± 7.20	26.18 ± 3.10	24.61 ± 4.04
C18:1n9C	1.16 ± 0.11	0.64 ± 0.23	1.09 ± 0.08	1.02 ± 0.15
C18:1n9T	1.48 ± 0.39	0.86 ± 0.47	1.49 ± 0.10	1.40 ± 0.11
C18:2n6C	1.60 ± 1.22	0.87 ± 0.60	1.35 ± 0.11	1.27 ± 0.21
C18:3n3	0.27 ± 0.47	0.14 ± 0.23	0.14 ± 0.25	0.15 ± 0.26
C18:3n6	0.17 ± 0.30	0.08 ± 0.15	0.25 ± 0.09	0.24 ± 0.09
C20:0	0.41 ± 0.08	0.23 ± 0.09	0.39 ± 0.05	0.37 ± 0.06
C20:3n6	0.03 ± 0.04	0.02 ± 0.02	0.01 ± 0.02	0.01 ± 0.02
C20:4n6	0.02 ± 0.04	0.01 ± 0.02	0.07 ± 0.01	0.07 ± 0.01*
C20:5n3	8.94 ± 5.57	5.66 ± 4.66	14.70 ± 2.63	13.71 ± 2.11
C21:0	0.09 ± 0.02	0.05 ± 0.02	0.08 ± 0.01	0.08 ± 0.01
C22:0	0.22 ± 0.11	0.13 ± 0.07	0.24 ± 0.09	0.22 ± 0.08
C22:1N9	0.15 ± 0.03	0.09 ± 0.05	0.11 ± 0.01	0.11 ± 0.01
C22:6N3	2.11 ± 1.88	1.18 ± 0.93	2.64 ± 0.34	2.48 ± 0.39
C24:0	0.96 ± 0.68	0.56 ± 0.38	0.89 ± 0.26	0.83 ± 0.24
C24:1	0.29 ± 0.31	0.17 ± 0.16	0.26 ± 0.08	0.24 ± 0.08
Total FA	100	56.21 ± 22.18	100	93.90 ± 8.58
PUFA	13.15 ± 8.41	7.96 ± 5.74	19.17 ± 2.76	17.94 ± 2.44*
MUFA	11.28 ± 5.10	6.47 ± 3.35	13.67 ± 2.00	12.90 ± 1.92*
SFA	75.47 ± 13.26	41.22 ± 15.87	67.06 ± 4.74	62.22 ± 8.75

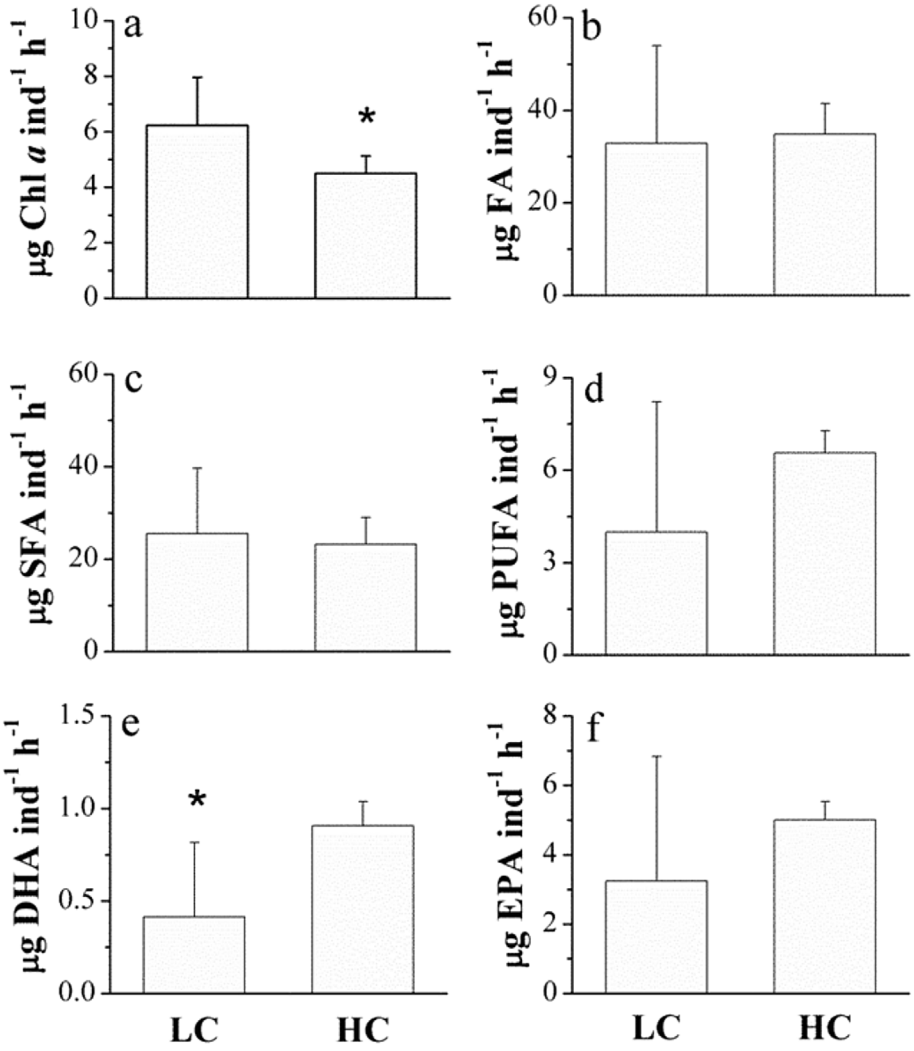

Fig. 7. The grazing/intake rates of mesozooplankton on phytoplankton from the LC controls and HC treatments normalized to (a) Chl *a*, and (b) total FA, (c) SFA, (d) PUFA, (e) DHA, (f) EPA. The symbols "*" in the panel show significant differences ($p < 0.05$) between LC controls and HC treatments, and data are means ± SD (n = 4 - 6).

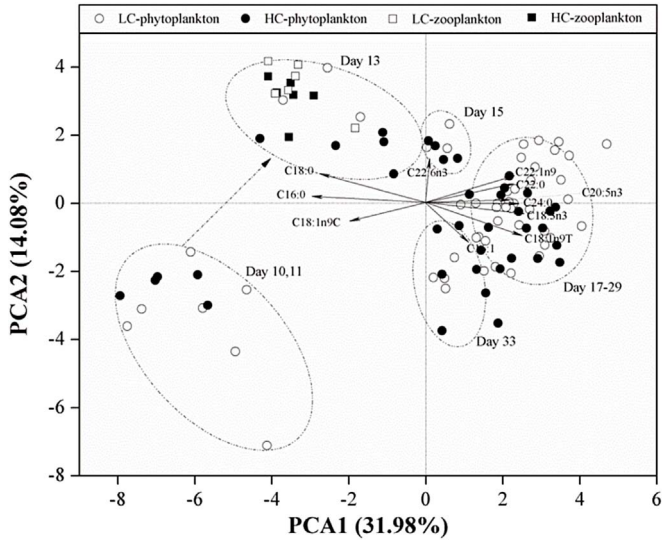

Fig. 8. The principal component analysis (PCA) of fatty acids based on mean compositions of LC and HC mesocoms. The PCA1 and PCA2 explain 31.89% and 14.08% of variation, respectively. The oval circle shows samples from different sampling time. The solid arrow shows the importance of FA in explain the variances in length.

significant effect on either FA or phytoplankton community composition; evidence that elevated pCO_2 had little physiological effects of on FA content among the dominant dinophyta species present. However, we show that elevated pCO_2 can induce physiological-driven shifts in FA composition among at least the dominant diatom species tested here, suggesting that future ocean acidification may affect FA production through both altered cell physiology as well as changing phytoplankton community composition.

The mesozooplankton grazing experiments were timed to coincide with the end of the exponential phase to study OA effects at the peak of the bloom. Comparatively short (3 day) incubation times were used here for two reasons. First, it limited the potential for significant changes in the measured phytoplankton FA composition at the start of the incubation. Second, warm water copepods have much faster turnover times than high latitude species, typically feed continuously, storing less FA, and using ingested food immediately for growth and reproduction. These characteristics mean there is tighter coupling between food and copepod FA composition than would occur in high latitude species, so the 3 day experiments here should be sufficient to for FA composition to shift to reflect the dietary composition.

Long chain FA in phytoplankton are a key nutritional constituent in the diets of mesozooplankton (Brett and Müller-Navarra, 1997), so it was surprising that OA-induced changes in phytoplankton FA composition had no discernable effect on grazer FA composition (Fig. 6). This result seems at odds with earlier studies showing a strong linkage between phytoplankton and copepods (Brett et al., 2006; Rossoll et al.,

2012; Bermúdez et al., 2016a). However, mesozooplankton grazing rates here were significantly lower in copepods fed with the HC treated cells, which likely contributed to the weak coupling between phytoplankton and mesozooplankton FA contents. The reason for the lower grazing rates is not understood. It seems unlikely that the mesozooplankton would have experienced short term OA "shock" when placed into the HC water because this lower pH is within the range these organisms experience with vertical migration in the eutrophic bay. Previous work also has shown that grazing rates of mesozooplankton from this region are not affected by similar decreases in pH Li and Gao (2012). However, pH was not regulated during the 3-day grazing experiment, beyond adding fresh mesocosm water every day, so it is possible that pH decreased below optimal levels for these copepods. Alternatively, phytoplankton FA concentrations can change rapidly with OA stress (Rossoll et al., 2012), so small pH fluctuations during the grazing incubation might explain the suggested decoupling of phytoplankton and mesozooplankton FA contents. It also is possible that the differences in phytoplankton FA contents measured here were too small to be detectable in the mesozooplankton FA pool.

5. Conclusions

The observed higher contents of PUFA and MUFA in late exponential phase under elevated pCO_2 indicate increased nutritional value for zooplankton and higher trophic levels. Our findings show that these selected phytoplankton species have a relatively high tolerance to acidification in terms of FA production, and that local mesozooplankton in these subtropical coastal waters appear to maintain their FA composition under end of century ocean acidification conditions.

Finally, the vast majority of work done examining the linkages between phytoplankton and mesozooplankton FA have focused on high latitude, cold water copepods, which develop large lipid storage reserves. Low latitude, warm water copepods have higher metabolic rates, and closer coupling between feeding and reproduction. Future studies are needed to evaluate whether the apparent lack of coupling of FA composition in our experiments results from unique stress factors in culturally eutrophic waters, or to inherent differences between trophic interactions between phytoplankton and zooplankton in high and low latitude coastal waters.

Author contributions

K. Gao designed the experiment; T. Wang conducted the measurement of phytoplankton and mesozooplankton fatty acid and mesozooplankton grazing rates and drafted the paper. S. Tong measured POC/PON. N. Liu measured the Chl *a*. F. Li measured the pH and silicate nutrient. All authors reviewed the paper. K. Gao and M. L. Wells were involved in data analysis and paper writing.

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